CHEMICAL INVESTIGATION OF INDIAN MEDICINAL PLANTS AND STUDIES ON SOME OXIDATIVE AND REDUCTIVE REACTIONS ON TRITERPENOIDS

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY (SCIENCE) OF THE
UNIVERSITY OF NORTH BENGAL
1984

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:o: ACKNOWLEDGEMENT :o:

The present thesis embodies the results of research carried out by the author at the Department of Chemistry, University of North Bengal, Dist; Darjeeling, West Bengal.

The author takes this opportunity to record his grateful thanks to Dr.B.P.Pradhan, M.Sc.,Ph.D., Reader in Chemistry, University of North Bengal for supervising the work with his valuable suggestions and affectionate guidance.

The author is indebted to Dr.J.N.Shoolery, Varian Associates, Palo Alto, California, U.S.A., for most of the NMR spectra recorded in the thesis, to Dr.P.M.Scopes, Imperial College, London, for the NMR, CD and optical rotations, to Dr.M.L.Sharma, Chandigarh, to Dr.S.Tyagrajan, Director, R.R.L., Hyderabad for some of the NMR spectra, to Dr.Allen Duffield, Biomedical Dept., University of New South Wales, Australlia, to the Director, C.D.R.I., Lucknow for the mass spectra recorded in the thesis, to Dr.S.K.Sengupta, E.I.P.W. for optical rotations and to authorities of Alfred Bernhardt Mikroanalytiches Laboratorium, West Germany, for the microanalysis recorded in the thesis.

The author is also grateful to Prof.D.H.R.Barton, Director, Institut de Chimie des substances Naturelles, C.N.R.S., Gif-Sur-Yvette, France for sending some valuable reprints and suggestions.

The author is thankful to the University Grants Commission, New Delhi for awarding a J.R.F.

The author will remain ever indebted to Dr.S.L.Dutta (formerly instrumentation officer) and Dr.S.K.Saha (formerly Scientific Officer), N.B.U. for the IR spectra recorded in the thesis.

The author is grateful to the authorities of Sukna forest, Darjeeling, for the collection and identification of plant materials.

The author expresses his warmest thanks to the fellow research workers, N.B.U. for their kind cooperation and encouragement during the progress of the work. Thanks are also due to Dr.S.N.Bose, Lecturer in Chemistry and Dr.T.K.Roy, Research Assistant at the Department for helpful discussions.

The author is indebted to his teachers, Dr.A.K. Ghosh, Dr.S.C.Roy and Dr.S.K.Majumder for their constant inspiration and encouragement.

The author's thanks are also due to Dr.S.K.Roy, Director and Dr.A.C.Das Gupta, S.S.O., Central Drugs Laboratory for their constant inspiration.

Thanks are also due to Mr.S.K.Mitra, Dr.M.K. Bhattacharjee, C.D.L. and Dr.B.Banerjee for their help in the compilation of the thesis and also to Mr.C.C.Chakraborty, Bengal Immunity Research Institute, Calcutta for typing the manuscript.

The author is thankful to his younger brother, Dr.Malay Kumar Chakraborti (M.B.B.S.), to his sisters, sister-in-law and other relatives for their constant inspiration. Finally the author expresses his deep regards to his parents for their inspiration and encouragement.

Dilip Kumar Chakraborti.

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S U M M A R Y

The work embodied in the present thesis has been divided into four parts.

PART—I

STUDIES ON THE REDUCTION WITH LITHIUM-ETHYLENEDIAMINE ON TRITERPENOIDS

CHAPTER—I

It gives a short review of metal dissolving reactions in presence of base.

CHAPTER—II

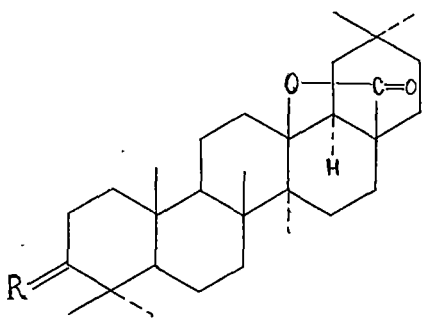
This chapter deals with the studies on the reduction of triterpenoid lactones (secondary & tertiary) with different sterical hindrances; 3-keto; isopropenyl double bond and sterically hindered esters.

S E C T I O N—A

(a) Studies on tertiary lactones:

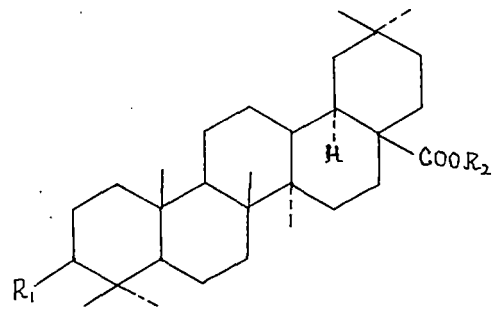
This section deals with the products obtained on reduction of 3-acetyl oleanan-18α-H-28→13β-olide 1 with Li-ethylenediamine. The products obtained after

refluxing for two hours under N_2 atmosphere have been separated by column chromatography. The first eluted, m.p. 270—71°, $C_{30}H_{50}O_2$, $[\alpha]_D$ +8.8°, has been characterised as oleanan-18-α-H-28-oic acid 2a, by means of PMR and mass spectral analysis. The second compound 2b, $C_{30}H_{50}O_3$, m.p. 295—96°, $[\alpha]_D$ +15°, (M^+ 458), methyl ester 2c, $C_{31}H_{52}O_3$, m.p. 198° (M^+ 472), acetyl acid 2d, $C_{32}H_{52}O_4$, m.p. 290°—91° (M^+ 500), was obtained.



1, R = OAc / H

1a, R = O



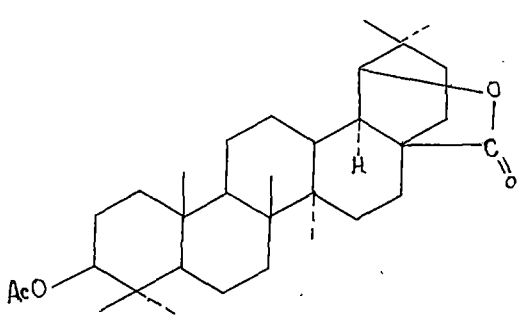
2a, R_1 = H ; R_2 = H
2b, R_1 = OH ; R_2 = H
2c, R_1 = OH ; R_2 = CH_3
2d, R_1 = OAc; R_2 = H

From the study of IR, NMR and mass spectral analysis, the second compound has been identified as 3-hydroxyoleanan-18α-H-28-oic acid 2b.

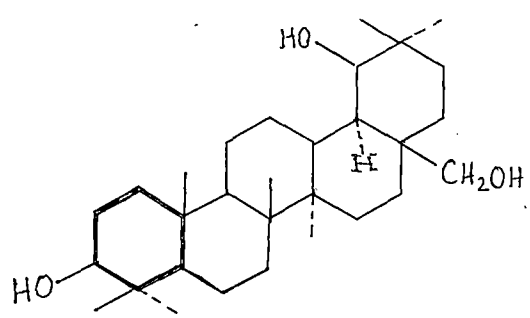
The reaction was repeated on 3-oxo-oleanan-18α-H-28→13β-olide 1a. The product isolated contained only one compound which was obtained in 85% yield and had the m.p. 295—96°. It has been characterised as 3β-hydroxy-oleanan-18α-H-28-oic acid 2b by preparation of its methyl ester and the acetate derivatives.

(b) Studies on secondary lactones:

The third compound that was studied is 3-acetyl-oleanan-18α-H-28→19β-olide 3. The products obtained after reduction were separated into acid and neutral parts. The acid part on chromatographic separation yielded the first compound, m.p. 269—70°, $[\alpha]_D$ +8.8°, that was characterised as oleanan-18α-H-28-oic acid 2a (by mmp and GC—IR comparison). The next polar compound from the acid part had m.p. 295—96°, $[\alpha]_D$ +14.5°. The compound was characterised as 3-hydroxy-oleanan-18α-H-28-oic acid 2b, by preparation of its methyl ester and the acetate derivatives. The neutral part afforded a compound (yield 15%) that had the



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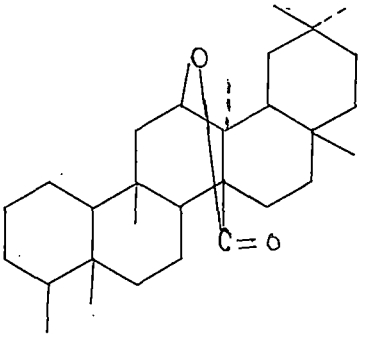


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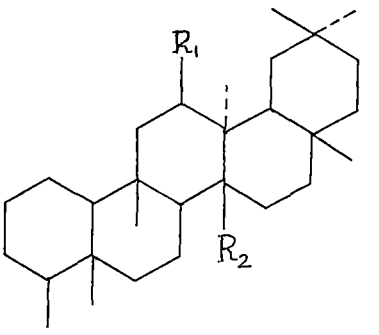
m.p. 290—92°, $[\alpha]_D$ +23°, m/e 442 ($M^+ - H_2O$), TNM test negative, was characterised as olean-18α-H-3β, 19β, 28-triol 4, by NMR and mass spectral analysis and preparation of its acetate derivative (triacetate), m.p. 211—12°.

(c) Studies on sterically hindered secondary lactones:

(i) 3-deoxy-adolactone—friedelan-26→12β-olide 5, on reduction with lithium-ethylenediamine afforded two different compounds. The acidic component, m.p. 290—91°, $[\alpha]_D$ +28.57° (M^+ 442), methyl ester of the compound had the m.p. 180°, $[\alpha]_D$ +30.3° (M^+ 456), has been characterised as methyl-3-deoxy-trichadenate 6b, by study of mass and NMR spectral analysis.



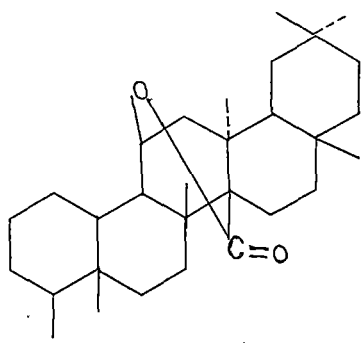
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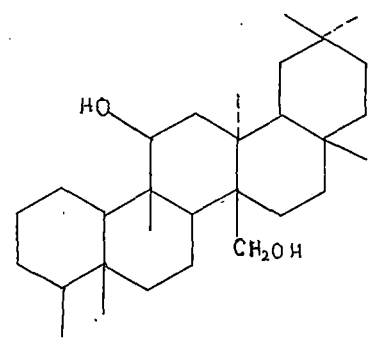
6a, R_1 = H; R_2 = COOH
6b, R_1 = H; R_2 = $COOCH_3$
6c, R_1 = OH; R_2 = CH_2OH

The neutral part of the above reaction product afforded a compound, $C_{30}H_{50}O_2$, m.p. 240—41°, m/e 426 (M^+—H_2O), PMR signals at 0.724 (d, J = 6Hz), 0.797, 0.899, 0.967, 0.983, 1.002, 1.244 ppm for seven methyl groups, 4.0 (2H, AB, q), 3.88 (1H, m) ppm, has been characterised as friedelan-12 β ,26-diol 6a.

(ii) Reduction of friedelan-3→ 11 β -olide 7, with lithium-ethylenediamine on reflux for two hours afforded one acidic component and one neutral component. The acid component on crystallisation furnished a compound, $C_{30}H_{50}O_2$, m.p. 293—94°, the methyl ester had the m.p.180°, has been characterised as 3-deoxy-trichadenic acid 6a.



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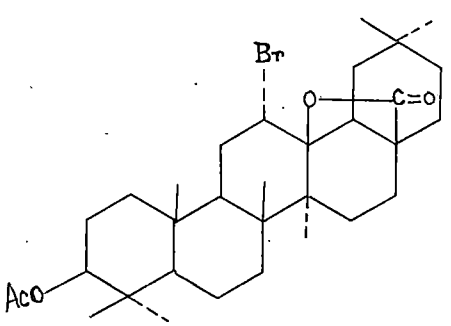
The neutral fraction on crystallisation furnished a solid (75%), m.p. > 350°, m/e 426 (M^+—H_2O), has been

characterised as friedelan-11 β , 28 diol 8 from mass and NMR spectral analysis.

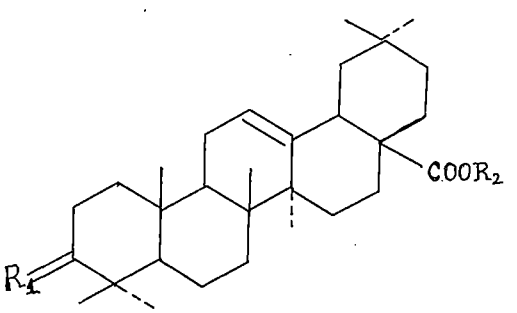
(d) Studies on triterpenoid bromolactones:

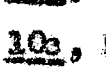
In order to examine the effect of bromine on the nature and yield of the products formed on lithium-ethylenediamine reduction the following compounds have been selected and studied for the purpose:

(1) Reaction on 3-acetyl-12 α -bromo-oleanan-28⟶13 β -olide 9, with lithium-ethylenediamine furnished the acidic components. The first one, m.p. 265—66°, was characterised as 3-deoxy-oleanolic acid 10a, by preparing its methyl ester 10b, m.p. 169°, TNM test positive.



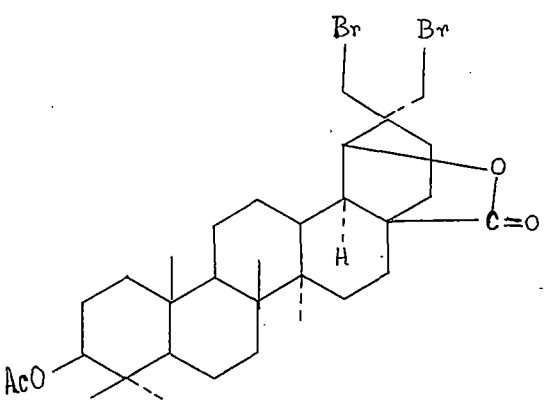
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10a, $R_1 = H_2$; $R_2 = H$
10b, $R_1 = H_2$; $R_2 = CH_3$
10c, $R_1 =$  ; $R_2 = H$
10d, $R_1 =$  ; $R_2 = CH_3$
10e, $R_1 = O$; $R_2 = CH_3$

The second compound isolated was analysed for $C_{30}H_{48}O_3$, m.p. 303—4°, was characterised as oleanolic acid 10c, by preparing its methyl ester 10d, m.p. 198—99°, (M^+470). FNM test was found to be positive.

(ii) Reaction on 3-acetyl-29,30-dibromo-oleanan-18α-H-28→19β-olide 11, with lithium-ethylenediamine furnished two acidic compounds. The first one, m.p. 270—71°, has been identified as oleanan-18α-H-28-oic acid 2a and the second one was identified as 3-hydroxy-olean-18α-H-28-oic acid 2b, (by mmp and CO—IR comparison with authentic samples).



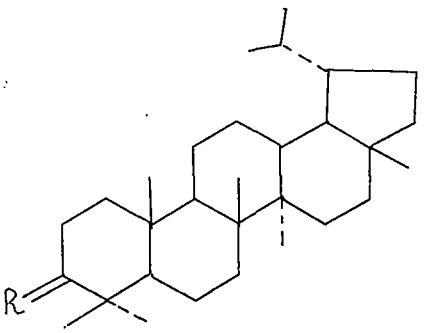
11

S E C T I O N—B

(a) Studies on 3-keto compounds:

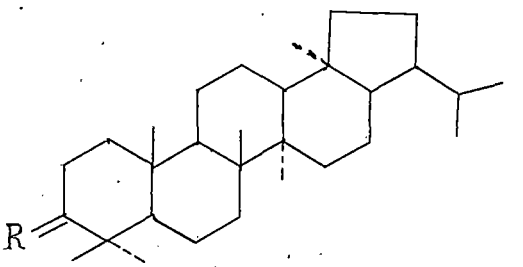
(i) Reaction of lupanone 12, with lithium-ethylenediamine furnished a single compound, m.p. 205°,

$[\alpha]_D$ -17.8°, that was identified as lupanol 12a.



12, R = O

12a, R = OH / H



13, R = O

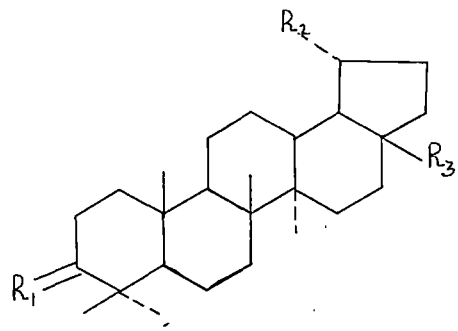
13a, R = OH / H

(ii) Reaction on moretanone 13 with lithium-ethylenediamine furnished a single compound, m.p. 223—24°, that was identified as moretanol 13a.

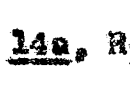
(b) Studies on hindered esters:

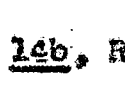
(i) Esters containing 3-keto and isopropenyl groups:

Reduction of methyl betulonate 14 with lithium-ethylenediamine furnished dihydrobetulinic acid 14a (yield 80%), m.p. 323—24°, $[\alpha]_D$ +26.0° (M^+ 458), confirmed by preparing acetyl derivative 14b, m.p. 310—11°, $[\alpha]_D$ -11.5° (M^+ 500) and comparison with authentic sample.



14, R_1 = O; R_2 = ⟨ ; R_3 = $COOCH_3$

14a, R_1 = ; R_2 =  ; R_3 = COOH

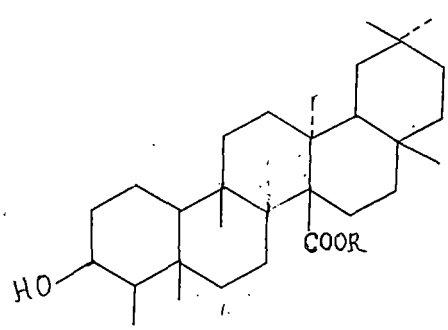
14b, R_1 = ; R_2 =  ; R_3 = COOH

(ii) Ester containing 3-keto, 12-13 double bond:

Reaction of methyl oleanonate 10a with lithium-ethylenediamine furnished a single compound, m.p. 301—302°. It was identified as oleanolic acid 10c (by map and IR comparison with authentic specimen).

(iii) Sterically hindered ester:

Reaction of methyl trichadenate A 15, with lithium-ethylenediamine furnished a single compound, m.p. 330—32°, $[\alpha]_D$ +35°, identified as trichadenic acid A 15a (by comparison with authentic sample).



15, R = CH_3

15a, R = H

S E C T I O N—C

C o n c l u s i o n:

From the results obtained on reduction of lactones it may be concluded that the tertiary lactyl oxygen is cleaved at C—O bond to furnish the acids. These observations are in accordance with those that reported by Barton and his coworkers on the reduction of esters where the carbonyl group is attached to the tertiary carbon atom. It is noteworthy to mention here if the bromine is suitably attached to the carbons α to the lactyl oxygen then the acid produced generates a double bond at the carbon atom to which lactyl oxygen is attached, whereas the other tertiary lactones, furnished saturated acids.

The reduction of secondary lactones 3 and 7 furnished comparatively larger amounts of diols (4 and 8) compared to the acids suggesting the facile cleavage of $-\overset{O}{\overset{\|}{C}}-O$ bond of lactone ring, showing that these lactones are comparatively less sterically hindered whereas the lactones 5 and 11 gave more of the acids and negligible amount of diols, suggesting that these secondary lactones are sterically much hindered thereby causing deoxygenation at carbon where lactyl oxygen is attached.

The 3-keto compounds on reduction gave the thermodynamically more stable alcohols with 100% purity. The hindered esters easily underwent reduction to give acids. The isopropenyl group has also been reduced to give the dihydro compound. A compound containing ester group, keto functional group, isopropenyl double bond can be reduced in a single step as exemplified in the case of methyl betulonate 14.

CHAPTER—III

Experimental portion has been discussed in this chapter.

PART—II

ACTION OF HYDROGEN PEROXIDE ON TRITERPENOID ALLYLIC ALCOHOLS

CHAPTER—I

This chapter gives a short review on the oxidative transformation on pentacyclic triterpenoids.

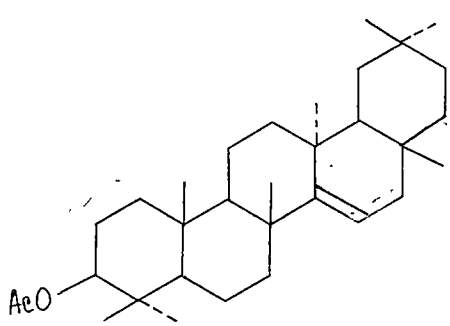
CHAPTER—II

This chapter deals with the action of hydrogen-peroxide in presence of p-toluene sulfonic acid on triterpenoid allylic alcohol in ring C and ring A.

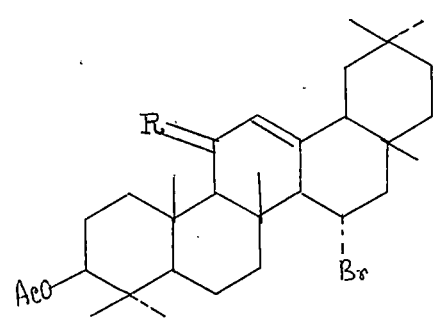
S E C T I O N—A

This section deals with the action of hydrogen peroxide on olean-12, 15-dien-3,11 diol 21, which has been prepared from taraxeryl acetate 16.

Tarexeryl acetate 16 with NBS afforded a compound 17, $C_{32}H_{51}O_2Br$, (M^+ 546, Br 81), m.p. 180—82°, $[\alpha]_D$ +47.4°, characterised as 15-bromo- β -amyrin acetate 17. Treatment of 17 with sodium dichromate in acetic acid furnished 11-oxo-15-bromo- β -amyrenyl acetate 18, $C_{32}H_{49}O_3Br$, m.p. 240—41°, $[\alpha]_D$ +88°.



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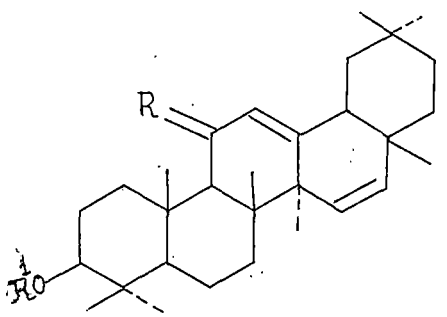


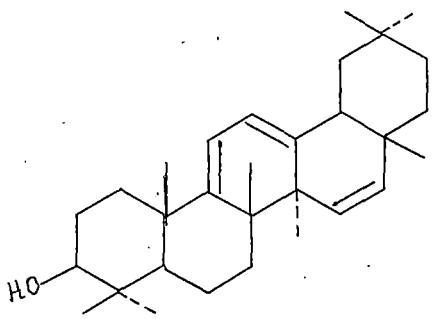
17, R = H, H

18, R = O

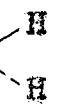
Attempts to dehydrobrominate 18 with dimethyl aniline/collidine gave the starting compound 18. A reverse route was followed to prepare the 11-oxo-12,15-diene by

dehydrobrominating 17, which furnished compound 19, $C_{32}H_{50}O_2$, m.p. 199—200°, $[\alpha]_D$ +42°. The diene 19 was oxidised to give the desired 11-oxo-12,15-diene 20, $C_{32}H_{43}O_3$, m.p. 243—45° $[\alpha]_D$ +28.6°, λ_{max} 244 nm.

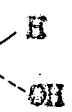


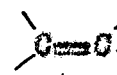


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19, R =  ; R^1=Ac

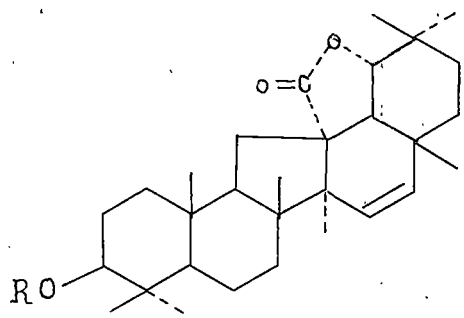
20, R = O ; R^1=Ac

21, R =  ; R^1=H

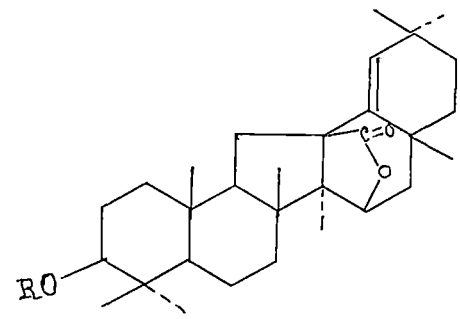
LAH reduction of the 11-oxo-diene 20, furnished a diene-diol 21, $C_{30}H_{48}O_2$, m.p. 198—200°, ν_{max} 3460 (—OH), 820 - () cm^{-1}; purification of the diol by chromatography yielded a homoannular diene 22, $C_{30}H_{46}O$, m.p. 189—90°.

The diol 21 was directly treated with hydrogen peroxide containing p-toluene sulfonic acid following the procedure adopted by Corey and his coworkers. The product furnished two compounds 23 and 25 of the same molecular formula $C_{30}H_{46}O_3$.

The compound 23, m.p. 240—41° on acetylation gave 24 and the structure of 24 was assigned as 3 β -acetate —C—12-nor-olean-13 en-13 α -carb—► 19 α -olide on the basis of ^{1}H, ^{13}C NMR and mass spectral analysis.

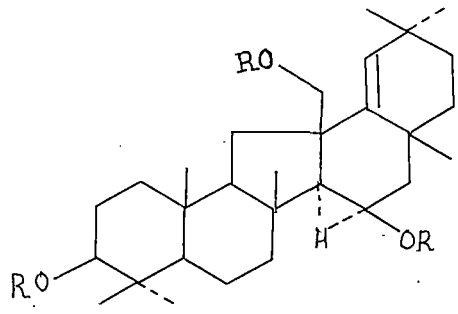


23, R = H
24, R = CH_3CO



25, R = H
26, R = CH_3CO

Another isomeric compound 25, m.p. 256—57°, obtained, was also acetylated and the acetylated product 26, $C_{32}H_{48}O_4$, m.p. 228—29° was assigned structure as 3β -acetate —C—12—nor—olean-18(19) en-13 β -carb—► 15 β -olide on the basis of ^{1}H NMR of the hydroxy lactone 25 and ^{1}H NMR and mass spectral analysis of 26. The structure of 25 was finally proved



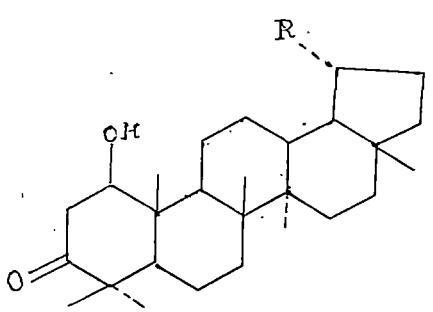
27, R = H
28, R = CH_3CO

by LAH reduction of the lactone that yielded a triol 27, $C_{30}H_{50}O_3$, m.p. 280—82°, acetylation of which gave a triacetate 28, $C_{30}H_{56}O_3$, m.p. 190—93°. The structure of the triacetate was proved by ^1H NMR, ^{13}C NMR and mass spectral analysis.

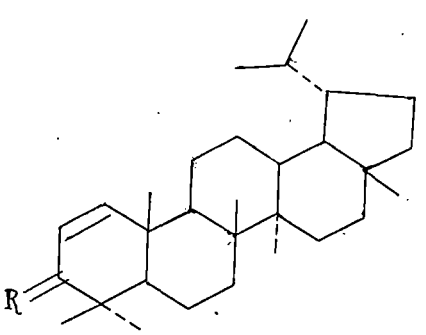
The mechanism of formation of two lactones has been also discussed.

S E C T I O N—B

The same reaction of H_2O_2 in presence of p-toluene-sulfonic acid on lup-1(2)-en-3 β -ol 31 has been carried out. Preparation of lup 1(2) en-3 β -ol has been discussed. Glochidonol 29 isolated from Glochidion accuminatum was hydrogenated using pt-catalyst to give the keto alcohol 29a. The compound 29a was dehydrated with acetic anhydride/pyridine to give



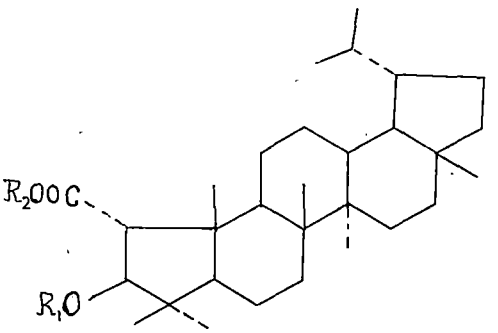
29, R = 
29a, R = 



30, R = O
31, R = 

lup-1(2)-en-3-one 30, m.p. 177—78°, $C_{30}H_{48}O$. LAH reduction of 30 gave the desired allylic alcohol, lup-1(2)-en-3 β -ol 31, $C_{30}H_{50}O$, m.p. 214—15°.

The allylic alcohol was treated with hydrogen peroxide in presence of p-toluene sulfonic acid in t-butanol. After reaction and work up followed by chromatography furnished a compound 32 , $C_{30}H_{50}O_3$; IR showed acidic function at 1695 cm^{-1}. It was esterified and gave the methyl ester 32a, $C_{31}H_{50}O_3$, m.p. 265—66°. The structure of the ester was characterised by ^1H NMR and mass spectral analysis. The ester was acetylated to give 32b, $C_{33}H_{54}O_4$, m.p. 250—51°.



32, R_1 = H ; R_2 = H
32a, R_1 = H ; R_2 = CH_3
32b, R_1 = $COCH_3$; R_2 = CH_3

The mechanism of ring A contraction has also been discussed.

Chapter III describes the experimental portion.

PART—III

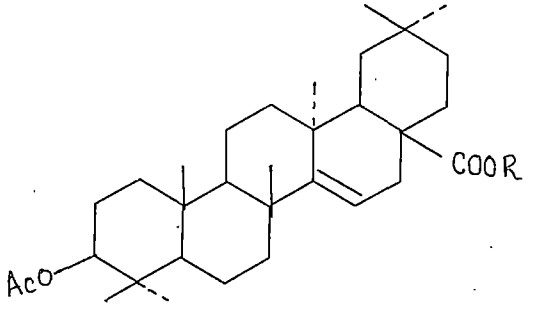
ACTION OF N-BROMOSUCCINIMIDE ON TRITERPENE ACIDS AND ESTERS

CHAPTER—I gives a short review on NBS reaction on triterpenoids.

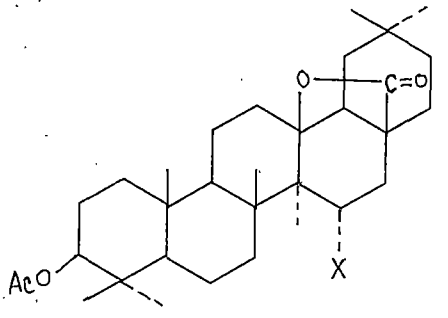
CHAPTER—II

(i) Treatment of NBS on acetyl methyl aleuritolate 33a:

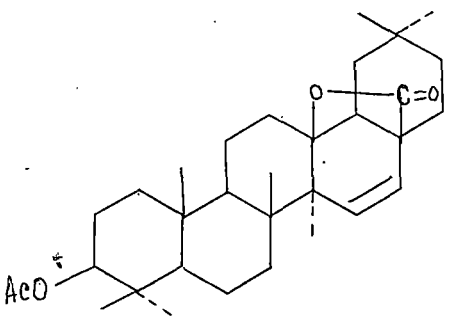
Acetyl methyl aleuritolate 33a was treated with NBS in DMSO in dark for 12 hours, followed by chromatography and crystallisation furnished a solid, analysed for $C_{32}H_{49}O_4Br$, m.p. 280—82°, $[\alpha]_D$ +19.51°, was found to be a γ -lactone from IR spectrum (ν_{max} 1780 cm^{-1}). It's structure as 34 was proved by PMR spectrum. Beilstein test was positive.



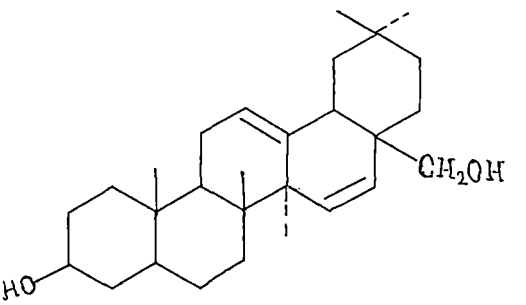
33a, R = CH_3
33b, R = H



34, X = Br
35, X = H



35



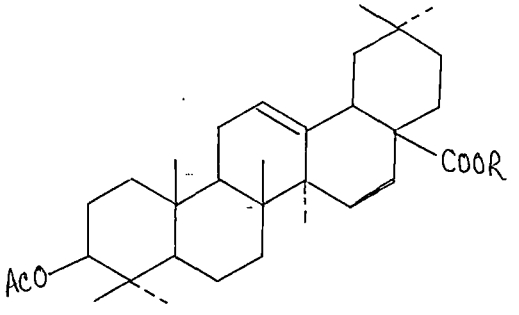
37

Repeatation of the reaction with acetyl aleuritolic acid 33b, furnished the same bromolactone 34 while compared with an authentic specimen. The structure of the bromolactone has been established as 34, on the fact that the compound on dehydrobromination with dimethylaniline afforded 15,16-dehydrolactone 35, $C_{32}H_{48}O_4$, m.p. 308—10°, which on catalytic hydrogenation afforded 3 β -acetyl-oleanan-28⟶13 olide 36, $C_{32}H_{50}O_4$, m.p. 293—94°, identical with authentic specimen (mmp and CO—IR). The compound 35 on LAH reduction furnished aegeceradiol 37, $C_{30}H_{48}O_2$, m.p. 235—36°, identical with an authentic sample (mmp and CO—IR comparison).

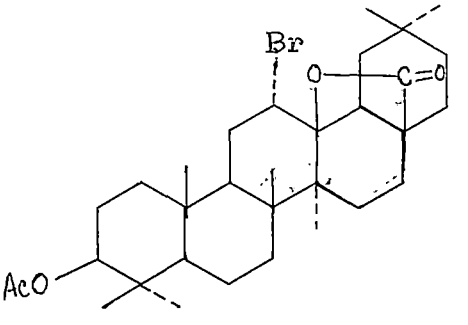
(ii) Treatment of NBS on acetyl methyl oleanolate 38a:

Acetyl methyl oleanolate 38a when treated with NBS following the same procedure afforded a compound 39, $C_{32}H_{49}O_4Br$, m.p. 215—16°, IR showed that it contains a γ -lactone; Beilstein

test for halogen was positive, was identified as 3 β -acetyl-12 α -bromo-oleanan-28$\rightarrow$13-olide 32, when compared with an authentic specimen.



38a, R = CH_3
38b, R = H

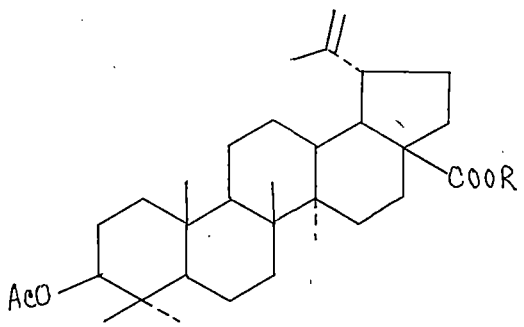


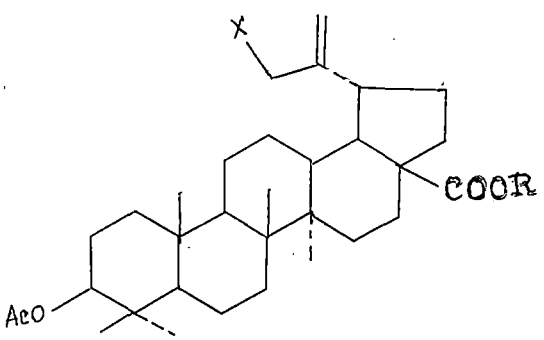
39

Acetyl oleanolic acid 38b furnished the same bromo lactone when treated with NBS/DMSO (compared with authentic specimen)

(iii) Treatment of NBS on methyl-3β-acetyl-betulenate 40a:

Methyl-3 β -acetyl-betulenate 40a on similar reaction with NBS in DMSO afforded three different bromo compounds, separated by column chromatography. The less polar one, 41a, $C_{33}H_{51}O_4Br$, m.p. 235--36°, $[\alpha]_D$ +42.55°, was identified as methyl-30-bromo-3 β -acetyl betulenate on the basis of IR,



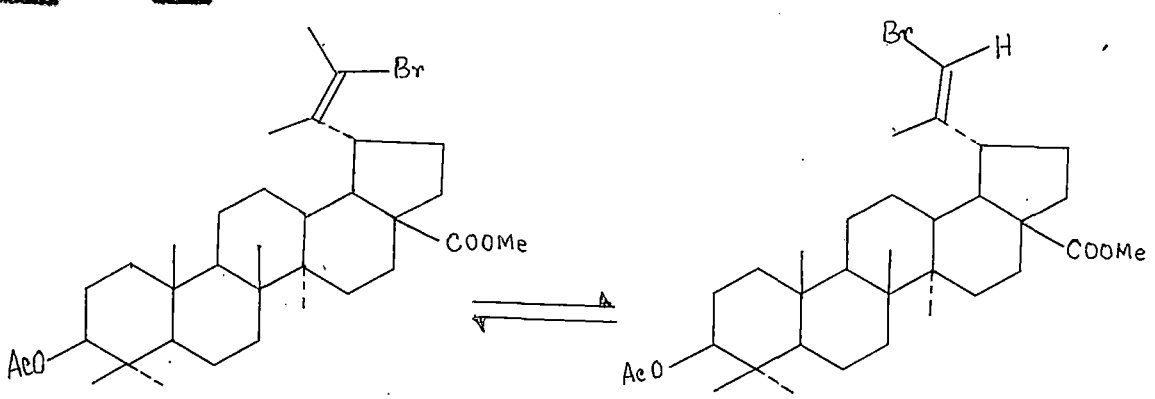


40a, R = CH_3
40b, R = H

41a, R = CH_3 ; X = Br
41b, R = H ; X = Br

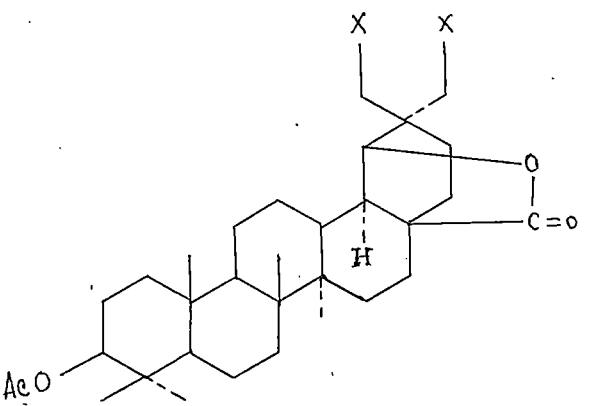
PMR spectra and further proved by debrominating 41b, with Zn/AcOH to give methyl-3β-acetyl betulenate 40a.

In the same polarity another bromo compound, $C_{31}H_{51}O_4Br$, m.p. 228--30°, $[\alpha]_D$+50° was obtained. PMR spectrum gave two singlets at 1.7 and 1.8 ppm for three protons suggested the presence of methyl group on a double bond. The structure was assigned as a mixture of cis-trans isomers 42a and 42b on the basis of total PMR spectra.



42a 42b

The third compound 43 (10%) was a dibromo lactone, $C_{32}H_{48}O_4Br_2$, m.p. 303—4°, $[\alpha]_D$ +47°, CD(n-hexane) 218 nm (ϵ = -0.99). The structure of the compound was assigned as 3 β -acetyl, 29,30, dibromo-18 αH-oleanan-28⟶ 19 β -olide 43a on the basis of PMR, ^{13}C NMR and mass spectral analysis. It resisted dehydrobromination with dimethylaniline but on debromination with Raney Nickel—hydrogen gave 43b, $C_{32}H_{50}O_4$, m.p. > 360°, $[\alpha]_D$ +59°, identical with 3 β -acetyl-oleanan-28⟶ 19 β -olide (compared with authentic specimen).



43a, X = Br
43b, X = H

3 β -acetyl betulenic acid 40b also furnished the same bromo lactone 43a on treatment with NBS in DMSO and 3 β -acetyl-30-bromobetulenic acid 41b, which was converted to the methyl ester 41a. These two compounds were identified as 43a and 41a when compared with authentic specimen.

Chapter-III describes the experimental portion.

PART—IV

CHEMICAL INVESTIGATION OF THE ROOT OF LEUCAS ASPERA SPRENG

Chapter—I gives the introduction and Chapter—II gives the morphological features of Lebiatae family and Leucas Aspera spreng

Chapter—III : Section A and B(i), deals with the extraction of the powdered root of Leucas Aspera with the ethyl alcohol, hydrolysis of the alcohol extract with 7% H_2SO_4 and extraction of the neutral fraction with ether. Column chromatography of the residue from the neutral part furnished two compounds. The first compound, m.p. 154—56°, $[\alpha]_D$ -50°, molecular formula $C_{28}H_{43}O$, was identified as stigmasterol by preparation of its acetate, $C_{31}H_{50}O_2$, m.p. 137—39°, $[\alpha]_D$ -34° (M^+ 454) and compared with authentic specimen.

Section B(ii) deals with the purification and characterisation of a new dihydroxy lactone-leucolactone, $C_{30}H_{48}O_4$. The second component eluted by benzene:ether = 4:1, on crystallisation and purification from chloroform-methanol mixture furnished crystalline solid, analysed for $C_{30}H_{48}O_4$ (M^+472), m.p. 310°. IR spectrum showed two hydroxyl groups and a γ-lactone ring at ν_{max} 3607, 3450 and 1750 cm^{-1} respectively. The compound has been designated as leucolactone 44.

Section B(iii) deals with the preparation of acetyl derivative 45, which has been analysed for $C_{34}H_{52}O_6$ (M^+ 556), m.p. 284—85°. It showed two acetate groups at ν_{max} 1740, 1729, 1235 and 1218 cm^{-1} and γ-lactone at 1768 cm^{-1}.

Section B(iv) deals with the preparation of diketo derivative 46 by oxidation with CrO_3—Py. The diketo leucolactone so formed, was analysed for $C_{30}H_{44}O_4$ (M^+ 468), m.p. 323—24°. IR spectrum showed a broad carbonyl absorption at 1705—1715 cm^{-1} and γ-lactone ring at 1760 cm^{-1}.

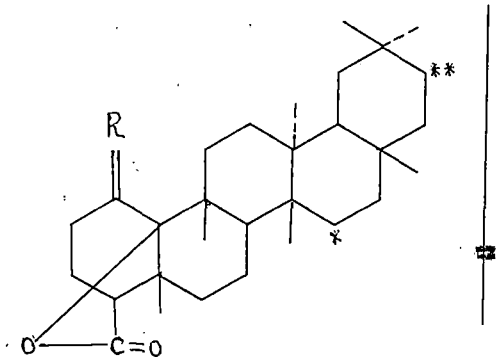
Section C(i) gives the PMR spectral data of leucolactone 44. The peaks were observed at 0.85 (1,t CH_3), 0.92 (3t-CH_3), 0.95 (1t, CH_3), 1.18 (1t CH_3), 1.28 (1t CH_3), 2.6 (1H, m), 3.18 ($W_{\frac{1}{2}}$ = 8Hz), 3.85 ($W_{\frac{1}{2}}$ =11Hz).

Section C(ii) deals with the NMR spectral analysis of the leucolactone acetate. Tertiary methyl signals at 0.85, 0.92, 1.18, 1.28 ppm showed the presence of seven methyl groups; the acetoxy methyl protons at 2.05 and 2.14 ppm. A proton α to the carbonyl group of the lactone ring showed up at 2.8 ppm had double doublets with J value of 8Hz indicating the equatorial orientations of α-proton and axial nature of the lactone carbonyl. The triplet centred at 4.45 ppm with

J value 18Hz was due to the methine proton, geminal to one of the acetate groups, which must be axially oriented. A doublet appeared at 4.9 ppm with coupling constant 8Hz indicated that the second acetoxy group is attached to a carbon containing a proton which is equatorially oriented having either only one or two neighbouring protons, the dihydral angle of one of which would be around 90°, thereby causing minimum coupling with the acetoxy methine proton. ^{13}C NMR showed the presence of 34 carbon atoms, the lactone carbonyl appeared at 176.9 ppm and the carbon containing the lactyl oxygen appeared at 90.2 ppm as singlet showing that the lactone ring is terminated at the tertiary carbon atom. Off resonance CW decoupled experiments showed the presence of 9—CH_3, 10—CH_2, 5—CH, 10—C— carbon atoms in leucolactone acetate.

Section C(iii) contains discussion on NMR spectral analysis of diketoleucolactone. XL—300 NMR showed the presence of 7-tertiary methyls at 0.82, 0.93, 1.02, 1.06, 1.10, 1.18 and 1.30 ppm. The multiplets between 2—3 ppm showed the presence of 5-protons α- to the carbonyl groups indicating that the two ketonic groups contain four α-protons, the lactone carbonyl having one α-proton. The doublets centred at 2.18 and 2.78 ppm are coupled with gem coupling separated by 0.6 ppm probably due to anisotropic effect of the adjacent

carbonyl. These two protons have no other neighbouring proton. The proton that appeared at 2.78 ppm showed a very low coupling ($J = 0.5$ Hz) with the methyl at 1.18 ppm suggesting that this proton being situated very close to a tertiary methyl group at 1.1 ppm. The absence of a singlet for a proton eliminates the position 18 for the oxygen function. The multiplet of the eight lines between 2.4 to 2.5 ppm showed that the CH_2 group next to the second carbonyl have two protons as CH_2 neighbour. This tied down one of the oxygen functions at C—1 positions, the α-protons of which have two neighbouring protons situated at C—3 position. The methyl peak that appeared at 1.18 ppm has been assigned to the C—28 methyl of friedelane skeleton by comparison with previously known friedelaneskeleton. Since it couples with one of the α-protons of the oxo group the second oxygen function is probably situated at C—15 or C—21 positions. Thus from the above spectral data the following probable structure is suggested.



= Either * or **
may contain second R group.

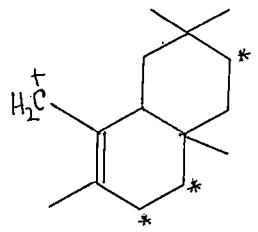
44, R = H, OH

45, R = H, OCOCH$_3$

46, R = O

S E C T I O N—D

Mass spectral analysis of leucolactone, leucolactone acetate, and diketo leucolactone suggested the formation of fragments a, b, c and d, typical of friedelane skeleton. The existance of fragment c and d excluded the possibility of existance of second oxygen function at 11, 12, 15, 16 position.



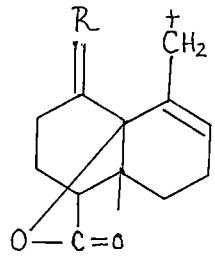
a

* Any one of the positions containing group R,

when R = OH, a = m/e 220

R = CH_3COO, a = m/e 262

R = O , a = m/e 218

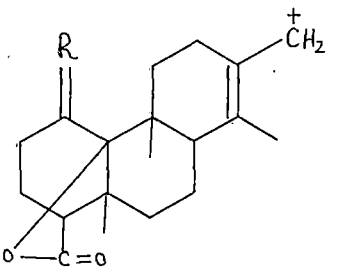


b

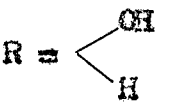
when R =  , b = m/e 221

R =  , b = m/e 263

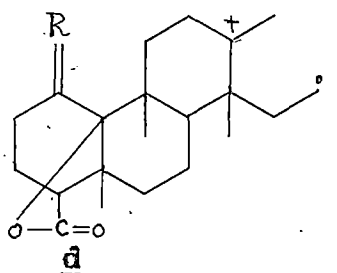
R = O , b = m/e 219



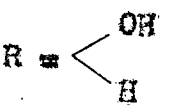
c

R =  , c = m/e 303

R = O , c = m/e 301

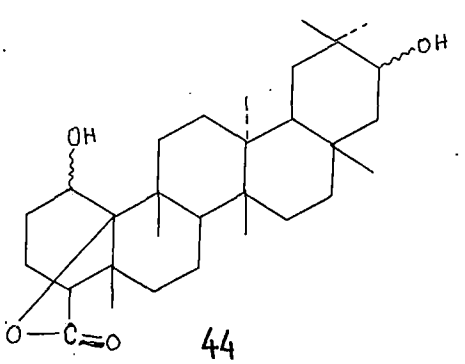


d

R =  , d = m/e 332

R = O , d = m/e 330

Thus from the mass spectra it is concluded the second oxygen is situated at C—21 position. Hence the probable structure of leucoleotone may be written as 44 without the stearochemistry of the two hydroxyl groups.



44

Further work to establish the stearochemistry of the two hydroxyl groups and some chemical transformation is in progress.

Chapter—IV describes the experimental portion.

LIST OF PUBLICATIONS

1. ACTION OF HYDROGEN PEROXIDE ON OLEAN-12,15-DIEN, 3,11-DIOL : PREPARATION OF C-NOR-TRITERPENE LACTONES.

   B.P.Pradhan, M.M.Mukherjee, D.K.Chakraberti and J.N.Shoolery, Tetrahedron, 39, 2819 (1983).

2. ACTION OF N-BROMOSUCCINIMIDE ON TRITERPENE ACIDS AND ESTERS IN DIMETHYL SULFOXIDE.

   B.P.Pradhan, M.M.Mukherjee, D.K.Chakraberti and J.N.Shoolery, Indian Journal of Chemistry, 22B, 12 (1983).

3. NOVEL METHOD FOR THE LACTONE RING OPENING.

   Bhim. P. Pradhan, Abul Hassan, Dilip K. Chakraberti and Sikha Dutta (Communicated).

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PART — I

STUDIES ON THE REDUCTION WITH LITHIUM-ETHYLENEDIAMINE ON TRITERPENOIDS

CHAPTER—I

A SHORT REVIEW ON THE METAL DISSOLVING REACTIONS IN PRESENCE OF BASES:

Metals in presence of bases are known as a reducing agent for the organic compounds since early years. While considering this type of reagents, the first example which should be taken under consideration is the well known Birch reduction. Birch reduction[1—5] is the name given to the reaction of unsaturated organic compounds with alkali metals and alcohols in liquid ammonia. This method was first used for aromatic compounds in 1937 by Wooster[6], who showed that benzene and its derivatives were reduced by sodium in liquid ammonia in the presence of an alcohol, while this reaction did not take place in absence of alcohol. However, the general recognition and broard application of this reaction was achieved only after a series of investigations by Birch[7] published from 1944 onwards. In the performance of the Birch reduction, a number of experimental variants have been proposed which differ from one another by the ratio and order of addition of the reagents — the alkali metal, the ammonia, and the alcohol — and also by the presence or absence of supplementary solvents. Since the reaction was carried out in

liquid ammonia, its temperature range was determined by the boiling and solidification points of ammonia (from -34°C to -80°C). According to Birch method[8], the alkali metal — sodium or potassium was added to a well stirred mixture of an alcohol (taken in stoichio-metric amount with respect to the metal), liquid ammonia, and the substance to be reduced. The method was applicable predominantly to simple aromatic compounds; for more complex compounds (methyl ester of hexestrol and estradiol) the yield fell sharply[9] mainly because of the low solubility of the compounds to be reduced. The modification has been found applicable mainly for the reduction of polycyclic derivatives of anisole, primary steroid estrogens.

Regel and coworkers[10] observed that aromatic systems could be reduced by lithium in presence of ethylene-diamine. This was the first method where alkali metal was used in presence of base without taking alcohol as solvent. The authors observed that in this reducing system aromatic rings were reduced to mono olefins and to cyclo paraffin. It reduced phenols, cleaved ethers, reduced ketones to alcohols, acetylenes, terminal and internal olefin to alkanes. The reaction was studied in different condition changing the amount of lithium or ethylene-diamine. It was observed that the extent of reduction increased with an increase in the

amount of lithium used and again addition of the lithium in portions increased the amount of reduction but in a very slow rate caused a decrease in the yield of reduced material. The authors applied the reaction on a number of compounds as shown in the following (table—1):

Table—1

Starting material	Comp. Moles	M per mole comp. moles	Ethylene diamine (ml.)	Products	Yields%
$\Delta^{9,10}$-Octalin	0.20	4.0	375	trans decalin	36.9
Phenanthrene	0.05	14.4	200	decahydro and dodecahydro phenanthrene	90
Anthracene	0.05	28.0	300	dodecahydro and tetradecahydro-anthracene	95
Benzene	0.30	4.8	375	cyclohexene cyclohexane	51.0 1.4
Benzyl alcohol	0.20	16.0	400	hexahydro benzyl alcohol	38.5
Phenol	0.40	4.4	375	phenol Δ^{2}-cyclohexenone cyclohexanone cyclohexanol cyclohexene cyclohexane	53.9 6.5 1.6 1.1 1.5 1.6
Heptanone-3	0.30	4.0	375	heptanol-3	29.6
Di-n-hexyl ether	0.20	8.0	400	hexene-2	30.5

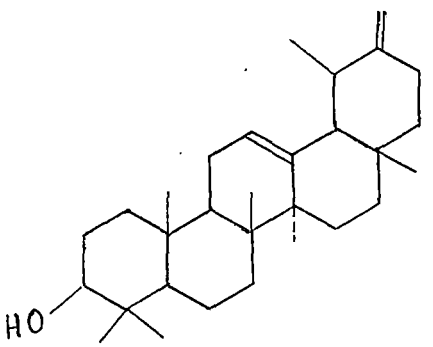
Table—1(Contd.)

Starting material	Comp. Moles	Li per mole comp. moles	Ethylene diamine (ml.)	Products	Yields %
Heptene-1	0.32	1.0	300	heptane heptene-2	41 13
Decene-1	0.30	2.4	150	decane decenes with internal double bond	47.2 13.8
Heptene-2	0.40	4.0	375	heptane heptene-1 heptenes with internal double bond	81.8 absent 2.6
Heptyne-1	0.20	10.0	300	heptane heptenes heptyne	50.9 absent absent
Octene-1	0.40	3.0	375	octane octenes with internal double bond	84.8 3.4
Octene-1	0.40	3.0	375	octane octenes with internal double bond	76.5 12.5

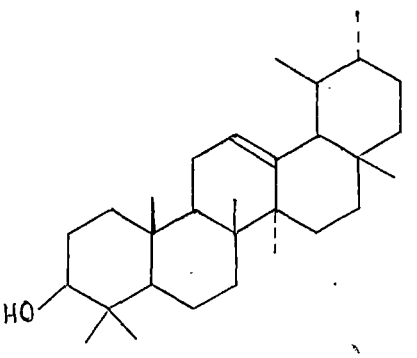
From table-1 it was found that terminal acetylenes and both terminal and internal olefins were reduced to alkanes by lithium and ethylene diamine. In contrast to sodium in liquid ammonia, it reduced acetylenes to olefins but not non conjugated internal olefins at all and reduced terminal olefins only in the presence of a proton donar. Under conditions, lithium in ethylene diamine where terminal olefin was

not completely reduced but entirely isomerised to internal olefins. It was also shown that the isomerisation was catalysed by the amide $H_2N—CH_2—CH_2—\overset{-}{N}H\overset{+}{L}i$.

Corey and coworkers[11] applied the reaction on triterpenoid observed that selective reduction of olefinic double bond occurred by the use of lithium in presence of ethylene diamine. They obtained compound 2 from compound 1.



1



2

Regel and coworkers[12] studied a number of reactions using lithium in presence of ethylene diamine on different organic compounds containing double bonds. The authors observed isomerisation of olefinic double bond as well as dehydrogenation occurred during reaction condition.

Isomerisation of terminal olefins to internal olefins[12]:

When metallic sodium was added to ethylene-diamines at a temperature of 80°—115°, there was a rapid reaction with evolution of hydrogen gas. A dark blue material formed at the surface of the metal; when the solution was stirred the colour quickly spreaded throughout the liquid. On continued heating the blue colour gradually faded and disappeared leaving a colourless or pale yellow solution. They thought that lithium in presence of ethylene diamine formed first N-lithiodiamine 3.

$$Li + H_2N\,CH_2CH_2NH_2 \longrightarrow \tfrac{1}{2}H_2 + H_2NCH_2CH_2NH\,Li$$

3

The N-lithio-ethylene diamine 3 thus formed was responsible for isomerisation as catalyst. When 1-octene was heated with a solution of 3 in ethylene diamine, the material which was recovered (90% yield) consisted entirely of internal olefins. Neither 1-octene nor octane was detected. In the same manner 4-methyl-cyclohexene was isomerised to 1-methyl cyclohexene.

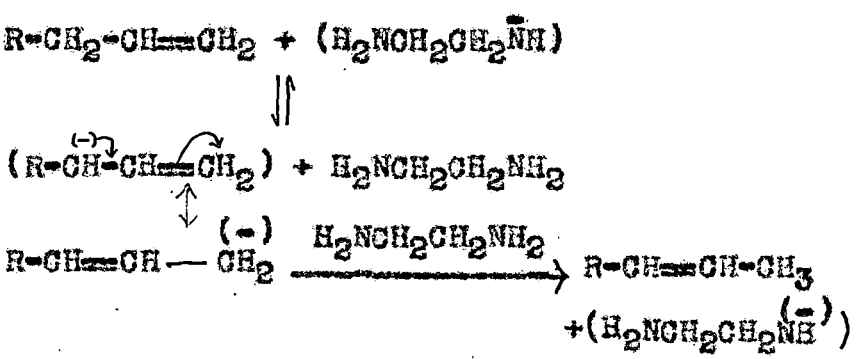
• dienes

Dehydrogenation of cyclic dienes[12]:

Regel and coworkers[12] observed that when 4-vinyl cyclohexene 4 was added to a solution of N-lithioethylene diamine 3, there was a rapid evolution of hydrogen gas. The product obtained in 67% yield, consisted entirely of ethyl benzene 7. The dehydrogenation reaction was apparently general; d-limonene (1-methyl-4 isopropyl pentyl cyclohexene) and α -phellandrene (2-methyl-5-isopropyl-1,3-cyclohexadiene) both yielded p-cymene. The dehydrogenation reaction with 3 was essentially quantitative in a few minutes at 100°C. The reaction was done with sodium in ethylene-diamine also but the rate was found to be slower. There the compound $H_2NCH_2CH_2NHNa$ 6 was formed as an intermediate.

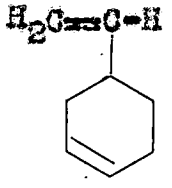
Mechanism suggested for two reactions:

The authors suggested the following mechanisms for the isomerisation as well as dehydrogenations:

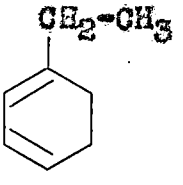


They were unable to describe the mechanism in view point for the role of metal ion. It did not seem likely that the structure of lithium and sodium compounds were different or that the high viscosity of the solution of 6 would be so great for an effect upon the rate of isomerisation. The reaction was discussed in an incomplete view point which considered only anion part.

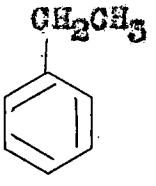
The mechanism for dehydrogenation of diene, the first step for 4-vinylcyclohexene 4 was considered to be the isomerisation to a conjugated diene 5



4



5

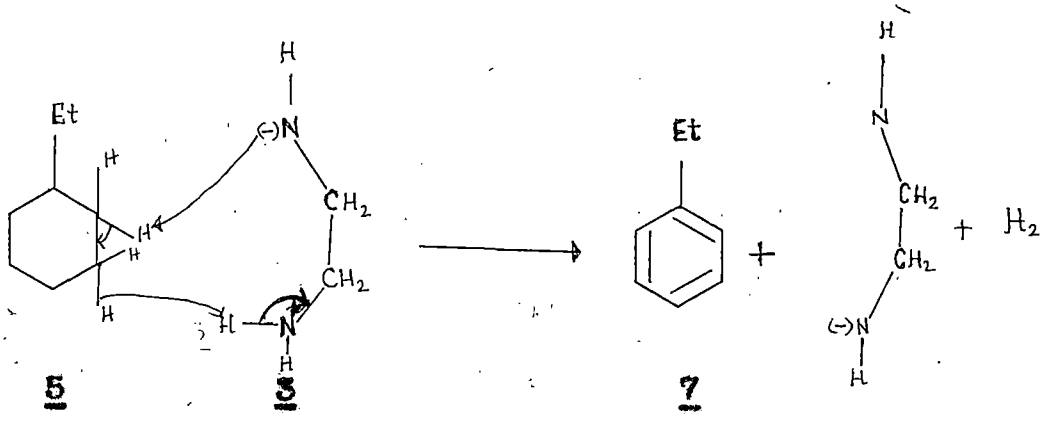


7

This was suggested by the fact that 6 would not dehydrogenate d-limonene (where the bonds must first isomerise to a conjugated system), but would dehydrogenate ∝-phallendene (where the double bonds were already conjugated). Both 3 and 6 readily removed protons from cyclic conjugated diene where by 6 must be a slow process.

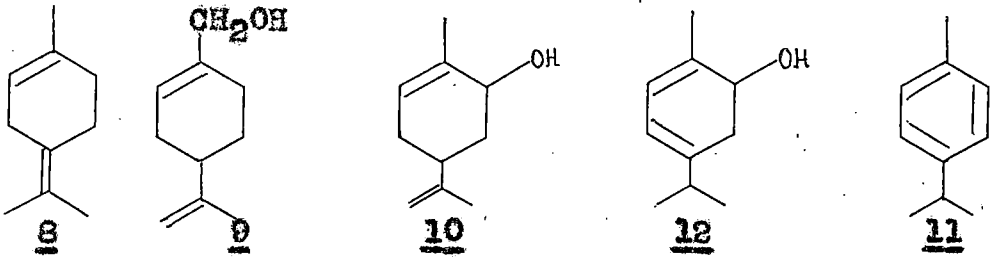
The path from 5 to ethyl benzene 7 was explained by the bidentate character of ethylenediamine made it

possible to visualize a concerted reaction in which 5 and 3 formed a cyclic intermediate.



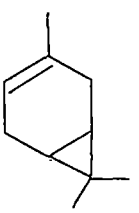
5 3 7

Tyagi, Ghatge and Bhattacharyya[13] applied the reaction of lithium in ethylenediamine on different terpenes. They found terpinolone 8, perillyl alcohol 9, and carveol 10 when treated with the reagent gave p-cymene 11 in good yield. Formation of p-cymene 11 from carveol 10 presumably proceeded through the conjugated alcohol 12 followed by dehydration. In case of perillyl alcohol 9 because of the absence of an ∝-hydrogen, the allylic double bonds must have isomerised followed by dehydration and rearrangement to form 11.

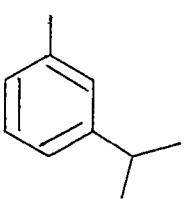


8 9 10 12 11

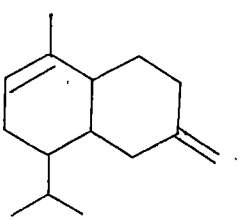
The authors[13] also examined the behaviour of the reagent on many terpenoids containing cyclo-propane and cyclobutane rings. Δ^3 Carene 13 gave in quantitative yields of cymenes which from comparative IR, analysis was found to be a mixture of p-cymene 11 and m-cymene 14 i.e. the cyclo-propane ring having been opened in two ways in conformity with the reactivity of Δ^3-carene.



13

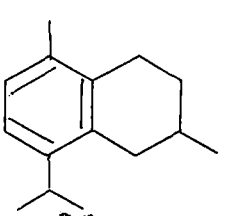


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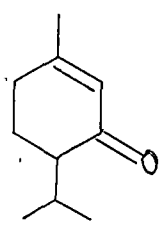
15

The sesquiterpenes like γ_1-cadinene 15 when treated with the same reagent converted to calaminine 16, presumably with migration of the methylenic double bond across a ring followed by aromatization of one of the rings. Aromatization of the other ring could not be effected by repeating the treatment or using excess of the reagent.

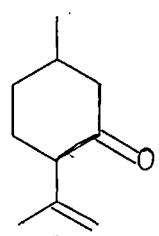


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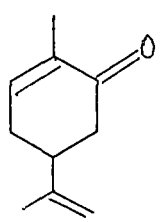
The reaction of lithium in ethylenediamine was extended to terpene ketonic systems. The possibility of using a monoethenoid ketone through its potential enolic form to give a phenol on dehydrogenation was examined. The conjugated ketone dl-piperitone 17 on treatment with this reagent remained unaltered and did not form any thymol as might have been anticipated. The unconjugated ketone isopulegone 18 partly isomerized to the conjugated ketone 19. Carvone 20 containing two double bonds, however, gave a quantitative yield of carvacrol 21. From that it was clear that aromatization of a ketonic system of the p-menthone series, the presence of two double bonds was essential. It was also noted that though isopulegone 18 partially isomerised to the conjugated ketone the double bond in the corresponding alcohol, isopulegol 22 did not migrate; the migration appeared to have prevented by the hydroxyl group.



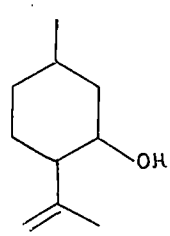
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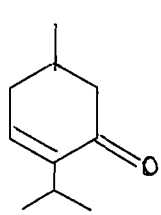
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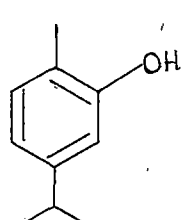
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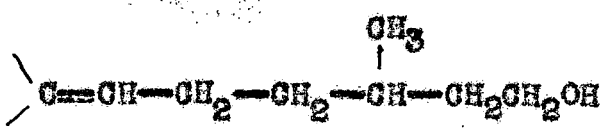


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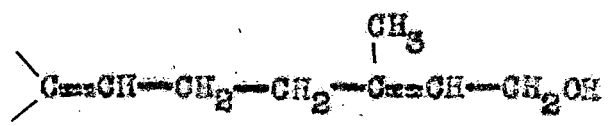


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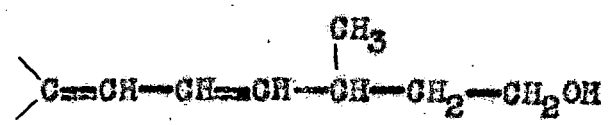
The reaction of lithium in ethylenediamine was also examined by the same authors[13] on the acyclic systems. The monoethenoid alcohol citronellol 23 remained unaltered. No migration of double bond to give rise to an allylic alcohol was noticed. In the case of geraniol 24 the allylic double bond actually migrated away from the alcoholic group to furnish the conjugated diene alcohol 25.



23



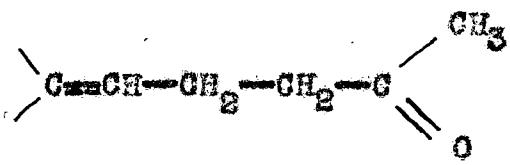
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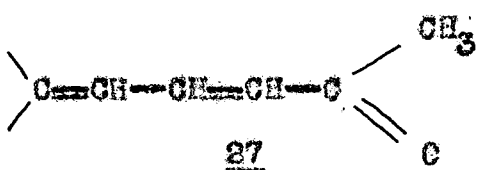
25

Lithium in ethylenediamine reaction was applied on one acyclic ketone 26, which gave the fully conjugated dienone 27 evidently formed through dehydrogenation, but

because of its unstable nature, it could not be isolated in pure form. The impure specimen gave characteristic colour reaction, showed expected U.V. absorption — λ_{max} 223 nm (ϵ 4385) and 266 nm (ϵ 557).



26



27

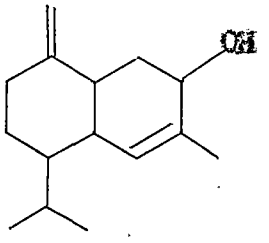
Smith and coworkers[14] observed the dehydrogenation of aliphatic and aromatic alcohols to carbonyl compounds in the presence of lithium metal in ethylenediamine. The result of different reactions are summerized in table-2.

Table—2

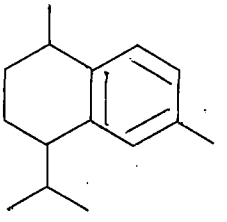
Alcohol	Carbonyl product	% yield
Cyclohexanol	Cyclohexanone	54
2-methyl cyclo-hexanol	2-methyl cyclohexanone	38
n-pentyl alcohol	n-valeraldehyde	66
2-hexanol	n-butyl methyl ketone	45
2-phenyl alcohol	Phenyl acetaldehyde	25
1-phenyl ethanol	Acetophenone	24
Benzyl alcohol	Benzaldehyde	80

Tyagi, Ghatge and Bhattacharyya[15] observed that lithioethylenediamine was an excellent reagent for the partial aromatization of cadinenic terpenens. The crystalline alcohol khusinol 28 on treatment with this reagent yielded the hydrocarbon 29; the purity of which was evidenced by a single peak on the VPC column. As the formation of the hydrocarbon 29 involved the ellimination of the hydroxyl group of khusinol, it must be represented by the structure 29 and not by 16, a product

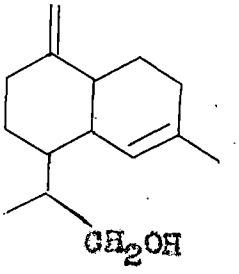
which was previously obtained by the dehydrogenation of γ_1-cadinene 15 by this reagent. Similarly on treatment with the reagent the crystalline alcohol khusol 30 afforded the partially aromatized alcohol 31. Formation of such a product retaining the hydroxyl group intact was not normally possible during dehydrogenation using conventional reagents.



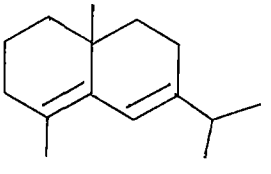
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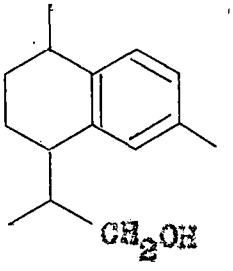
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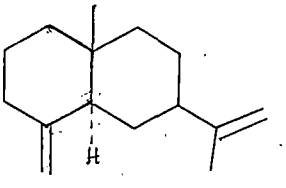
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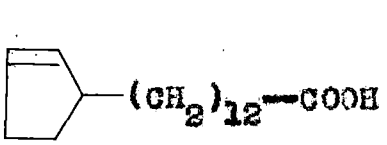


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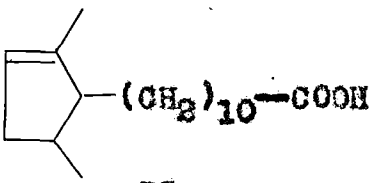
β-selinene 32 under the same condition showed facile transformation to the heteroannuler diene (+) δ -selinene 33. This diene contained only one assymetric centre with a

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β-oriented methyl group at C-10. Chaulmoogric acid 34 having disubstituted double bond was completely converted



34



35

to isochaulmoogric acid 35 having trisubstituted double bond, when reacted with lithioethylenediamine.

Baumel and Harris[16] observed that lithiated derivatives of diamines could be used to metalate a variety of weekly acidic compounds. N-lithioethylenediamine reacted smoothly with a variety of amines like aniline, diphenylamine and hydrocarbons like florene, indene, phenyl acetylene etc. at room temperature. In general the product isolated was a 1:1 crystalline solid complex of lithiated derivative with ethylenediamine. Exceptions were observed in the case of acetylenes. When acetylene was introduced into a slurry

$$RH + H_2NCH_2CH_2NHLi \rightleftharpoons RLiNH_2CH_2CH_2NH_2$$

of N-lithioethylenediamine above the decomposition temperature of lithium acetylide — ethylenediamine a solid product obtained which contained 87% dilithium acetylide and 13% ethylenediamine.

The basis for the reaction between hydrocarbon and amine with N-lithioethylenediamine depends on their relative acidities. In the absence of other effects, the most acidic hydrogen atom of the hydrocarbon or amine must be acidic than the most acidic hydrogen on ethylenediamine.

$$RH + H_2NCH_2CH_2NHLi \rightleftharpoons RLi + H_2NCH_2CH_2NH_2$$

Complex formation was also considered to be an important factor in the overall reaction:

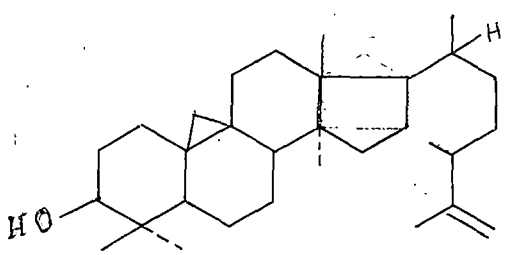
$$RLi + H_2NCH_2CH_2NH_2 \longrightarrow RLiNCH_2CH_2NH_2$$

The complex might be prepared in an one step process from lithium metal in hydrocarbon solvents, since the N-lithioethylenediamine could be prepared in situ. While using one step process reduction of the hydrocarbons could complete with metalation because of the potentially reductive nature of Li-diamine system. But indene and fluorene could be reduced by lithium in dimethoxyethane. If indene was present initially in the preparation of indenyllithumdiamine complexes varying amounts of reduction to indene

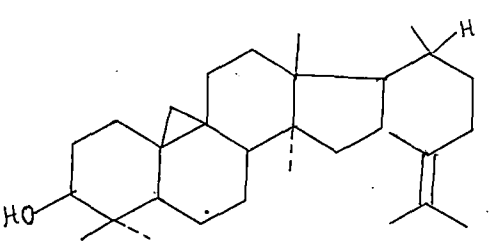
could occur depending on the diamine employed. Indene reduction could be avoided by preparing lithiated diamine before introducing indene to the system.

Sodium powder instead of lithium powder was also used for metallation in presence of ethylenediamine. Apparently N-sodioethylenediamine formed at the metal surface making it resistant for further attack by ethylenediamine. If however an appropriate RH compound was present to react with the formed N-sodioethylenediamine, fresh metal surface was exposed and overall reaction proceeded to completion. Acetylene hydrocarbons reacted with sodium and ethylenediamine in toluene at 35°C to form the corresponding sodium acetylide. Amines would not react with sodium and ethylenediamine under any condition tested.

Narula and Sukh Dev[17] applied the reaction of Li in presence of ethylenediamine for the isomerisation of cycloaudenol 36 on exposure to N-lithioethylene diamine at 120°-125° gave the isopropyledene isomer 37 in 92% yield



36



37

Barton and coworkers[18] reported that sterically hindered alcohols were conveniently and efficiently converted into the corresponding alkanes by metal amine reduction of the derived esters with carboxyllic acid. The only side reaction was the regeneration of the starting alcohol. Different reaction which were done are summerized in table-3. The readily available acetate esters were admirable substrate. The authors used mainly lithium in ethylamine as the reducing system, but other metals (Na, K) and other amines were also effective.

Table-3

Starting Material	Product (% yield)
1. 3β, 6β-diacetoxy-5α-cholestane	5α-cholestane-3β-ol (46) 5α-cholestane-3β, 6β-diol(35)
2. 3β,6β-diacetoxy-5α-cholestane	5α-cholestane-3β-ol (60) 5α-cholestane-3β,6β-diol(25)
3. 3β,6β-dibenzoyloxy-5α-cholestane	5α-cholestane-3β,6β-diol(80)
4. 3β,6β-diformyloxy-5α-cholestane	5α-cholestane-3β,6β-diol(86)
5. 3β,6β-diisobutyryloxy-5α-cholestane	5α-cholestane-3β-ol(16) 5α-cholestane-3β,6β-diol (55)

Table—3(contd.)

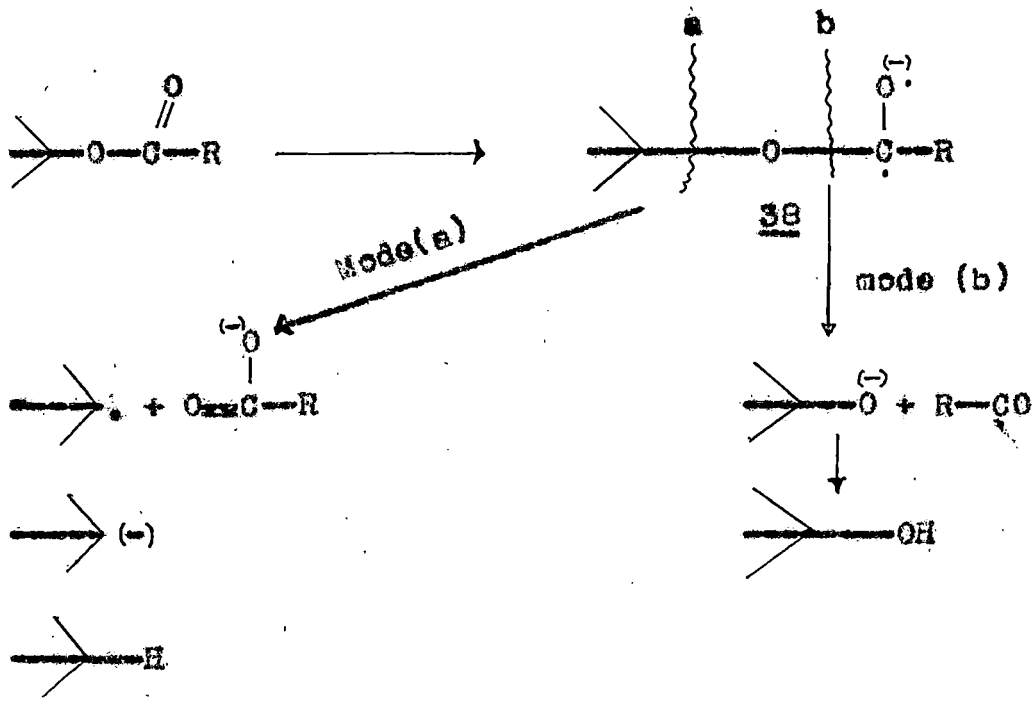
Starting Material	Product (% yield)
6. 3β,6β-diformyloxy-5α-cholestane	5α-cholestane-3β-ol (71) 5α-cholestane-3β,6β-diol(19)
7. 3β,6β-dipropanoyloxy-5α-cholestane	5α-cholestane-3β-ol (15) 5α-cholestane-3β,6β-diol (61)
8. 3β,6β-dipivaloyloxy-5α-cholestane	5α-cholestane-3β-ol (73) 5α-cholestane-3β,6β-diol(16)
9. 3β,5α-diacetoxycholestane	5α-cholestane-3β-ol (66) cholestane-3β,5α-diol (8)
10. 3β-5α-6β-triacetoxy cholestanol	Cholesterol (81) Cholestane-3β,5α,6β-triol
11. 3β,12-α-diacetoxy-13α-oleanane	13α-oleanane-3β-ol (85)
12. 3β,25-diacetoxy-5α-lanost-8ene	5α-lanost-8-en-3β-ol (75) 5α-lanost-8-en-3β-25-diol (10)
13. Caryolan-1-ol-acetate	Caryolane (80)

Except for entries 2,4,6 and 8 (K-Bu^t NH_2-18-crown-6), Li-EtNH_2 was used.

From the above table-3, it was clear that acetates of sterically hindered secondary alcohols and of tertiary alcohols were reduced by lithium in ethyl amine to afford predominantly the corresponding alkanes rather than the parent alcohols.

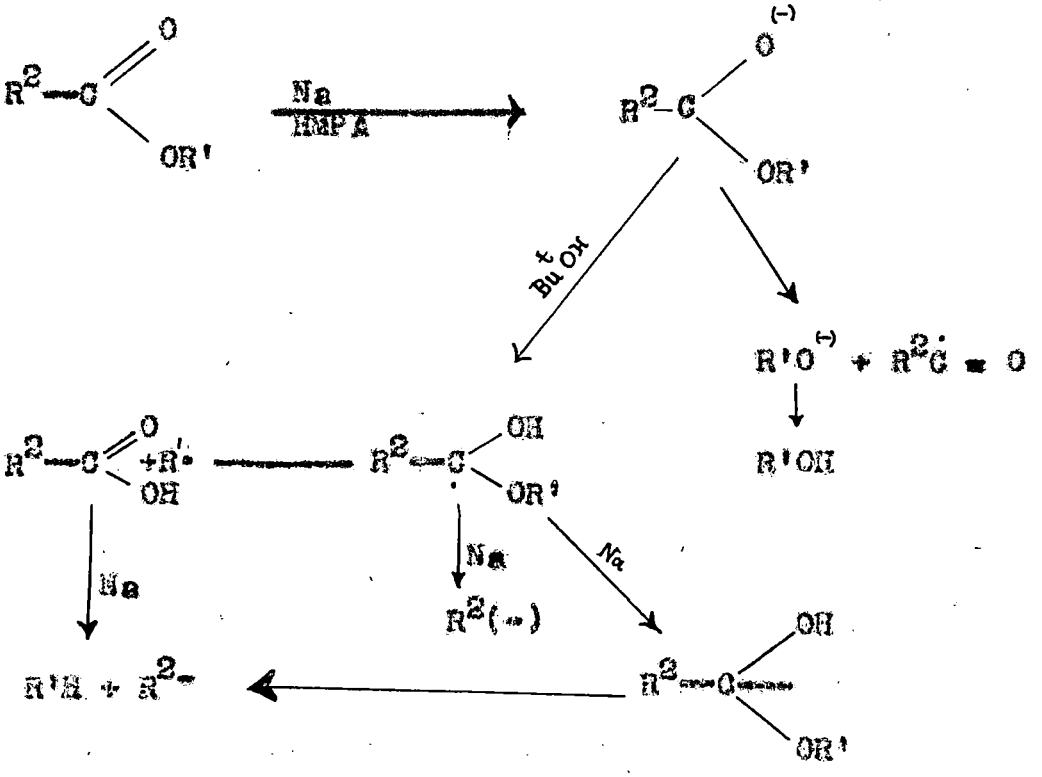
Reduction of esters of tertiary alcohols by the metals in presence of bases suggested the authors to assume in favour of a mechanism of the reactions involving radical fragmentation of the initially formed radical ion 38 in Scheme 1. Mode (a) and thence deoxygenation, evidently became the favoured process when cleavage of the C—O bond is attended by a sufficient release of unfavourable steric interactions. Otherwise mode (b) was preferred and the alcohol is regenerated. Under the reaction condition, reduction of radicals to the corresponding carbanions must be rapid. Thus the formation of cholesterol by the reduction of 3β,5α,6β-triacetoxy-cholestane was probably the result of displacement of the acetate group from C—6 by a carbanion at C—5. The fact that reduction only occurred with esters of sterically hindered alcohols confirms upon this method of selectivity for deoxygenation process.

Scheme—1



Deshyayes and Pete[19] reported a number of reduction examples of alkyl esters to alkanes by using sodium metal in presence of hexamethyl phosporic acid. The allyl esters of nonyl, heptyl, cyclohexyl, bornyl was examined by the workers. The mechanism which they proposed similar to the previous workers are shown in Scheme—2.

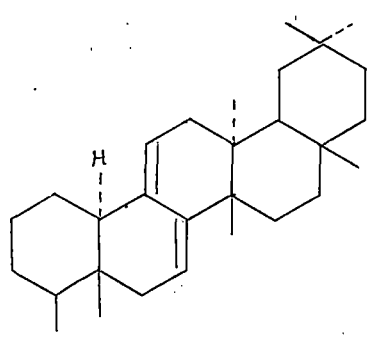
Scheme—2



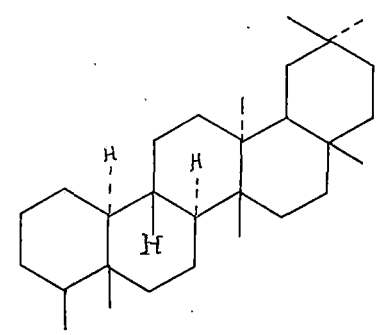
Tertiary butyl alcohol was added in the reaction mixture as a proton source. Reduction of tertiary esters gave the corresponding alkane quantitatively whereas primary and secondary esters lead to mixture of the corresponding alkane and alcohols in good yield.

Sengupta and coworkers[20] investigated the reaction of lithium in ethylendiamine on triterpenoids hetero-annuler 1,3 diene. Reduction of 25-norfriedolean-7,9(11),

diene 39 (0.15 g) was done in a solution of ethylenediamine (20 ml) with lithium (0.2 g) added in small pieces with stirring under nitrogen atmosphere and the mixture was refluxed for three hours. Solid ammonium chloride was added and the mixture was acidified with cold 6N HCl. After work up they isolated 25-nor-fried-oleanane, 40. It showed negative TNM test showing the absence of any double bond which was supported by NMR and IR spectrum. So the diene system was completely reduced.

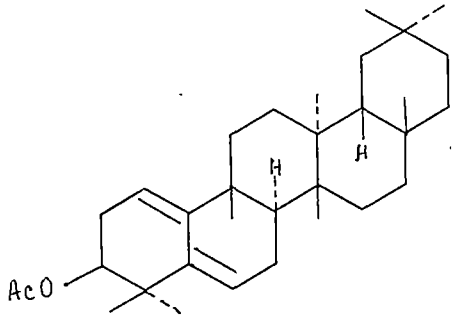


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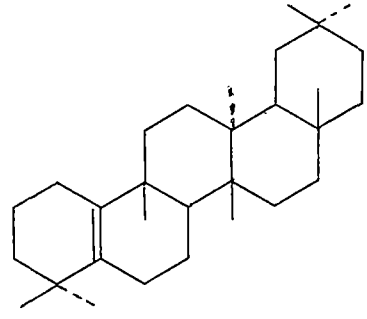


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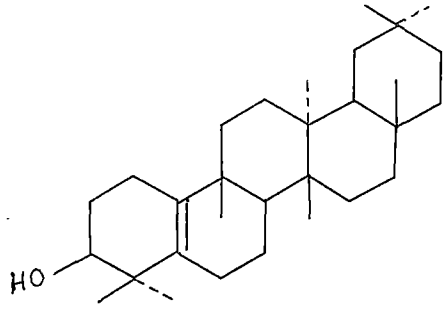
Reaction with lithium in ethylendiamine was reported by Sengupta et al on gluta-1(10), 5 dienyl-3β-acetate 41 and the products obtained were a mixture of deoxygenated and hydrolysed products. The products were respectively glut-5(10)-ene 42 and glut-5 (10) en—3β-ol 43. When multiflora-7,9(11)-dienyl-3β-acetate 44 were similarly reduced they isolated two compounds isomultiflorene 45 and iso— multiflorenol 46.



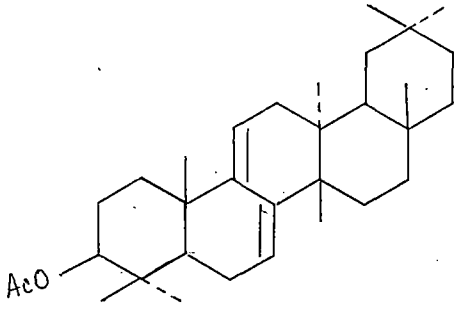
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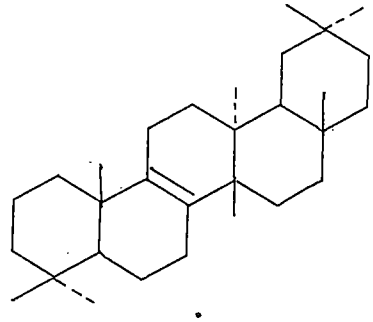
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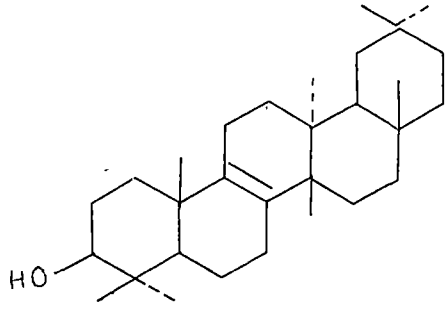
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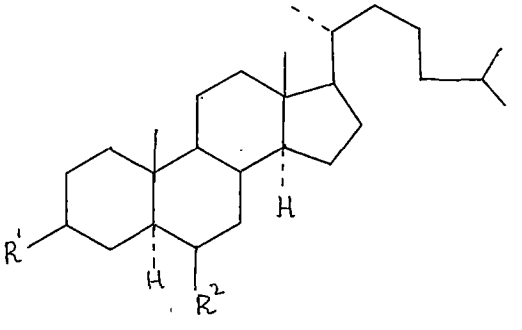


45



46

Barton and coworkers[21] studied the deoxygenation of carboxylic esters by alkali metals in organic bases. In the previous works by the workers[18] it was observed that typical diesters 47a and 47b gave 5∝-cholestan-3β-ol 47c (60 and 79% yield respectively) where the more hindered axial (6β) ester was selectively deoxygenated.



47a , $R^1 = R^2 =$ OAc

47b , $R^1 = R^2 = Bu^tCO_2$

47c , $R^1 =$ OH, $R^2 =$ H

47d , $R^1 = R^2 = R^3CO_2$

47e , $R^1 = R^2 =$ H

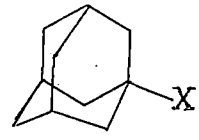
47f , $R^1 =$ H, $R^2 =$ OH

47g , $R^1 = R^2 =$ OH

47h , $R^1 = R^3CO_2$, $R^2 =$ H

The recent works by the authors[21] showed that the aliphatic or allycyclic esters normally react by mode (a)[18] provided that the medium is nucleophile-free.

Adamantane-1-carboxylic esters of sterols were taken for study. Adamentane carboxylate esters 48a and cyclic carbonates 48a and 49b were reduced by their addition in



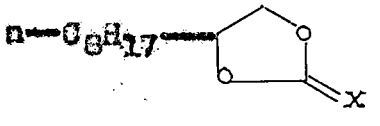
$\equiv R^S X$

48a, X = COCl	48e, X = CHO
48b, X = CO_2H	48f, X = $CH(-\bar{O})\bar{O}$ or $CH(-\bar{O})\bar{N}Et$
48c, X = CH_2OH	48g, X = CO_2Et
48d, X = CONHEt	48h, X = CO_2 $\lceil CH_2 \rceil_{17}$ Me

tetrahydrofuran to potassium and 18-crown -6 in t - butylamine. The products which were obtained in the reduction of esters are summerized in table—4. In table—4 reactions 1,2 and 4—7 were carried out using potassium and 18-crown-6 in t-butylamine at room temperature and reaction 3 with lithium and ethylamine at 17°C.

Table—4

Substrate	Products (% yield)
1. 47d	47a (45), 47c (27), 47g (8) 47f (6), 46b (92)
2. 48b	No reaction
3. 48b	48e (51), 48b (10), 48c (9)
4. 48g + 47e	47c (69), 47e (15), 48b (85)
5. 48h	$Me(CH_2)_{17}OH$ (53), $Me(CH_2)_{14}Me$ (41), 48b (90)
6. 49a	$n\text{-}C_8H_{17}CH(X)CH_2Y$, X=Y=OAC (67); X=H, Y=OAC + X=OAC, Y=H (5)
7. 49b	$n\text{-}C_8H_{17}CH(X)CH_2Y$, X=Y=OAC (35); X=H, Y=OAC (26); X=OAC, Y=H (8); $n\text{-}C_8H_{17}CH{=}CH_2$ (5)



49a, X = O
49b, X = S

In the reduction of the ester 47h the products which were obtained are summerized in table—5. In table—5,

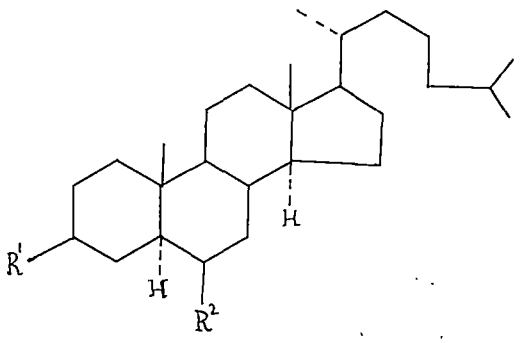
reactions were carried out at 46° (1,2), 20° (3—5), 17 (9—11) or -73°C (8) in t-butylamine and THF (1—3, 6,7), t-butylamine with potassium (4), 1,2-dimethoxyethane and iodomethane (5), THF and t-butylacetate (11).

Table—5

Amount/m. mol of Ester (47h), metal, 18 crown-6	% yield of products				
	47e	47c	48b	48c	48d
1. 1.04, 38, 11	43	57	96	2	-
2. 0.84, (34+18), (6+4)	32	68	81	0	-
3. 1.03, 13, 4	45	37	84	0	-
4. 1.03, 14, 4.5	45	44	93	7	-
5. 1.20, 28, 7	30	57	92	0	-
6. 1.09, 36, 6	27	66	77	5	-
7. 1.04, 36, 6	15	81	71	0	-
8. 1.17, 29, 0	1	93	0	69	0
9. 1.15, 22, 0	7	85	4	4	92
10. 1.17, 27.2, 0	4	84	4	65	0
11. 1.03, 65, 0	5	92	2	29	51
12. 0.45, 100, 0	0	58	0	66	0

In all cases reduction of ester 47b gave 5α-cholestane 47e and acid 48b with the latter substantially predominating. The possibility that this difference resulted from competative hydrolysis by adventitious water was unlikely since vigorous drying was used and in entry 5, (table—5) iodomethane was added before the ester to scavenge any water, the ratio of 47e : 48b was not increased. The yields of acid 48b and alkane 47e were decreased at lower temperature. Deoxygenation was a minor pathway on lithium-ethylamine reduction giving 47c and 48d. In the presence of excess electrons or at low temperature both transacylation (giving 48d) and radical anion fragmentation (giving 47e) were suppressed and the two electron Bouveault-Blanc products 47c and 48c formed. Entry 4 of table—4 showed that ester deacylation by an alkoxide competed with reduction. In entry 7 of table—4 predominance of primary acetate was consistant with deoxygenation via the radical anion, not the dianion.

Barton and coworkers[22] studied that primary and secondary alcohols could be easily deoxygenated by the reduction of their dialkylaminothiocarbonyl derivatives with potassium and 18-crown-6 to produce the corresponding alkanes.



47a, $R^1=R^2=OAc$,

47c, $R^1=OH$, $R^2=H$,

47e, $R^1=R^2=H$

47f, $R^1=H$, $R^2=OH$

47g, $R^1=R^2=OH$

50a, $R^1=R^2=MeS.CS.O$,

50b, $R^1=R^2=EtNH.CS.O$

50c, $R^1=R^2=CH_2-(CH_2)_3-N(CS.O)$

50d, $R^1=CH_2-(CH_2)_4-N-CS.O$, $R^2=H$

50e, $R^1=Et_2NCSO$, $R^2=H$

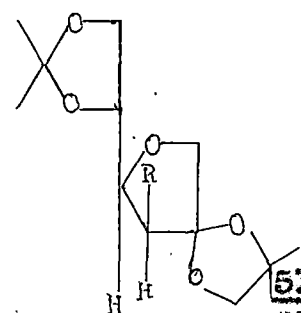
50f, $R^1=Me_2NCH_2CH_2N(Me)CS.O$, $R^2=H$

Reduction of dithiocarbonate 50a and thiocarbamates 50b, 50c, 50d, 50e, 50f and 51a were done with potassium in t-butylamine solubilised by 18-crown-6. The products which were obtained are shown in table—6.

Table—6

Entry	Starting material	Products (% yield)
1	50a	47e(38), 47c(19), 47g(8), 47f(2)
2	50b	47g(45), 47c(18)
3	50c	47c(62), 47f(15), 47c(12), 47g(5)
4	50d	47e(74), 47c(14)
5	50e	47e(86), 47c(6)
6	50e	47e(58), 47c(40)
7	50f	47e(83), 47c(12)
8	$Me(CH_2)_{17}O.CS.NEt$	$Me(CH_2)_{16}Me(87)$, $Me(CH_2)_{17}$-OH(12)
9	51a	51b(56), 51c(14)

All reductions were done at room temperature except entry-7 which was done at -30°C

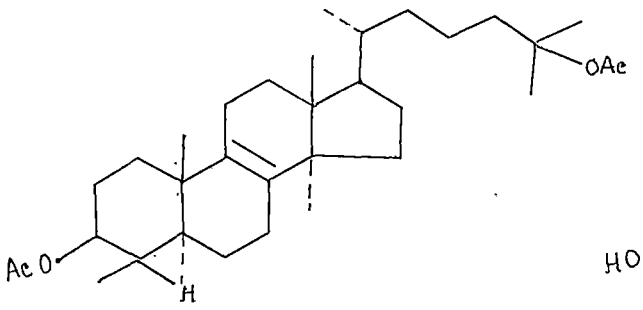


51a, $R=Et_2N.CS.O$
51b, R=OH
51c, R=H

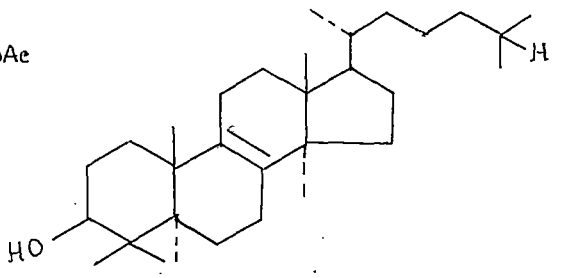
The primary and secondary alcohols were thus easily deoxygenated by this method of Barton and his co-workers[22] since the thiocarbamate derivatives were easily obtained from the corresponding alcohols.

Sengupta and coworkers[23] examined the reaction of Li in ethylenediamine on triterpene hindered esters. They observed methyl esters of oleanolic acid, crategolic acid, prodocarpic acid, ursolic acid were hydrolysed during the reaction condition and the corresponding acids were obtained.

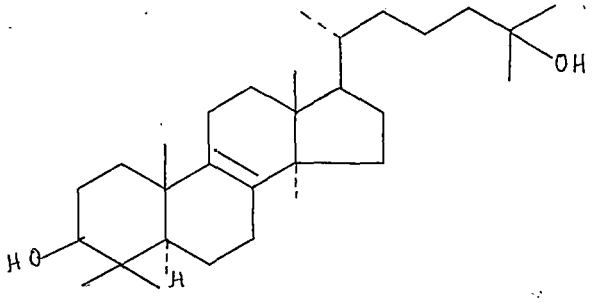
Barton and coworkers[24] observed from the previous works[21,22] and with some additional works[24] that sterically hindered esters were deoxygenated in presence of group IA metal in bases while the non hindered esters produced the corresponding alcohol. At ambient temperature the principal pathway on the dissolving metal reduction on alkyl carboxyllic esters was the formation of alkane and carboxyllic acid anion. They described the pathway as the alkyl-oxygen cleavage of the ester radical ion. They studied the reduction on sterol system where diesters were taken in sterically different environments. The compounds 52 and 53 when treated with lithium in ethylamine the products 54, 55 and 56 were obtained respectively. In compound 52 and 53 the acetoxy groups were in different sterical environments.



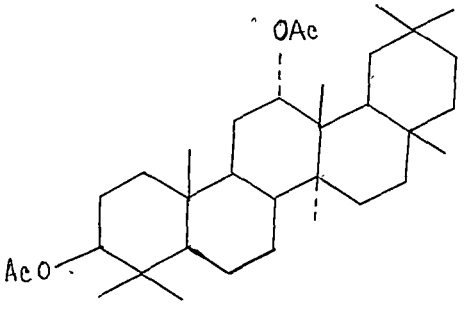
52



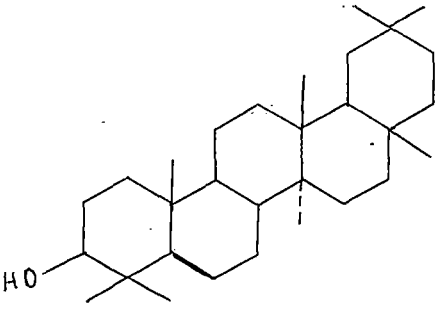
54 (75%)



55 (10%)



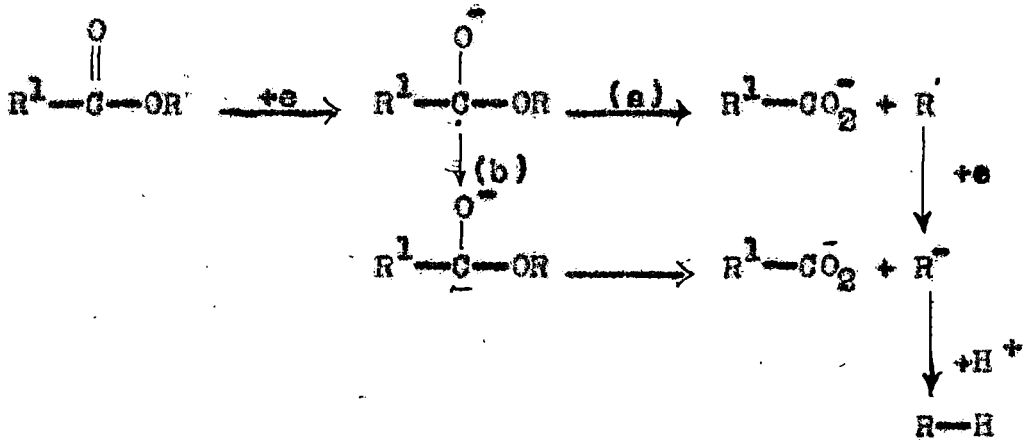
53



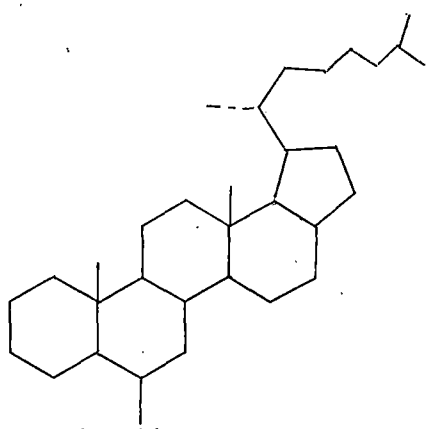
56

Possible mechanisms for the deoxygenation are shown in Scheme—3. In pathway (a) the initially formed radical anion fragments to give an alkyl radical and carboxylate anion. Alternatively, in pathway (b) the radical anion was further reduced to the dianion which then collapsed to yield a carbanion and carboxylate anion.

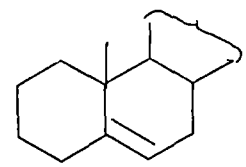
Scheme—3



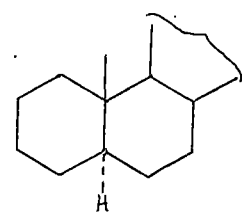
Reduction of 3β,5α-cyclocholesta-6β-yl acetate 57 gave cholest-5-ene 58 (85%) and 3β,5α-cyclocholestane 59 (15%). This result was consistant with the occurrence of pathway (a), since the rapid rearrangement of the 3β, 5α-cyclocholestan-6-yl radical would form stable cholest-5 en 3-yl, radical



57



58



59

The formation of cholest-5 ene 58 was presumably the result of displacement of the acetoxy group from C—6 by a carbanion at C—5.

These reductions supported the fact that the deoxygenation occurred predominatly, via the fragmentation of the radical anion (scheme—3).

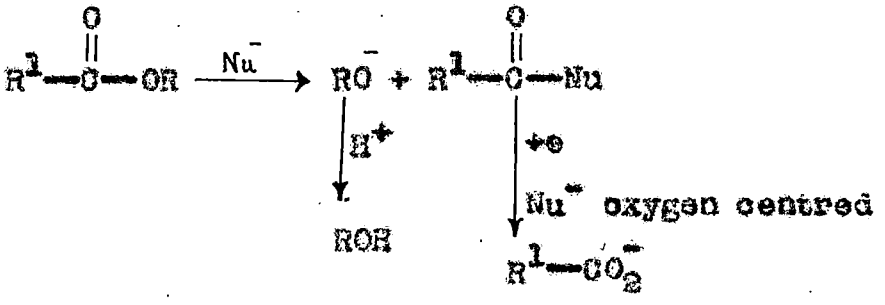
Both pathways in scheme—3 required that the acyl component of the ester was released at the carboxylate oxidation level. This point was established by the experiment viz. the reduction of adamantane-1-carboxyllic acid 48b

was studied. Controlled reduction of the compound was stable to treatment with potassium and 18-crown-6 in t-butylamine. Prolonged reduction of the compound 48b gave adamantane-1-carbaldehyde 48a and adamanten-1 yl methanol 48c, in addition to recovered acid 48b (10%).

The reduction of 5α-cholestane-3β,6β-diyl-bis (adamantane-1-carboxylate) 47d gave adamantane-1-carboxyllic acid 48b in 92% yield. The yield of the acid was higher than that of deoxygenation products. Competitive hydrolysis of the ester by adventitious water could have accounted for this fact, but seemed improbable in view of the precautions taken to ensure anhydrous conditions.

The process which competes with deoxygenation and in which the starting alcohol was regenerated assumed to be the deacetylation of the ester by some other neucleophile Nu^-. The process is shown in Scheme—4.

Scheme—4

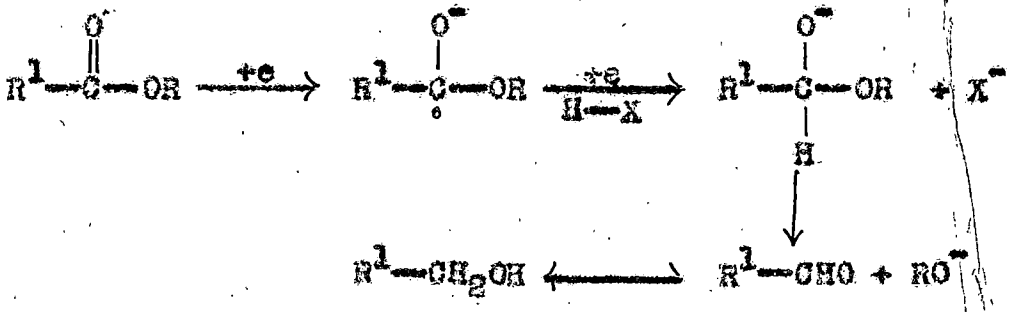


The newly formed ester where the nucleophile was oxygen centred, subsequent reduction of the ester, $R^1—\overset{O}{\overset{\|}{C}}—Nu$ took place and generation of carboxylate anion was observed. To prove the postulate the workers studied the reaction of a mixture of ethyl- adamantane-1-carboxylate 48g or ethyl acetate and 5α-cholestan-3β-ol 47c and isolated 5α-cholestane 47e. Reduction of 5α-cholestan-3β-yl adamantane-1-carboxylate 47h under various condition was studied. With lithium in ethylamine there was little deoxygenation or formation of adamantane-1-carboxyllic acid 48b. This was due to the occurrence of transacylation. This problem was overcome by the use of t-butylamine as solvent. At lower temperature transacylation was suppressed as was radical-anion fragmentation.

The source of nucleophile was established by the fact, tetrahydrofuran was fragmented by strong bases. 18-crown-6was broken down by treatment with potassium in t-butylamine. Deoxygenation by dissolving metal reduction of carboxylic esters was limited by competitive deacylation by solvent or crown ether derived nucleophiles. The selective deoxygenation of sterically hindered esters was a result of the suppression of this competitive deacylation.

The radical ion derived from the esters normally fragment to give the carboxylate anion and radical made the Bouveault-Blanc and acyloin reaction curiosities.[6] The Bouveault-Blanc reaction by protonation of ester radical anion concerted with the second electron transfer. This was shown in Scheme—5 which allowed the acyl-oxygen fission to take place with formation of the alkoxide and the aldehyde. Further reduction and protonation of the latter afforded the primary alcohol.

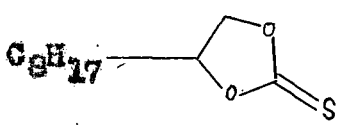
Scheme—5



The radical ion did not fragment immediately in ethanol solution was thought to result from solution of the anion by the solvent. Ester radical anions were sensitive to solvation and to temperature. At elevated temperatures in the presence of finely divided droplets of metal the reaction must be surface reaction in which

the ester radical anions remained bonded to the metal before coupling. While using liquid ammonia (-33°C) the radical ion which was formed could be trapped by alkylation as did not fragment quickly because of the low temperature.

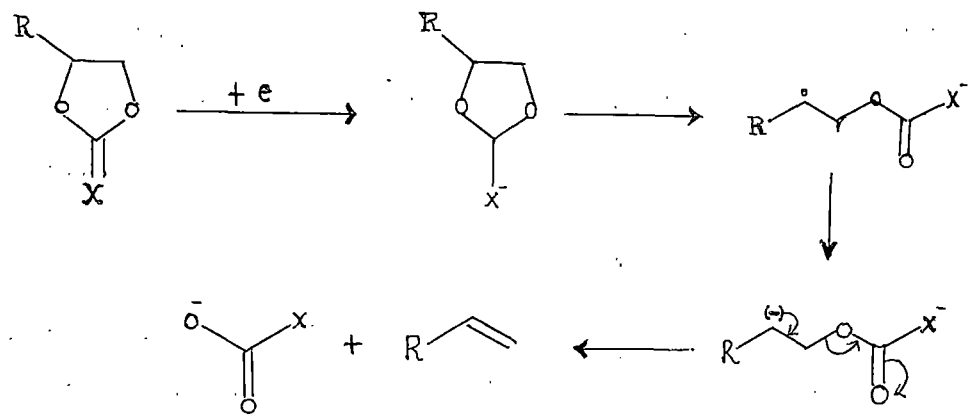
Barton and his coworkers[25] established a new method for deoxygenation of alcohols by reduction of the corresponding NN-dialkylamino thiocarbonyl oxyalkanes derivative with lithium in ethyl amine or potassium solubilised by 18-crown-6 in t-butylamine. Reduction of the 1,3-dioxolan-2-one or 2-thione derivatives were preparatively unsatisfactory. However formation of more decan-1-ol than decan-2-ol from the thions 60 was consistent with ring fragmentation at the radical- anion stage as shown in Scheme—6.



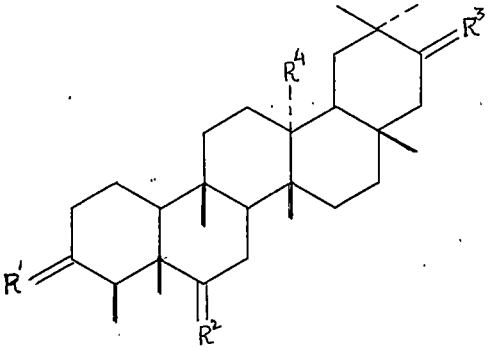
60

In general with the exception of xanthates and thiocarbamates all the alcohol derivatives were not efficiently deoxygenated.

Scheme—6



Gunatilak, Nanayakkara and Sultanbawa[26] used lithium-ethylenediamine reaction to establish the structure of kokzeylanol 61 and kokeyzeylmonol 62. Lithium-ethylene diamine reduction of kokzeylanol diacetate 63 followed by oxidation (CrO_3-pyridine) furnished friedelin 64 and similar treatment of kokzeylanol diacetate 65 gave friedelin-3,21-dione 66.



61, R^1=O; R^2= β-OH, α-H; R^3=H_2; R^4=CH_2OH

62, R^1=R^3=O; R^2= β-OH, α-H; R^4=CH_2OH

63, R^1=O; R^2= β-OAC, α-H; R^3=H_2; R^4=CH_2OAC

64, R^1=O; R^2=R^3=H_2; R^4=CH_3

65, R^1=R^3=O; R^2= β-OAC, α-H; R^4=CH_2OAC

66, R^1=R^3=O; R^2=H_2; R^4=CH_3.

CHAPTER—II

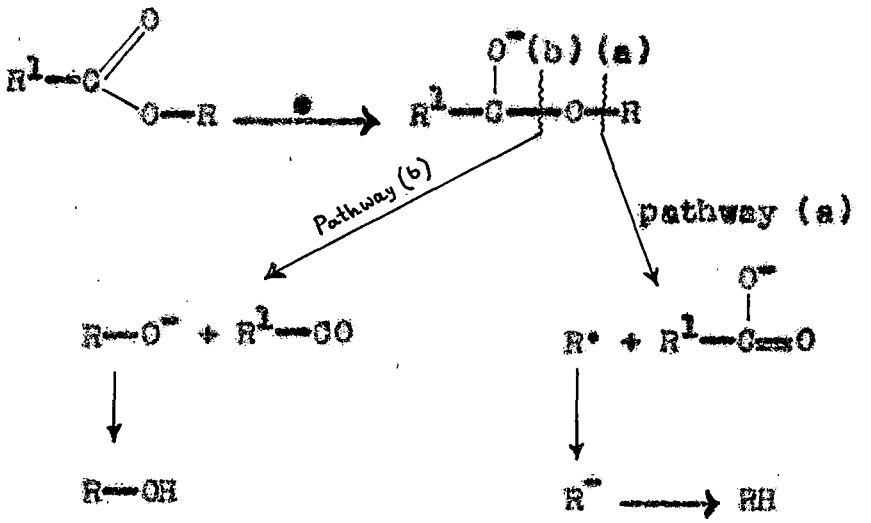
RESULTS AND DISCUSSION

SECTION—A

STUDIES ON THE REDUCTIVE ACTION OF LITHIUM ON TRITERPENOID LACTONES IN ETHYLENEDIAMINE

INTRODUCTION:

Barton and coworkers[21,22 & 24] reported that group 1A metal and alkyl amine reduced the esters and gave different products in different reaction condition and temperature. The mechanism so proposed consisted of two pathways. In pathway (a) deoxygenation took place and alkane was obtained and in pathway (b) deacylation took place and alcohol was obtained. The alcohols could be deoxygenated by making their different derivatives when treated with group 1A metal and alkylamine.



It was observed by Barton and coworkers[21] that the ester of hindered alcohols furnished more of the deoxygenated products (by pathway- a) while esters of non hindered alcohols produced the original alcohol (pathway- b).

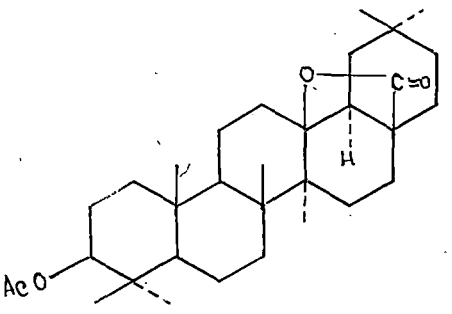
Interested by these results we tried to apply this type of reactions on triterpenoids. Our first attempt was on triterpenoid lactones. Since lactones can be considered as the intramolecular esters, we applied the reduction reaction on them. We took secondary and tertiary lactones of different sterical hindrance. The usual method for lactone ring opening lead to the introduction of

a functional group at the carbon where the lactyl oxygen is attached. The most common reagents used for such purposes are acid, alkali and lithium aluminium hydride but some of the lactones are stable to the former two while reduction with the last one may not always be desirable.

Sengupta and coworkers[20] studied the reaction on triterpene acetate using lithium in presence of ethylenediamine. We also used the same reagent for the reaction of triterpene lactones. Besides, lactones we have also done some reactions on triterpene keto compounds and triterpene hindered ester which has been discussed in section B.

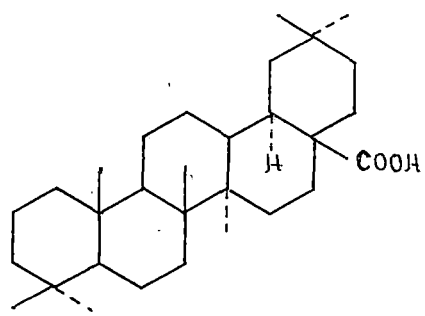
(a) <u>Treatment of lithium-ethylenediamine on triterpene tertiary lactones:</u>

While studying the reactions of lithium in presence of ethylenediamine the first model compound was taken as a tertiary lactone <u>viz.</u> 3β-acetoxy-oleanan-18α-H-28⟶13-olide[27] <u>67</u> .



<u>67</u>

The lactone 67 was refluxed with a mixture of lithium and dry ethylenediamine in an atmosphere of nitrogen gas for 2 hours. Excess lithium was destroyed by addition of ammonium chloride. After usual work up the mass was extracted with ether and treated with 10% NaOH to separate neutral and acid parts. After separation and removal of solvent from neutral part showed no residue indicating the absence of formation of neutral compounds, while acid part gave a gummy mass which was chromatographed over silical gel column. The less polar fraction which gave negative TNM test was analysed for $C_{30}H_{50}O_2$, m.p. 270°—1° $[\alpha]_D$ + 8.8°, was identified as oleanan-18α-H-28 oic acid 68. The yield of this compound was found to be 40%. The structure of the acid as 68 was established on the basis of IR, PMR and mass spectral analysis.



68

IR spectrum (Fig.1) of the compound showed peaks at 1695 cm^{-1} (—COOH function). No peak for hydroxyl functional group was observed in the IR spectrum.

PMR spectrum (Fig.2) of the compound 68 showed the presence of seven tertiary methyl groups in the region 0.69 to 0.94 ppm.

Mass spectrum (Fig.3) of the compound showed moleculer ion peak at m/e 442, other prominent peaks at m/e 427, 398, 397, 261, 205, 191 (base). Cleavage[28] of ring C yielded the fragments a and b, at m/e 205 and 191 respectively. The peaks at m/e 398, 397 was assumed to be the loss of CO_2 and —COOH from moleculer ion peak. The peak at m/e 427 might be due to loss of one CH_3 group from M^+ ion (Chart—1).

With the increase of polarity of solvents in the chromatographic separation, the second compound was obtained in 40% yield. The compound after crystallisation from $CHCl_3$—MeOH, was analysed for $C_{30}H_{50}O_3$, m.p. 295—6°, $[\alpha]_D$ +16°. IR spectrum (Fig.4) of the compound showed the presence of a hydroxyl group in the region 3450 cm^{-1}. Peak at 1690 cm^{-1} was assumed for the presence of a carboxyllic acid functional group. The structure of

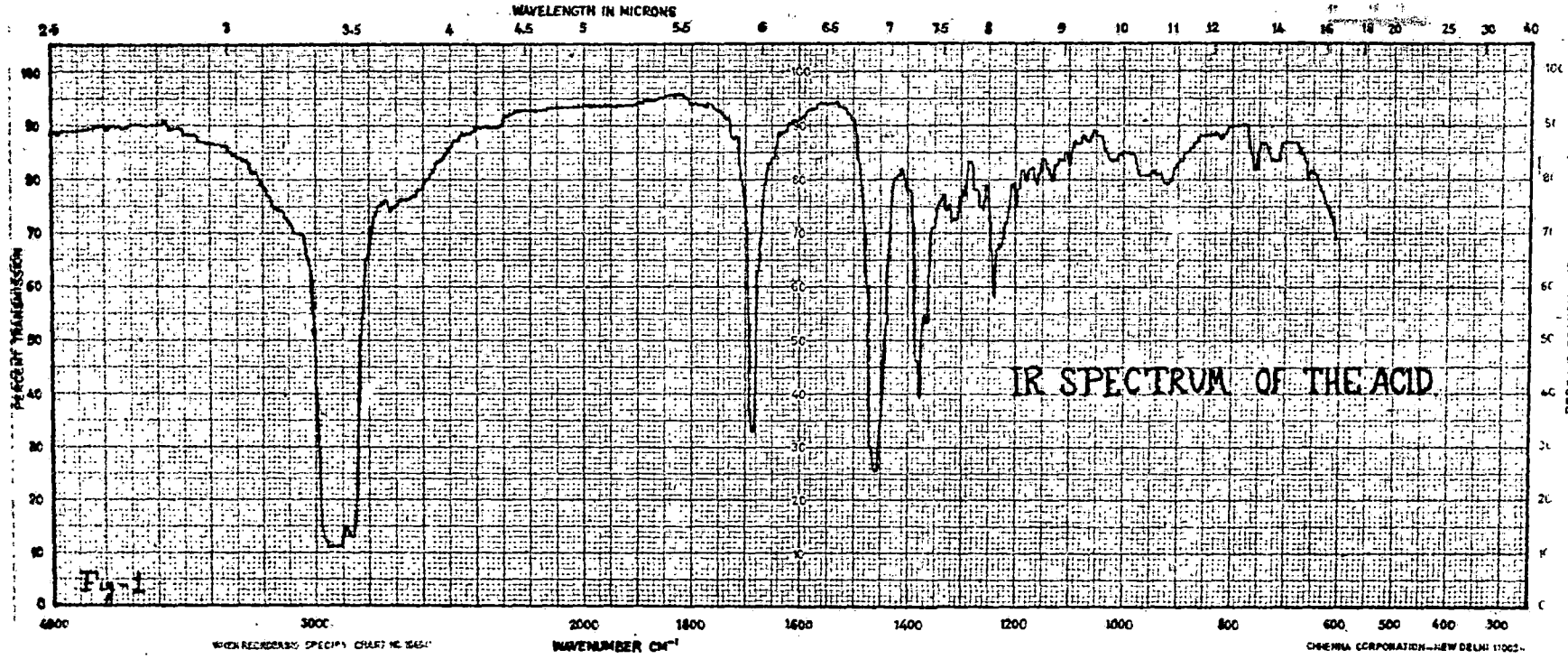


Fig.1: IR spectrum of oleanan-18∝-28-oic acid, 68.

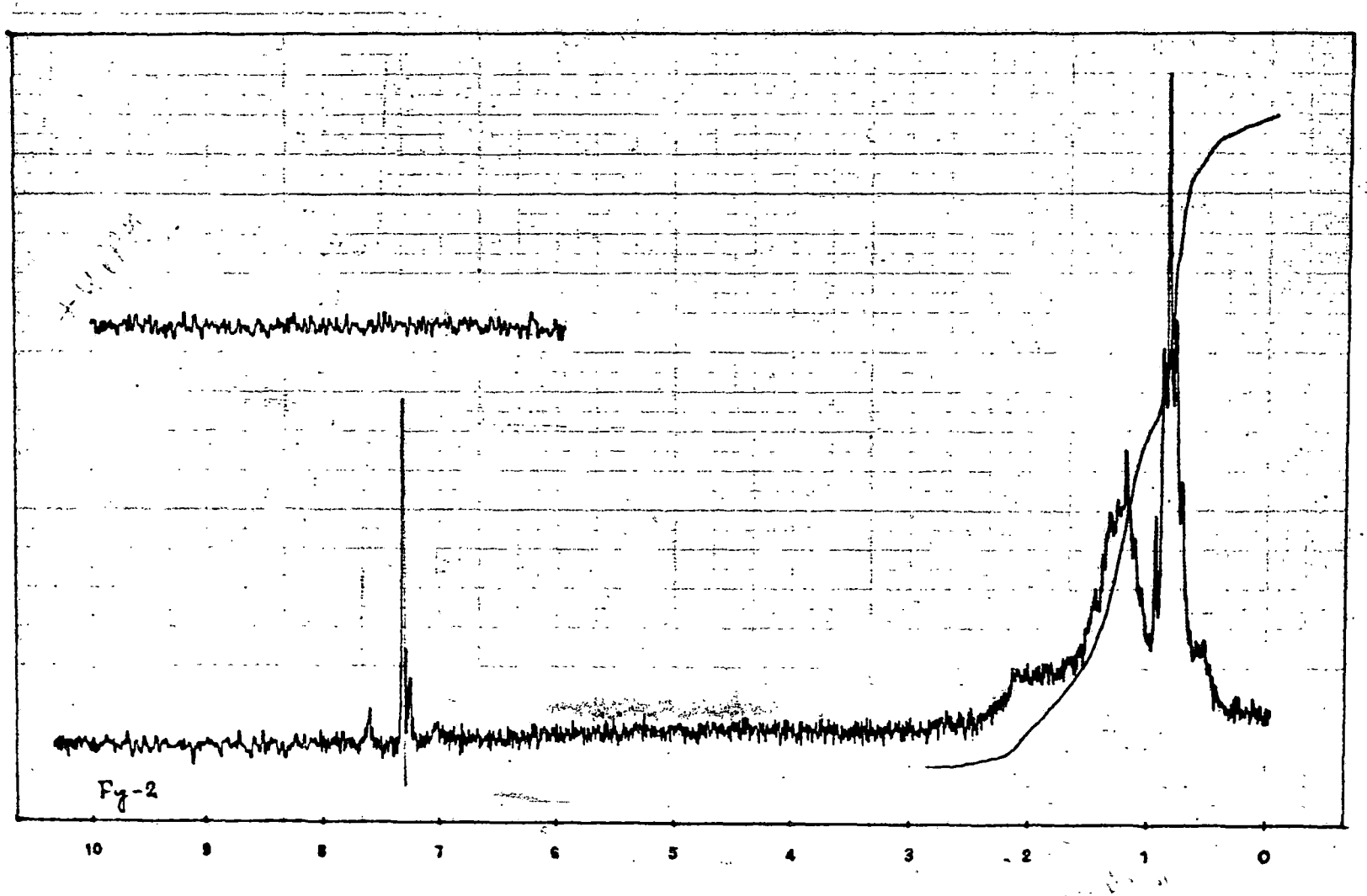


Fig.2: PMR spectrum of oleanan-18∝-28-oic acid, 68.

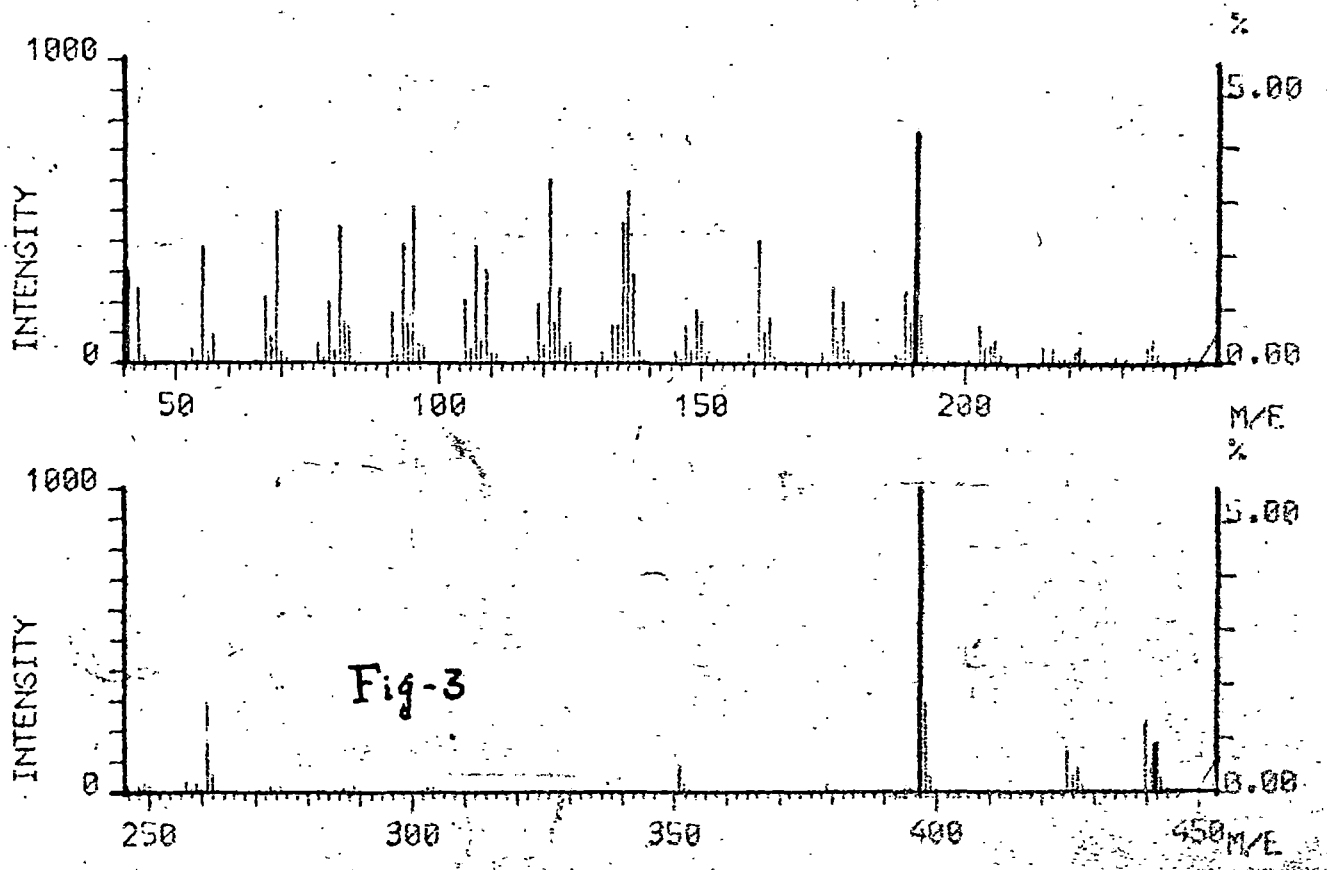


Fig-3

Fig.3: Mass spectrum of oleanen-18∝-28-oic acid, 6B.

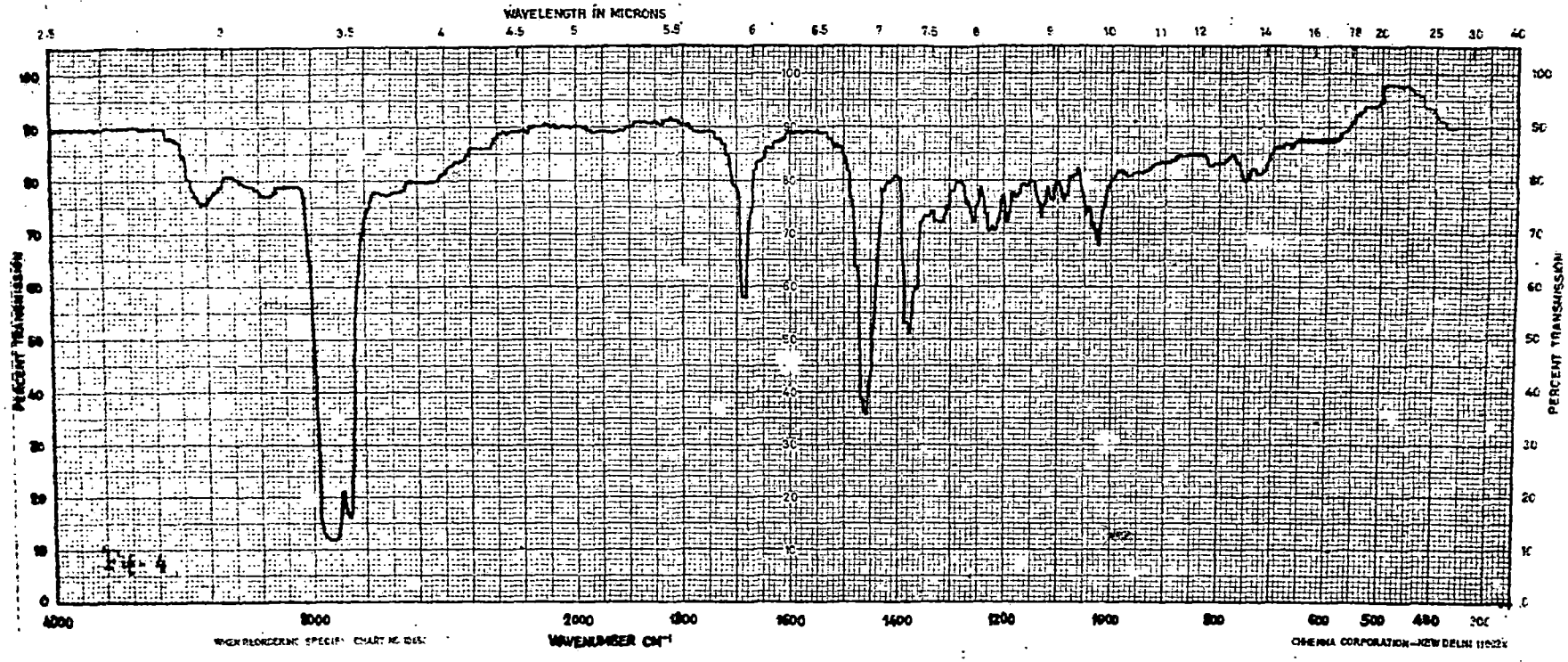
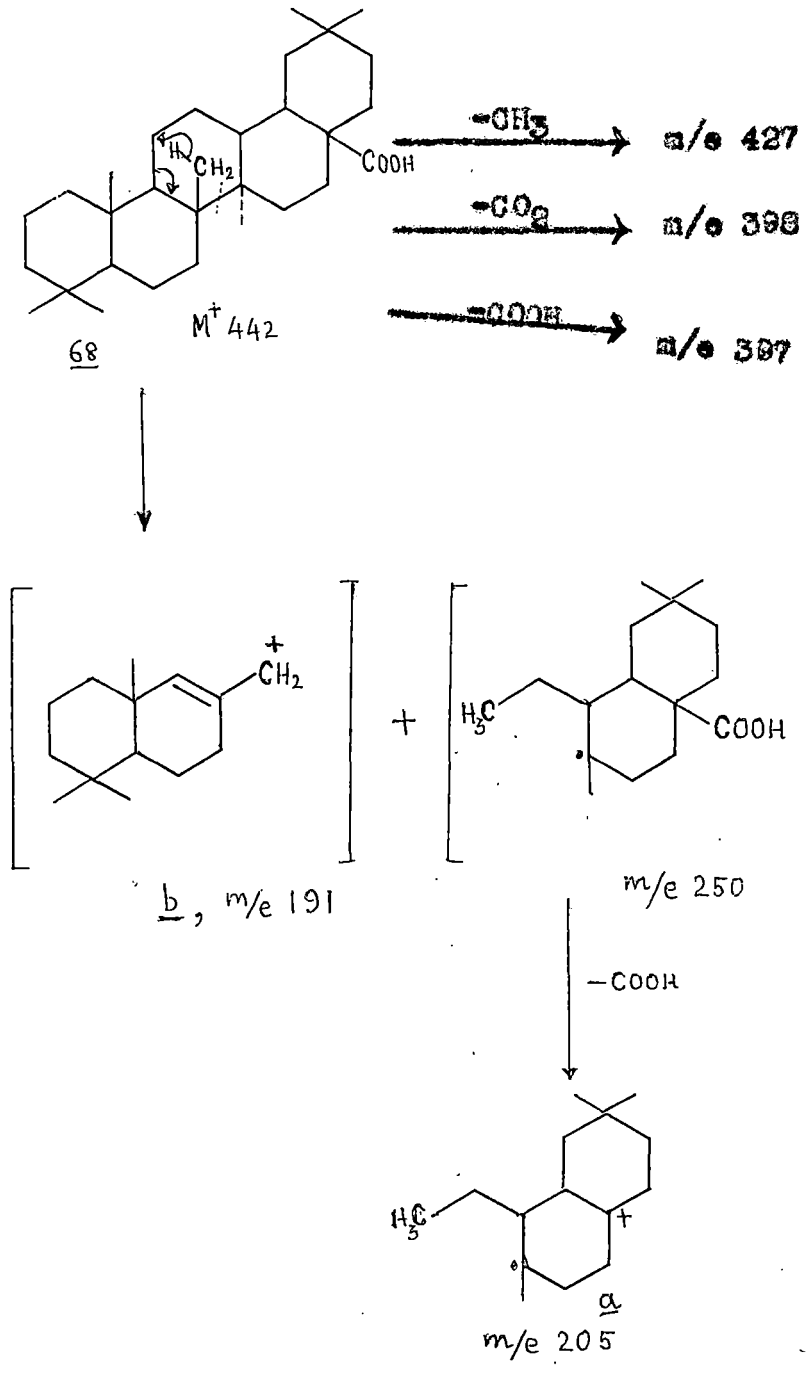
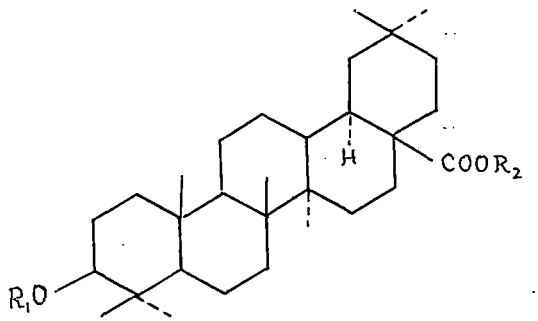


Fig.4: IR spectrum of 3-hydroxy-oleanan-18 α-H-28-oic acid, 69a.

Chart—1


-CH3
m/e 427
-CO2
m/e 398
-COOH
m/e 397
M+ 442
68
CH2
COOH
b, m/e 191
H3C
COOH
m/e 250
-COOH
H3C
a
m/e 205

the compound 69a was established from its mass spectra and also from the IR, NMR and mass spectral analysis of its acetate and ester derivatives.



69a, R_1=H, R_2=H

69b, R_1=H, R_2=CH_3

69c, R_1=AC, R_2=H

Mass spectrum (Fig.5) of the compound 69a exhibited a molecular ion peak at m/e 458 which was consistent with the molecular formula $C_{30}H_{50}O_3$. The most substantial peaks in its mass spectrum appeared at m/e 443 (M^+—CH_3), 440 (M^+—H_2O), 425 (M^+—H_2O—CH_3), 414 (M^+—CO_2)and 413 (M^+—COOH), 250, 235, 205, 205 and 189. The formation of m/e 205 and 189 are characteristic of a saturated oleanan

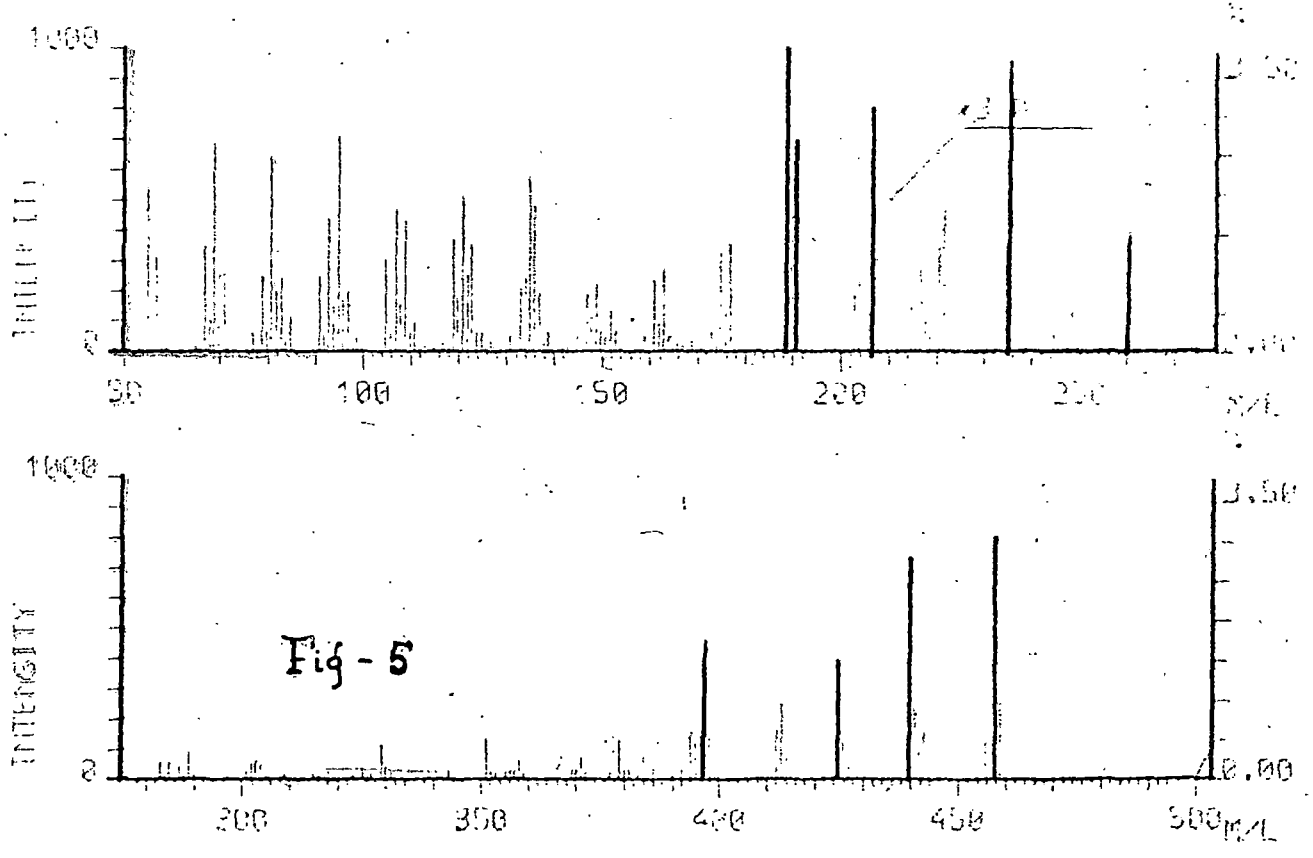
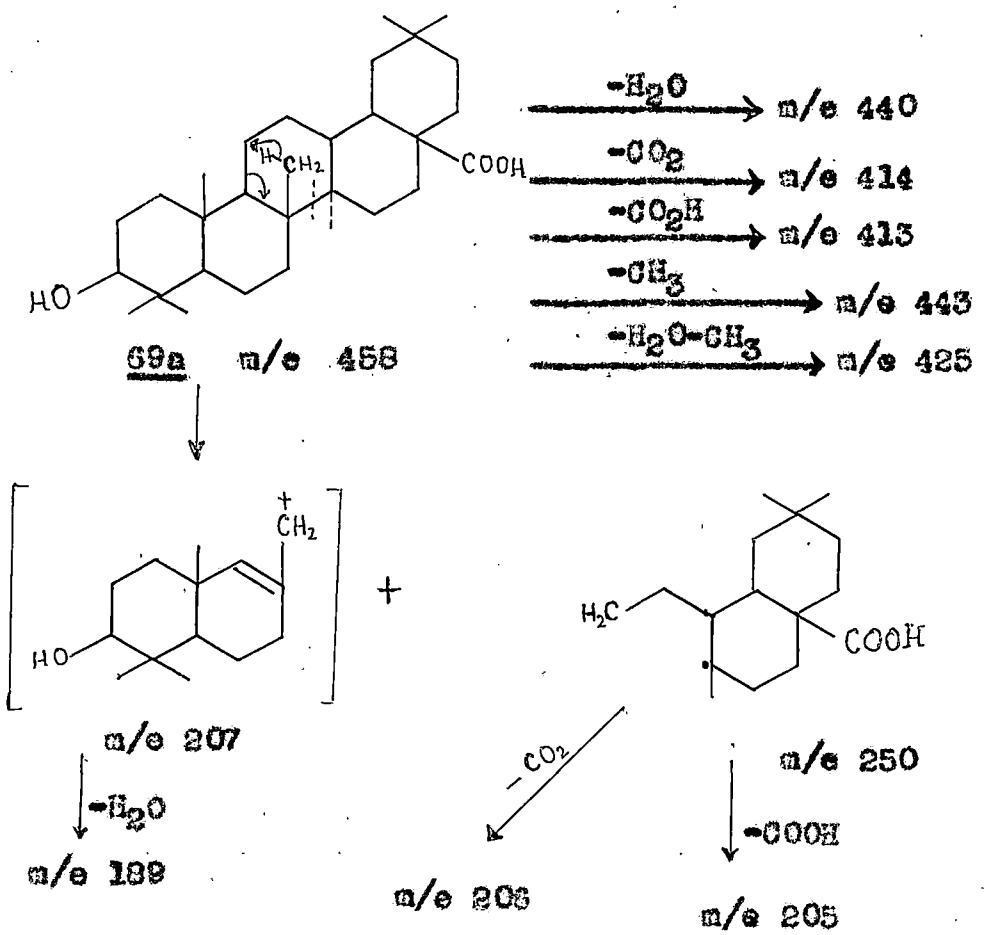


Fig.5: Mass spectrum of 3-hydroxy-oleanan-18∝-H-28-oic acid, 69a.

skeleton[28]. Cleavage of ring c formed two fragments at m/e 205 and 207. The fragment at m/e 250 by the loss of CO_2 or COOH formed the peak at m/e 205 and 206. The peak at m/e 189 might be due to loss of water from the fragment at m/e 207. The whole situation can be represented by Chart—2.

Chart—2



The compound 69a was esterified with an ethereal solution of diazomethane and the hydroxy ester so formed was crystallised and analysed for $C_{31}H_{52}O_3$, m.p. = 198°, $[\alpha]_D$ + 17°. IR spectrum (Fig.6) of the hydroxy ester 69b showed the presence of a hydroxyl functional group in the region 3250 cm^{-1} and the carbomethoxy functional group at 1725 cm^{-1}. PMR spectrum (Fig.7) of the compound showed the presence of seven tertiary methyl protons in the region 0.76 to 0.96 ppm. The proton geminal to C_3—OH appeared at 3.20 ppm as a quartet (—CHOH). The three protons of carbomethoxy functional group (—COOCH_3) appeared at 3.64 ppm. Mass spectrum (Fig.8) of the compound showed a molecular ion peak at m/e 472 which was consistent with the molecular formula $C_{31}H_{52}O_3$. Other peaks at m/e 454 (M^+-H_2O), 439 (M^+-H_2O-CH_3), 413 (M^+—COOCH_3), 395 (M^+—H_2O—COOCH_3). The peak at m/e 262 and 207 (base peak) were assumed to be formed by the cleavage of ring C as shown by Djerassi and coworkers[28] for saturated oleanan skeleton. The peak at m/e 189 obtained by the loss of water from m/e 207 and peak at m/e 203 which was prominent was assumed to be due to the loss of —COOCH_3 from m/e 262 (Chart—3).

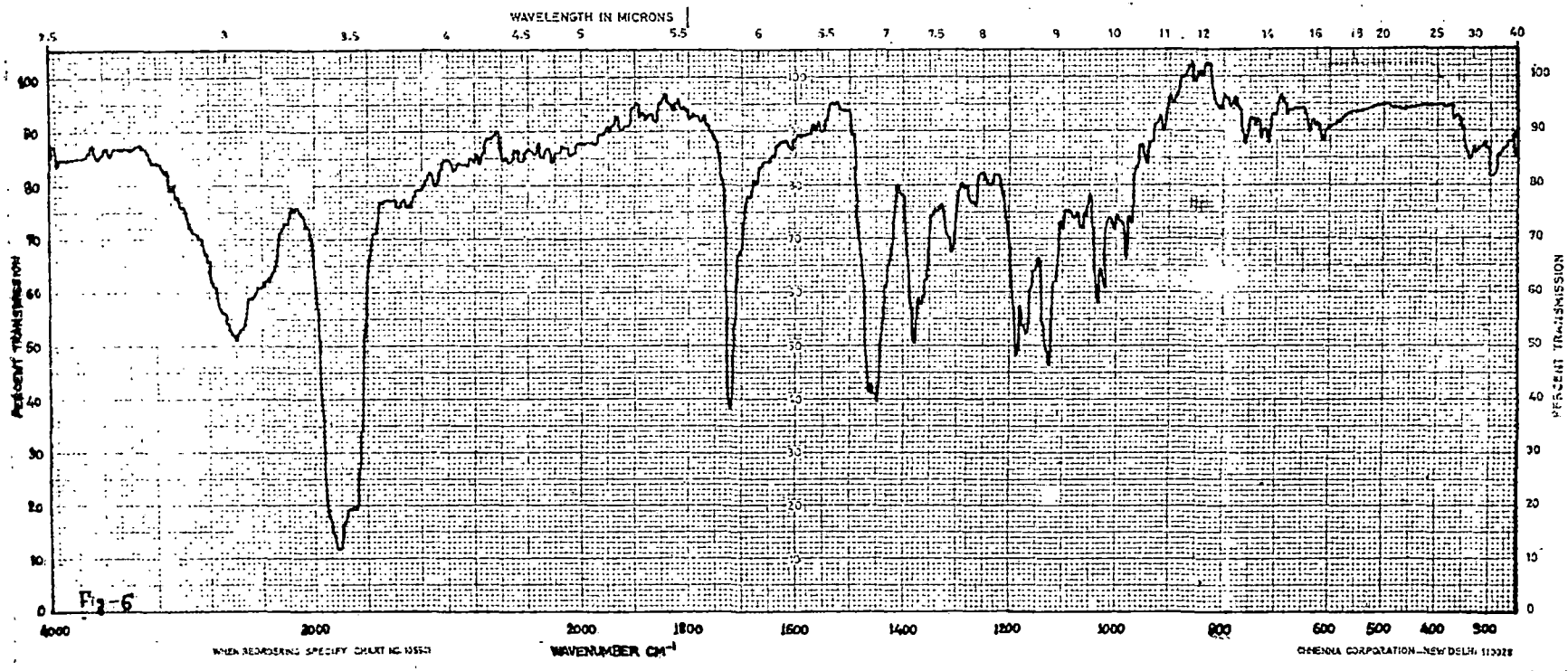


Fig.6: IR spectrum of methyl-3-hydroxy-oleanan-18∝-H-28-oate, 69b.

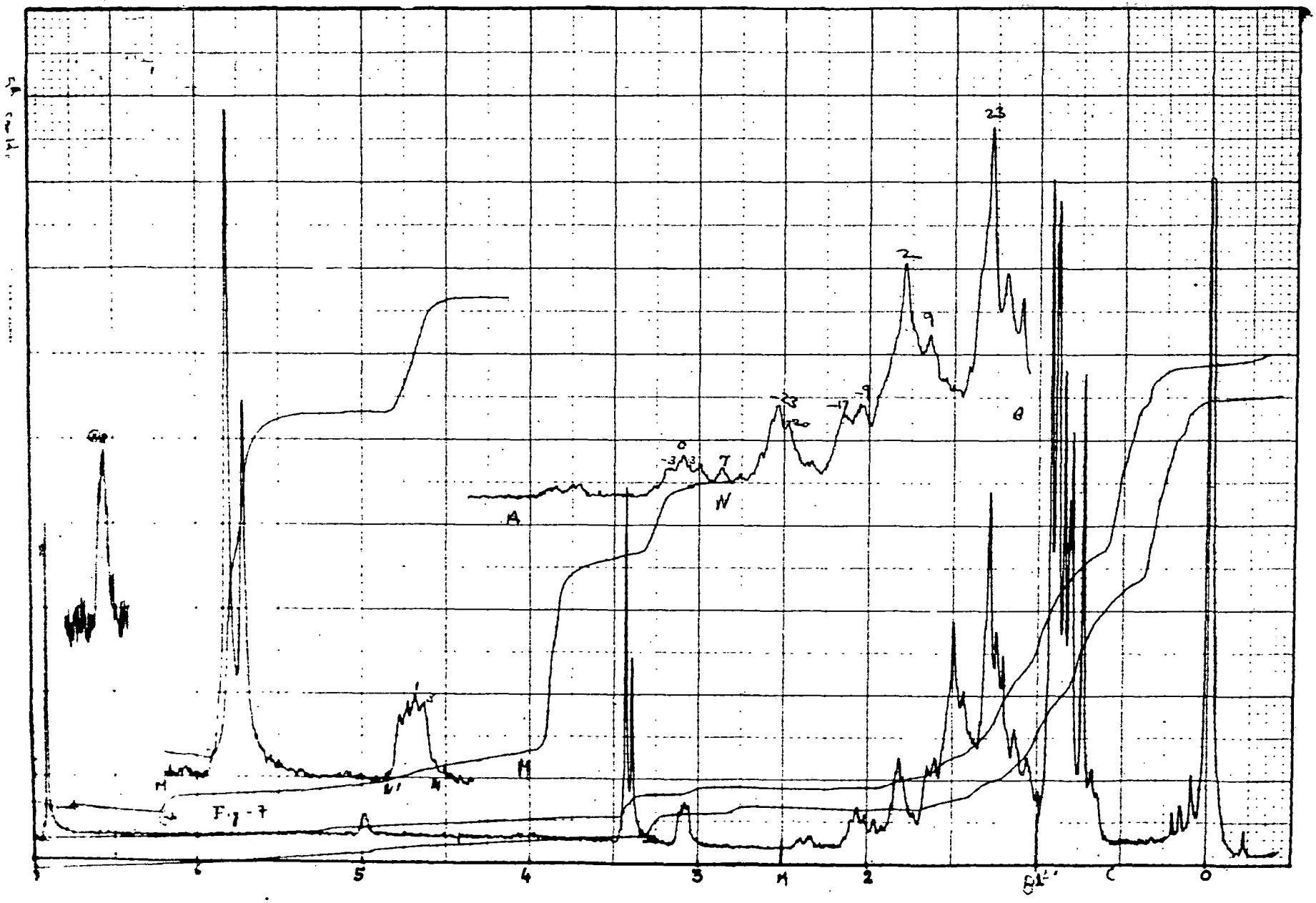


Fig.7: PMR spectrum of methyl-3-hydroxy-olean-18 α-H-28-oate, 69b.

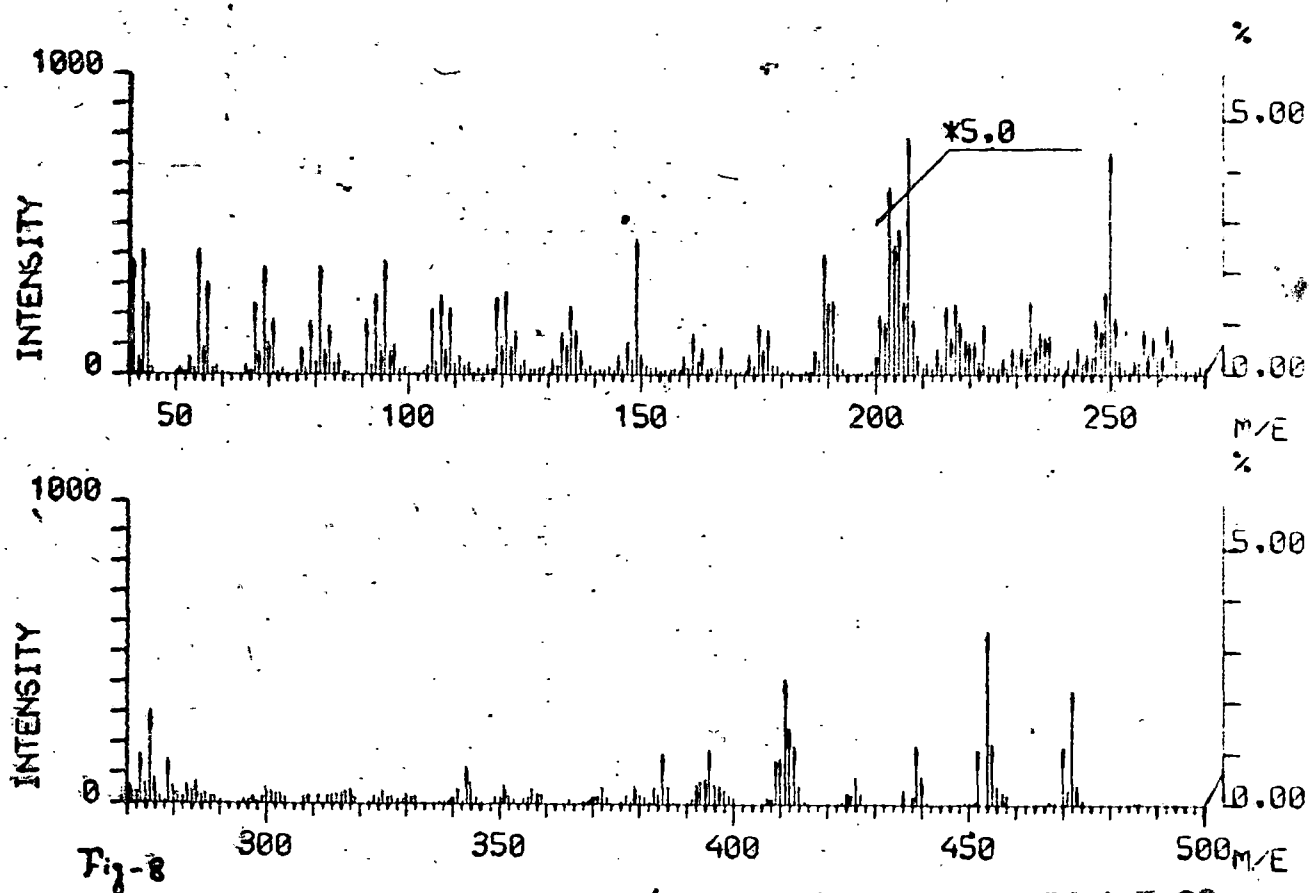
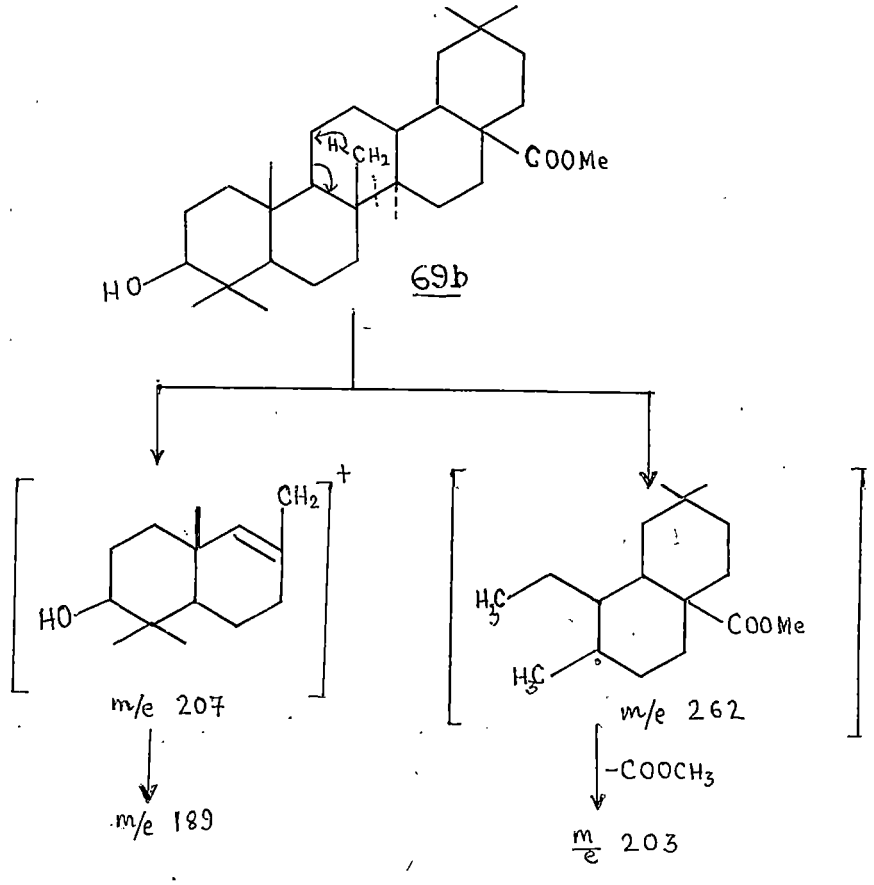


Fig-8

Fig.8: Mass spectrum of methyl-3-hydroxy-oleanan-18∝-H-28-oate, 69b.

Chart—3



The hydroxy acid 69a on acetylation with acetic anhydride and pyridine afforded a solid which was crystallised from chloroform and methanol to afford a crystal of m.p. 290—91°, $[\alpha]_D$ + 15°. Elemental analysis showed the molecular formula $C_{32}H_{52}O_4$. The structure of the compound was assigned as 69c on the basis of IR, PMR and mass spectral analysis.

IR spectrum (Fig.9) of the compound showed the presence of carboxylic acid function at 1695 cm^{-1} and peaks at 1725 cm^{-1} and 1245 cm^{-1} indicated the presence of an acetoxy functional group in the molecule. PMR spectrum (Fig.10) of the compound showed the presence of seven tertiary methyl groups in the region 0.81 to 0.94 ppm; peak at 1.96 ppm that appeared as a singlet was due to three protons of acetoxy group ($-OCOC\underline{H}_3$). The triplet at 4.3 ppm suggested the presence of a proton at C—3 geminal to acetoxy group ($-\underline{H}-C-O-COCH_3$) coupled with the two protons at C—2 position. On the basis of PMR spectra the structure of the acetoxy acid 69c can be assigned as 3β-acetoxy oleanan-18α-H—28—oic acid. The structure was corroborated by its mass fragmentation pattern. Mass spectrum (Fig.11) of the compound showed molecular ion (M^+) peak at m/e 500 which is consistent with the molecular formula $C_{32}H_{52}O_4$. Other peaks at m/e 455 (M^+-COOH), m/e 440 (M^+-CH_3COOH), m/e 425 ($M^+-CH_3COOH-CH_3$). The peaks at m/e 250 and 249 was assumed to form by the cleavage of ring C. Peak at m/e 205 are formed by the loss of —COOH group from m/e 250. Peak at m/e 189 might be explained by the loss of acetic acid fragment from m/e 249. The fragmentation is shown in Chart—4.

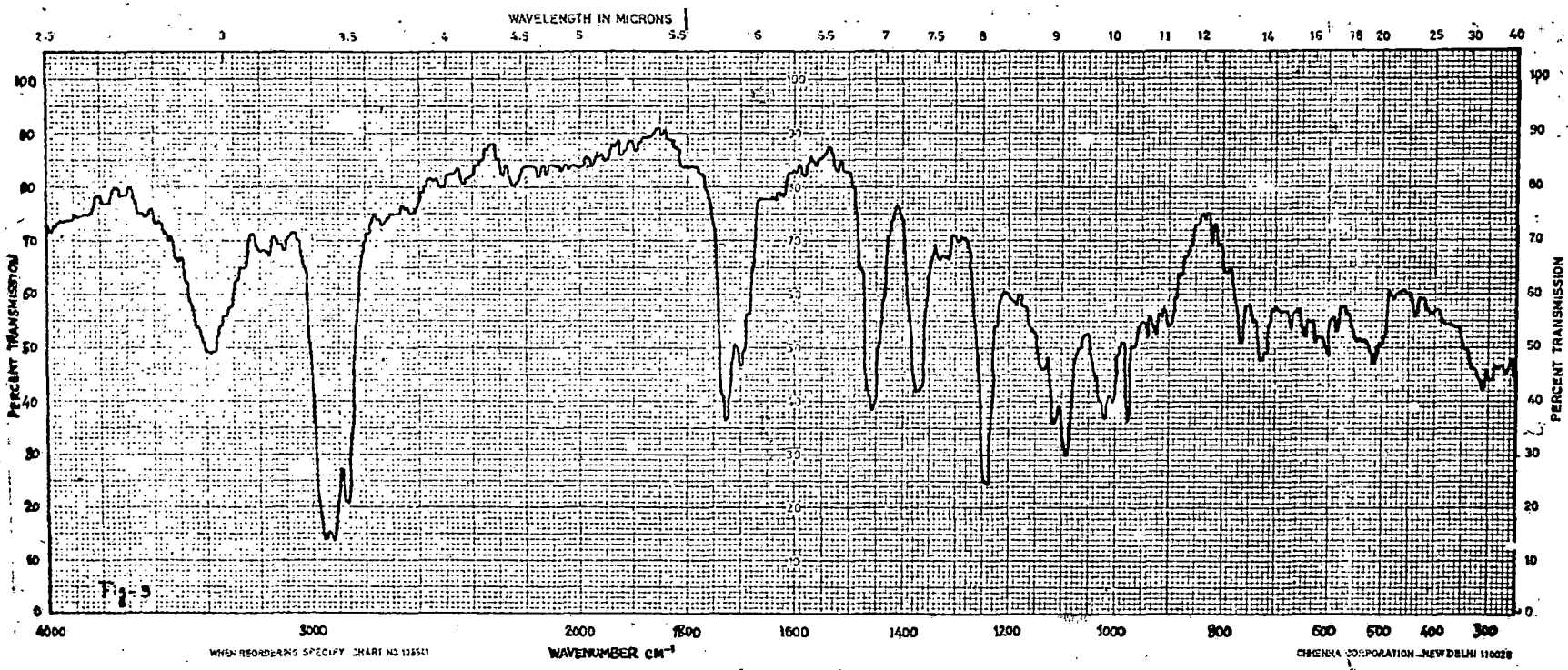


Fig.9: IR spectrum of 3-acetyl-oleanan-18 ∝-H-28-oic acid.

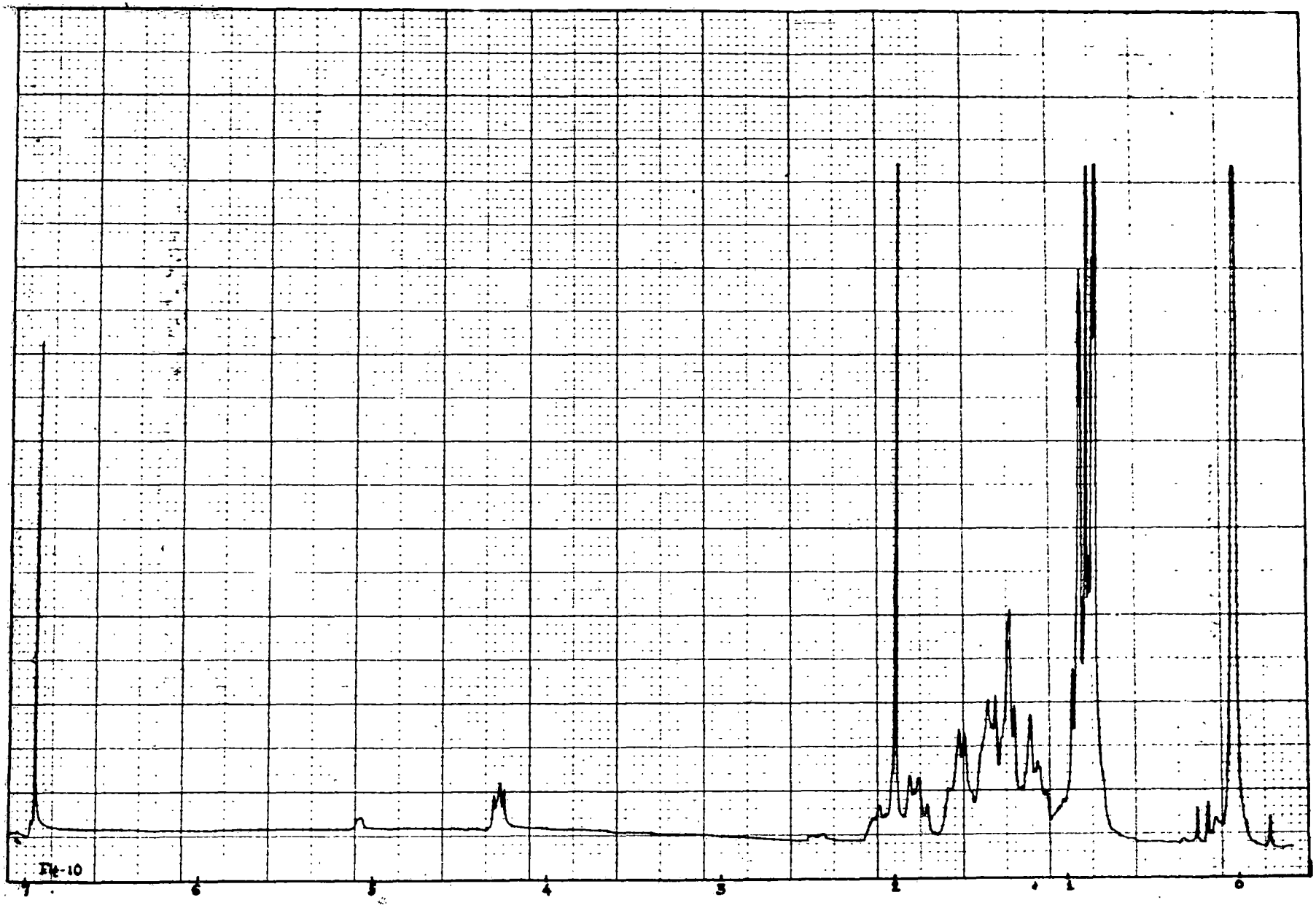


Fig.10: PMR spectrum of 3-acetyl-oleanan-18α-H-28-oic acid, 69c.

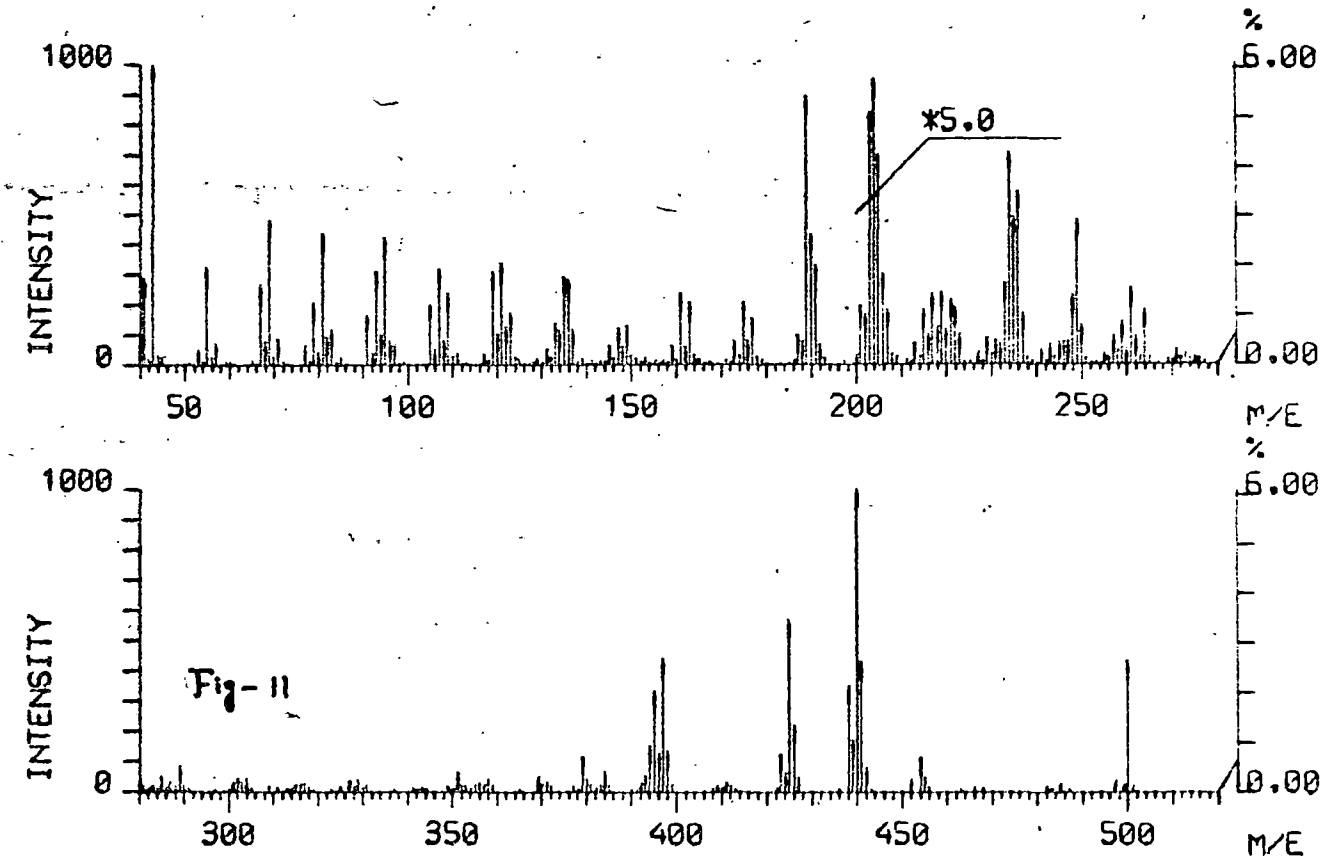
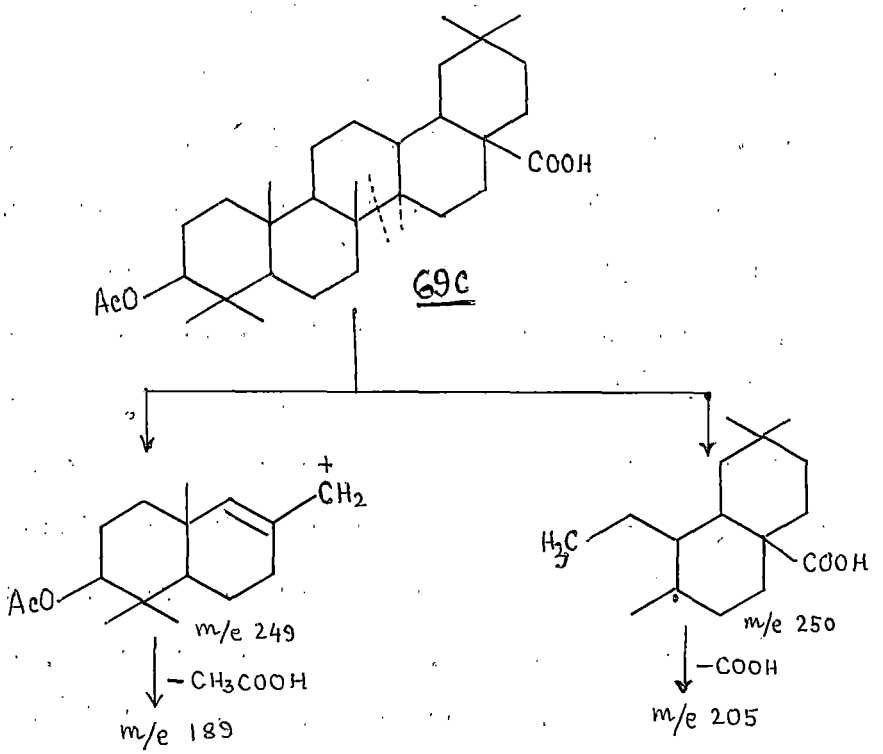


Fig-11

Fig.11: Mass spectrum of 3-acetyl-oleanan-18∝-H-28-oic acid, 69c.

Chart—4



From the above discussion on different spectral analysis of the second compound 69a and its derivatives obtained from lithium ethylenediamine reduction on 3β-acetoxy-oleanan-18α-H 28 ⟶ 13β-olide 67, it can be definitely assigned the structure of 69a as 3β hydroxy oleanan-18α-H—28 oic acid.

Thus lithium in ethylenediamine was found to be novel method for lactone ring opening. In the compound 3β-acetoxy oleanan-18α-H—28 ⟶ 13β olide the lactyl oxygen

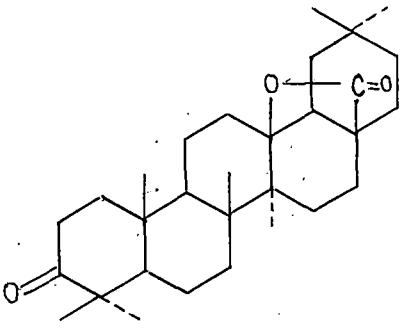
attached in 13 position was tertiary. The cleavage of C—13—O bond is in accord with the mechanism given by Barton and coworkers[21,22 and 24]. Since the lactyl oxygen at C—13 is attached to the tertiary carbon it was assumed that complete deoxygenation at C—13 would give the saturated acid without introduction of any group at C—13 position. The acetate group at C—3 position was found to be partially deoxygenated as observed by Sengupta and coworkers[20].

So tertiary lactones when treated with lithium in presence of ethylenediamine gave saturated acid by deoxygenation on the carbon where the lactyl oxygen is attached. We have studied the same reaction on another tertiary keto lactone viz. 3-oxo-oleanan-18α-H-28⟶13β-olide[29] 70. Carbonyl group at C—3 position was taken instead of acetate to avoid the mixture of products as was seen in the earlier case.

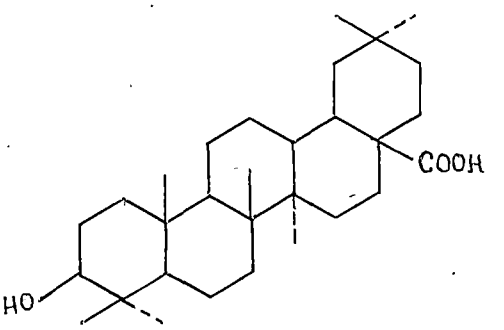
Treatment of lithium in presence of ethylenediamine on 3-oxo-oleanan-18α-H-28⟶13β-olide[29] 70.

The compound ketolactone 70 was treated with lithium in presence of ethylenediamine in N_2 atmosphere for 2 hours. After usual work up and separation of acid and

neutral part no material was obtained from the neutral part. The acid part was chromatographed which afforded only one compound in 85% yield, m.p. 295—6°, $[\alpha]_D$ + 15° was analysed for $C_{30}H_{50}O_3$. The compound was found to be identical with 3β-hydroxy oleanan-18α-H-28-oic acid 69a (mmp and IR comparison) and also preparation of methyl ester derivative confirmed the structure.



70

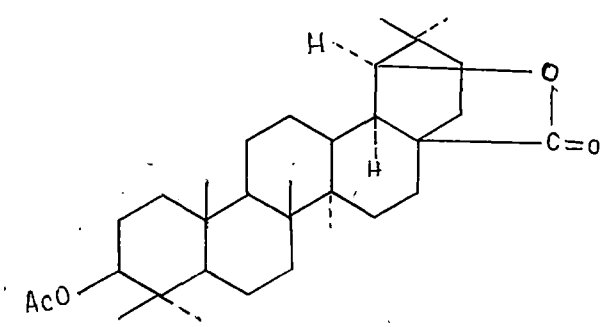


69a

The tertiary lactone here also gave the saturated acid with deoxygenation on carbon where the lactyl oxygen was attached. Moreover the carbonyl group at C—3 position was reduced to the thermodynamically more stable hydroxyl group. The identity of the compound was correctly established by preparing corresponding methyl ester derivatives and compared with the previously prepared compounds mentioned in the preceeding paragraphs.

(b) Reduction of triterpene secondary lactones with lithium-ethylenediamine:

We have discussed the results of reaction of lithium in presence of ethylenediamine on tertiary lactones. Now we concentrate our attention on the reaction of lithylenediamine on triterpene lactone where the lactyl oxygen is attached to a secondary carbon. For the model compound we have elected 3β-acetyl-oleanan-18α-H-28 ⟶ 19β olide[30] 71 prepared from acetyl betulenic acid.



71

The lactone 71 was refluxed with a mixture of lithium and dry ethylenediamine in an atmosphere of nitrogen gas for 2 hours. Excess lithium was destroyed with solid ammonium chloride. After usual work up the mass obtained was washed with 10% sodium hydroxide to separate the acid and neutral fractions.

The acid fraction was chromatographed over a silica gel column and two products thus separated were crystallized from chloroform-methanol mixture separately. The less polar compound, m.p. 269—70°, $[\alpha]_D$ + 8.8° had the molecular formula $C_{30}H_{50}O_2$ was found to be identical with olean-18α-H-28-oic acid 68 (30% yield). Similarly the more polar fraction furnished a solid (35% yield), m.p. 295—6°, $[\alpha]_D$ + 14.5°, that was analysed for $C_{30}H_{50}O_3$. Preparation of the ester and acetate derivatives confirmed the compound as 3β-hydroxy-olean-18α-H-28-oic acid 69a. The compounds 68 and 69a was identified by comparison with the authentic specimens prepared from the previous reactions (mmp and GC-IR comparison).

The neutral fraction was chromatographed over a column of deactivated alumina. A compound was obtained in 15% yield while the elution of solvent was benzene:ether (3:2). The compound was crystallized from a mixture of chloroform-methanol and the crystallised product 72a had the molecular formula $C_{30}H_{52}O_3$, m.p. 230—32°, $[\alpha]_D$ +23°; it gave negative TNM test showing absence of olefinic double bond. IR spectrum (Fig.12) of the compound showed broad peak in the region 3350—3400 cm^{-1} for hydroxyl groups present in the molecule. No other significant peak was found in IR spectrum. The structure of the compound was proved by PMR

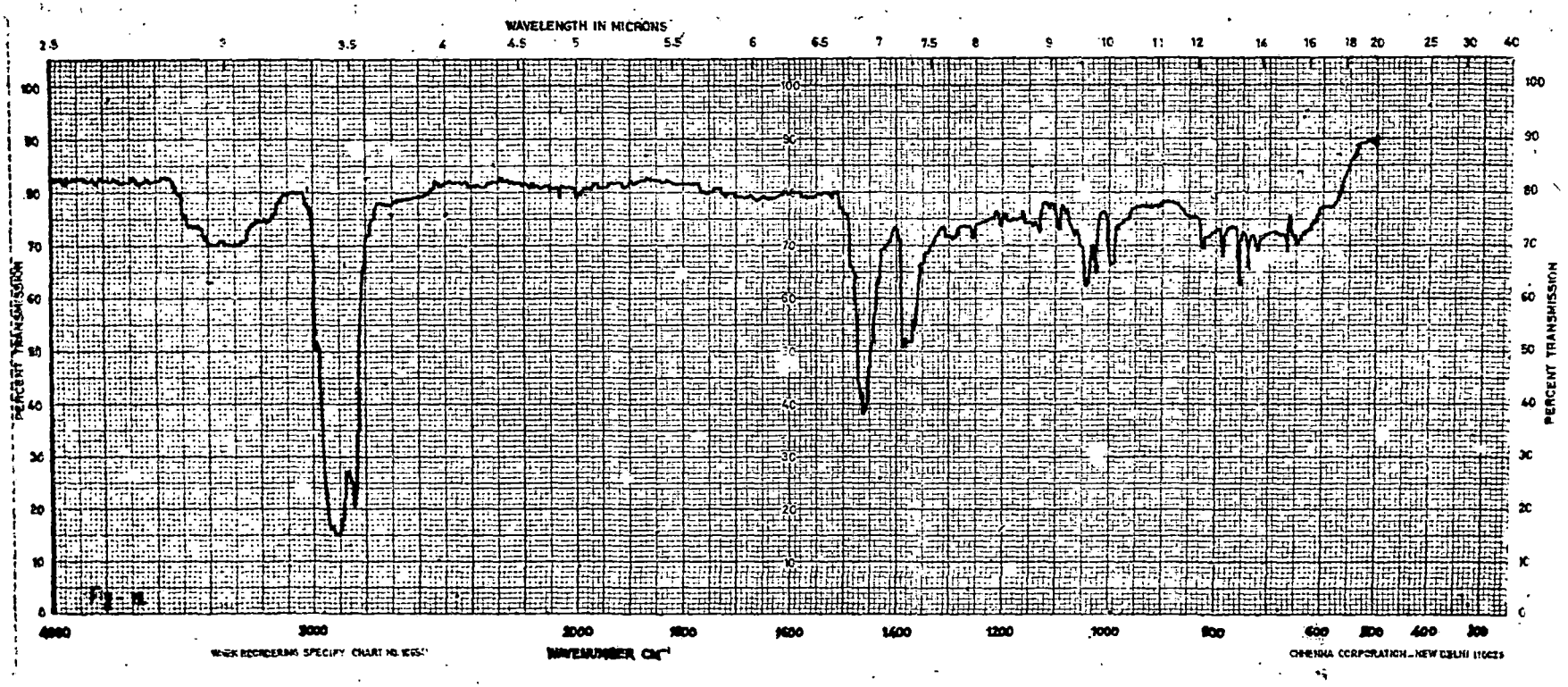
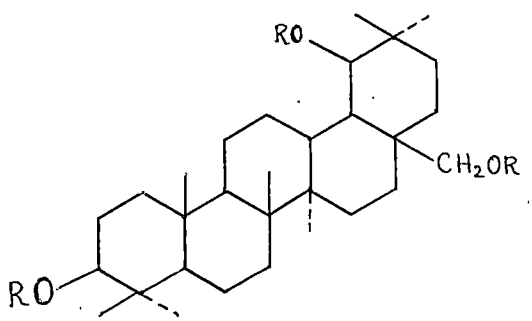


Fig.12: IR spectrum of oleanan-18α-H-3β,19β,28-triol, 72a.

and mass spectral analysis. PMR spectrum (Fig.13) of the compound showed seven tertiary methyl protons at the region 0.8 to 1.3 ppm. At the region 3.5 ppm peaks obtained for two methylene protons geminal to the hydroxy group. The peak



72a, R = H

72b, R = OAc.

at 4.13 ppm as a multiplet was assumed for the proton geminal to C—3 - hydroxyl group. Another peak at 4.26 ppm might be due to the proton geminal to C—18 hydroxyl group. The structure of the trihydroxy compound was finally proved by its mass fragmentation pattern. The compound gave a peak at m/e 442 (Fig.14) which was assumed to form by the loss of one molecule of water from M^+ 460. Other peaks at m/e 424 (m/e 442—H_2O), 409 (m/e 424—CH_3), 394 (m/e 424—CH_2OH), m/e 381 (base peak) which can be shown from the genesis (Chart—8). The peaks at m/e 189 and 203 can be

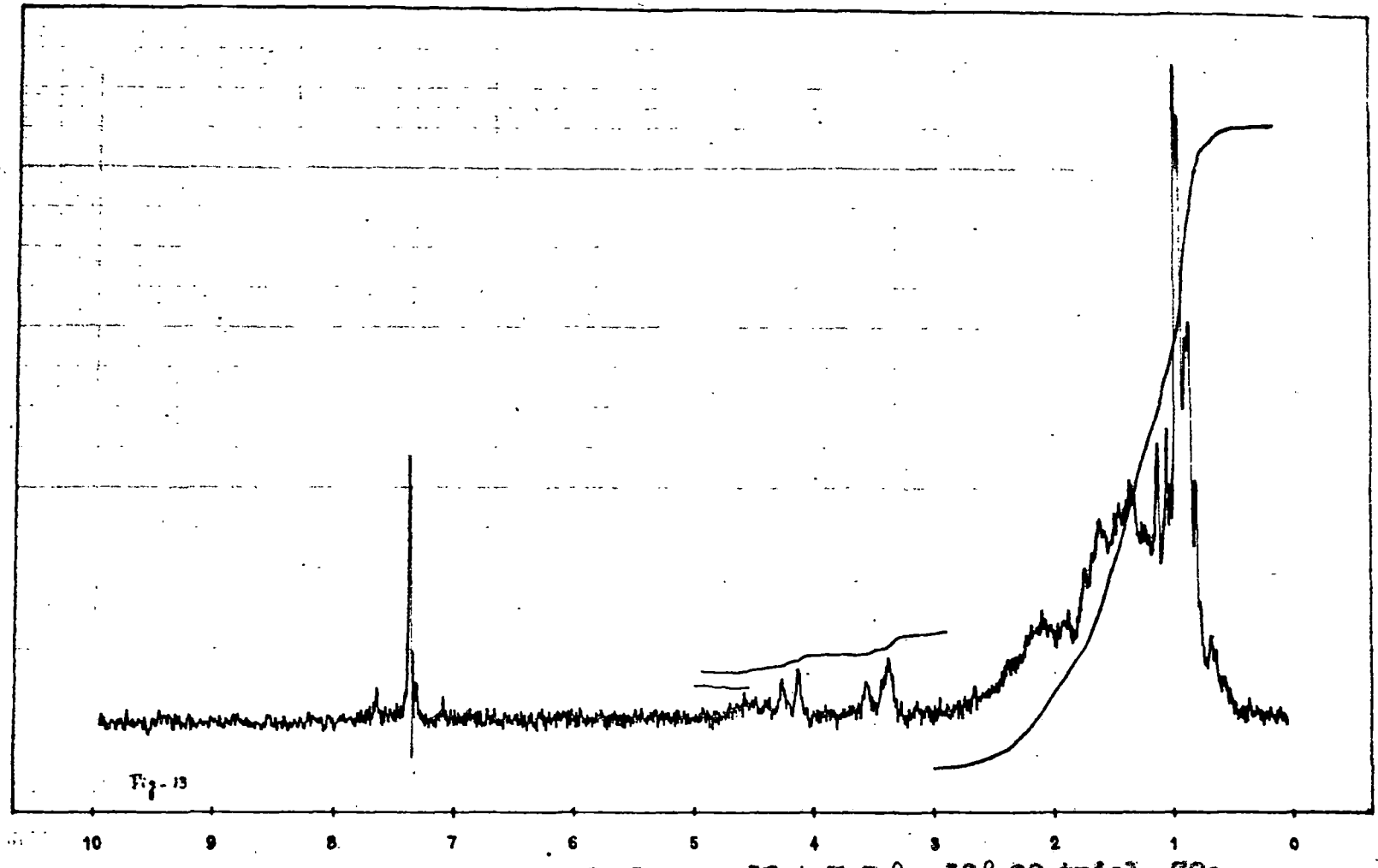


Fig.13: PMR spectrum of oleanan-18α-H,3β, 19β,28-triol, 72a.

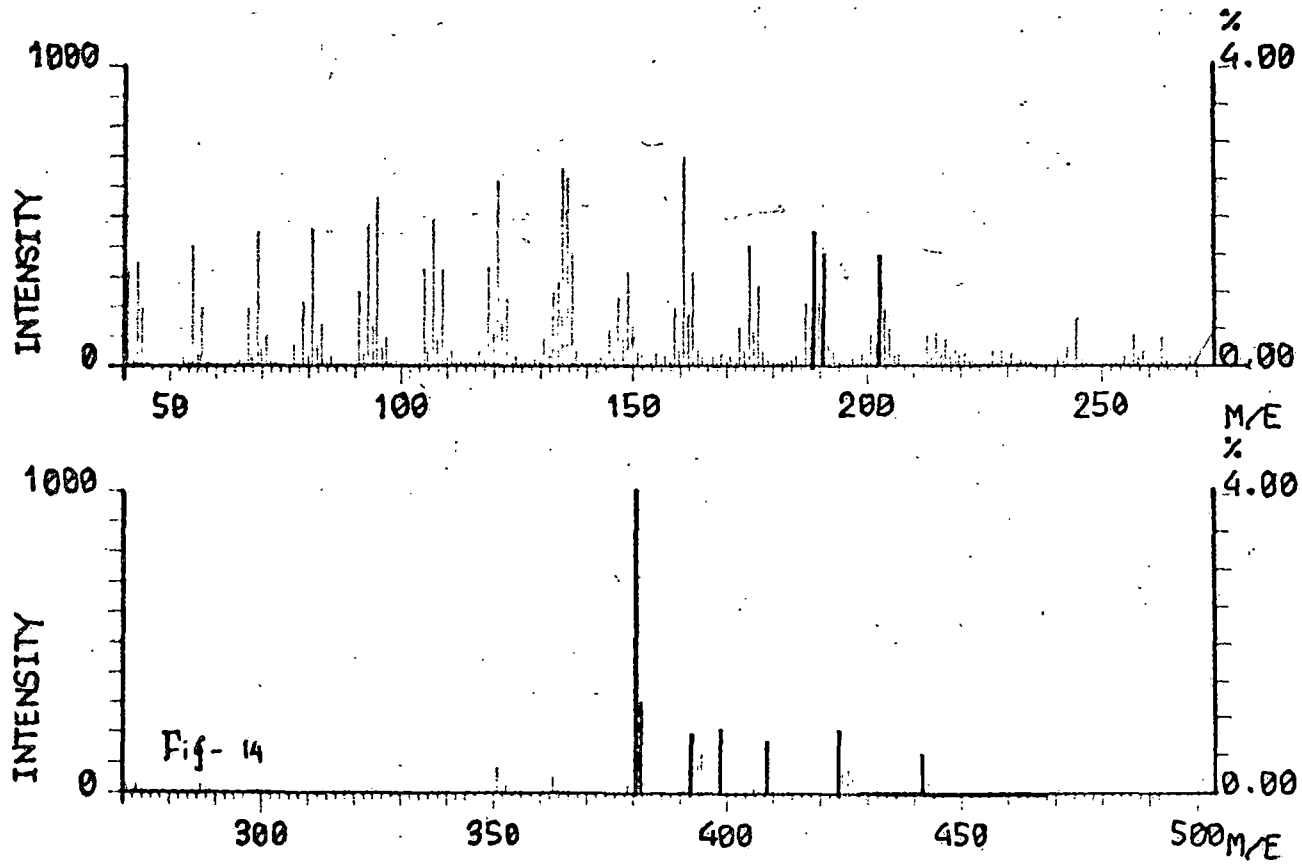
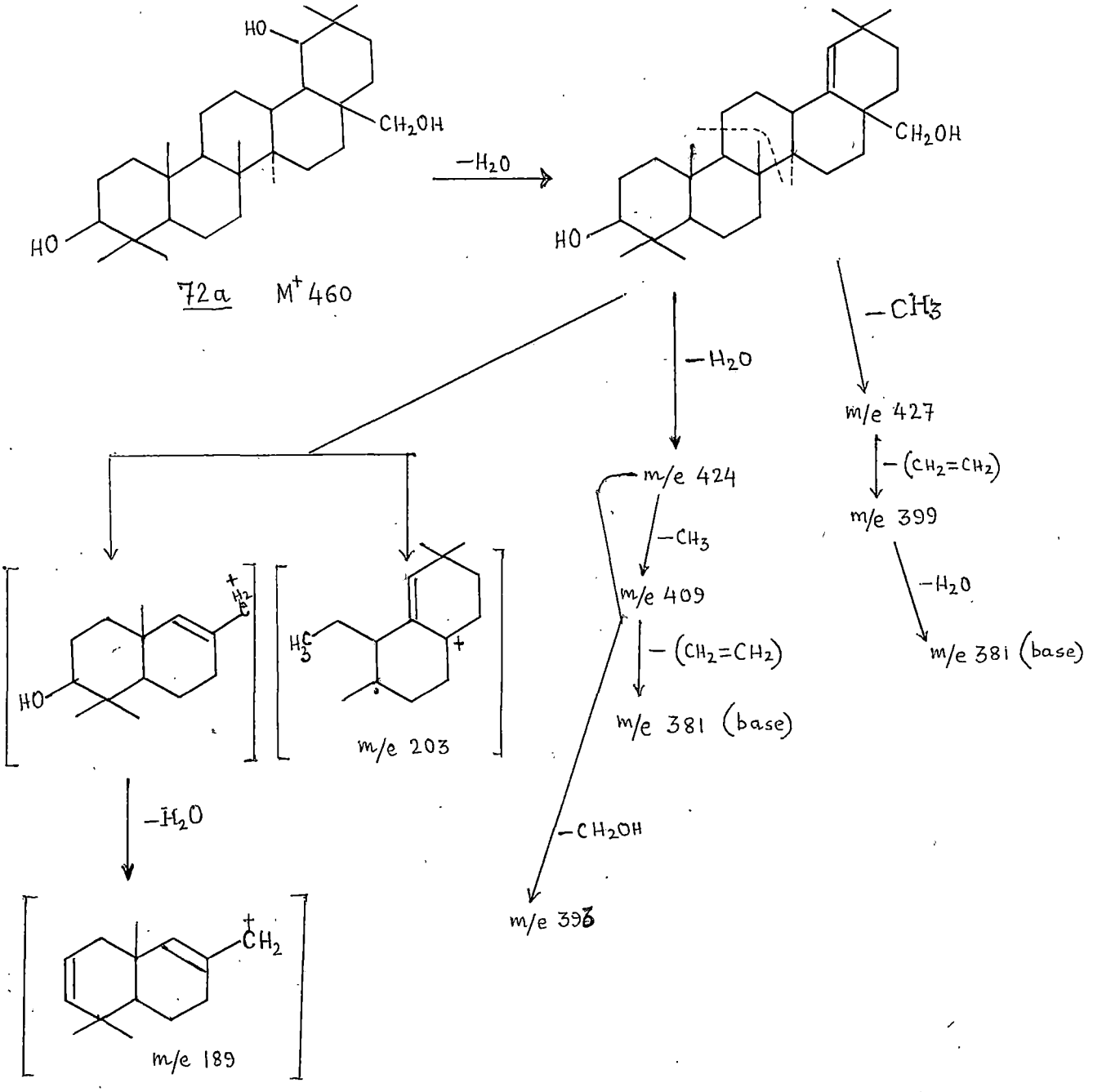


Fig- 14

Fig.14: Mass spectrum of oleanan-18α-H-3β,19β,28-triol, 72a.

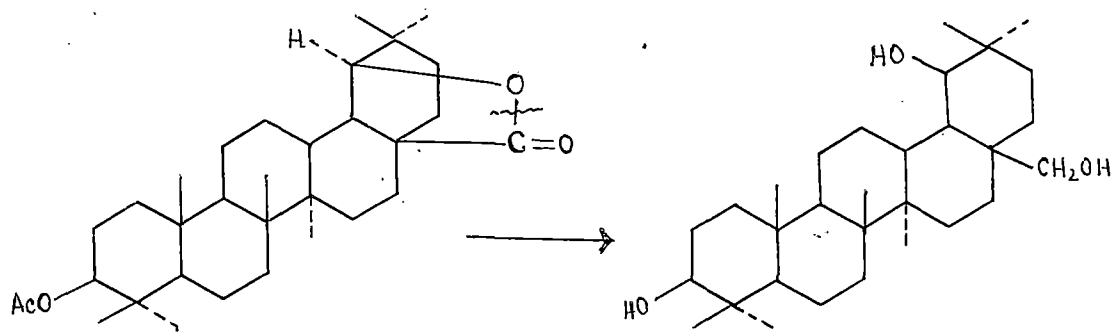
explained by the cleavage of ring C of a saturated oleanen skeleton[28], as shown in Chart—5.

Chart—5



So the structure of the trihydroxy compound can be assigned as oleanan-18α-H, 3β, 19β, 28 triol[31]. The structure was finally confirmed by preparing the corresponding triacetate[31] derivative 72b, m.p. 211°—12°, which was analysed for $C_{36}H_{58}O_6$. IR spectrum showed broad peaks at the region 1720—1730 cm^{-1} and 1240—1250 cm^{-1} which confirmed the presence of acetate groups in the molecule. The finding in the reaction of lithium in presence of ethylenediamine on 3β-acetyl-oleanan-18α-H-28 ⟶ 19β olide 1e. a secondary lactone is different to some extent that of a tertiary lactone. In the case of a secondary lactone we got three products viz. oleanan-18α-H-28-oic acid 68, 3β-hydroxy-oleanan-18α-H-28-oic acid 69a, oleanan-18α-H-3β, 19β, 28-triol 72a. The formation of third compound although in lower yield can be explained by assuming that the secondary lactyl oxygen is less sterically hindered and hence the cleavage —O—C(=O)— of the lactone was possible as showed by Barton and coworkers[18] in case of a less sterically hindered esters. So for the triterpene secondary lactone which can be considered less sterically hindered than the tertiary lactones gave partially O—C(=O)— cleaved product to generate the dihydroxy functional group as shown in Scheme—7.

Scheme—7

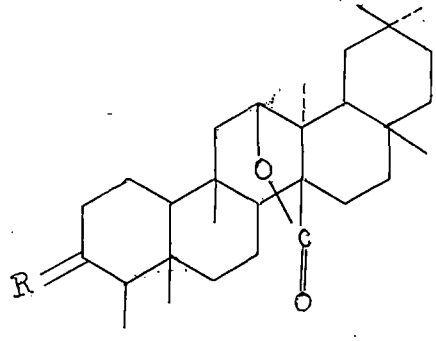


71 72a

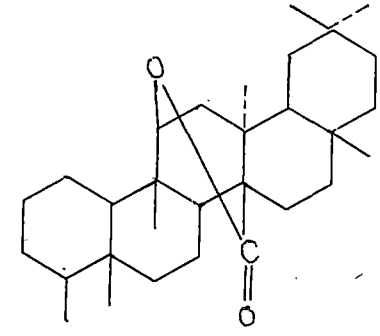
(c) Lithium-ethylenediamine reaction on sterically hindered triterpene lactones:

Pradhan and coworkers[32] recently isolated 3-oxo-friedelan-26 ⟶ 12β-olide 73 from the plant Gymnocordia-Odata and found that the lactone was too much sterically hindered. The lactone was however found stable to acid or alkali hydrolysis. The 3-oxo-friedelan-26 ⟶ 12β-olide 73 was too much sterically hindered lactone because the lactone when subjected to Huang-Minlon reduction of 3-keto function, the lactone ring did not open even under drastic

condition while it only isomerised[33] from C—26 ⟶ 12 to C—26 ⟶ 11 position to give a mixture of isomeric lactones 74a and 74b. So the isomerization of friedelan-



73, R=O
74a, R=H_2



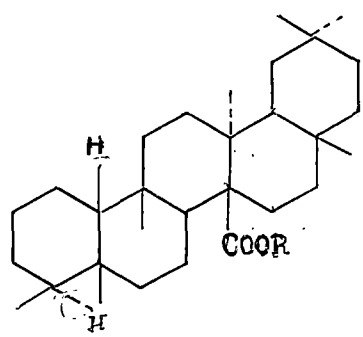
74b

26 ⟶ 12β -olide 74a to friedelan-26 ⟶ 11β -olide 74b in presence of strong basic condition was observed. Hence it was assumed that C—26 ⟶ 12β lactone is more sterically hindered than C—26 ⟶ 11β lactone. On this two lactones the effect of reduction with lithium in presence of ethylenediamine was studied.

(i) Li-ethylenediamine reduction on friedelan-26→ 12β-olide 74a:

A mixture of friedelan-26 ⟶ 12β-olide 74a in ethylenediamine was treated with lithium in an atmosphere of N_2 gas for about 2 hours. Excess lithium was destroyed by ammonium chloride and then usual work up followed by treatment with 10% sodium hydroxide furnished neutral and acid fractions.

The acid fraction was chromatographed over a silica gel. Column and only one compound was isolated in 75% yield. The compound was crystallised from chloroform-methanol mixture and the crystal was analysed for $C_{30}H_{50}O_2$, m.p. 290—91°, $[\alpha]_D$ +28.57°. The compound was assigned as 3-deoxy trichadenic acid[34] 75a on the basis of IR, PMR and mass spectral analysis of the compound and its ester. IR spectrum (Fig.15) of the compound showed peaks



75a, R = H
75b, R = CH_3

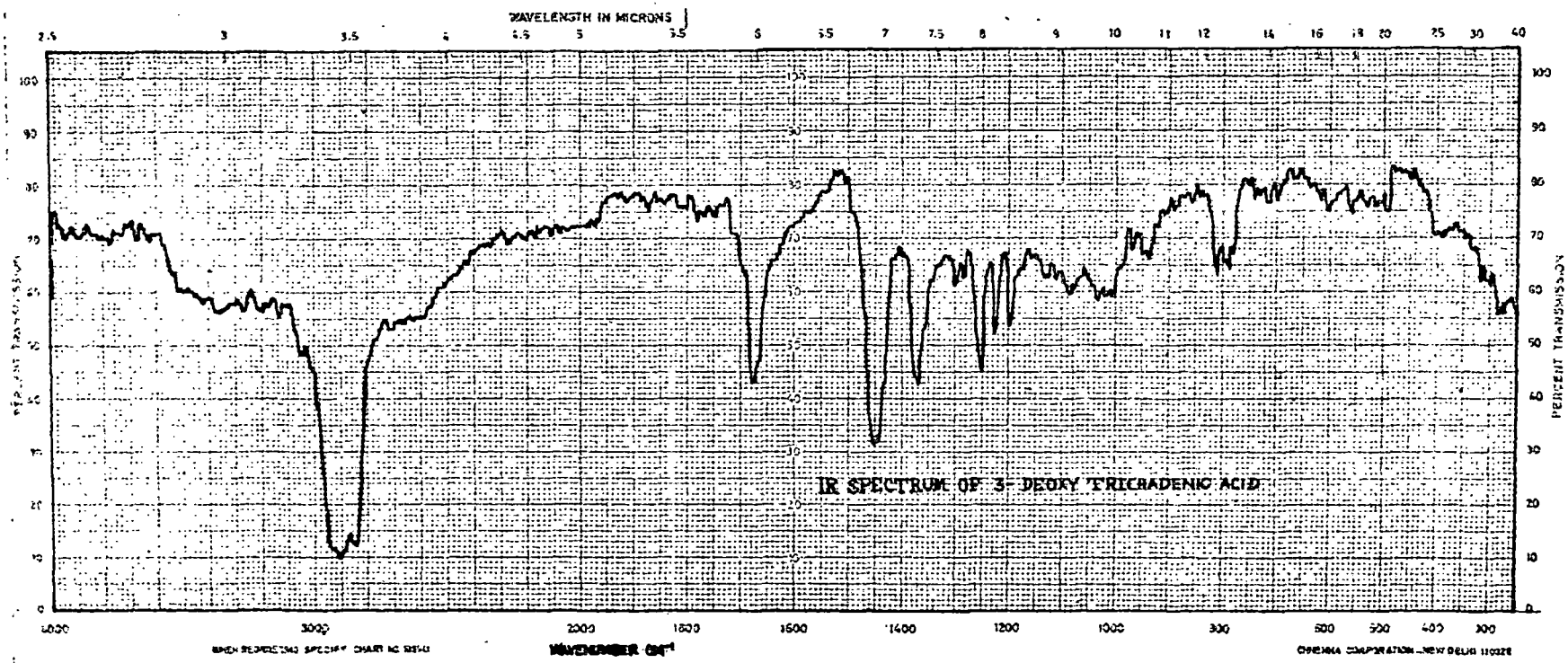


Fig.15: IR spectrum of 3-deoxy-trichadenic acid, 75a.

at 1680 cm^{-1} for —COOH functional group. Mass spectrum (Fig.16) of the compound showed a molecular ion peak at m/e 442 (M^+) which is consistent with the molecular formula. Other peaks at m/e 427 (M^+—CH_3), 397 (M^+—COOH), 371 (m/e 397—CH_3), 291, 290, 274, 273, 260, 259, 257, 231, 217, 205, 191, 149 (base) which are characterised peak of saturated friedelan skeleton[28]. The compound as 3-deoxy-trichadenic acid was finally confirmed by preparing its methyl ester 75b. The methyl ester so formed was crystallised to afford the crystal of 75b, m.p. 180°, $[\alpha]_D$ +30.3°, which was analysed for $C_{31}H_{52}O_2$. IR spectrum showed presence of carbomethoxy functional group at 1735 cm^{-1}. PMR spectrum (Fig.17) of the compound showed the presence of seven tertiary methyl groups from 0.76 to 1.22 ppm. The three protons of —$COOCH_3$ group appeared as singlet at 3.72 ppm. Mass spectrum (Fig.18) of the compound showed molecular ion peak at m/e 456 (M^+), 425, 424, 409, 397, 396, 317, 303, 305 (base). The above physical data are identical with those of methyl-3-deoxy-trichadenate[34].

Neutral fraction was chromatographed over a column of deactivated alumina and at the elution of benzene only one compound was isolated in 5% yield. The compound was crystallised from methanol while the crystallised

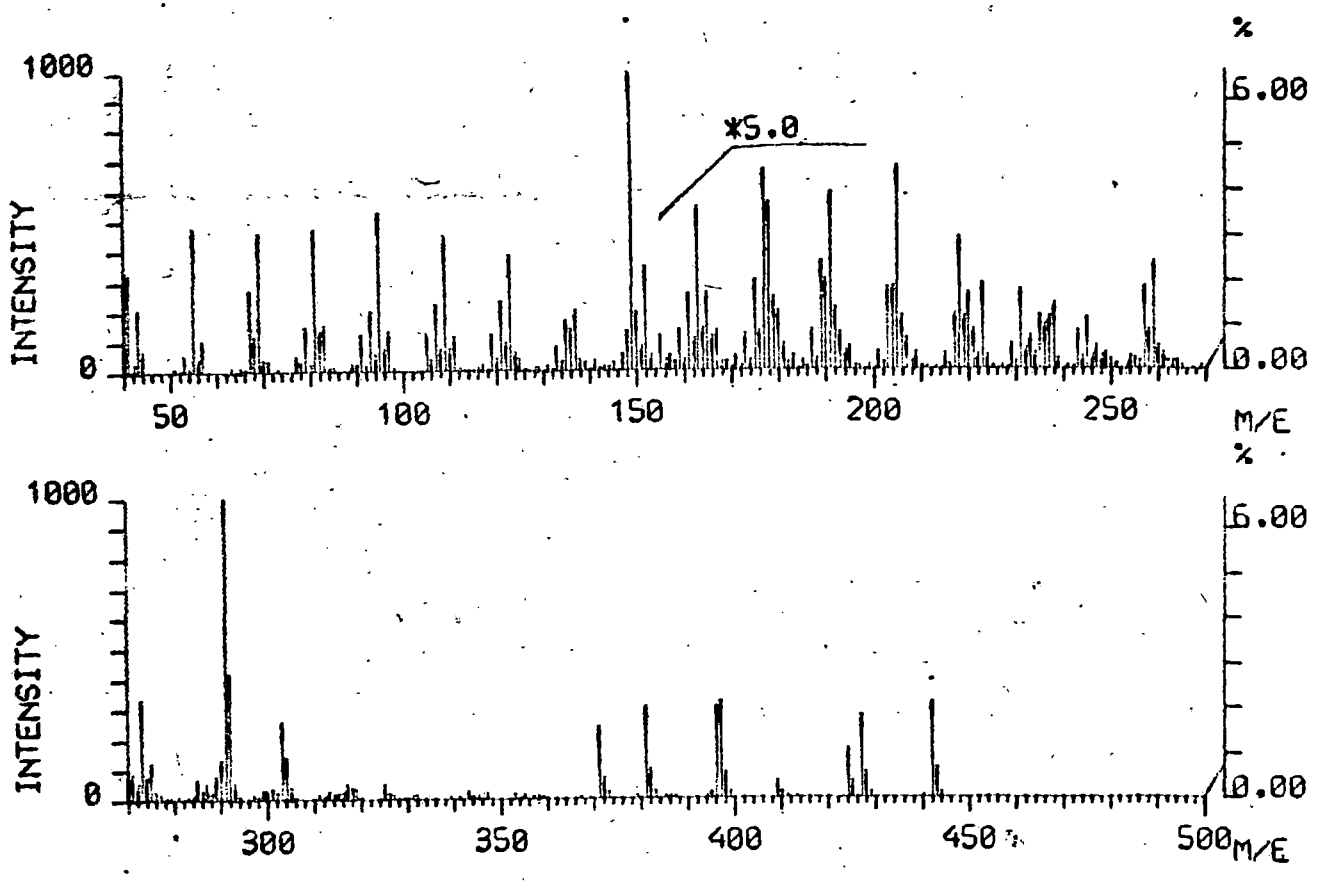


Fig.16: Mass spectrum of 3-deoxy-trichadenic acid, 75a.

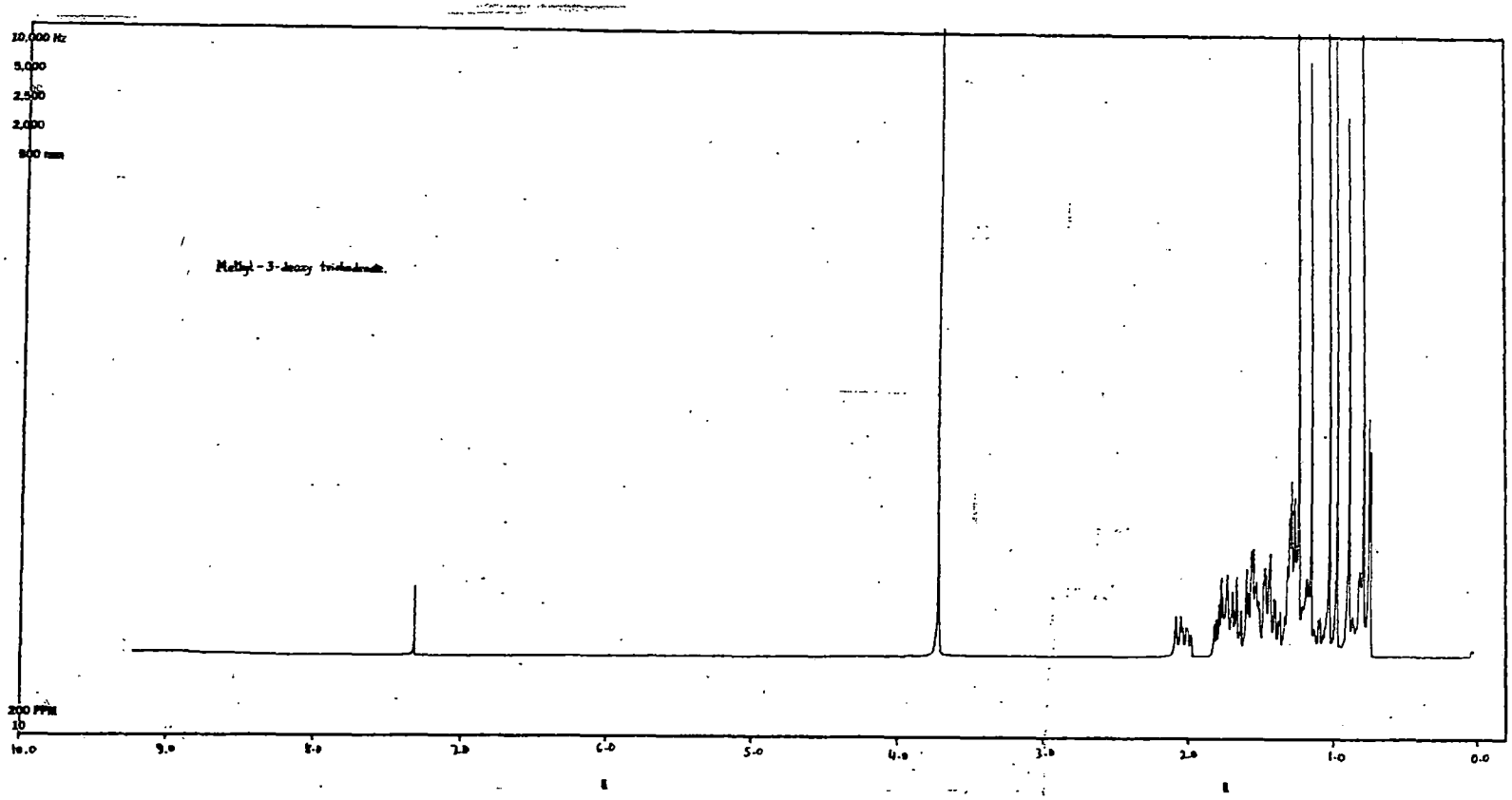


Fig.17: PMR spectrum of methyl-3-deoxy-trichadenate, 75b.

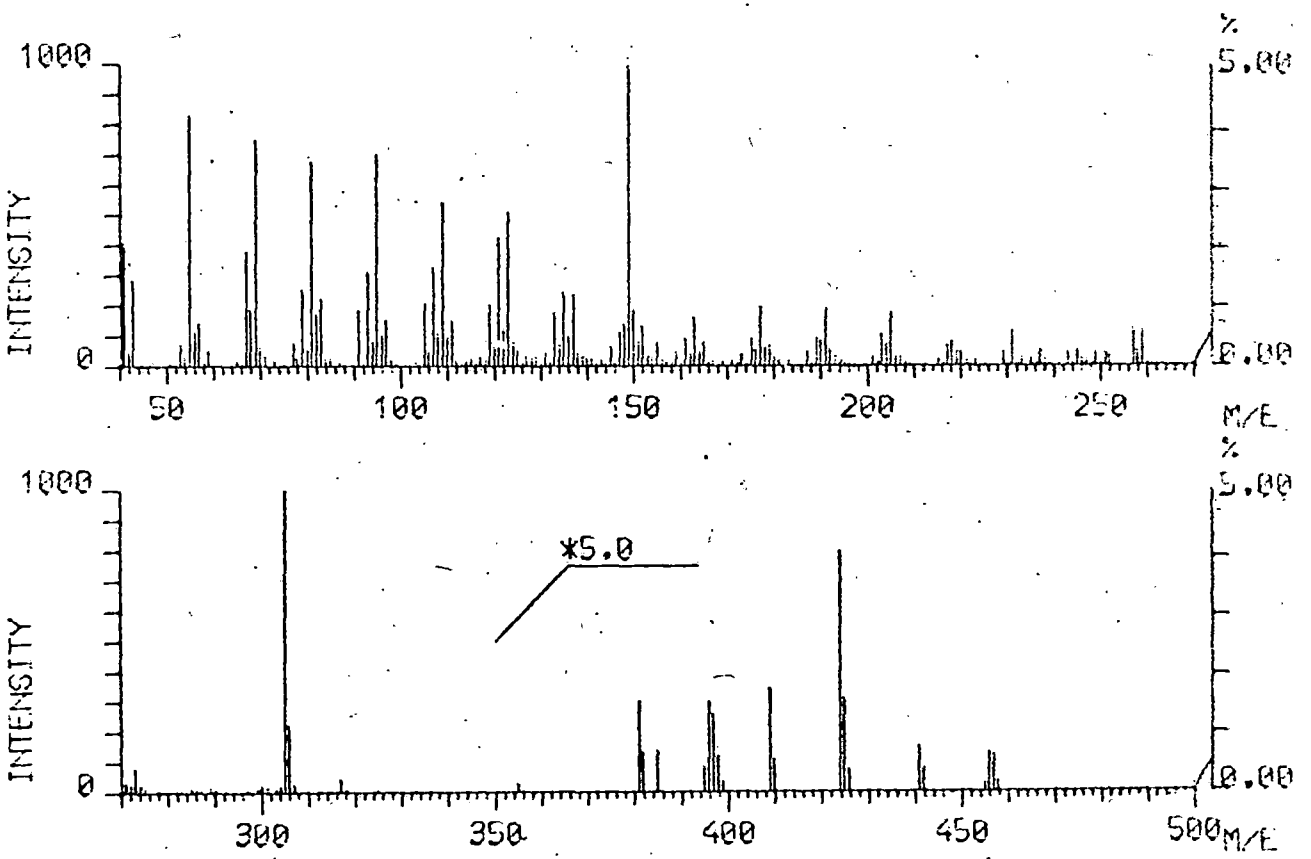
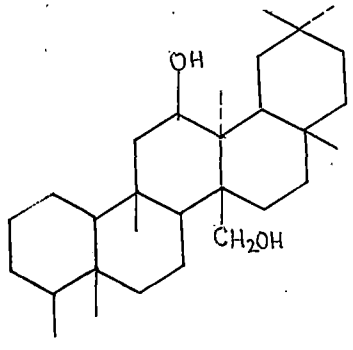


Fig.18: Mass spectrum of methyl-3-deoxy-trichadonate, 75b.

product 76 was analysed for $C_{30}H_{52}O_2$, m.p. 240—1°. The compound was assigned as friedelan-12β, 28-diol 76 on the basis of IR, PMR and mass spectral analysis. IR spectrum



76

(Fig.19) of the compound showed broad peak at ν_{max} 3250—3350 cm^{-1} showing the presence of hydroxyl groups in the molecule.

PMR spectrum (Fig.20) of the compound showed a doublet centered at 0.724 ppm with J value 6H_3 for a methyl group at secondary carbon atom, a singlet at 0.7976, 0.899, 0.9677, 0.9836, 1.002 and 1.244 ppm for six other methyls on tertiary carbon atoms. It showed A—B quartet centred at 4.0 ppm integrable for two protons and

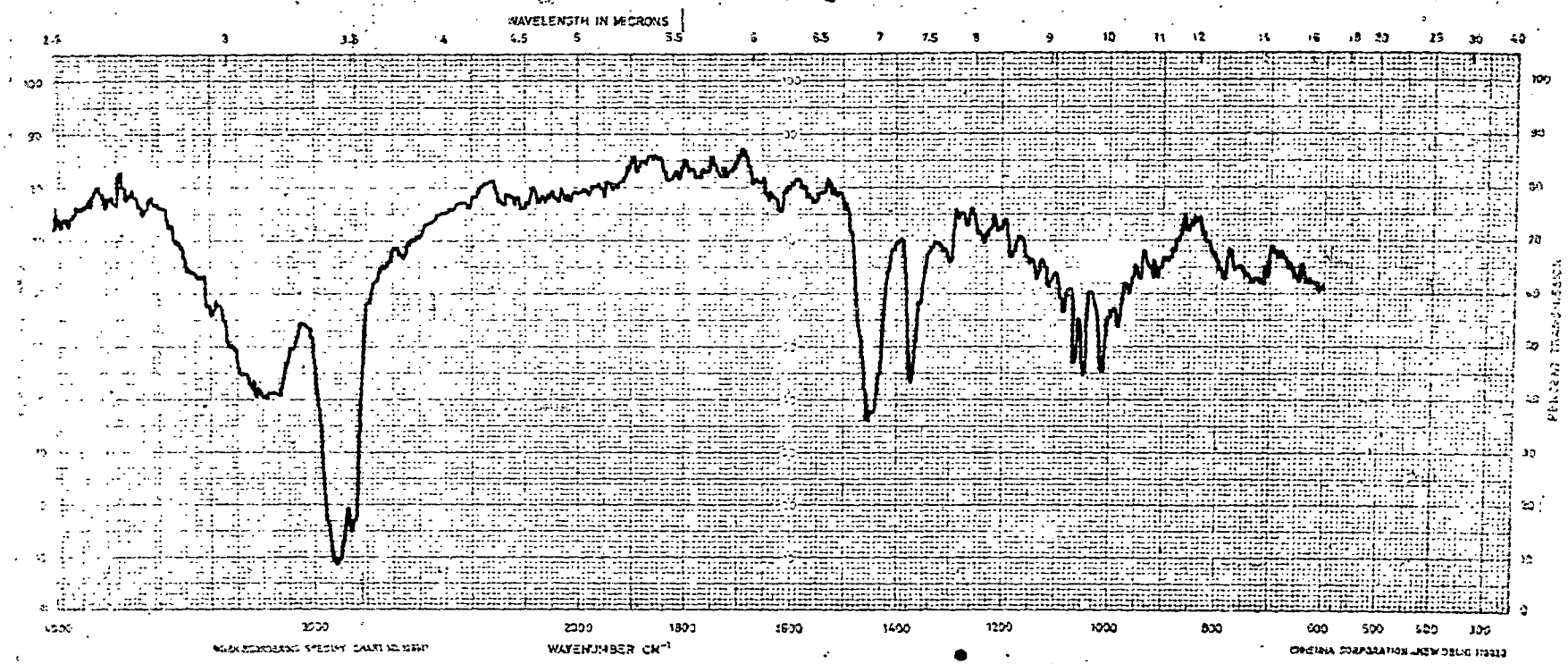


Fig.19: IR spectrum of friedelan-12β,28-diol, 76.

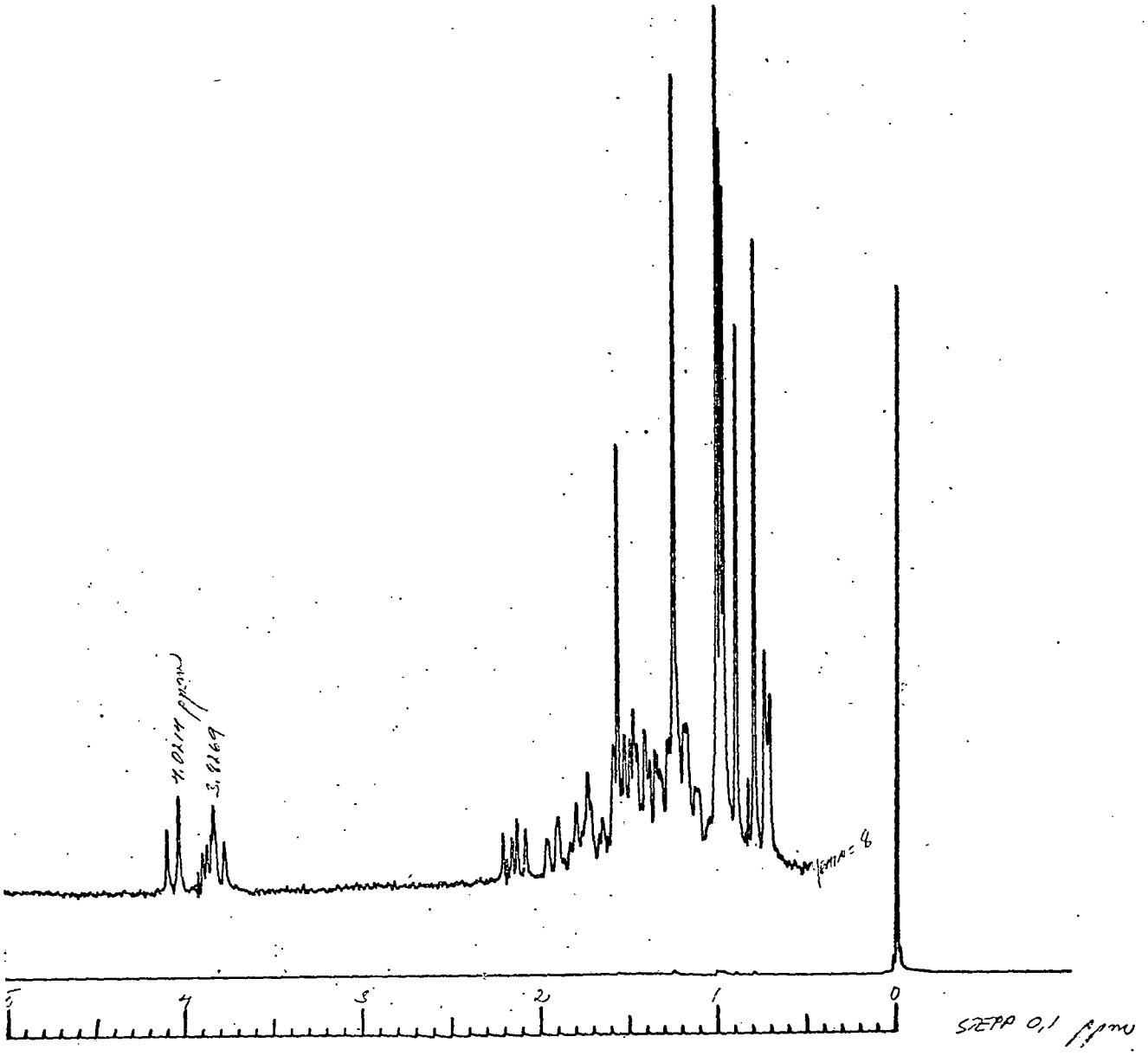
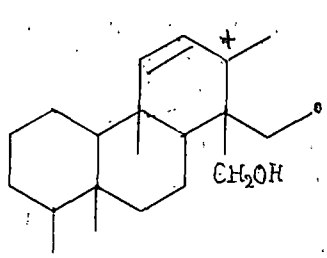


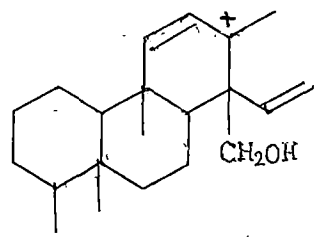
Fig.20: PMR spectrum of friedelan-12β,26-diol, 76.

a multiplet at 3.68 ppm integrable for one proton. The A—B quartet must be due to C—26—H_2—OH and the multiplet is due to C—12—H—OH. Mass spectrum (Fig.21) of the compound showed peak at m/e 426($M-H_2O$), 395 for the loss $-CH_2OH$ (31 mass unit) from fragment m/e 426. Peaks at m/e 302, 301 for the fragments a' and b', other prominent peaks at m/e 220, 203, 189, 177 (base peak). Hence the above fragments confirms the structure of the diol as 76.



m/e 302

a'



m/e 301

b'

The deoxylactone 74a on LAH reduction afforded the same diol as indicated by m.m.p., CO—IR and CO—TLC comparisons.

So the reaction of lithium in presence of ethylenediamine on friedelan-26→12β -olide 74a, which was considered as sterically too much hindered lactone afforded two compounds. The formation of 3-deoxy-trichadenic acid 75a

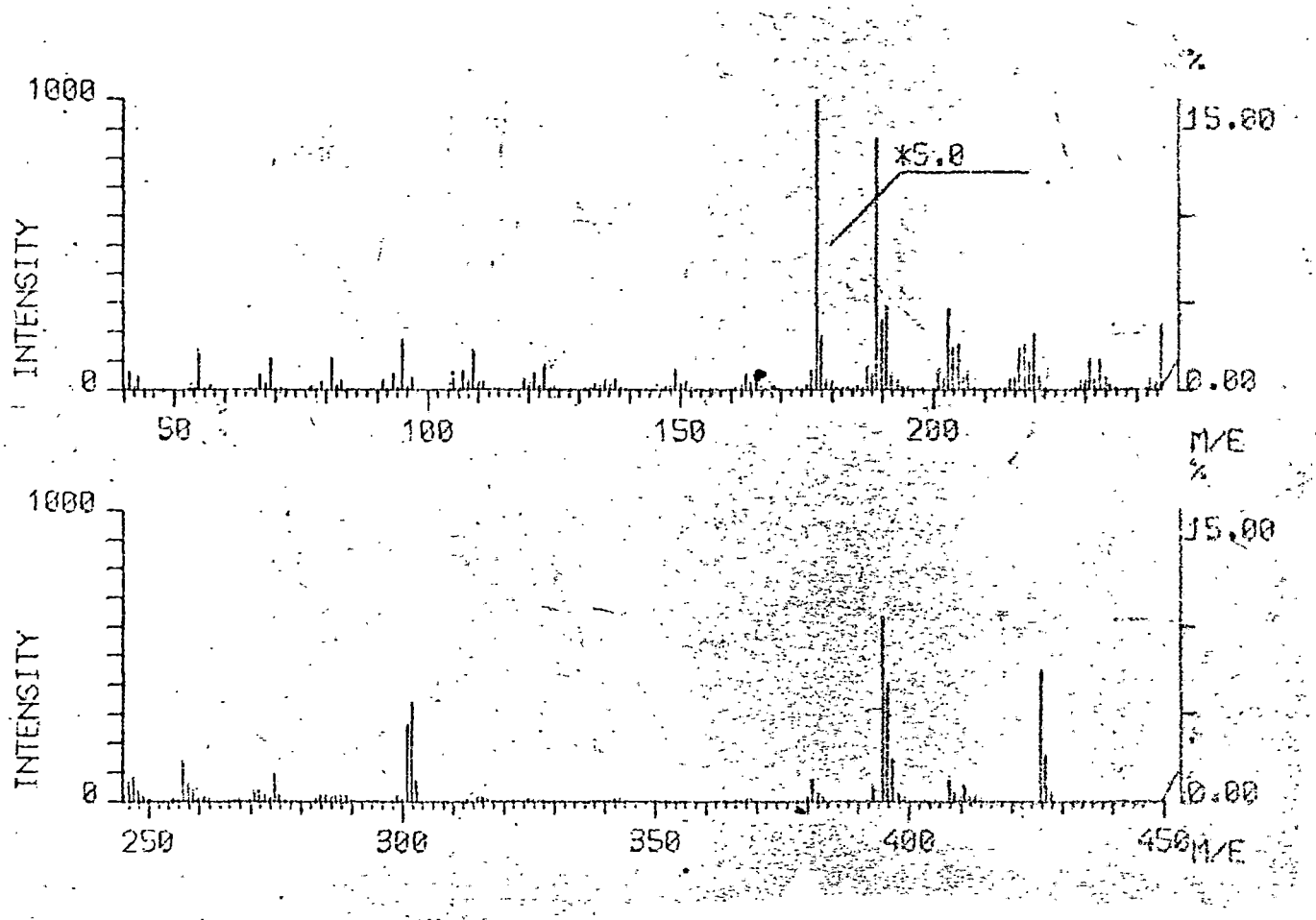


Fig.21: Mass spectrum of friedelan-12β,28-diol 76.

in higher yield may be explained by the deoxygenation on C—12 where lactyl oxygen was attached. Again the formation of friedelan-12β, 26-diol 76 in lower yield might be due to deacylation of lactone by the cleavage of —O—C(=O)— bond. So deoxygenation was favoured in case of more sterically hindered lactone. To be more sure of the fact we had carried out the same reaction on less sterically hindered lactone friedelan-26⟶ 11β -olide 74b which was more stable.

(ii) Li-ethylenediamine reaction on friedelan-26⟶ 11β -olide 74b:

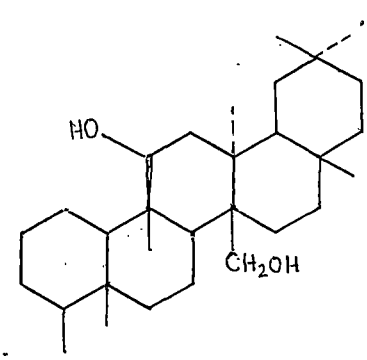
The lactone 74b in ethylenediamine was refluxed with lithium in an atmosphere of nitrogen gas was followed by usual work up and separation into acid and neutral fractions by 10% sodium hydroxide.

The acid fraction was chromatographed over a column of silica gel and only one compound was isolated in 5% yield. The compound was crystallised from chloroform-methanol mixture and the crystallised compound was analysed for $C_{30}H_{50}O_2$, m.p. 293—94°. The compound was identified as 3-deoxy-trichadenic acid 75a when compared with an authentic specimen (m.m.p and CO IR comparison). It was

again confirmed by preparing its methyl ester derivative 75b, m.p. 180°, was analysed for $C_{31}H_{52}O_2$. The ester was identified as methyl-3-deoxy-trichadenate when compared with an authentic specimen.

The neutral fraction was chromatographed over a column of deactivated alumina and only one compound was isolated in benzene elution in 75% yield. The compound was crystallised from methanol and the crystallised compound 76, which was analysed for $C_{30}H_{52}O_2$, m.p. $>$ 350°. The compound was characterised as friedlane-11β-26-diol 76a on the basis of IR, PMR and mass spectral analysis. IR spectrum (Fig.22) of the compound showed peak at 3200—3300 cm^{-1} showing the presence of hydroxyl groups in the molecule.

PMR spectrum (Fig.23) showed the presence of a doublet centred at 0.8 ppm for a methyl group on carbon containing a proton and another six singlets between 0.9—1.28 ppm for six methyls on tertiary carbon atom. A multiplet at 3.9 ppm integrable for two protons indicated the presence



76a

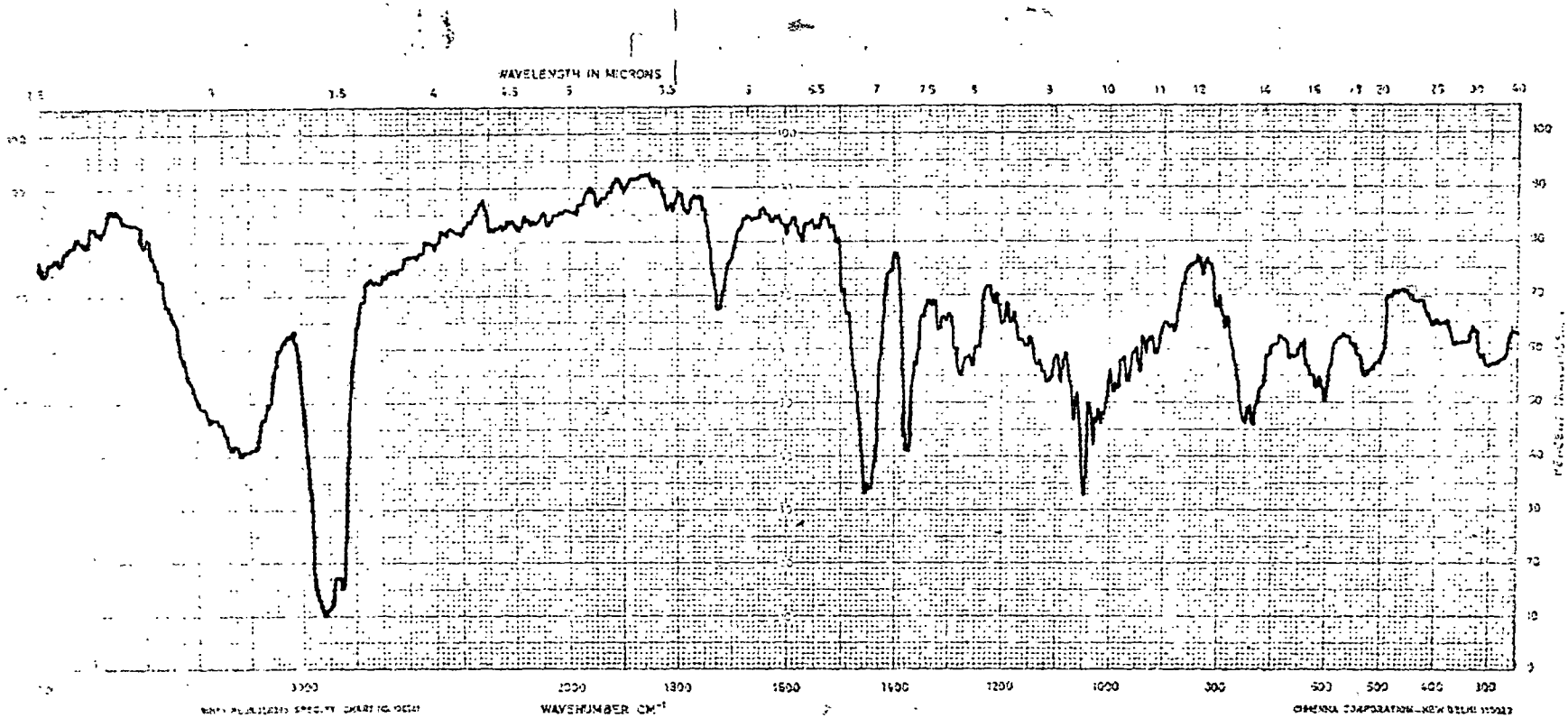


Fig.22: IR spectrum of friedelan-11β,26-diol, 76a.

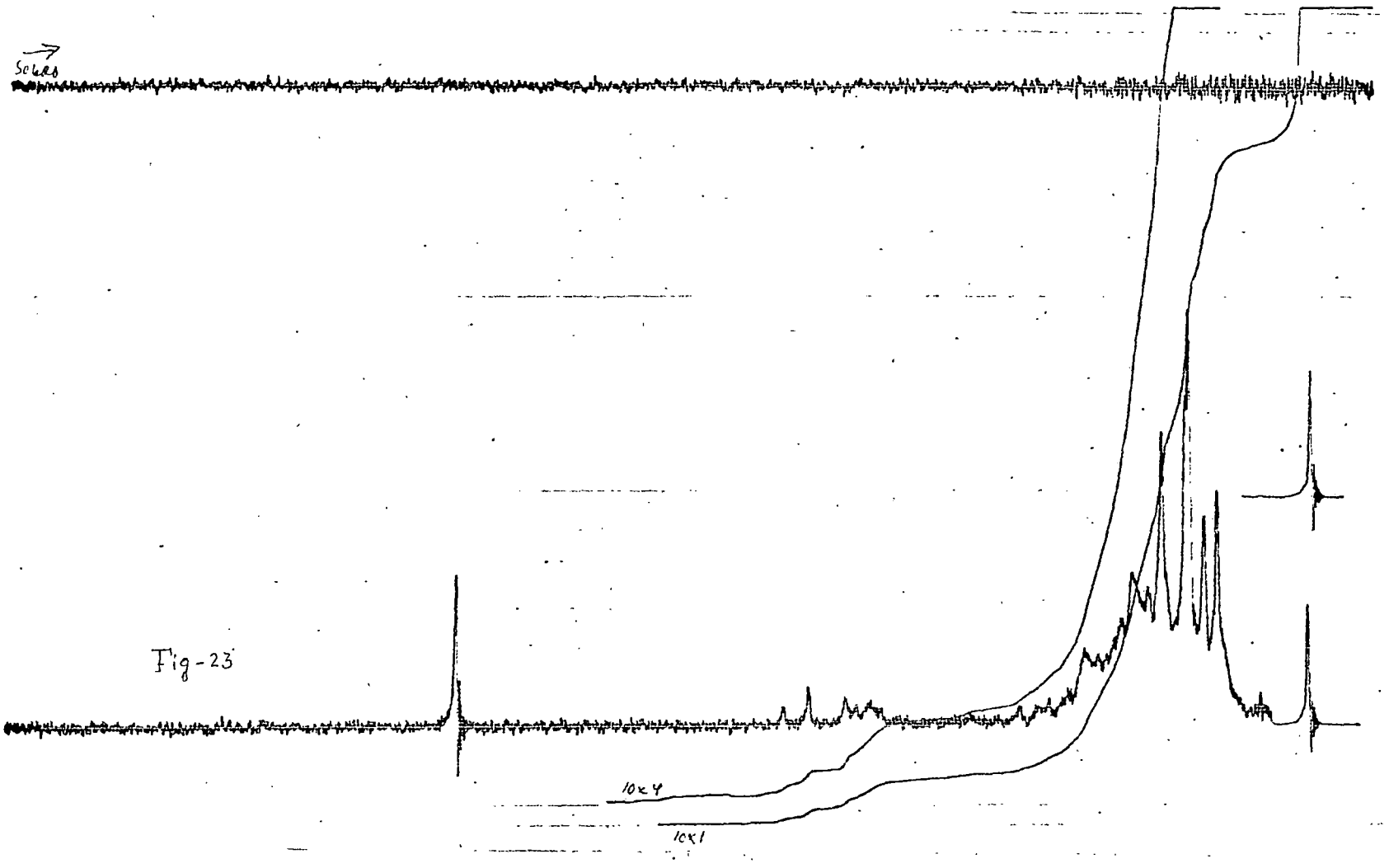


Fig.23: PMR spectrum of friedelan-11β ,26-diol, 76a.

of C—26—H_2—OH and a multiplet at 4.35 ppm integrable for one proton indicated the presence of —C—11—H—OH.

Mass spectrum of the compound 76a showed peak at m/e 426 (M^+—H_2O), 395 for the loss of —CH_2OH group (31 mass unit) from fragment at m/e 426. The presence of peaks at m/e 302 and 301 may be due to the fragments a' and b' characterised for saturated friedelane skeleton[28]. The other prominent fragments were observed at m/e 189 and m/e 177 (base peak). Hence the above fragmentation confirms the structure of the diol 76 which are typical of friedelane skeleton[28].

Thus lithium in presence of ethylenediamine when treated on less sterically hindered lactone viz. friedelan-26⟶ 11β olide 74b, the percentage yield of friedelan-26, 11β-diol 76a was greater than that of 3-deoxy trichadenic acid 75a. Friedelan 26, 11β-diol was obtained by the deacetylation from the C—11 position by the cleavage of acyl-oxygen of the lactone producing the diol whereas the formation of 3-deoxy-trichadenic acid can be explained by the deoxygenation from C—11 position. So in this reaction deacylation was favoured than deoxygenation as was found by Barton and coworkers[21,22 & 24] on esters of alcohols.

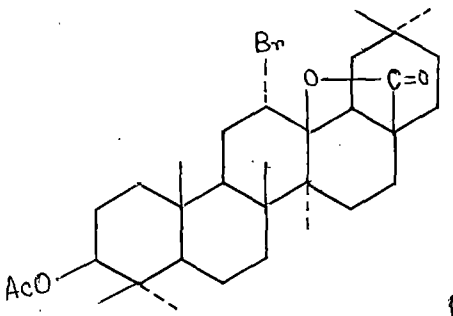
So from above findings it is clear that the ring opening of lactones, in different sterical environments, gives products in differents yields. The more hindered lactones give the corresponding saturated acids in higher yield by deoxygenation at carbon where lactyl oxygen is attached and the less hindered lactones give the corresponding diols in higher yield by deacetylation at the carbon where lactyl oxygen is attached.

(d) Li-ethylenediamine reaction on triterpenoid bromo lactones:

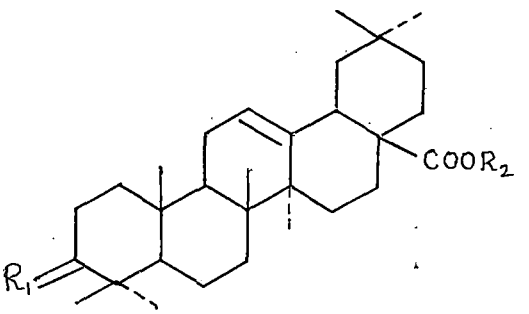
In order to examine the effect of bromine on the nature and yields of products formed on Li-ethylenediamine reduction the following compounds have been selected and studied for the purpose:

(i) Li-ethylenediamine reduction on 3-acetyl-12α-bromo-oleanan-28⟶ 13-olide[35]:

The bromo lactone, 3-acetyl-12α-bromo-oleanan-28⟶ 13-olide 77, which is a tertiary lactone was refluxed in ethylenediamine with lithium for two hours in an atmosphere of N_2. After refluxing for two ½ hours excess lithium was destroyed with solid ammonium chloride. After



77



78a, $R_1=H_2$; $R_2=H$,
78b, $R_1=H_2$; $R_2=CH_3$
78c, $R_1=\beta$-OH, α-H; $R_2=H$
78d, $R_1=\beta$-OH, α-H; $R_2=CH_3$
78e, $R_1=O$; $R_2=CH_3$

work up it was separated into acid and neutral parts by treating with 10% sodium hydroxide solution.

The acid fraction was chromatographed over a column of silica gel and two compounds were isolated. The less polar compound was analysed for $C_{30}H_{48}O_2$, m.p. 265—66° was identified as 3-deoxy oleanolic acid 78a. It was confirmed by preparing its methyl ester[36] 78b, analysed for $C_{31}H_{50}O_2$ had m.p. 169°. IR spectrum (Fig.24a) of the ester 78b showed the presence of carbomethoxy

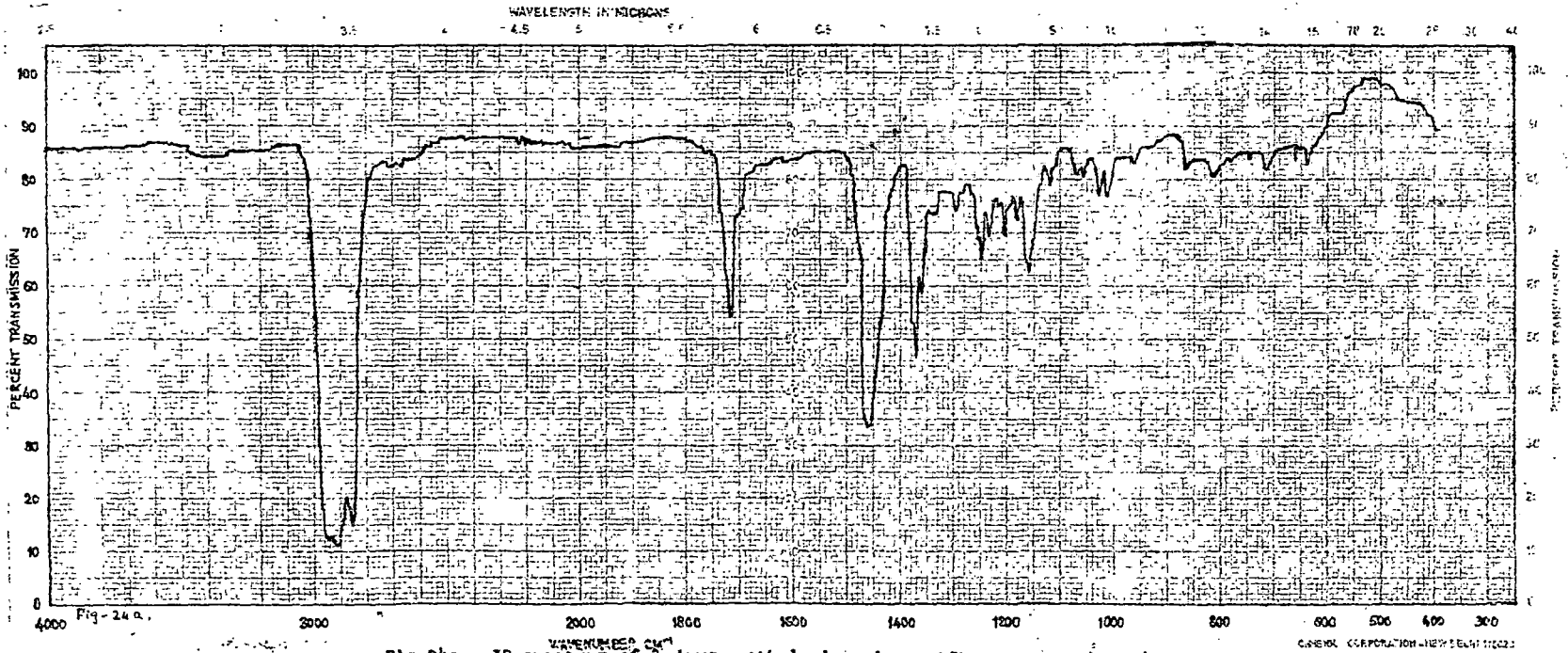


Fig 24a : IR spectrum of 3-deoxy-methyl-olesnolate, 78b.

functional group at 1730 cm^{-1}. The high polar compound was isolated in 35% yield. The compound 78b was crystallised from methanol was analysed for $C_{30}H_{48}O_3$, m.p. 303—4°. IR spectrum of the compound showed peaks at 3450 cm^{-1} (hydroxyl function) and 1690 cm^{-1} (—COOH function). The structure of the compound, 78c was established by the IR, NMR and mass spectral analysis of the corresponding methyl ester. Esterification of the acid, 78c with diazomethane followed by purification yielded the ester 78d, analysed for $C_{31}H_{50}O_3$, m.p. 198—9°. IR spectrum (Fig.24) of the compound 78d showed the presence of a hydroxyl group at ν_{max} 3280 cm^{-1} and an ester group at 1730 cm^{-1}. PMR spectrum (Fig 25) showed 7 methyl protons at the region 0.56 to 0.96 ppm. A singlet at 3.5 ppm showed three protons of carbomethoxy group (—COOCH_3). A multiplet at 3.8 ppm assumed for the C—3 proton geminal to hydroxyl group. A triplet at 5.16 ppm showed the olefinic proton at C—12 position. Mass spectrum (Fig.26) of the compound showed a molecular ion peak (M^+) at m/e 470, which was consistent with the molecular formula $C_{31}H_{50}O_3$. The other peaks appeared at m/e 452 (M^+—H_2O), 455 (M^+—CH_3), 411 (M^+—COOCH_3), 262, 207, 203 (base peak) and 189 were typical of methyl oleanolate[28]. The fragmentation is shown in Chart—6.

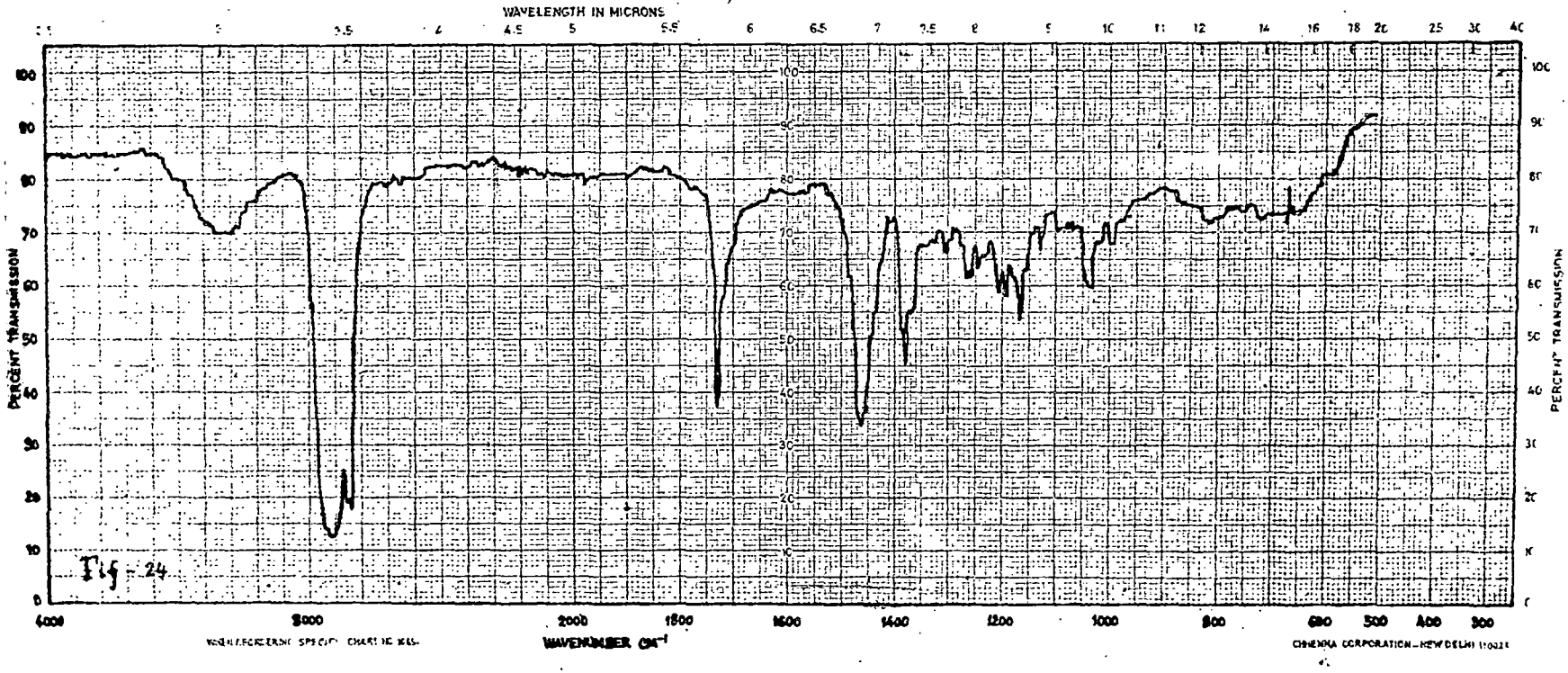


Fig.24: IR spectrum of methyl oleanolate, 78d.

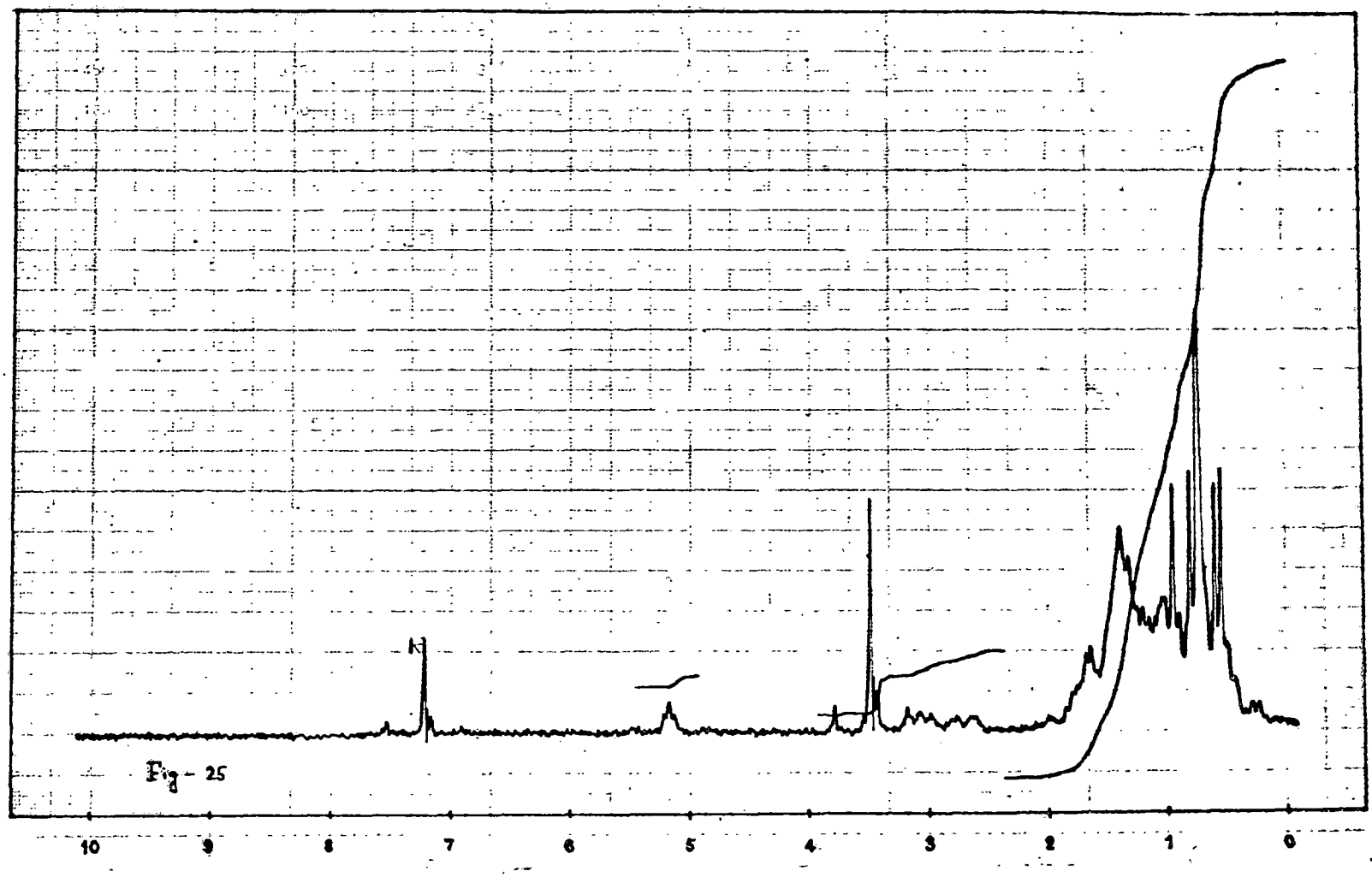


Fig.25: PMR spectrum of methyl oleanolate, 78d.

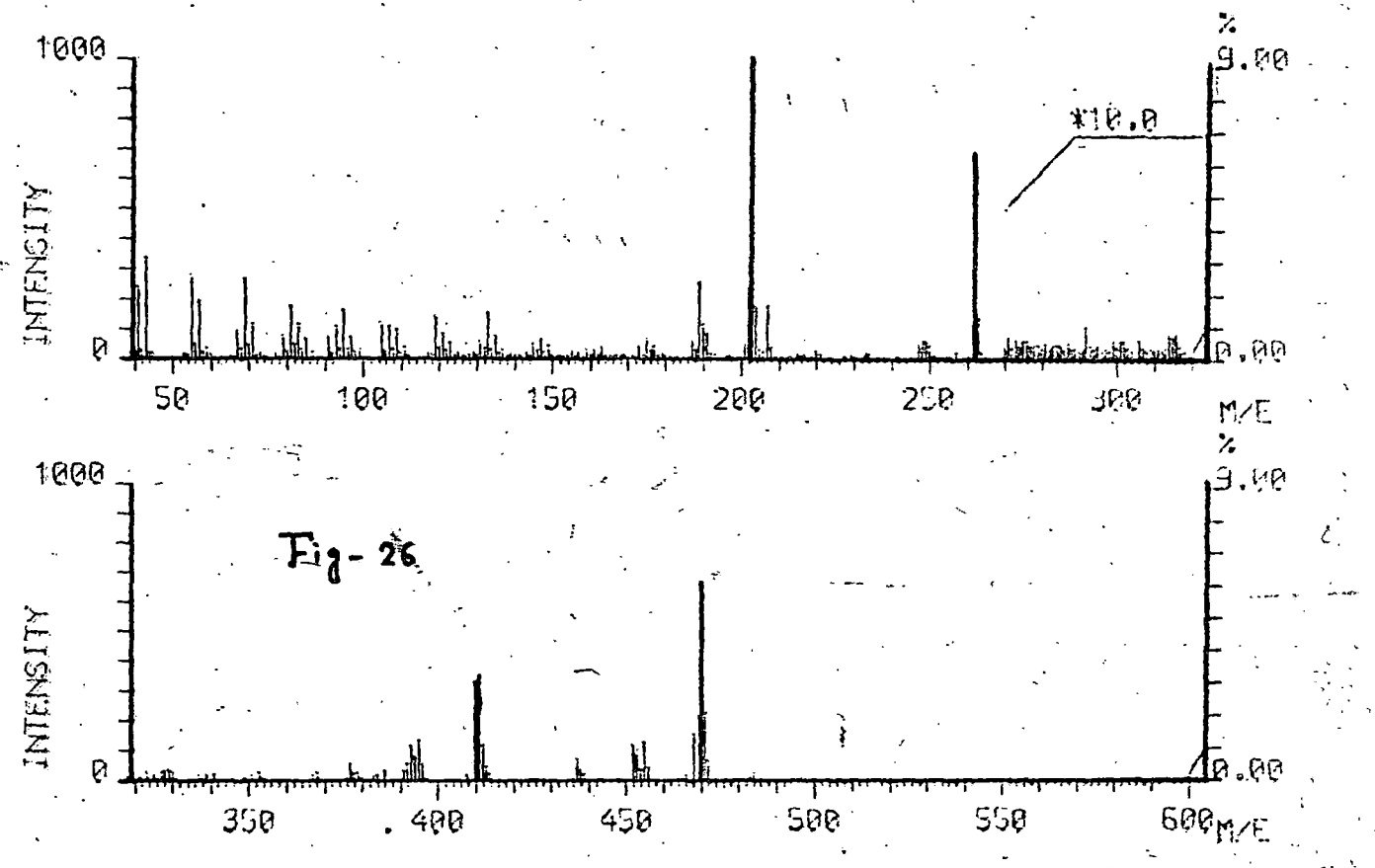
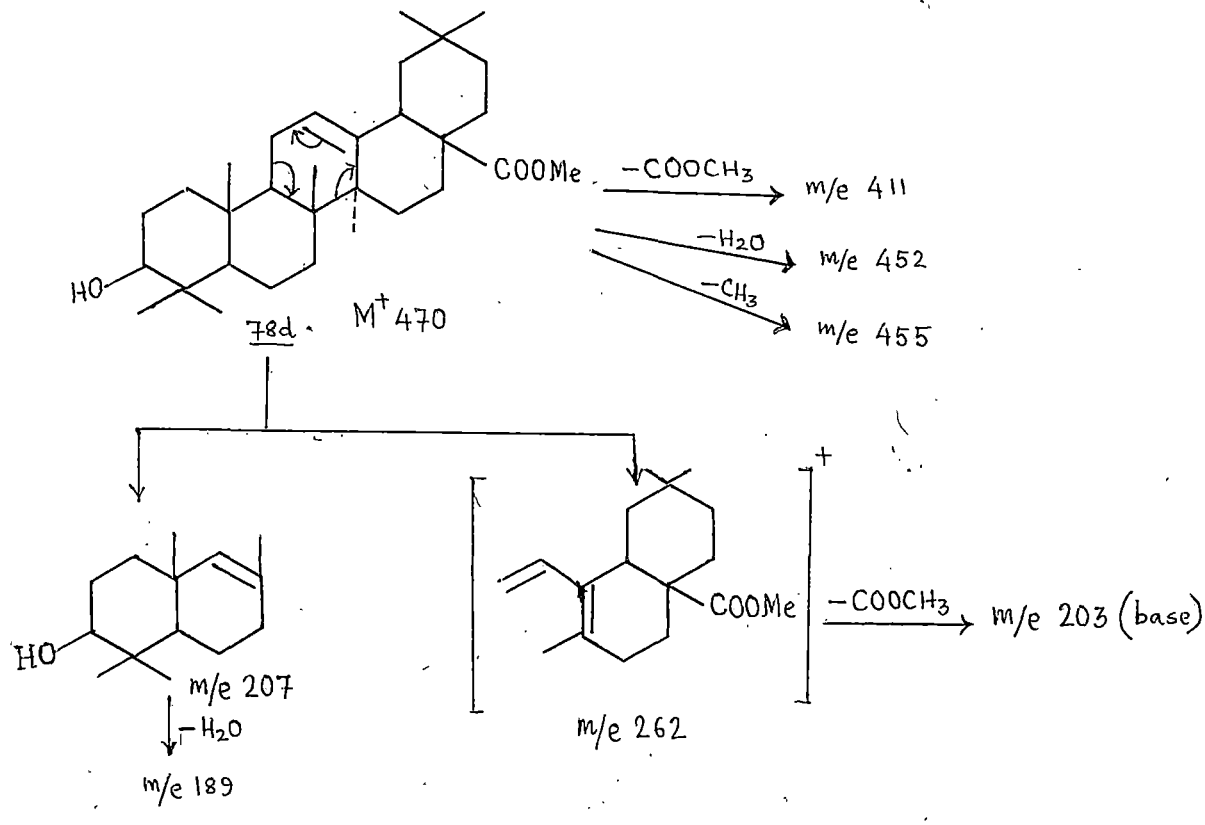


Fig-26

Fig.26: Mass spectrum of methyl oleanolate, 78d.

Chart—6



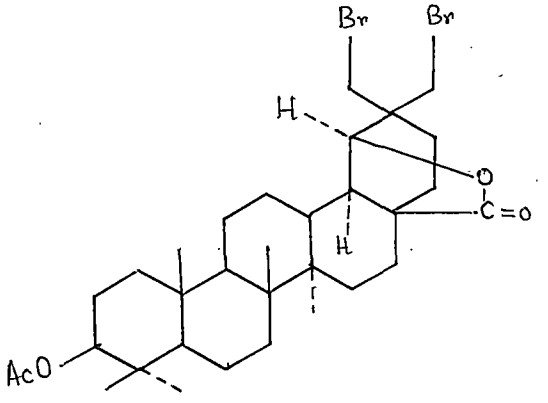
From the above data the compound was proved as methyl oleanolate[36] which was confirmed by comparison with an authentic specimen (m.m.p and GC—IR comparison). So the compound which was obtained originally from the reaction of Li-ethylenediamine on 3-acetate of 12α-bromo-oleanan-28⟶ 13β-olide was confirmed as oleanolic acid 78c. The result was contradictory than those obtained from the previous reaction which formed e saturated acid

by deoxygenation. Here acid was formed with a double bond at the carbon where the lactyl oxygen was attached. This might be occurred due to the presence of bromine atom in the molecule. Further work on bromolactones is in progress to establish the mechanism in our laboratory.

From neutral fraction no compound was isolated.

(ii) Li-ethylenediamine reduction on 3β-acetyl-29,30-dibromo-18α-H-oleanan-28⟶ 19β-olide[37] 79:

The compound 3β-acetyl-29-30-dibromo-18α-H-oleanan-28⟶ 19β olide 79, on which the reaction of Li-ethylenediamine has been carried out is a secondary lactone and was anticipated that the both deoxygenated and deacetylated product should be obtained as was found in the previous case (reaction on 71).



79

The dibromolactone 79 was refluxed with a mixture of lithium and ethylenediamine in an atmosphere of N_2 for about 2 hours. Following the same procedure for work up and mixture was separated into acid and neutral fractions by treatment with 10% sodium hydroxide.

The acid fraction was chromatographed over a silica gel column. The less polar compound (40% yield) was crystallised and the product was analysed for $C_{30}H_{50}O_2$, m.p. 270—1°, was found to be identical with oleanan-18-α-H-28-oic acid 68 (compared with authentic specimen). The more polar one was obtained in 40% yield, was crystallised and analysed for $C_{30}H_{50}O_3$, m.p. 295—6°. It was identified as 3β-hydroxy oleanan-18α-H-28-oic acid 69a, when compared with an authentic specimen (m.m.p. and CO—IR). For both the compounds Bielstein tests were negative showed bromine was completely debrominated. The neutral fraction did not afford any solid material.

Thus the reaction on dibromolactone 79 in ethylenediamine with lithium was somehow different from the 3β-acetyl oleanan-18α-H-28⟶ 19β olide 71, while both were secondary lactones. The compound 3β-acetyl-oleanan-18-α-H-28⟶ 19β olide 71 on reduction afforded three

compounds, two of which are saturated acids 68 and 69a, resulted from the deoxygenation at C—19 position and third was a triol 72 formed by deacylation of the lactone ring at C—19 position. But in the case of dibromo secondary lactone viz 3β-acetyl-29, 30-dibromo-oleanan-18α-H-28⟶ 19β olide 79, the two acids 68 and 69a were obtained but no formation of triol was observed ie. no deacetylation (pathway b by Barton & coworkers[18]) took place. This was probably due to steriçal crowding of two bromine atoms at C—29 and C—30 position in the molecule which favoured deoxygenation from C—19 position rather than deacetylation. Further work on bromolactones are in progress in our laboratory to establish the facts.

SECTION—B

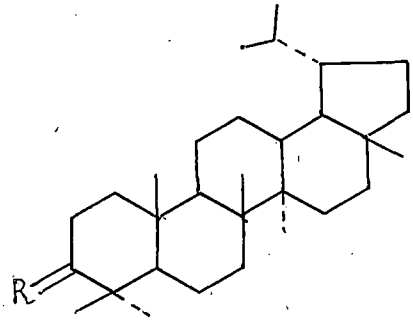
STUDIES ON THE ACTION OF LITHIUM ON TRITERPENOIDS CONTAINING (a) 3-KETO (b) STERICALLY HINDERED ESTERS WITH OR WITHOUT ISOPROPENYL GROUP IN ETHYLENEDIAMINE

(a) Studies on 3-keto triterpenoids:

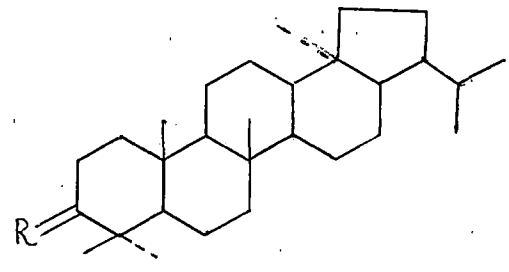
Reactions of Li-ethylenediamine on 3-keto compounds were studied taking lupanone 80a and moretanone 81a as model compounds.

(1) Li-ethylenediamine reaction on lupanone[38]:

Lupanone 80a in dry ethylenediamine was treated with lithium as mentioned earlier. After usual work up followed by crystallisation the compound 80b obtained in



80a, R = O
80b, R = H_2



81a, R = O
81b, R = H_2

80% yield, was analysed for $C_{30}H_{52}O$, m.p. 206°, $[\alpha]_D$ -17.6°. IR spectrum (Fig.27) showed peaks at 3330 cm^{-1} for hydroxyl functional group. The compound was identified as lupanol[39] 80b by comparison (m.m.p. & CO—IR) with an authentic specimen of lupanol.

(ii) Li-ethylenediamine reaction on moretanone[40] 81a:

Moretanone 81a in dry ethylenediamine was treated with lithium and after usual work up a compound was obtained which on chromatography and crystallisation afforded a crystal, $C_{30}H_{52}O$, m.p. 223—24°. IR spectrum (Fig.28) of the compound showed peak at 3320 cm^{-1} for hydroxyl function. The compound was identified as moretanol[40] 81b by comparison with an authentic specimen (m.m.p. and CO—IR comparison).

Thus from the above two reactions on triterpene keto compounds it was proved that the triterpene keto compounds can be reduced to the thermodynamically stable alcohol by the action of lithium in presence of ethylenediamine.

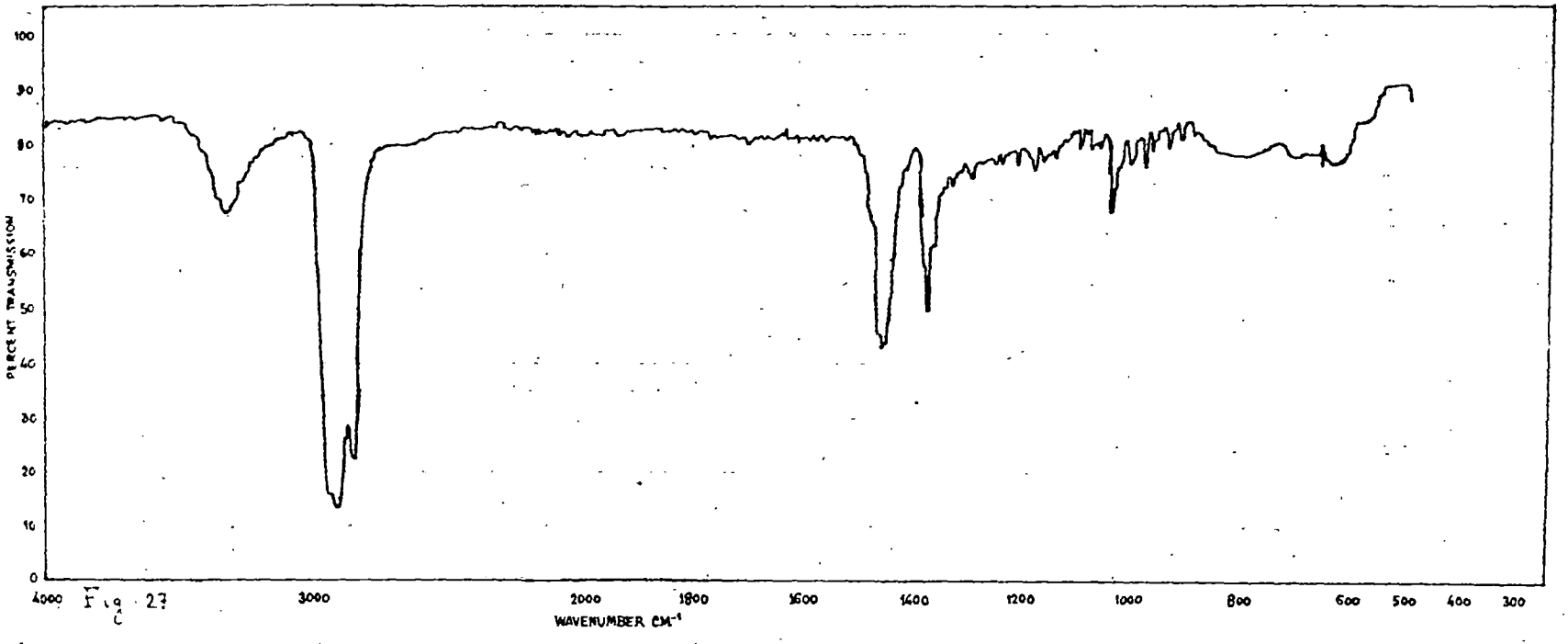


Fig.27: IR spectrum of lupanol, 80b.

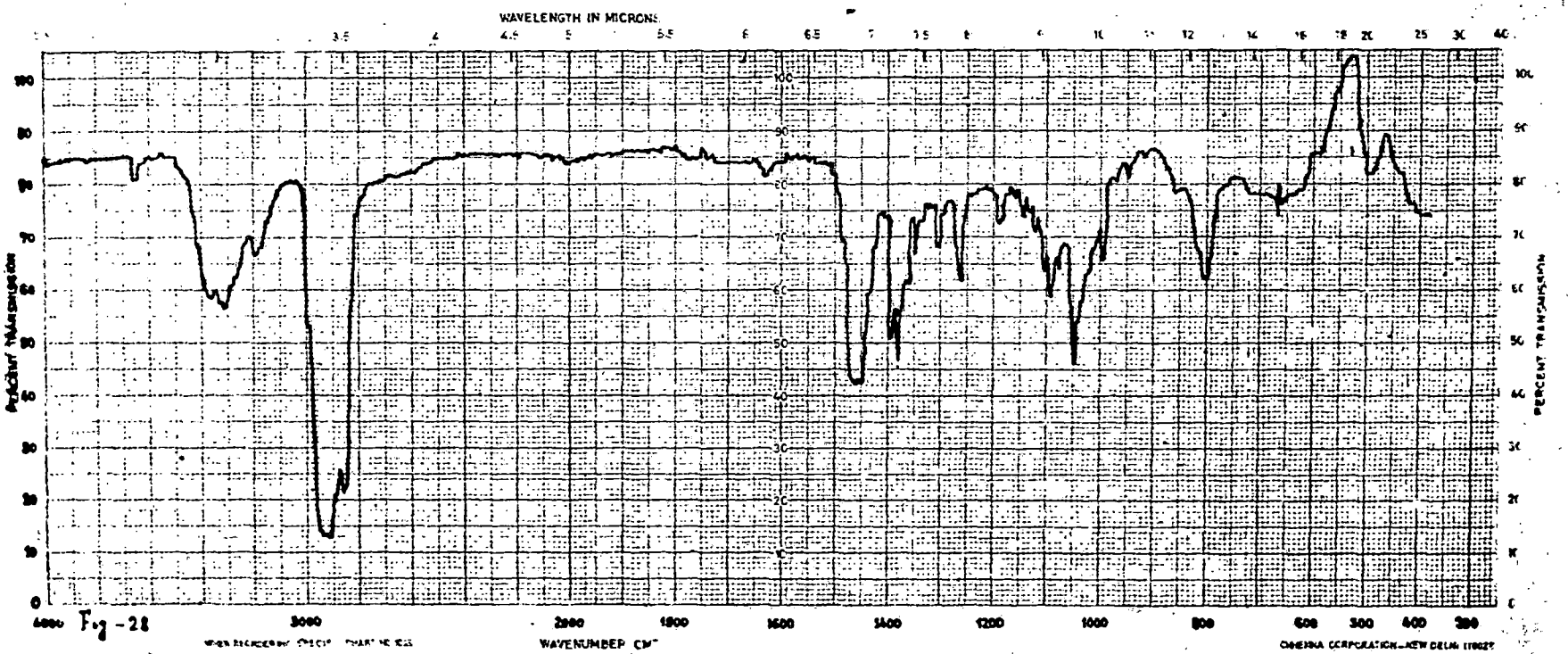


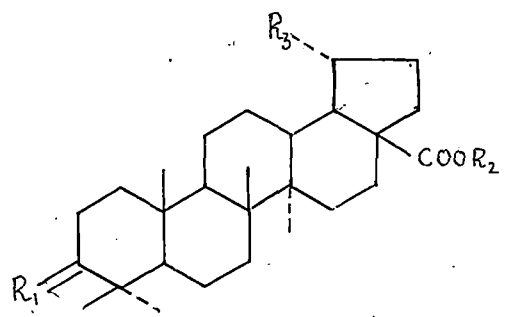
Fig.28: IR spectrum of moretanol, 81b.

(b) Studies of Li-ethylenediamine reaction on triterpenoid hindered esters:

Sengupta and coworkers[23] have reported the action of lithium-ethylenediamine on some hindered esters. We have also carried out some reactions on hindered esters with different functional groups present in the molecules which are discussed in the following:

(i) Esters containing 3-keto and isopropenyl groups:

The compound for the purpose was selected methyl betulonate[41] 82a. A mixture of methyl betulonate, dry ethylenediamine and metalic lithium was refluxed in



82a, R_1=O, R_2=CH_3, R_3= $\begin{matrix} CH_3 \\ \diagdown\!\!\diagup \\ CH_2 \end{matrix}$

82b, R_1= $\begin{matrix} OH \\ \diagdown \\ H \end{matrix}$, R_2 = H, R_3= $\begin{matrix} CH_3 \\ \diagdown \\ CH_3 \end{matrix}$

82c, R_1= β-OAC, α-H, R_2 = H, R_3= $\begin{matrix} CH_3 \\ \diagdown \\ CH_3 \end{matrix}$

an atmosphere of N_2 gas for 2 hours. After usual work up the mass was treated with 10% sodium hydroxide to separate into acid and neutral fractions. The acid fraction was chromatographed over a column of silicol gel and only one compound was isolated in 80% yield. The compound 82b, after crystallisation furnished crystals which analysed for $C_{30}H_{50}O_3$, m.p. 323—24°, $[\alpha]_D$ -28.0°. IR spectrum (Fig.29) of the compound revealed that the compound contained a hydroxyl functional group (at ν_{max} 3440 cm^{-1}) and —COOH functional group (ν_{max} at 1680 cm^{-1}). It gave negative TNM test showing the absence of double bond present in the starting ester. Mass spectrum (Fig.30) showed molecular ion peak at m/e 458 (M^+) which is consistent with the molecular formula. Other peaks at m/e 443 (458—CH_3), 415 (M^+- isopropyl), 413 (M^+- COOH), 207, 189 (base peak).

The hydroxy acid so formed was acetylated with acetic anhydride and pyridine. The usual work up and crystallisation from chloroform-methanol afforded crystals of 82c, m.p. 310—11°, $[\alpha]_D$ -11.5°, was analysed for $C_{32}H_{52}O_4$. IR spectrum (Fig.31) of the compound showed peaks at ν_{max} 1735 cm^{-1} and 1240 cm^{-1} for acetate functional group and peak at 1690 cm^{-1} revealed the presence of —COOH functional group. PMR spectrum (Fig.32) of the compound

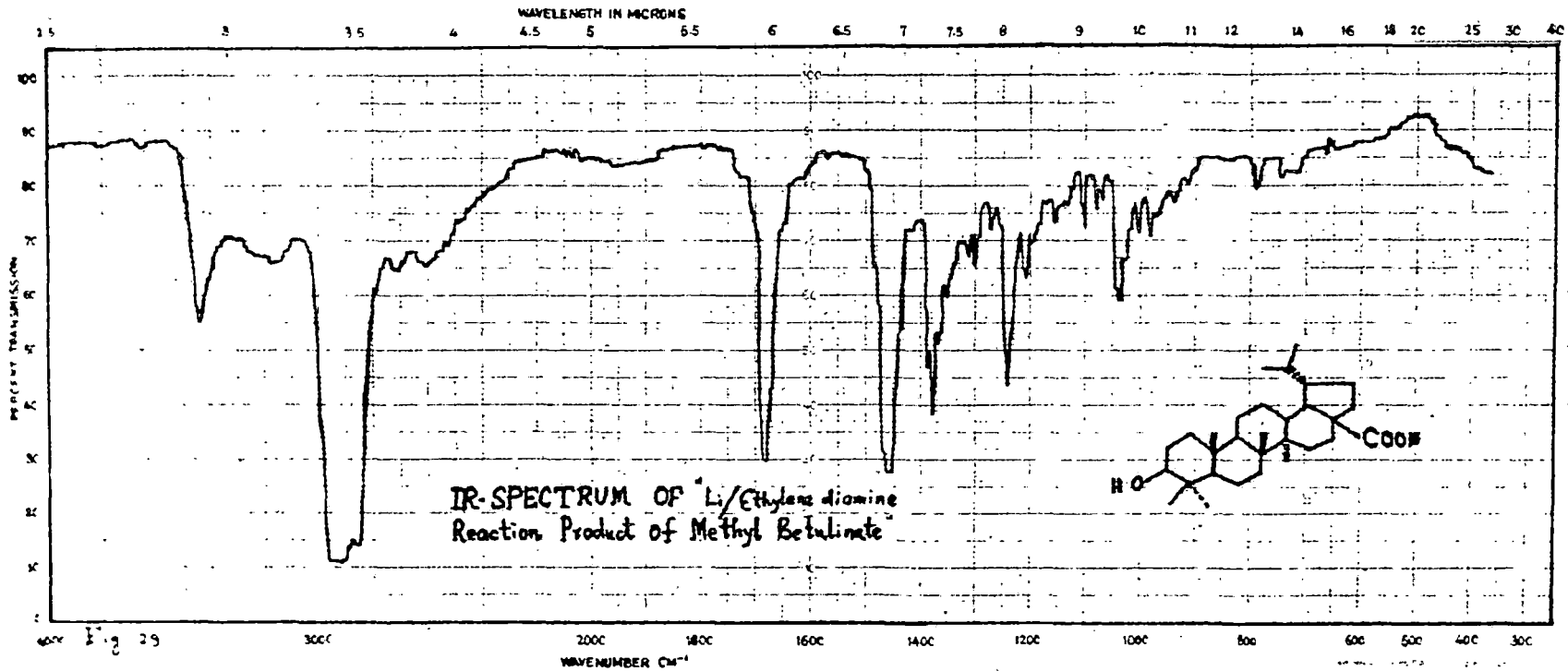


Fig.29: IR spectrum of dihydro betulenic acid, 82b.

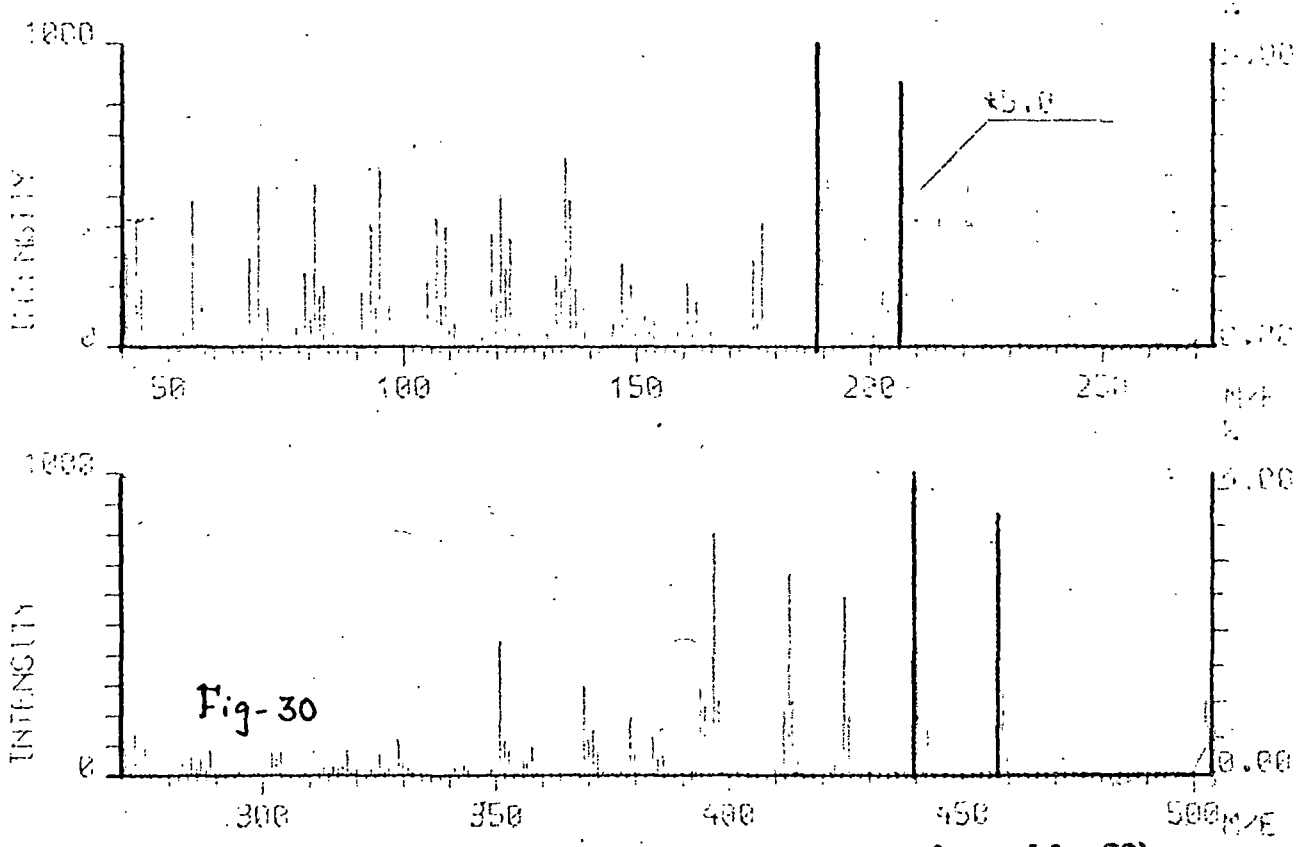


Fig-30

Fig.30: Mass spectrum of dihydro betulenic acid, 82b.

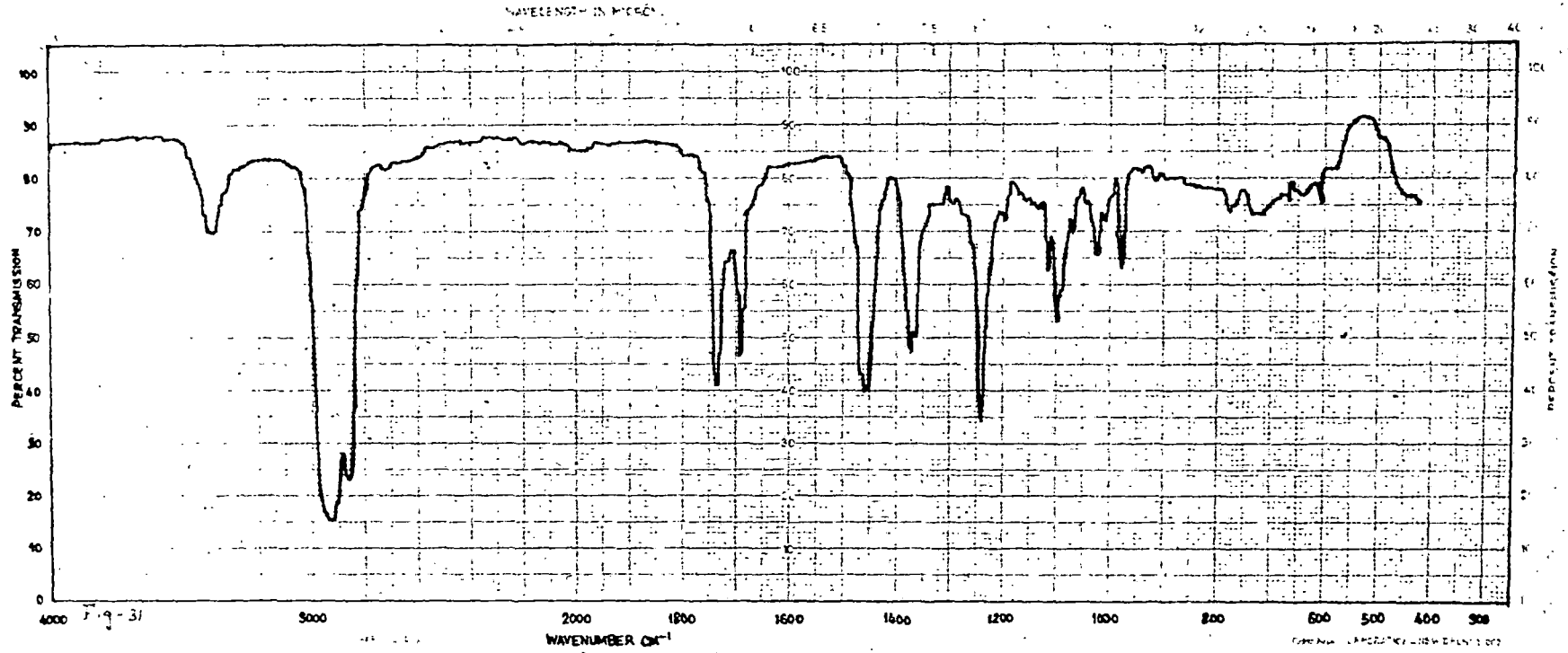


Fig.31: IR spectrum of acetyl betulanic acid, 82c.

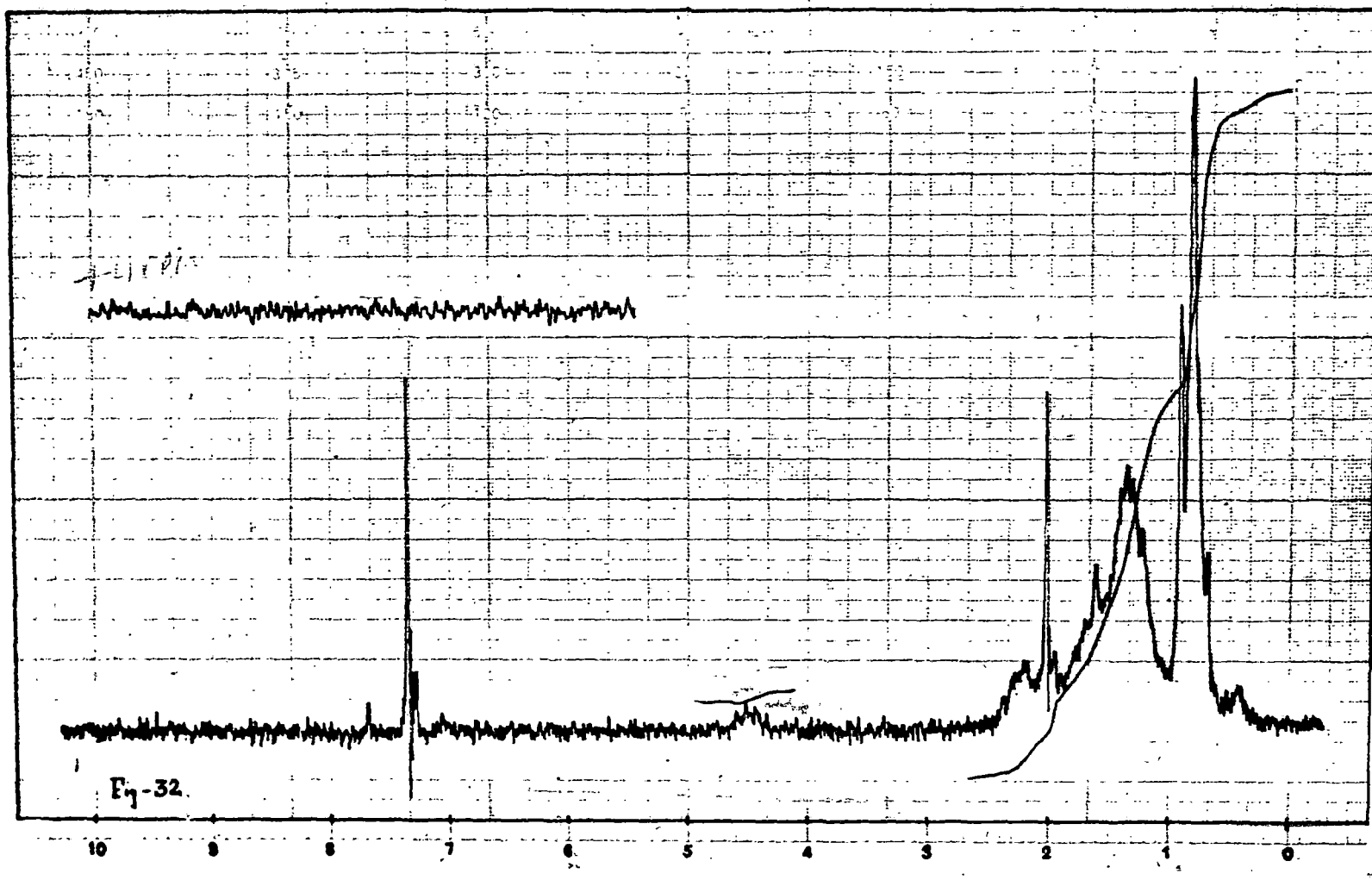


Fig.32: PMR spectrum of acetyl betulanic acid, 82c.

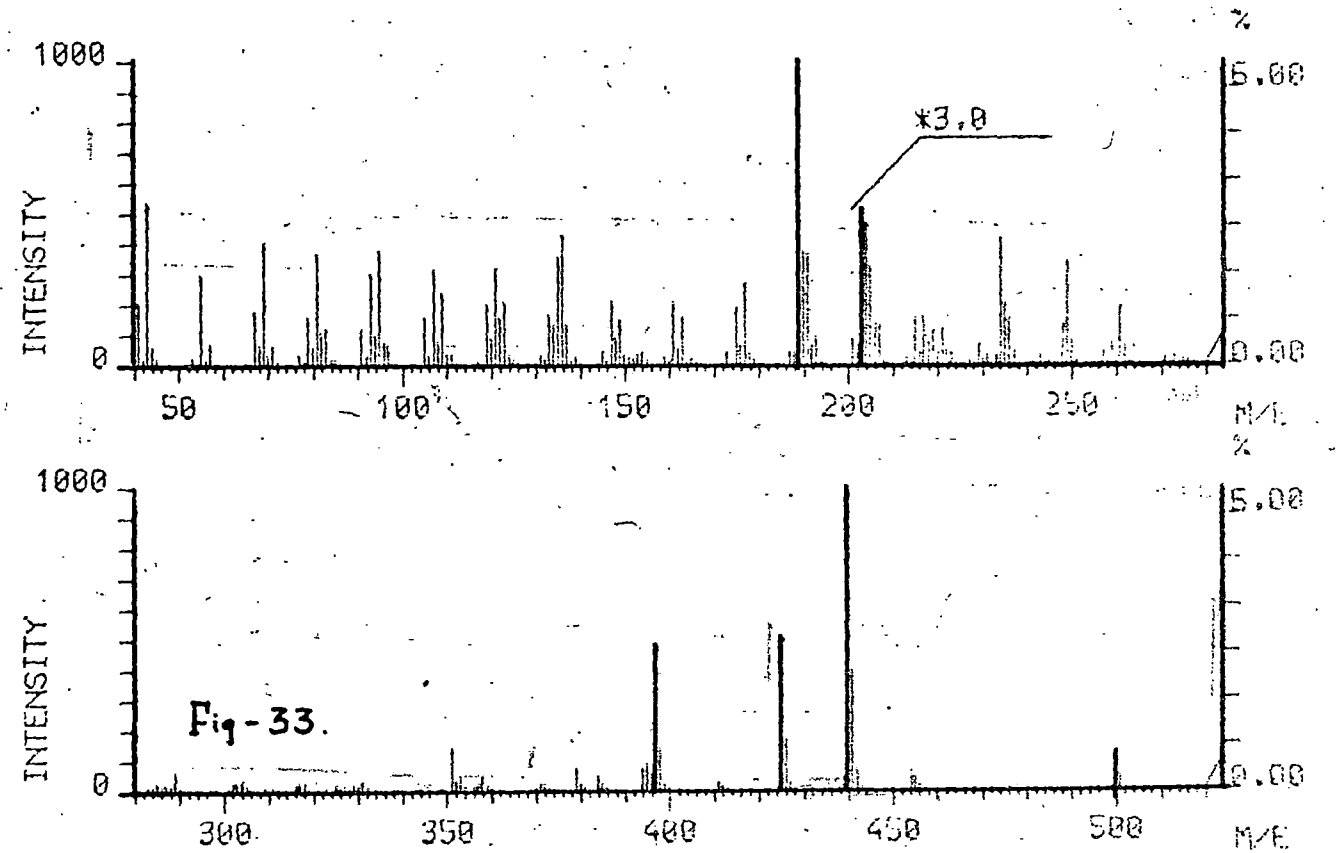


Fig-33.

Fig.33: Mass spectrum of acetyl betulamic acid, 82c.

showed seven tertiary methyl groups at the region 0.66 to 0.93 ppm. A singlet at 2.03 ppm showed the presence of three protons of the acetate functional group (—OCOCH_3). A multiplet at the region 4.5 ppm integrated for one proton for the hydrogen geminal to C—3 acetate group (—C—O—COCH_3). Mass spectrum (Fig.33) of the compound 82c was similar to that of acetyl dihydro betulonic acid. The M^+ ion peak at m/e 500 was consistent with the molecular formula $C_{32}H_{52}O_4$. The other substantial peaks at m/e 440 (M^+—CH_3COOH), 455 (M^+—COOH), 249 and 189 (base) are characteristic peaks[28] of acetyl betulanic acid.

From the above mentioned data it was clear that when methyl betulonate 82a was subjected to Li-ethylenediamine reaction the ester group was hydrolysed along with the hydrogenation of the isopropenyl double bond. The formation of dihydrobetulonic acid[42] 82b, and its acetyl derivative[42] 82c, was finally confirmed by comparison with authentic specimens (m.m.p. and CO—IR).

(ii) Esters containing C-12-13 double bond:

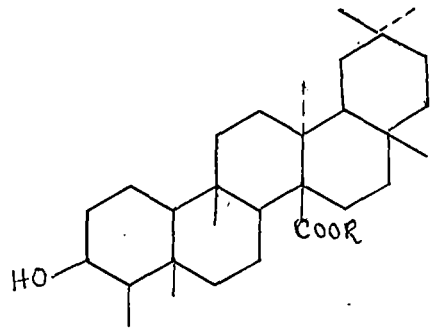
The model compound taken for the reaction of Li-ethylenediamine was methyl oleanonate[56] 78e. The compound was treated with lithium in ethylenediamine resulted a mass which was separated into acid and neutral fractions.

The acid fraction showed only one compound (from TLC) which gave positive TNM test showing double bond was present. The compound (80% yield) was crystallised and the crystal analysed for $C_{30}H_{48}O_3$, m.p. 301—2°, $[\alpha]_D$ +75.3°. IR spectrum (Fig.34) showed the presence of hydroxyl functional group at ν_{max} 3400 cm^{-1} and at 1685 cm^{-1} for —COOH functional group. The compound was identified as oleanolic acid[36] 78c, when compared with an authentic sample of oleanolic acid [m.m.p. and CO—IR (Fig.35)].

So methyl oleanonate gave the hydrolysed product without reducing the double bond.

(iii) Sterically hindered esters:

Reaction of methyl trichadenate[34] A 83a, with lithium in ethylenediamine furnished a single compound 83b,



R=CH_3, 83a
R=H , 83b

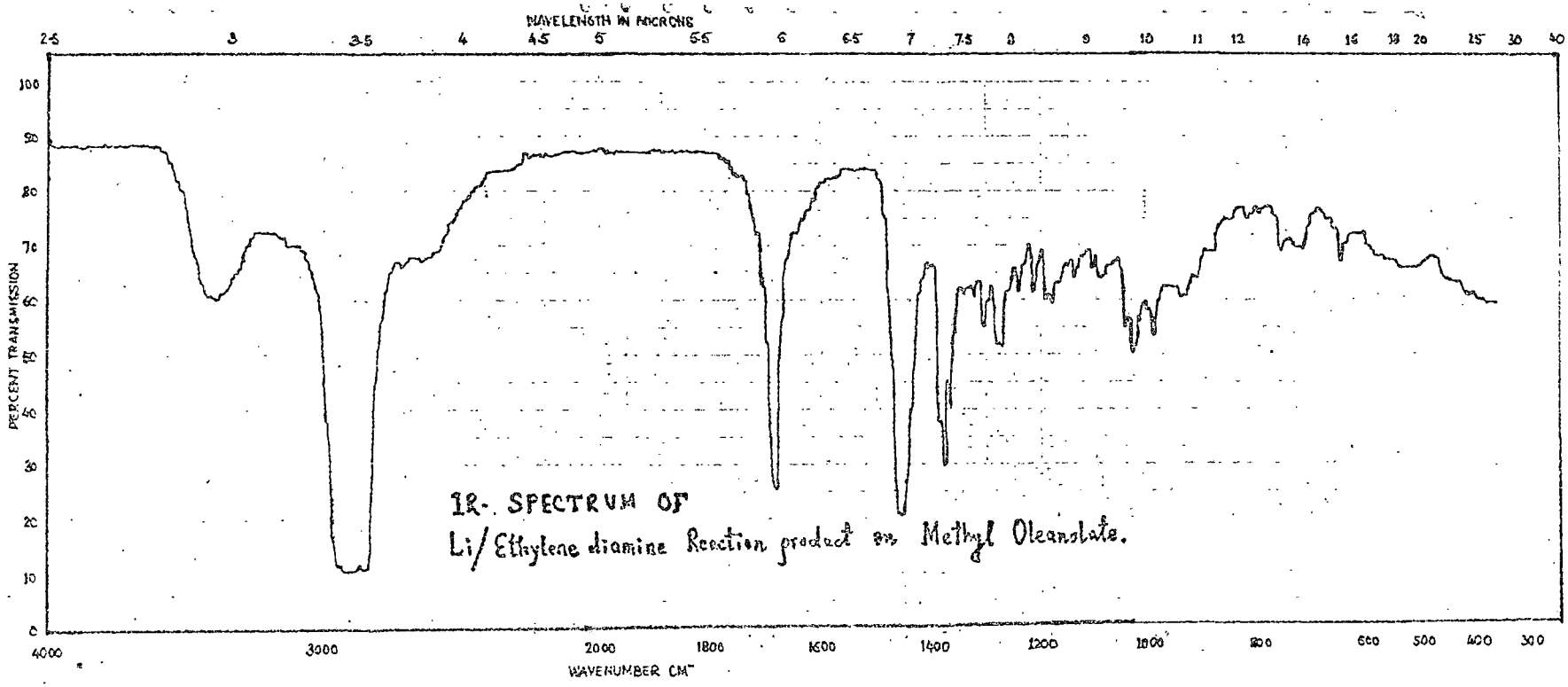


Fig.34: IR spectrum of the compound, 78c.

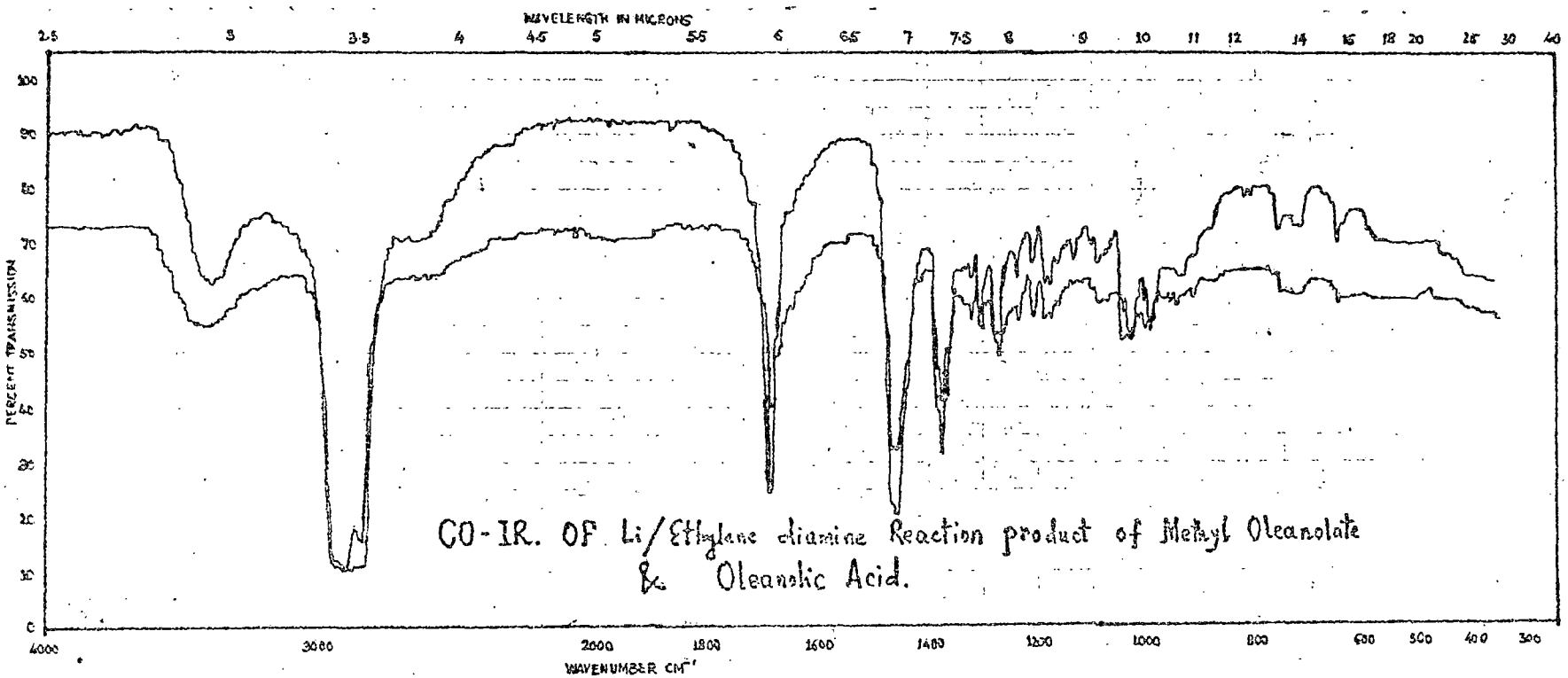


Fig.35: CO—IR spectrum of compound 78e and oleanolic acid.

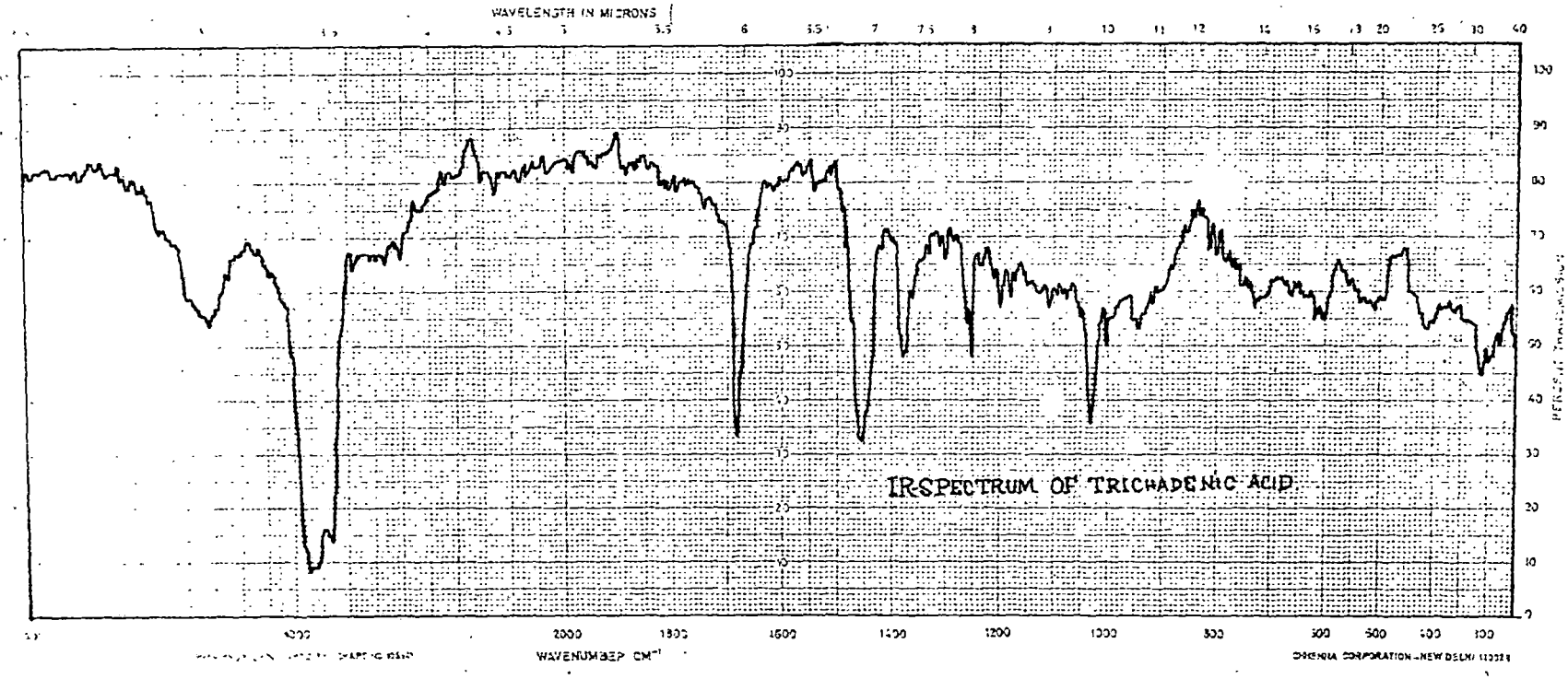


Fig.36: IR spectrum of trichadenic acid A.

m.p. 330—32°, $[\alpha]_D$ +35°, was analysed for $C_{30}H_{50}O_3$. The compound was identified as trichadenic acid[34] A 83b when compared with an authentic specimen (m.m.p. and CO—IR). IR spectrum (Fig.36) showed the hydroxyl and carboxylic acid function at 3440 and 1690 cm^{-1} respectively.

SECTION—C:

Conclusions:

From the results obtained in the reduction of lactones (Section—A) it may be concluded that it is possible to cleave a lactone ring to the relative amounts of the acid to diols indicating the sterical hindrance of the lactone ring. From section [B] we conclude that it is possible to reduce the 3-keto group to thermodynamically more stable alcohol in 100% purity. The isopropenyl double bonds can be reduced to isopropyl group. The sterically hindered double bonds are not affected as observed by Corey and coworkers[43]. The hindered triterpenoid esters can be smoothly be hydrolysed to acids. It may be mentioned that the hydrogenolysis of isopropenyl group takes place even in the crude compounds compared to the high purity requirements in the case of catalytic hydrogenation.

CHAPTER—III

E X P E R I M E N T A L

Melting points are uncorrected. The petroleum used throughout had the b.p. 60—80°. Brockmann alumina (S.Merck) and silical gel (BDH) were used for column chromatography. TLC plates were coated with silica gel G (acc to Stahl) having the thickness of about 0.2 mm and the spots located by exposing to iodine vapour. All the optical rotations were determined in chloroform solution. IR spectra were recorded as nujol mull on a Beckmann IR—20 and Perkin-Elmer spectrophotometer and the UV spectra in methanol on a Beckmann DU-2 spectrophotometer. NMR spectra were recorded in Varian A—60, XL—200, XL—300 NMR instruments using $CDCl_3$ as solvent containing TMS as internal standard. Mass spectra were recorded by solid probe CI/CH_4 method.

SECTION—A

Treatment of 3β-acetyl-oleanan-18-α-H-28⟶ 13β-olide 67 in ethylenediamine with lithium; isolation of two acids 68 and 68a:

300 mg of the lactone 67 was taken in 100 ml dry ethylenediamine. To the solution 300 mg lithium was added in small portions with stirring in an atmosphere of N_2 gas. After the addition of lithium, the reaction mixture was refluxed for two hours. The reaction mixture was cooled and solid ammonium chloride was added to destroy any excess lithium. Then the mixture was acidified with 6N HCl and extracted with solvent ether. The organic layer was washed with 10% sodium hydroxide. The ether layer was then washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent did not afford any solid material. The alkali extract was acedified and extracted with ether. The ether layer was washed with water till neutral and the mass obtained after evaporation of solvent was chromatographed over a column of silica gel (40 gms). The following solvents were used for chromatography:

Table—7

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—20	White solid m.p.260—65°
Petroleum ether:benzene (1:4)	21—25	Nil
Benzene	26—30	Nil
Benzene:solvent ether (4:1)	31—40	White solid m.p. 285—90°

Further elution with more polar solvents did not afford any solid material

Isolation of oleanan-18α-H-28-oic acid 68:

Fractions 13—20 (table—7) were mixed. The residue (120 mg) was crystallised from chloroform-methanol to afford crystals of oleanan 18-α-H-28-oic acid 68, m.p. 270—1°.

Analysis report:

Found, C, 81.45% ; H, 11.4%

Calculated for $C_{30}H_{50}O_2$, C, 81.39% ; H, 11.38%

IR(nujol) : ν_{max} at 1695 cm^{-1} (—COOH function)
Fig.1

PMR : 0.83—0.94 ppm (7-tertiary methyl protons)
Fig.2

Mass : m/e 442 (M^+), 427, 393, 337, 281, 205, 191 (base)
Fig.3

Isolation of 3β-hydroxy-oleanan-18-α-H-28 oic acid 69a

Fractions 31—40 (table—7) were combined while the solid residue 120 mg was obtained. It was crystallised from chloroform-methanol mixture. The crystal obtained was

identified as 3β -hydroxy-oleanan-18α-H-28-oic acid 69a, m.p. 295—6°.

Analysis report:

Found, C, 78.48% ; H, 10.98%

Required for $C_{30}H_{50}O_3$, C, 78.55% ; H, 10.99%

IR (nujol) : ν_{max} at 3460 cm^{-1} (—OH group)
1690 cm^{-1} (—COOH group)
Fig.4

Mass : m/e 458 (M^+), 443, 440, 425, 414, 413, 250, 235, 203, 203 and 189 Fig.5

Esterification of 3β -hydroxy-oleanan-18α-H-28-oic acid 69a:

To 50 mg of the hydroxy acid 69a dissolved in dry ether (50 ml) was added a solution of diazomethane in ether (prepared from 50 mg nitrosomethyl urea and 50% NaOH solution) and was kept over-night. On the following day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and was then dried over anhydrous sodium sulphate. Evaporation of solvent gave a solid (40 mg) which was crystallised

from a mixture of chloroform and methanol when crystals of $C_{31}H_{52}O_3$, m.p. 198° , $[\alpha]_D$ +17° were obtained.

Analysis report:

Found, C, 78.69% ; H, 11.08%

Required for $C_{31}H_{52}O_3$, C, 78.76% ; H, 11.09%

IR (nujol) : ν_{max} at, 3250 cm^{-1} (—OH group)
1725 cm^{-1} ($—COOCH_3$ group)
Fig.6.

PMR : 0.76—0.96 ppm (seven tertiary methyl protons), 3.20 ppm (—CHOH), 3.64 ppm ($—COOCH_3$)
Fig.7

Mass : m/e 472 (M^+), 454, 439, 413, 395, 262, 207, 205, 189 Fig.8

The above physical data of the compound characterised as methyl-3β-hydroxy-oleanan-18αH-28-oate 69b.

Acetylation of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a; preparation of 3β-acetyl-oleanan-18α-H-28-oic acid 69c:

50 mg of the hydroxy acid 69a was dissolved in 1 ml pyridine. This was treated with 1 ml of acetic-anhydride. The reaction mixture was kept on water bath for 4 hours. It was cooled and poured into ice cold water

and extracted with solvent ether. The ethereal layer was washed several times with water and then dried over anhydrous sodium sulphate. Removal of solvent afforded a gummy mass (50 mg) which was chromatographed over a silica gel (10 gms) column. The following solvents were used for elution.

Table—8

Eluent	Fraction collected 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—18	White solid

Further elution with more polar solvents did not afford any solid material

Fractions obtained from 13—18 (table—8) were combined and the residue (40 mg) was crystallised from chloroform-methanol mixture, when crystals of m.p.290-91°, $[\alpha]_D$ +15° were obtained.

Analysis report:

Found, C, 76.77% ; H, 10.47%

Required for $C_{32}H_{52}O_4$, C, 76.75% ; H, 10.47%

IR (nujol) : ν max at, 1695 cm^{-1} (—COOH)
1725 and 1245 cm^{-1} (acetate)
Fig.9

PMR : 0.81—0.94 ppm (methyl groups),
1.96 ppm (s, —OCO$\underline{CH_3}$), 4.3 ppm (t,-$\underline{H}$-CO-COCH_3)
Fig.10

Mass : m/e 500 (M^+), 455, 440, 425, 250, 249,
236, 205 Fig.11

The compound was identified as 3β-acetyl-oleanan-18α-H-28-oic acid 69c.

Li-ethylenediamine reaction on 3-oxo-oleanan-18α-H-28 $\longrightarrow$ 13β-olide 70; isolation of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a:

200 mg of the lactone 70 was taken in 75 ml of dry ethylenediamine. To the solution 200 mg of lithium metal was added in small pieces with stirring in an atmosphere of nitrogen gas. After addition the reaction mixture was refluxed for a period of two hours. Then it was cooled and solid ammonium chloride was added till excess lithium

was destroyed. The mixture was acidified with 6N HCl and then extracted with solvent ether. After washing with water the ether layer was separated into neutral and acid fractions in the usual way by treatment with 10% sodium-hydroxide. The neutral fraction did not afford any solid material. The acid fraction was chromatographed over a column of silical gel (40 gms). The following solvents were used for elution:

Table—9

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—9	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—16	Nil
Petroleum ether:benzene (1:4)	17—20	Nil
Benzene	21—24	Nil
Benzene:ether (4:1)	25—32	White solid

Further elution with more polar solvents did not afford any solid material

Fractions 25—32 (table—9) were combined and the residue (170 mg) obtained was crystallised from a mixture of chloroform and methanol. The crystals of m.p. 295—8°, was identified as 3β-hydroxy-oleanan-18α-H-28-oic acid 69a, when compared with authentic specimen (m.m.p. and CO—IR).

Analysis report:

Found,	C, 78.54% ; H, 10.8%
Calculated for $C_{30}H_{50}O_3$	C, 78.55% ; H, 10.99%

Esterification of the above acid:

50 mg of the acid was dissolved in dry ether (80 ml) and a solution of diazomethane was added to it and kept for over-night. On the following day usual work up followed by crystallisation from chloroform-methanol mixture afforded crystals of m.p. 198°.

Analysis report:

Found,	C, 78.75% ; H, 11.07%
Calculated for $C_{31}H_{52}O_3$	C, 78.76% ; H, 11.09%

The compound was identified as methyl-3β-hydroxy-oleanan-18α-H-28-oate when compared with an authentic specimen (m.m.p. and CO—IR comparison).

Preparation of 3β-acetyl-oleanan-18α-H-28$\longrightarrow$19β-olide 71:

Acetyl betulenic acid (1 gm) was dissolved in benzene (10 cc) and a mixture of acetic acid (80 cc) and sulphuric acid (16 gm, d 1.84) was added with shaking.

After 2 days at 20°, the reaction mixture was diluted with water and taken in benzene. The benzene layer was washed several times with water till neutral and dried over anhydrous sodium sulphate. Removal of solvent gave a mass which was taken in solvent ether. The ether layer was washed with 5% cold sodium hydroxide solution and the washings were discarded. The ether layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a mass which was crystallised from chloroform-methanol mixture. The crystals of m.p. $>$ 350° (lit.m.p. $>$ 350°) was identified as 3β-acetyl-oleanan-18α-H-28$\longrightarrow$19β-olide.

Analysis report:

Found,	C, 77.09%; H, 10.05%
Calculated for $C_{32}H_{50}O_4$	C, 77.1% ; H, 10.04%

IR (nujol) : ν_{max} at 1765 cm^{-1} (γ-lactone)
1725 and 1240 cm^{-1} (acetate).

Treatment of 3β-acetyl-oleanan-18α-H-28$\rightarrow$19β-olide 71, in ethylenediamine with lithium; isolation of (i) olean-18α-H-28-oic acid 68, (ii) 3β-hydroxy-oleanan-18α-H-28-oic acid 69a (iii) oleanan-18α-H-3β, 19β, 28-triol 72a:

The lactone 71 (200 mg) was taken in dry ethylenediamine (100 ml). To the solution 500 mg of metallic lithium was added in small pieces with stirring in an atmosphere of nitrogen gas. Then the mixture was refluxed for a period of two hours. After cooling solid ammonium chloride was added until excess lithium was destroyed. The mixture was acidified with 6N HCl and then extracted with solvent ether. The ether layer was separated into acid and neutral fractions in the usual way by treatment with 10% sodium hydroxide.

Examination of acid fraction:

The alkaline solution was acedified with dilute HCl and then extracted with ether. The organic layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a residue which was chromatographed over a column of silica gel (50 gms). The chromatogram was developed with petroleum ether and the following solvents were used for elution of the column.

Table—10

Eluent	Fractions collected 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—20	White solid
Petroleum ether:benzene (1:4)	21—25	Nil
Benzene	26—30	Nil
Benzene:ether (4:1)	31—40	White solid

Further elution with more polar solvents did not afford any solid material

Isolation of oleanan-18α-H-28-oic acid:

Fractions 13—20 (table—10) were combined and the residue obtained (90 mg) was crystallised from chloroform-methanol mixture. The crystal of m.p. 270—1° was

found to be identical with oleanan-18α-H-28-oic acid 68, when compared with an authentic specimen (m.m.p. and CO—IR).

Analysis report:

Found, C, 81.37% ; H, 11.4%

Calculated for $C_{30}H_{50}O_2$ C, 81.39% ; H, 11.39%

IR (nujol) : ν max at, 1690 cm^{-1} (—COOH group)

Isolation of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a:

Fractions 31—40 (table—10) were combined and the residue obtained (105 mg) was crystallised from a mixture of chloroform and methanol. The crystallised product, m.p. 295—6°, was found to be identical with 3β-hydroxy-oleanan-18α-H-28-oic acid 69a, when compared with an authentic sample (m.m.p. and CO—IR).

Preparation of methyl ester of the above acid 69a:

To the ethereal solution of the acid (50 mg), ethereal solution of diazomethane was added and the mixture was kept over-night. On the following day the mixture was acidified with acetic acid to destroy any excess diazomethane present in the mixture. Then the ether layer

was washed with water and then dried over anhydrous sodium sulphate. Removal of solvent afforded a compound which was crystallised from chloroform and methanol. The crystals of m.p. 197—98° was identified as methyl-3β-hydroxy-oleanan-18∝-H-28-oate-acid 69b, when compared with an authentic specimen (by m.m.p. and IR comparison).

Analysis report:

Found, C, 78.77% ; H, 11.08%

Calculated for $C_{31}H_{52}O_3$ C, 78.76% ; H, 11.08%

IR (nujol): ν_{max} at 1735 cm^{-1} (—COOCH$_3$),
3450 cm^{-1} (—OH).

Preparation of acetyl derivative of the hydroxy acid 69a:

50 mg of the hydroxy acid 69a was dissolved in 1 ml pyridine and to the solution 1 ml of acetic anhydride was added and the reaction mixture was kept on water bath for four hours. Then it was cooled and poured on ice cold water and extracted with solvent ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Removal of solvent gave a compound which was crystallised from chloroform-methanol mixture. The crystals

of m.p. 290—1° was found to be identical with 3β-acetyl-oleanan-18α-H-28-oic acid 69c, when compared with an authentic specimen (m.m.p. and IR comparison).

Analysis report:

Found, C, 76.75% ; H, 10.5%

Calculated for $C_{32}H_{52}O_4$ C, 76.75% ; H, 10.47%

IR (nujol): ν_{max} at 1695 cm^{-1} (—COOH),
1725 and 1240 cm^{-1} (—OCOCH$_3$).

Examination of neutral fraction:

The neutral fraction obtained (50 mg) on removal of solvent was chromatographed over a column of alumina (20 gms) deactivated with 10% acetic acid. The chromatogram was developed with petroleum ether. The following solvents were used for elution of the column:

Table—11

Eluent	Fractions collected in 50 cc	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—16	Nil
Benzene	17—20	Nil
Benzene:ether (4:1)	21—30	White solid

Further elution with more polar solvents did not afford any solid material.

Isolation of oleanan-18α-H-3β, 19β, 28-triol 72a:

Fractions 21—30 (table—11) were combined and the residue (30 mg) crystallised from chloroform-methanol mixture. The crystals had m.p. 290—92°, $[\alpha]_D$ +23°.

Analysis report:

Found, C, 78.52% ; H, 10.96%

Required for $C_{30}H_{52}O_3$ C, 78.55% ; H, 10.99%

IR(nujol): ν_{max} at 3350-3400 cm^{-1} (hydroxyl groups)

Fig.12

PMR : 0.8—1.3 ppm (methyl groups), 3.5 ppm ($-C\underline{H}_2OH$), 4.13 ppm (m, $-C\underline{H}OH$), 4.26 ppm($-C_{16}-\underline{H}-OH$)

Fig.13

Mass : m/e 442 (M^+-H_2O), 424, 409, 399, 394, 381 (base peak), 205, 189.

Fig.14

The compound was identified as oleanan-18 -α-H-3β , 16β , 28-triol 72a. (lit. m.p. 296—298°. $[\alpha]_D$ +24°)

Preparation of the acetyl derivative of the above triol 72a:

20 mg of the triol 72a was taken in 1 cc pyridine and 1 cc acetic anhydride was added to the mixture. It was kept on water bath for four hours. Then it was cooled and poured into ice cold water and then extracted with ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Removal of solvent afforded a compound which was crystallised from $CHCl_3$—MeOH. The crystals had m.p. 211—12°.

Analysis report:

Found, C, 73.68% ; H, 9.87%

Required for $C_{36}H_{58}O_6$ C, 73.7% ; H, 9.89%

IR (nujol): ν_{max} 1720—1730 (b), 1240—1250 cm^{-1} (b).

The compound was identified as oleanan-12α-H-3β, 19β, 28-triacetate 72b (lit, m.p. 213—16°).

Treatment of friedelan-26⟶ 12β-olide 74a with Li-ethylenediamine; isolation of (i) 3-deoxy-trichadenic acid 75a, (ii) friedelan-12β, 26-diol 76.

150 mg of friedelan-26⟶ 12β-olide[33] 74a was taken 50 ml of dry ethylenediamine and to the solution 150 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. The reaction mixture was refluxed for two hours. After two hours it was cooled and on usual work up afforded a mass which was separated into acid and neutral fractions by treatment with 10% sodium hydroxide solution.

Examination of the acid fraction:

The alkali extract was acidified, extracted with ether. The ether layer was washed with water and dried

over anhydrous sodium sulphate. Removal of solvent afforded a residue (120 mg) which was dissolved in minimum amount of benzene and poured into a column of silica gel (20 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—12

Eluent	Fractions collected in 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—20	White solid

Further elution with more polar solvents did not afford any solid material.

Isolation of 3-deoxy-trichadenic acid 75a:

Fractions 13—20 (table—12) were combined and the residue (113 mg) was crystallised from chloroform-methanol mixture. The crystals of m.p. 290—91°, $[\alpha]_D$ +28.57°, was identified as 3-deoxy-trichadenic acid 75a [lit, m.p. 291—92°, $[\alpha]_D$ +4.2°].

Analysis report:

Found,	C, 81.35% ; H, 11.37%
Required for $C_{30}H_{50}O_2$	C, 81.39% ; H, 11.38%

IR(nujol): ν_{max} 1680 cm^{-1} (—COOH function)

Fig.15

Mass : m/e 442 (M^+), 427, 397, 371, 291, 290, 274, 273, 260, 259, 257, 231, 217, 205, 191, 149 (base peak)

Fig.16

Esterification of the above acid; preparation of 3-deoxy-methyl trichadenate 75b:

50 mg of the acid 75b was dissolved in 200 cc ether and a solution of diazomethane in ether (prepared from 50 mg nitrosomethyl urea and sodium hydroxide) was

added to it. The reaction mixture was kept for overnight. On the following day acetic acid was added to destroy any excess diazomethane present in the mixture. The ethereal solution was then washed with water and $NaHCO_3$. Then the organic layer was dried over anhydrous sodium sulphate. Removal of solvent gave a compound which was crystallised from chloroform-methanol mixture. The crystals of m.p. 180°, $[\alpha]_D$ +30.3° was identified as 3-deoxy-methyl trichadenate 75b. (lit, m.p. 180°, $[\alpha]_D$ -21.0°).

Analysis report:

Found, C, 81.54% ; H, 11.4%

Calculated for $C_{31}H_{52}O_2$ C, 81.57% ; H, 11.4%

IR (nujol): ν_{max} 1735 cm^{-1} ($-COOCH_3$)

PMR : 0.76—1.22 ppm (tertiary methyls), 3.72 ppm (s, $-COOCH_3$) Fig.17

Mass : m/e 456 (M^+), 425, 424, 409, 397, 396, 317, 306, 303 (base) Fig.18

Examination of the neutral part:

The neutral fraction obtained, on removal of solvent, was chromatographed over a column of alumina (20 gms), deactivated with 10% acetic acid. The chromatogram was developed with petrdeum-ether and was eluted with the following solvents:

Table—15

Eluent	Fraction collected 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Benzene:petroleum ether (1:4)	5—8	Nil
Benzene:petroleum ether (2:3)	9—12	Nil
Benzene:petroleum ether (3:2)	13—16	Nil
Benzene:petroleum ether (4:1)	17—20	Nil
Benzene	21—25	Nil
Benzene:solvent ether (4:1)	26—40	White solid

Further elution with more poler solvents did not afford any solid materials.

Isolation of friedelan 12β, 26 diol:

Fractions 26—30 (table—13) were combined and the residue (8 mg) was crystallised from $CHCl_3$—MeOH mixture. The crystals of $C_{30}H_{52}O_2$, had m.p. 240—1°.

Analysis report:

Found, C, 81.05% ; H, 11.75%

Required for $C_{30}H_{52}O_2$ C, 81.02% ; H, 11.79%

IR (nujol): ν max at 3250—3500 cm^{-1}(b) (hydroxyl group) Fig.19

PMR : 0.724 ppm (d), 0.7976, 0.899, 0.9677, 0.9836, 1.002 and 1.244 ppm (s) (Methyl groups), 4.0 ppm (A-B q, for C-26-$\underline{H}_2$-OH) 3.65 ppm (m, C-12-$\underline{H}$-OH) Fig.20

Mass : m/e at 426, 395, 302, 301, 220, 205, 189, 177 (base peak) Fig.21

The compound was identified as friedelan-12β-26-diol 76.

Lithium aluminium hydride reduction of friedelan-26⟶ 12β-olide 74a; preparation of friedelan-26, 12β-diol 76:

50 mg of friedelan-26⟶ 12β-olide was taken in 100 cc of dry ether and 50 mg of LAH was added to it.

The reaction mixture was stirred at 0°C for two hours. After two hours a saturated solution of Na_2SO_4 was added to it which separated the organic layer. The organic layer was washed with water and then dried over anhydrous Na_2SO_4. Removal of solvent afforded a compound which was crystallised from chloroform-methanol mixture. The crystals of m.p. 240—1° was found to be identical with friedelan-26, 12β-diol 76.

Treatment of friedelan-26⟶ 11β-dide 74b in ethylenediamine with lithium; isolation of (i) friedelan-11β-26-diol 76a (ii) 3-deoxy-trichadonic acid 75a:

150 mg of friedelan-26⟶ 11β olide 74b was dissolved in 50 ml of dry ethylenediamine. To the solution 150 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2 gas. Then the mixture was refluxed for two hours. The mixture on usual work up was separated into neutral and acid fractions by treatment with 10% aqueous sodium hydroxide solution.

Examination of the neutral part:

The neutral part was dissolved in minimum amount of benzene and was poured into a column of alumina (50 gms) deactivated with 10% acetic acid. The chromatogram was developed with petroleum-ether and the following solvents were used for elution:

Table—14

Eluent	Fractions collected 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—16	Nil
Petroleum ether:benzene (1:4)	17—20	Nil
Benzene	21—24	Nil
Benzene:ether (4:1)	25—32	White solid

Further elution with more polar solvents did not afford any solid material.

Isolation of friedelan - 12β-26-diol 76s:

Fractions 25—32 (table—14) were combined and the residue (112.5 mg) was crystallised from chloroform-methanol mixture and the crystal of $C_{30}H_{52}O_2$, m.p. $> 350^{\circ}$,

was identified as friedelan - 11β -28-diol 76a.

Analysis report:

Found, C, 81.05% ; H, 11.77%

Required for $C_{30}H_{52}O_2$ C, 81.02% ; H, 11.79%

IR(nujol): ν_{max} at 3200—3500 cm^{-1} (b)
(for hydroxyl group)
Fig.22

PMR : 0.8 (d), 0.9—1.28 ppm(s) (for seven methyls), 3.9 ppm (m, C—28-$\underline{H}_2$-OH), 4.35 ppm (m, C—11-$\underline{H}$-(OH)
Fig.23

Mass : m/e at 426 (M^+—H_2O), 395, 302, 301, 189 and 177 (base peak).

Examination of the acid fraction:

The acid fraction was dissolved in minimum amount of benzene and poured into a column of silica gel (20 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—15

Eluent	Fractions collected 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—20	White solid

Further elution with more polar solvents did not afford any solid material.

Fractions 13—20 (table—15) were combined and the residue obtained (7.6 mg) was crystallised from chloroform-methanol mixture. The crystals of $C_{30}H_{50}O_2$, m.p. 293—94° were obtained. The compound was identified as 3-deoxy-trichadenic acid (compared with an authentic specimen by m.m.p. & CO—IR).

Esterification of the above acid:

20 mg of the above acid was taken in 100 ml of solvent ether and an ethereal solution of diazomethane was added to it at 0°C. After keeping over-night at low temperature the reaction mixture was worked up in the usual way. After work up the solid obtained was crystallised from $CHCl_3$—MeOH mixture when the crystal was found to be identical with methyl-3-deoxy-trichadenate 75b, when compared with an authentic specimen (m.m.p. & CO—IR).

Preparation of 3β-acetyl-12α-bromo-oleanan-28⟶13β-olide 77; Treatment of 3β-acetyl oleanolic acid with NBS:

400 mg of 3β-acetyl oleanolic acid was taken in dry chloroform (25 ml), containing dimethyl sulfoxide (10 ml). To the solution 400 mg of NBS was added in one lot and the mixture kept in dark for 24-hours. The mixture was diluted with water, extracted with chloroform and the organic layer was dried over anhydrous sodium sulphate. The solvent was removed and the residue was again extracted with ether, stirred with aq. sodium hydroxide (5%) and separated into alkaline and neutral extracts. The alkali extract on acidification and extraction with water did not furnish any solid material. The neutral ethereal

portion was concentrated to give a residue (300 mg) which was homogeneous in TLC (single spot). It was crystallised from chloroform-methanol, m.p. 215—17° found identical with 3β-acetyl-12α-bromo-oleanan-28⟶13β-olide 77.

Treatment of 3β-acetyl-12α-bromo-oleanan-28⟶13β-olide 77 in ethylenediamine with lithium; isolation of 3-deoxy-oleanolic acid 78a and oleanolic acid 78c:

300 mg of 3β-acetyl-12α-bromo-oleanan-28⟶13β-olide 77, was taken in 100 ml of dry ethylenediamine and to the solution 300 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. The reaction mixture was refluxed for two hours and then on usual work up the mass obtained was separated into acid and neutral fractions by treatment with 10% aqueous sodium hydroxide solution. The neutral fraction did not furnish any solid material. The acidic fraction was chromatographed over a column of silica gel (40 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—16

Eluent	Fractions collected 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—18	White solid
Petroleum ether:benzene (1:4)	19—22	Nil
Benzene	23—26	Nil
Benzene:solvent ether (4:1)	27—34	White solid

Further elution with more polar solvents did not afford any solid material.

Isolation of 3-deoxy-oleanolic acid:

Fractions 13—18 (table—16) were mixed and crystallised from methanol when the crystal of $C_{30}H_{48}O_2$,

m.p. 265—66°, was identified as 3-deoxy-oleanolic acid 78a.

Analysis report:

Found, C, 81.76% ; H, 10.8%

Required for $C_{30}H_{48}O_2$ C, 81.8% ; H, 10.8%

Esterification of the above acid:

50 mg of the acid was dissolved in 100 ml of solvent ether and an ethereal solution of diazomethane (prepared from 50 mg of nitrosomethyl urea) was added to it. On the following day it was worked up and crystallised from chloroform-methanol mixture. The crystals had m.p. 169° was identified as methyl-3-deoxy-oleanolate (lit, m.p. 170°) 78b.

Analysis report:

Found, C, 81.95% ; H, 11.00%

Required for $C_{31}H_{50}O_2$ C, 81.93% ; H, 11.01%

IR(nujol): ν_{max} at 1730 cm^{-1} ($-COOCH_3$)

Fig.24a

Isolation of oleanolic acid 78c:

Fractions 27—34 (table—16) were combined and the residue (105 mg) obtained was crystallised from methanol. The crystals of $C_{30}H_{48}O_3$, m.p. 303—4°, were identified as oleanolic acid.

IR (nujol): ν_{max} at 3450 cm^{-1} (—OH),
1690 cm^{-1} (—COOH)

Preparation of methyl ester of the above acid; isolation of methyl oleanolate 78d:

50 mg of the above acid was dissolved in ether (50 ml), added a solution of diazomethane in ether prepared from nitrosomethyl urea (50 mg) and KOH and was kept over-night at low temperature. On the following day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and was then dried with anhydrous sodium sulphate. Evaporation of solvent gave a solid (40 mg) which was crystallised from a mixture of chloroform-methanol, when crystals of m.p. 196—9° were obtained. This was found to be identical with methyl oleanolate (m.m.p. CO—IR and CO—TLC comparison with authentic specimen).

TNM test : Yellow colouration.

Analysis report:

Found, C, 79.08% ; H, 10.67%

Calculated for $C_{31}H_{50}O_3$ C, 79.10% ; H, 10.71%

IR (nujol): ν max at 3260 (—OH) and 1730 cm^{-1} (—$OOCH_3$)

Fig.24

PMR : 0.58—0.96 ppm (methyl groups), 3.5 ppm (s, —$OOCH_3$), 3.8 ppm (m, —C<u>H</u>—OH), 5.18 ppm (t, >C=C<)

<u>H</u>

Fig.25

Mass : m/e at 470 (M^+), 452, 455, 411, 382, 207, 203, 189

Fig.26

Preparation of 3β-acetyl-29, 30-dibromo-oleanan-18-α-H-28—> 19β-olide 79; Treatment of 3β-acetyl betulenic acid with NBS:

To a solution of 3 gms of 3β-acetyl betulenic acid in 150 ml of chloroform containing 80 ml of dry dimethylsulfoxide, 6 gms of crystal NBS were added in lots of 300 mg each and the solution kept in dark for 24 hrs. The mixture was diluted with water and the organic layer was separated and washed several times with water. The chloroform layer was dried over anhydrous sodium sulphate and evaporation of solvent afforded a gummy mass which was

taken in ether and washed with 10% aq. sodium hydroxide solution to separate it into acid and neutral parts. The neutral part was washed with water and evaporation of solvent afforded a mass which after crystallisation from chloroform-methanol mixture to give the crystals of $C_{33}H_{51}O_4Br_2$, m.p. 235—6° $[\alpha]_D$ +42.55°. It was identified as 3β-acetyl-29, 30-dibromo-oleanan-18α-H-28⟶19β-olide 79, by comparison with an authentic specimen (mmp and CO—IR comparison). The yield was 300 mg.

Treatment of Li-ethylenediamine on 3β-acetyl-29,30-dibromo-oleanan-18α-H-28⟶19β-olide 79; isolation of compounds (i) oleanan-18α-H-28-oic acid 68, (ii) 3β-hydroxyoleanan-18α-H-28-oic acid 69a:

250 mg of 3β-acetyl-29,30-dibromo-oleanan-18α-H-28⟶19β-olide 79, was taken in dry ethylene-diamine (100 ml). To the solution 250 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. Then the reaction mixture was refluxed for two hours. The reaction mixture was cooled and on usual work up the mass obtained was separated into acid and neutral fractions by treatment with 10% sodium hydroxide. The neutral fraction did not afford any solid material. The acid fraction was dissolved in minimum amount of benzene and poured into a column of silica gel (40 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—17

Eluent	Fractions collected 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—20	White solid
Petroleum ether:benzene (1:4)	21—24	Nil
Benzene	25—28	Nil
Benzene:solvent ether (4:1)	29—40	White solid

Further elution with more polar solvents did not afford any solid material.

Isolation of oleanan-18αH-28-oic acid 68:

Fraction 13—20 (table—17) were combined and the residue (100 mg) was crystallised from chloroform-methanol mixture, while the crystal of m.p. 270—1°, was found to be identical with oleanan-18α-H-28-oic acid (comparison with authentic sample by mmp. CO—IR). Beilstein test negative.

Isolation of 3β-hydroxy-oleanan-18α-H-28-oic acid 89a:

Fractions 29—40 (table—17) were combined and the residue (100 mg) obtained was crystallised from $CHCl_3$—MeOH mixture. The crystal of $C_{30}H_{50}O_3$, m.p. 295—6°, was identified as 3β-hydroxy-oleanan-18α-H-28-oic acid 89a, when compared with an authentic specimen (mmp and Co—IR comparison). Beilstein test negative.

SECTION—B

Treatment of lupanone 80a in ethylenediamine with lithium; Isolation of lupanol 80b:

150 mg of lupanone 80a, was taken in dry ethylenediamine (50 ml). To the solution 150 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. The reaction mixture was refluxed for a period of 2 hrs. Then it was cooled and on usual work up followed by crystallisation a compound, which was not identical with the starting material (TLC & mmp), was obtained. The crystal, $C_{30}H_{52}O$, m.p. 206°, $[\alpha]_D$ -17.8°, was found to be identical with lupanol 80b (by mmp and Co—IR comparison with authentic specimen). The yield was 120 mg.

Analysis report:

Found,	C, 84.1%; H, 12.20%
Calculated for $C_{30}H_{52}O$	C, 84.04%; H, 12.22%

IR(nujol): ν_{max} at 3330 cm^{-1} (—OH)

Fig.27

Li-ethylenediamine treatment on moretanone 81a; isolation of moretanol 81b:

150 mg of moretanone 81a was taken in ethylenediamine (50 ml). To the solution lithium metal (150 mg) was added as in the earlier experiments in nitrogen atmosphere. After refluxing the reaction mixture for two hours and then usual work up a compound was isolated. The compound was found to be not identical with the starting material (comparison of TLC). The compound was crystallised from chloroform-methanol mixture when the crystal of m.p. 223—24° was obtained. It was found to be identical with moretanol 81b (compared with authentic specimen by mmp, CO—IR and TLC).

Analysis report:

Found, C, 84.02% ; H, 12.21%

Calculated for $C_{30}H_{52}O$, C, 84.04% ; H, 12.22%

IR(nujol): ν max at 3320 cm^{-1} (free hydroxyl group)

Fig.28

Li-ethylenediamine reaction on methyl betulonate 82a; isolation of dihydro betulenic acid 82b:

300 mg of methyl betulonate 82a was taken in 100 cc of dry ethylenediamine and lithium (300 mg) metal was added to the solution in small pieces with stirring in an atmosphere

of N_2. The reaction mixture was refluxed in N_2 atmosphere for 2 hrs. It was cooled and solid NH_4Cl was added to destroy excess lithium present in the mixture. It was acidified with 6N HCl and extracted with ether. The ether layer was washed with water and then treated with 10% sodium hydroxide. The neutral ether layer was evaporated. Absence of residue showed no neutral component was formed. The alkaline extract was acidified and extracted with ether. The ethereal solution was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a compound (TLC single spot) which was crystallised from chloroform-methanol mixture. The crystal, $C_{30}H_{50}O_3$, m.p. 323—24°, $[\alpha]_D$ +28.0° , was found to be identical with dihydrobetulenic acid. Yield of the acid was 240 mg. TNM test was negative.

Analysis report:

Found, C, 78.53% ; H, 10.99%

Calculated for $C_{30}H_{50}O_3$, C, 76.55% ; H, 10.99%

IR(nujol): ν_{max} at 3440 cm^{-1} (—OH group), 1680 cm^{-1} (—COOH group). Fig.29

Mass : m/e at 458 (M^+), 443, 415, 413, 189 (base peak) Fig.30

Acetylation of dihydro betulenic acid 82b; preparation of 3β-acetyl dihydrobetulenic acid 82c:

100 mg of the above prepared dihydrobetulenic acid was dissolved in 1 cc pyridine and 1cc acetic anhydride was added to the solution. The reaction mixture was kept on water bath for 4 hours. Then it was cooled and poured into ice cold water when a solid separated out which was filtered. The residue obtained was crystallised from chloroform-methanol mixture and the crystals of $C_{32}H_{52}O_4$ had m.p. 310—11°, $[\alpha]_D$ -11.5°. It was found to be identical with 3β-acetyl-dihydro-betulenic acid 82c, when compared with an authentic specimen (mmp and Co—IR comparison).

Analysis report:

Found, C, 76.74% ; H, 10.45%

Required for $C_{32}H_{52}O_4$, C, 76.75% ; H, 10.47%

IR(nujol): ν_{max} at 1735 and 1240 cm^{-1} (acetate)
1690 cm^{-1} (—COOH) Fig.31

PMR : 0.66—0.93 ppm (methyl groups), 2.03 ppm (s, —OCOCH_3), 4.5 ppm (m, —C$\underline{H}$—C—O—$\overset{O}{\overset{\|}{C}}$—C$H_3$)
Fig.32

Mass : m/e at 500 (M^+), 455, 440, 249, 189 (base peak)
Fig.33

Li-ethylenediamine reaction on methyl oleanonate 78e; isolation of oleanolic acid 78c:

200 mg of methyl oleanonate 78e was taken in 70 ml of dry ethylenediamine and 200 mg lithium was added as in the previous cases. It was refluxed under nitrogen atmosphere for two hours. After work up and seperation of acid and neutral fractions a compound was isolated from the acid part. It was crystallised from methanol while the crystal of $C_{30}H_{48}O_3$ had m.p. 301—32° $[\alpha]_D$+75.3°. It was found to be identical with oleanolic acid 78c, when compared with an authentic specimen (mmp and CO—IR comparison). It showed positive TNM test. The yield was 160 mg.

Analysis report:

Found,	C, 78.85% ; H, 10.58%
Calculated for $C_{30}H_{48}O_3$	C, 78.9% ; H, 10.59%

IR(nujol) : ν_{max} at 3400 cm^{-1} (—OH group),
1685 cm^{-1} (—COOH group).

Fig.34

Treatment of methyl trichadenate A 83a, in ethylenediamine with lithium; isolation of trichadenic acid A 83b:

200 mg of methyl trichadenate was taken in 50 ml of dry ethylenediamine and 200 mg of lithium metal was added with stirring in an atmosphereof H_2 gas. Then the reaction mixture was refluxed for about 2 hrs. It was then cooled and solid NH_4Cl was added and then acidified with 6N HCl. After extraction with ether the organic layer was washed with water and then dried over anhydrous sodium sulphate. Evaporation of solvent afforded a compound which was crystallised from chloroform-methanol mixture. The crystals had m.p. 330—32° $[\alpha]_D$ +35° was identified as trichadenic acid A, when compared with an authentic specimen. Yield was 100 mg.

Analysis report:

Found,	C, 78.53% ; H, 10.96%
Calculated for $C_{30}H_{50}O_3$,	C, 78.55% ; H, 10.99%

IR(nujol): ν_{max} at 3440 cm^{-1} (—OH),
1690 cm^{-1} (—COOH).

Fig.36.

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PART — II

ACTION OF HYDROGEN PEROXIDE ON TRITERPENOID ALLYLIC ALCOHOLS

CHAPTER—I

A SHORT REVIEW ON SOME OXIDATIVE REACTIONS OF PENTACYCLIC TRITERPENOIDS

In Organic Chemistry oxidative reaction can not be well defined very easily like in inorganic chemistry i.e. loss of electrons or increase in oxidation number. While electrons are directly transferred in some organic oxidation reactions the mechanism of most of these reactions do not involve a direct electron transfer. While considering for oxidation number it is easy to apply in some cases. As for example the oxidation number of carbon in CH_4 is -4, in most cases attempts to apply the concept lead to fractional values or apparent absurdities. Thus carbon in propene has an oxidation number of - 2.67 and in butane of - 2.5, though organic chemists considered these two compounds as being in different oxidation states. An improvement could be made by assigning different oxidation states to different carbon atoms in a molecule, depending on what is bonded to them. As for example two carbon atoms in acetic acid are obviously in different oxidation states. Assumption would be required to explain the phenomena since the oxidation number of an atom in a molecule is assigned on the basis of the oxidation numbers of the atoms

attached to it. Then the Organic Chemists defined oxidation as the conversion of a functional group in a molecule from one category to a higher one. Most oxidation in Organic Chemistry involve a gain of oxygen or a loss of hydrogen.

We have carried out reactions of hydrogen peroxide, which is known as an oxidising agent, on triterpenoids. It has been found that triterpenoids undergo molecular rearrangements with very simple reagents. Some important rearrangements take place under various oxidative conditions e.g. with mercuric acetate, lead tetracetate, chromic acid, organic peracids, hydrogen peroxide etc. Photochemical Oxidation and autoxidation on triterpenoids have also been observed. The following is a brief review of different transformational reactions on triterpenoids with the help of different oxidative reagents.

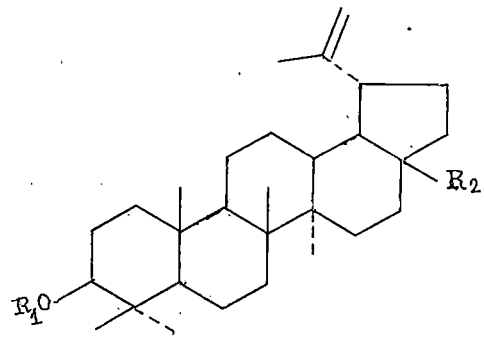
1. Mercuric acetate reaction in the lupane series[3,4]:

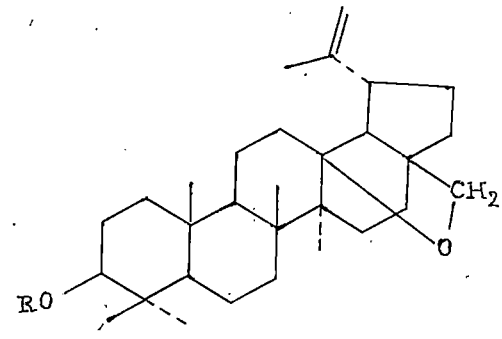
It has been found that mercuric acetate was used widely for the dehydrogenating agent for introducing an ethylenic double bond in conjugation with an existing double bond in the field of steroids.

Biedebach et al[1,2] first reported the Hg (II) acetate oxidation on pentacyclic triterpenes of lupane series.

Betulin and lupeol formed dehydro compounds of unknown structure. Since their esters also underwent dehydrogenation but not their dihydro derivatives, it was concluded that this dehydrogenation was associated with the presence of an olefinic bond.

Allison and coworkers[5] carried out mercuric acetate oxidation on betulin 1a and isolated a cylic ether 2a, IR ν max 1630, 886 cm^{-1} (Vinylidine group). NMR spectrum of the corresponding acetate 2b exhibited peaks at 63 and 89 cps ($-C-CH_2-O$). Hydrogenation of 2b followed by oxidation gave a lactone 3b, IR ν max 1770 cm^{-1} (γ -lactone).





1a $R_1 = H$, $R_2 = CH_2OH$

1b $R_1 = Ac$, $R_2 = COOH$

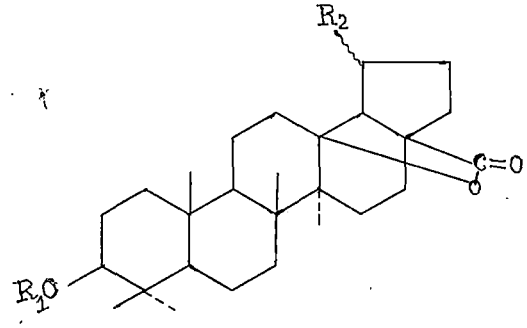
1c $R_1 = Ac$, $R_2 = COOCH_3$

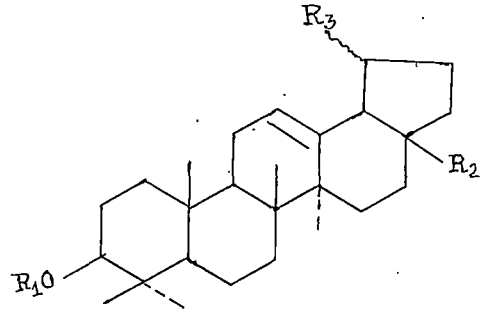
1d $R_1 = Ac$, $R_2 = CH_2OAc$

2a R = H

2b R = Ac

The authors advanced the following arguments in favour of their structure 3b for the lactone. The termination of the lactone could be at 13, 15, 19 or 21 position. Lithium aluminium hydride reduction product of 3b on acetylation produced a diacetate and not a triacetate, thus excluding positions 15 and 21 for the lactone termination and it was thought that one of the hydroxyl groups was tertiary in nature. The smooth dehydration of the diacetate with $POCl_3$ - pyridine confirmed the tertiary nature of the third hydroxyl group and the product obtained in this reaction was assigned structure 4e, λ max 208 nm (ϵ 7900), IR ν max 1650 and 3050 cm^{-1}. The decision





3a, R_1=Ac, R_2=isopropenyl
3b, R_1=Ac, R_2=isopropyl
3c, R_1=H, R_2=isopropenyl

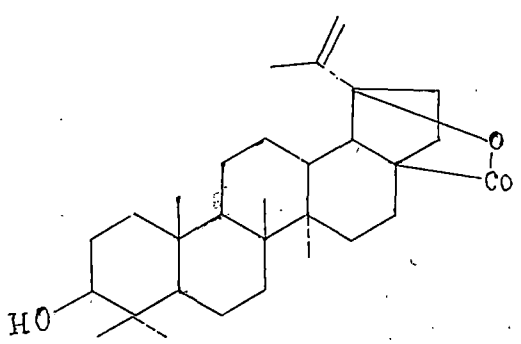
4a, R_1=Ac, R_2=CO_2Me, R_3=isopropenyl
4b, R_1=Ac, R_2=CO_2Me, R_3=isopropyl
4c, R_1=H, R_2=CO_2H, R_3=isopropyl
4d, R_1=Ac, R_2=CH_2OAc, R_3=isopropenyl
4e, R_1=Ac, R_2=CH_2OAc, R_3=isopropyl
4f, R_1=Ac, R_2=CH_3, R_3=isopropyl

regarding the lactone termination was made on the basis of experiments with betulinic acid and its methyl ester. Acetyl

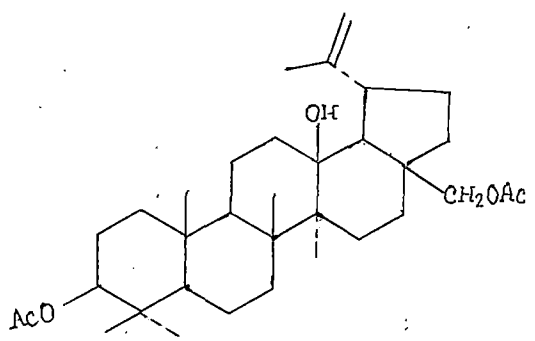
betulinic acid 1b with Hg (ic) acetate oxidation gave a γ-lactone assigned as 3a, IR ν max 1732 cm^{-1}, which on hydrogenation gave the same dihydrolactone 3b as that obtained from betulin. Consequently the lactone termination and the other linkage in 2b must be at the same point, either at C—13 or C—19. The lactone 3a was found to be different from thurberogenin which was assigned the C—17—O—19 lactone structure by Djerassi et al[6] in 1955. Nonidentity of thurberogenin with this lactone lead Allison and coworkers[5] to assign structure 3a (13 - 17 lactone) for the lactone.

It was found that when 3β-acetoxy methyl betulinate 1c on similar oxidation with Hg (ii) acetate gave an unconjugated diene λ max 208 nm (ϵ 7100), IR ν max 3078, 1634, 901 - (C = CH_2), 1730, 1250 cm^{-1} (—O.COCH_3) and was assigned structure 4a. The corresponding hydrogenated product 4b, λ max 205 nm (ϵ 5300), ν max 878 cm^{-1}, on hydrolysis with sodium ethoxide gave the acid 4c. The later on treatment with hydrogen chloride in chloroform gave the lactone 3c. Betulin diacetate 1d on Hg (ii) acetate oxidation gave a nonconjugated diene assigned as structure 4d, which was also prepared from the ester 4a and the lactone 3a. Reduction of the ester 4a with lithium aluminium hydride gave a diol which on acetylation gave the same dienyl acetate 4d. When the lactone 3a was reduced by lithium

aluminium hydride and then acetylation of the product gave the acetoxy alcohol 5. The acetoxy alcohol on dehydration gave the dienyl acetate 4d. Since 4d was formed by dehydration of the tertiary hydroxyl group at C—13 and after hydrogenation of it gave 4e, which showed no IR band for vinylidine group. But UV absorption of the compound showed a maxima at λ_{max} 206 nm (ϵ 7900), it was confirmed that the double bond was trisubstituted and should be placed at 12—13 position.

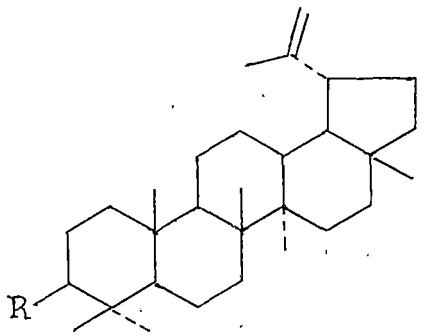


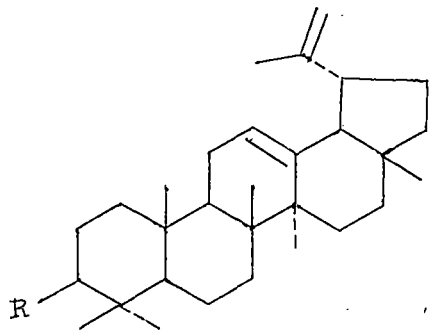
Thurberogenin



5

The authors also claimed that Hg (ic) acetate oxidation of lupeol 6a, lupenyl acetate 6b lupenyl benzoate 6c, lupenone 6d and α -lupene 6e under the same condition introduced a double bond at C, 12—13 position, the resultant

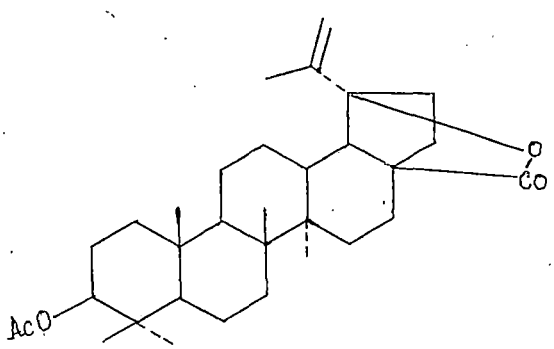




6a,	R = OH	7a,	R = OH
6b,	R = OAc	7b,	R = OAc
6c,	R = OBz	7c,	R = OBZ
6d,	R = O	7d,	R = O
6e,	R = H	7e,	R = H

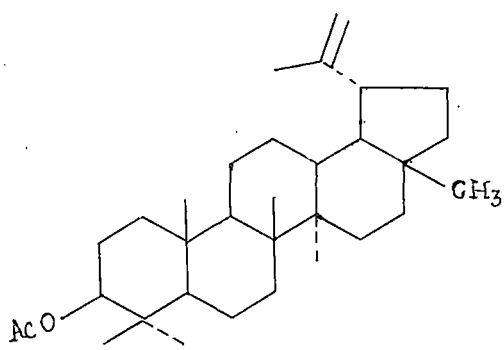
products being 7a, 7b, 7c, 7d and 7e respectively. The structure of the compound was evident from UV and perbenzoic acid titration.

Baddeley and his coworkers[7] proposed the structure of the lactone obtained from acetyl betulinic acid 1b would be like 8 and not as 3a.

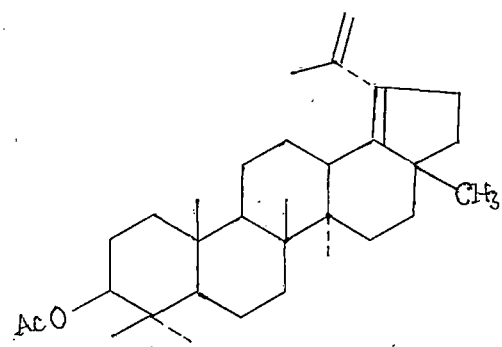


8

Almost simultaneously Baddeley et al[8] and Mclean and his coworkers[9] independently put forward spectroscopic and chemical evidences showing conclusively that in absence of any reactive substituent at C—17, mercuric acetate oxidation introduced a tetrasubstituted conjugated double bond at C—18, 19. Thus, 9 would produce 10 on mercuric acetate oxidation.

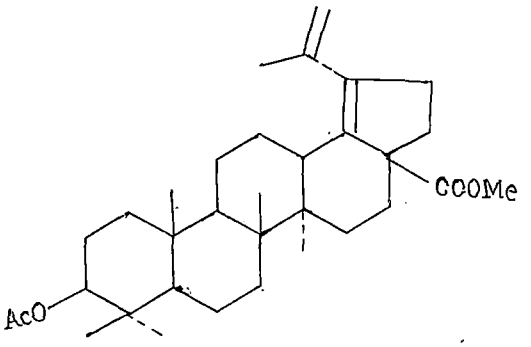


9

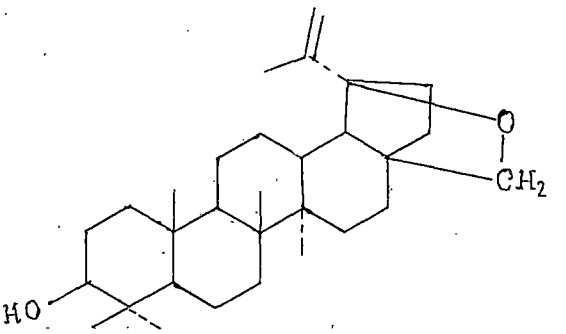


10

The structure of 10 was proved by the UV absorption of the compound for a conjugated diene system. It was again showed by Baddeley[10] the product of mercuric acetate oxidation on 1c, to be 11.



11



12

The structure of 11 was proved by its UV absorption for conjugated dienesystem and also by degradative evidence.

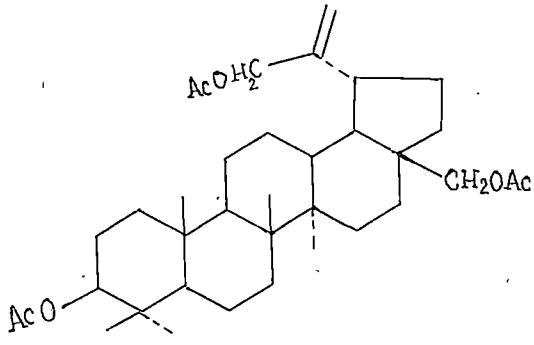
Vystrcil et al[11] have shown that the primary product of oxidation of betulin 1a with Hg (ic) acetate has got the structure 12 and not the 2a proposed earlier by Allison et al[5].

2. Lead tetraacetate reaction on some triterpenoids:

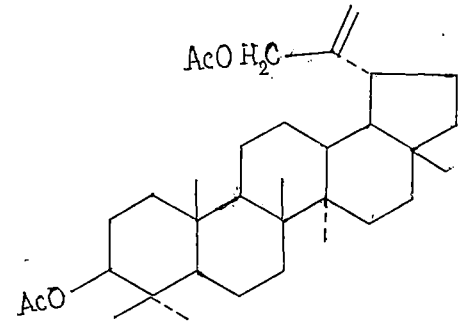
Lead tetraacetate was found to be an useful oxidising agent in the field of terpenes. It has been used in many important transformation of pentacyclic triterpenes.

While studying in the lupane series which contain an isopropenyl double bond has two allylic position at C—19 and C—30. It was expected that one of these two allylic

positions could be oxidised[12—15] by the reagent. It has been demonstrated[16] that in these compounds the C—30 position was so reactive that it reacted with lead tetraacetate in glacial acetic acid and the alternative C—19 position remained unaffected. Thus betulin diacetate 1d and lupeol acetate 6b gave products showing that only the C—30 position was attacked and the compounds 13 and 14 were isolated.

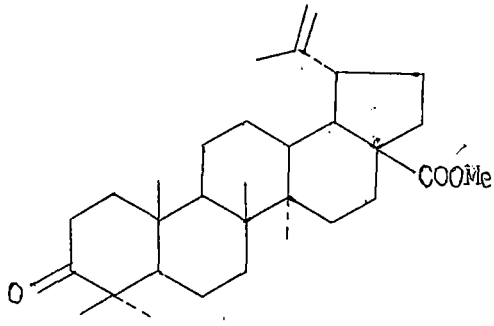


13

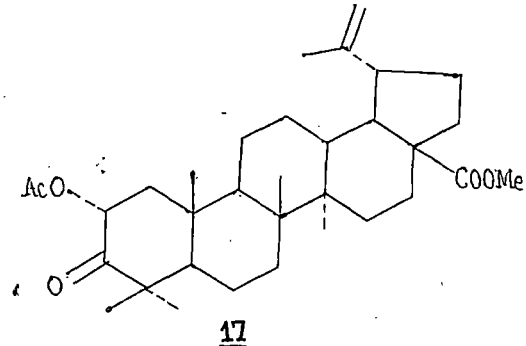


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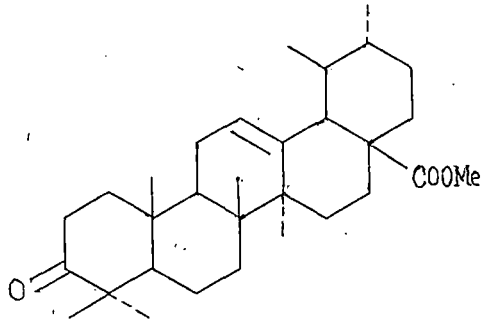
It was found that if the C—3 position of the triterpene occupied by a Keto functional group instead of a hydroxyl or acetate functional group the attack of acetoxy was found solely at C—2 position, not at C—30 position. When methyl betulonate 15 and methyl ursonate 16 was reacted with lead tetraacetate it was found that the corresponding 2∝-acetoxy 3-keto compounds[17] were obtained which were assigned as 17 and 18.



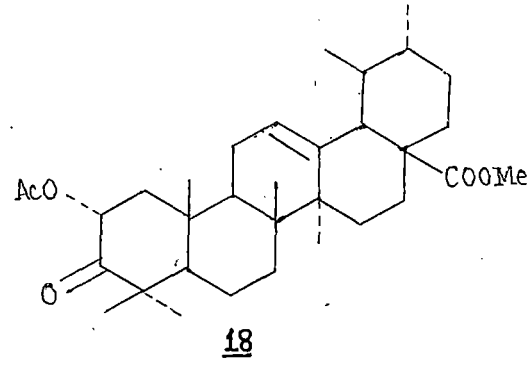
15



17



16

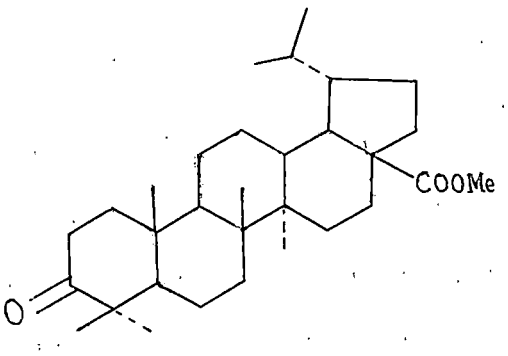


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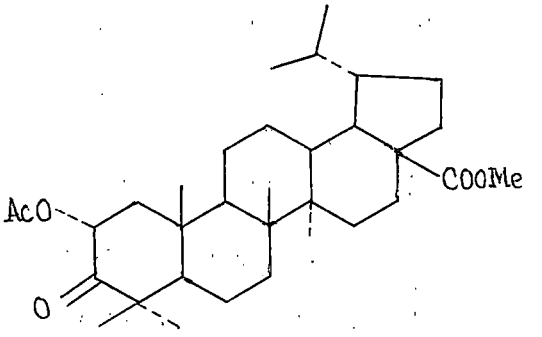
It was thought that the introduction of acetoxy group at C—2 position instead of C—30 was based on the fact that the α-hydrogens at C—2 position was much more reactive than the allylic hydrogens at C—19 or C—30 position due to the presence of a keto group at C—3 position.

It was also found that methyl dihydroalphitolate could be synthesised starting from betulinic acid[18].

Introduction of acetoxy group at the C—2, α-position to 3-oxo group of methyl-3-oxo-dihydrobetulonate 19, could be done very easily by lead tetraacetate[19] to form 20.



19

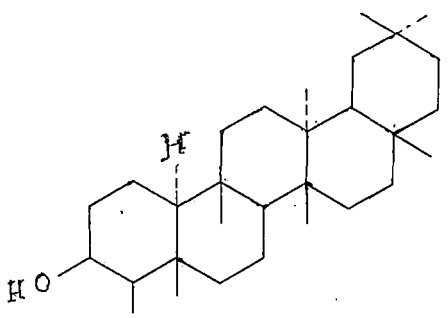


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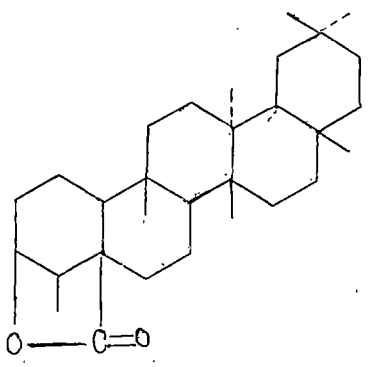
It was considered that in this reaction there was the formation of enol[20]. This enol was expected to give the desired product when attacked from less hindered α-side of the molecule. Under mild condition[21] in which boron trifluoride catalyst was used, an acetoxy ketone was obtained.

So, lead tetraacetate is a very useful reagent for the introduction of 2 α-oxygen function in 3 keto pentacyclic triterpenes.

Fukuda and coworkers[22] observed the action of lead tetraacetate in the presence of iodine and calcium carbonate under irradiation on 3β -friedelanol 21 produced a γ -lactone 22. The product was established by different physical methods.



21



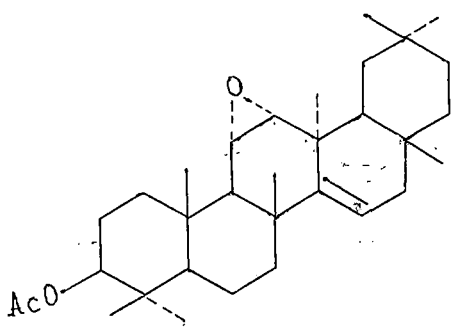
22

3. Oxidation of chromic acid on pentacyclic triterpene:

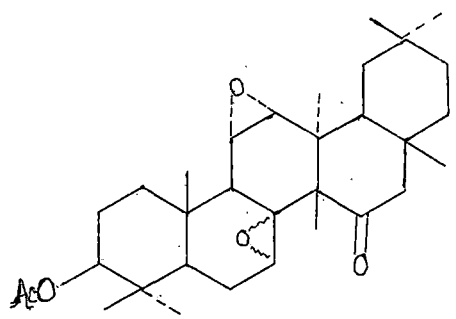
Chromic acid has got very good oxidising property in organic chemistry. With the help of this reagent various types of conversion occur among the pentacyclic triterpenes.

Conversion of epoxytaraxerol[23] to the multiflorenol series involved the oxidative rearrangement of the acetate 23 using chromic acid in the presence of a strong acid. These were conditions which in general seem to be less favourable to over-all allylic oxidation to a conjugated

enone and relatively more favourable to the formation of epoxides, glycols, or their further transformational products[24,25]. The product of the oxidation of the acetate 23 was dioxidoketo acetate $C_{32}H_{48}O_5$, the structure of which was assigned as 24. The structure of 24 was established on the basis of physical and chemical evidences.

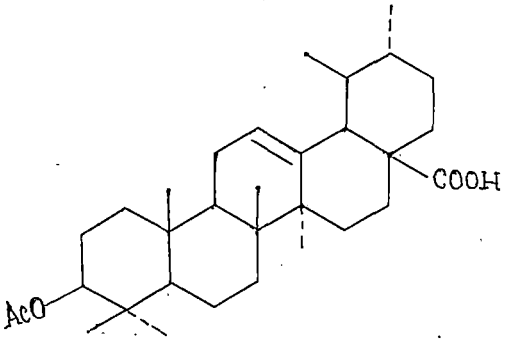


23

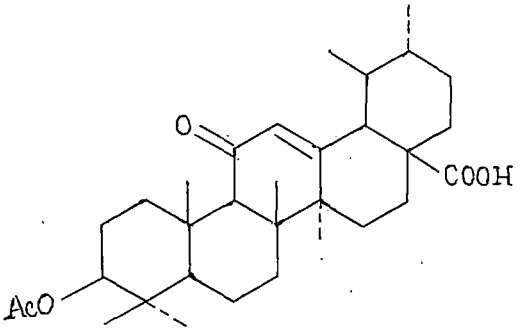


24

Spring and coworkers[26,27,28] have observed that when ursolic acid acetate 25 was treated with chromic acid in refluxing 97% acetic acid then introduction of 11-keto compound i.e. 3 β -acetoxy-11-oxo ursolic acid 26 was obtained. The compound has got a clear UV absorption in the region 228 nm for the presence of a α-β unsaturated ketone.

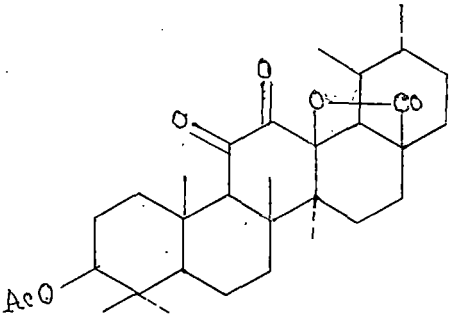


25



26

The other product of the reaction was too much interesting for the lactone with a di-one system in the molecule. The compound was formulated as 3 β -acetoxy-11, 12-dioxo ursan-28-oic—13-18-lactone 27. The structure was proved by chemical and physical evidences.

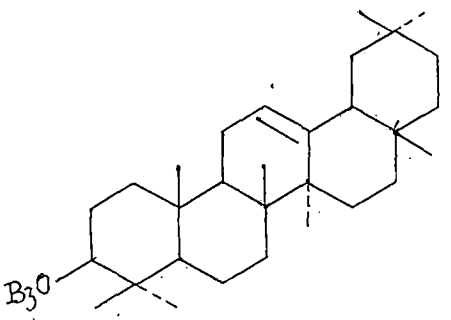


27

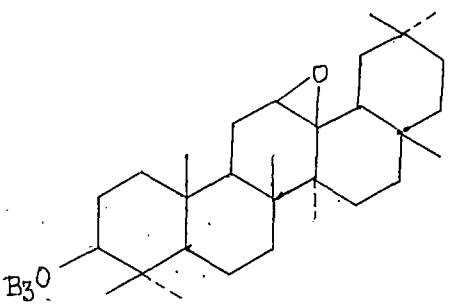
4. Reaction of triterpenoids with organic peracids:

Several transformation reactions were done with the help of organic peracids like peracetic acid, perbenzoic acid and m-chloro perbenzoic acid on the pentacyclic triterpenes. The products obtained in different cases were found to be interesting in all respects.

Treatment of β-amyrin benzoate 28 with perbenzoic acid in chloroform at 0° afforded epoxy compound[29]. The compound so obtained was assigned as 3β-benzoyloxy-12β-13-epoxy oleanane 29.



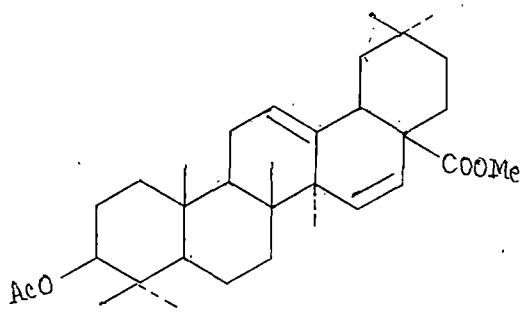
28



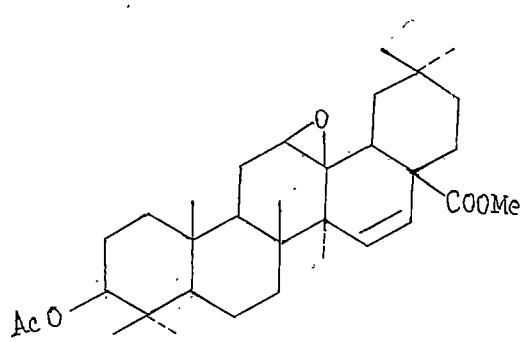
29

Barton and his coworkers[30] observed that when methyl 3β-acetoxy olean-12,15-dien-28-oate 30 was treated with perbenzoic acid (1.0 mol eq.) it formed an epoxy compound.

The compound so obtained was formulated as methyl 3β-acetyl-12β,18-epoxyolean-15-en-28-oate 31.



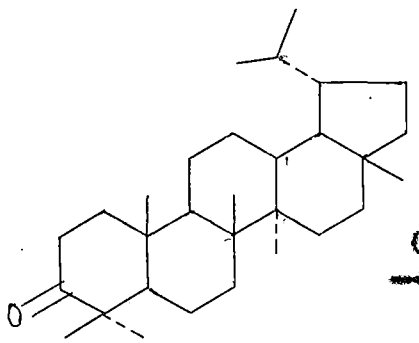
30



31

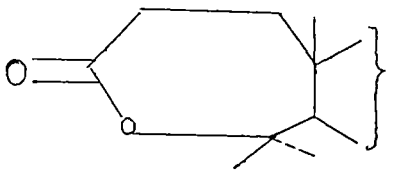
Another example of reaction of perbenzoic acid was the conversion of lupanone 32 to isodihydrocamaric acid 33. The product 33 was obtained[31] when lupanone 32 was treated with m-chloroperbenzoic acid in chloroform followed by alkaline hydrolysis of the product 4-oxe-A-homo-lupan-3-one 34 whereby ring A cleaved to yield 3,4-seco-lupan-4-ol-3-oic acid 35 which dehydrated with 10% sulphuric acid - acetic acid to give 33. So using this route (Scheme—1) synthesis of 3,4-seco acids could be done.

Scheme—1



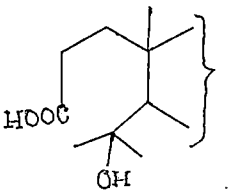
32

$Cl.C_6H_5CO_3H$ / $CHCl_3$



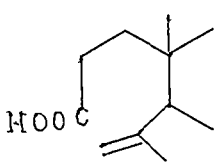
34

Alkaline hydrolysis



35

10% H_2SO_4-AcOH

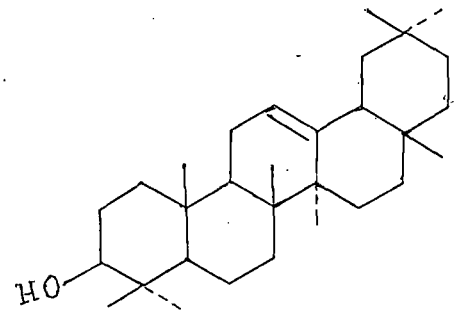


33

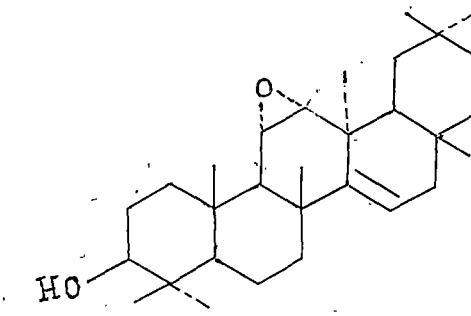
5. Action of hydrogen peroxide on pentacyclic triterpenes:

Hydrogen peroxide was found to be a very interesting oxidising reagent in the field of triterpenoids which formed different types of rearrangement products.

While studying the photooxidating reaction on α and β amyrins Corey et al[23] observed that Olean-12-en-3 ol 36 produced epoxy taraxerol 37.



36



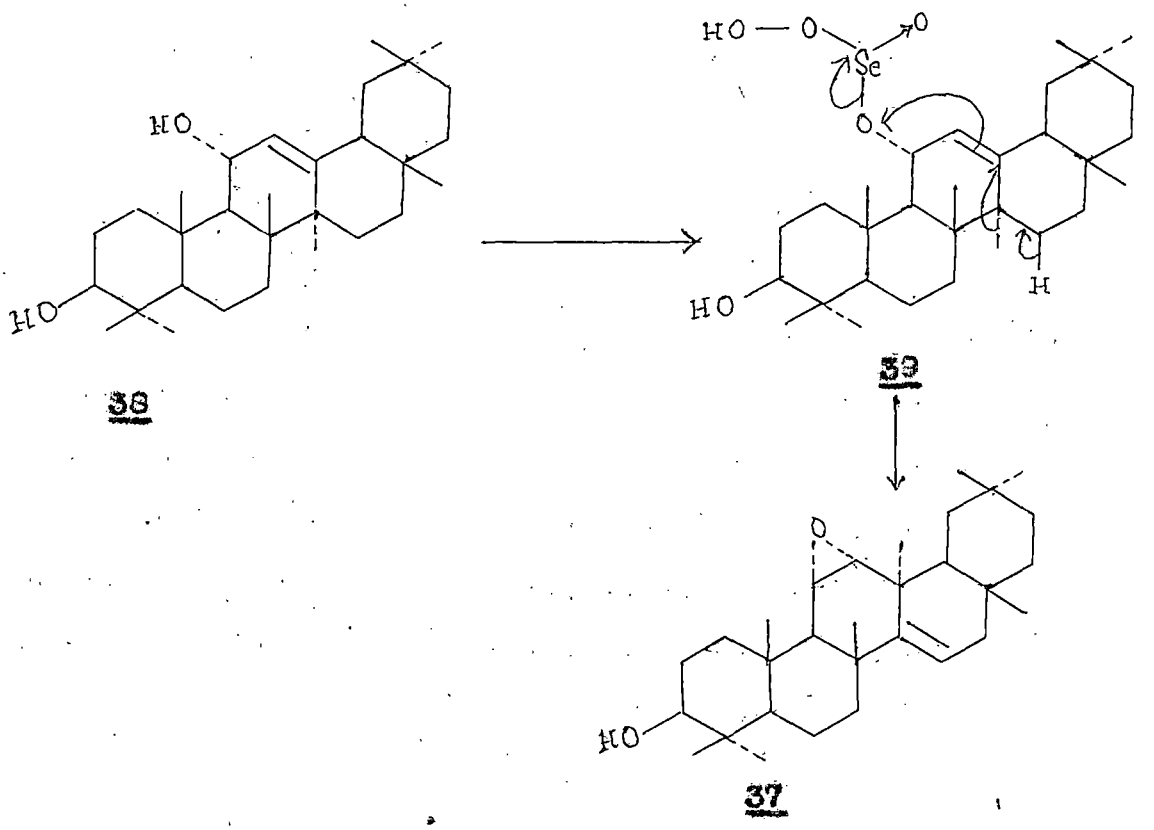
37

The structure of the epoxy taraxerol was established by the authors by different chemical and physical evidences. They observed that there was a skeletal change from more stable β-amyrin to taraxerene system. In the conversion of 36 to 37a therefore, there was an intrinsic driving force which would overcome the energetically

unfavourable change in the arrangement of carbon and hydrogen and the skeletal rearrangement could be considered as powered by the oxidation. The former could be effected by mere application of a suitable catalytic agent. While studying in β -amyrin series they obtained the same epoxy compound.

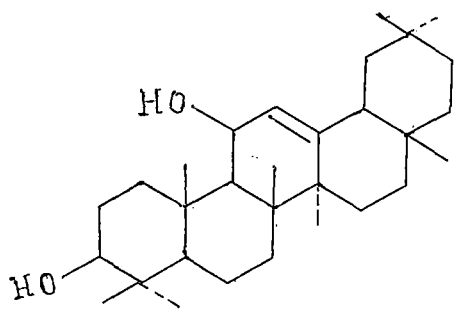
While studying individually with α -amyrin series for the synthesis of 37 , the first successful experiment involved the oxidation of olean-12-en-3 β ,11 α -diol 38 with a mixture of hydrogen peroxide and selenous acid in tert. butyl alcohol. This experiment produced 37 in 50—60% yield. The pathway which was first considered was the formation of the intermediate 39 as shown in Scheme—2.

Scheme—2



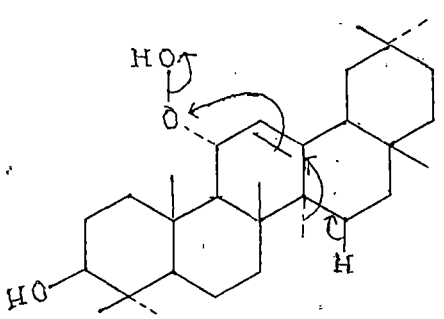
38 39 37

However, this was shown not to be the actual mechanism of the formation of 37 from 3β, 11α-diol 38 from the fact that the reaction of the 11-epimeric olean-12-en-3β, 11β-diol 40 with the hydrogen peroxide and selenous acid reagent also afforded the same epoxide 37 and not the epimeric epoxide.



40

So, evidently the C-11-O bond was broken during the reaction. They suggested an alternative mechanism in which the isomeric diols 38 and 40 yield the same C-11, 12, 13 allylic cation which reacts with the peroxide to form olean-12-en-3β-ol, 11α-hydroperoxide 41.



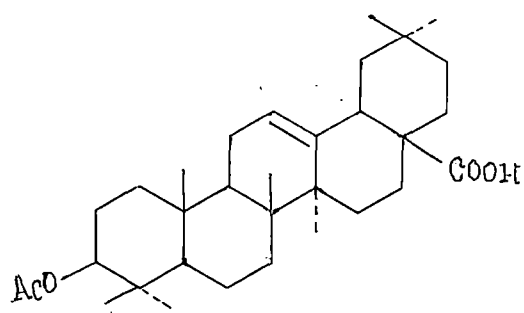
41

This hydroperoxide 41 in turn would undergo acid catalysed O—O fission and carbon rearrangement to give 37.

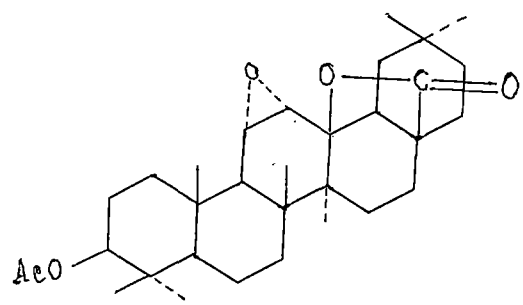
As expected from the above-mentioned fact it was found selenous acid merely functioned as an acid catalyst and the reaction could be done in presence of other acids like p-toluene sulfonic acid.

The reaction was extended to α -amyrin analogue ursan-12-en-3β , 11α-diol and observed similar type of products being formed.

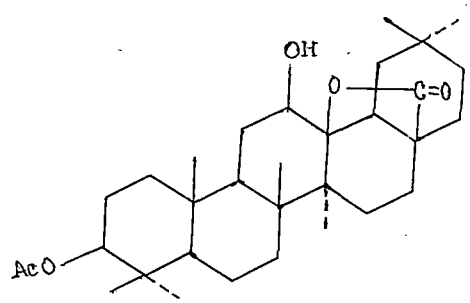
Majumder and coworkers[32] reported that when oleanolic acid acetate 42, was treated with H_2O_2 in presence of boiling acetic acid and epoxy γ -lactone 43 and 12-hydroxy-γ -lactone 44 was formed.



42

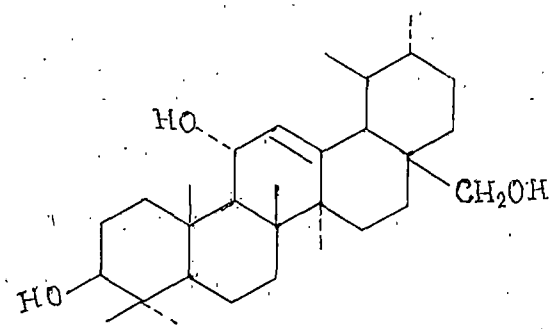


43

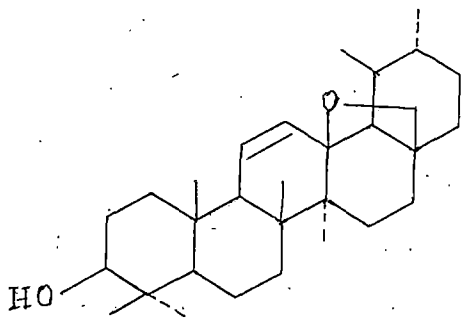


44

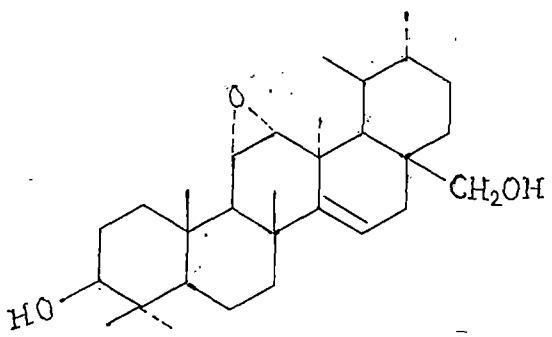
The similar type of reaction also observed in the case of ursane skeleton. It was observed that when 45 was treated with H_2O_2/ KOH, 46 and 47 were formed.



45



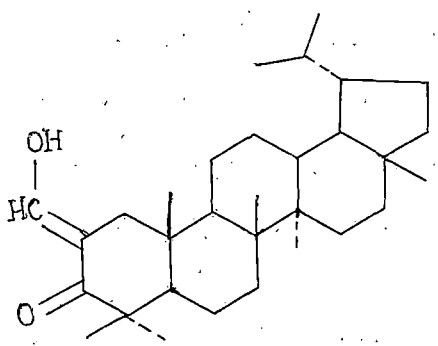
46



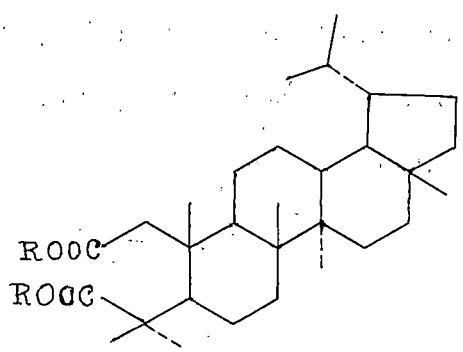
47

Another example of interest was the action of hydrogen peroxide on triterpenoids containing a hydroxymethylene ketone function. It has been observed that in absence of $\beta\gamma$-unsaturation and especially in alkaline media[33,34] oxidative

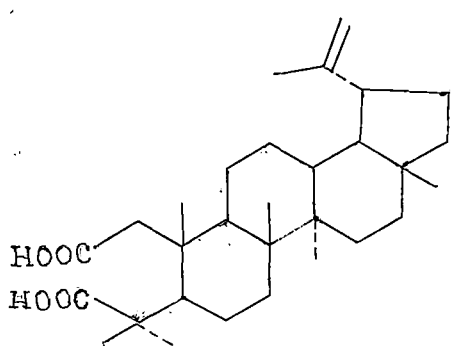
oleavage occurred producing 2,3-secodiacids. Thus the ketone 48 gave exclusively the diacid[35] 48a, characterised as the ester 48b which was also obtained from 48 by catalytic hydrogenation[36].



48



48a R = H
48b R = CH_3

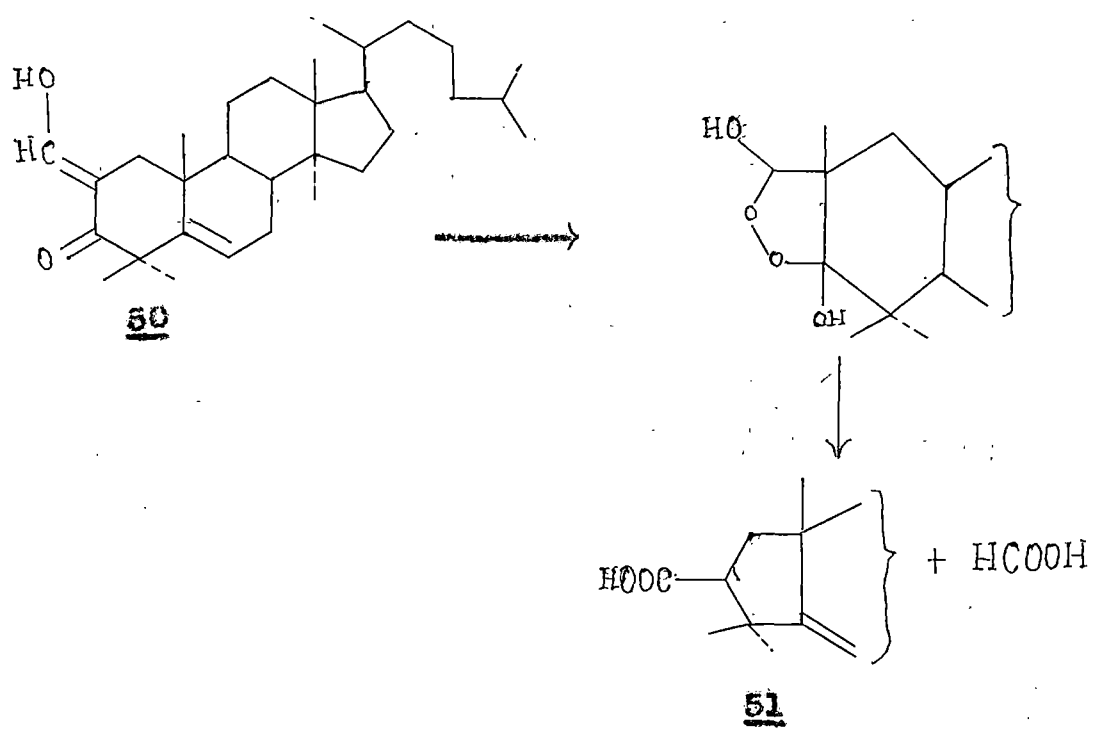


49

It has been found that the ring contraction occurred in the presence of a β-γ-unsaturation. Thus hydroxymethylene

anhydro dihydro litsemantone 50 would undergo rearrangement[37] in presence of hydrogen peroxide in alkaline media producing 51 as shown in Scheme—3.

Scheme—3

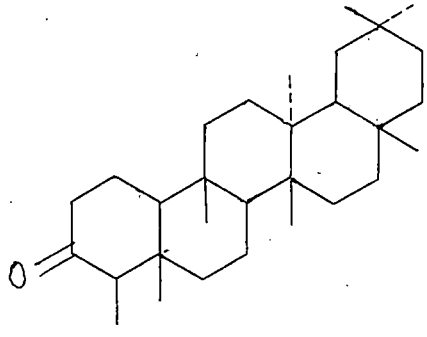


50

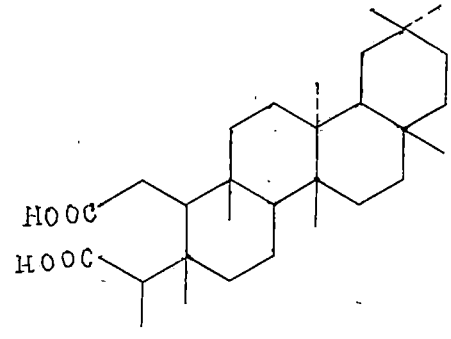
51

Pradhan et al[38] observed that when friedelin 52 was treated with hydrogen peroxide and selenium dioxide in

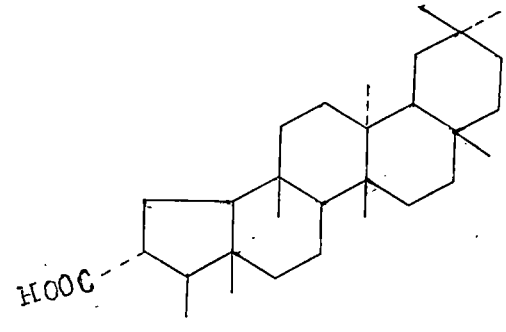
tertiary butanol, a number of products were formed which were assigned structures 53, 54, 55.



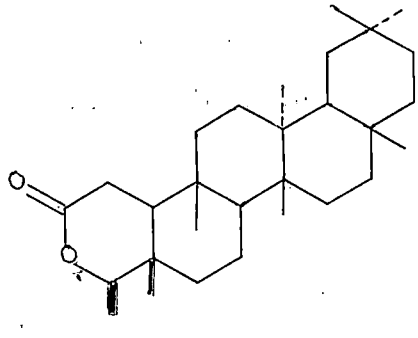
52



53



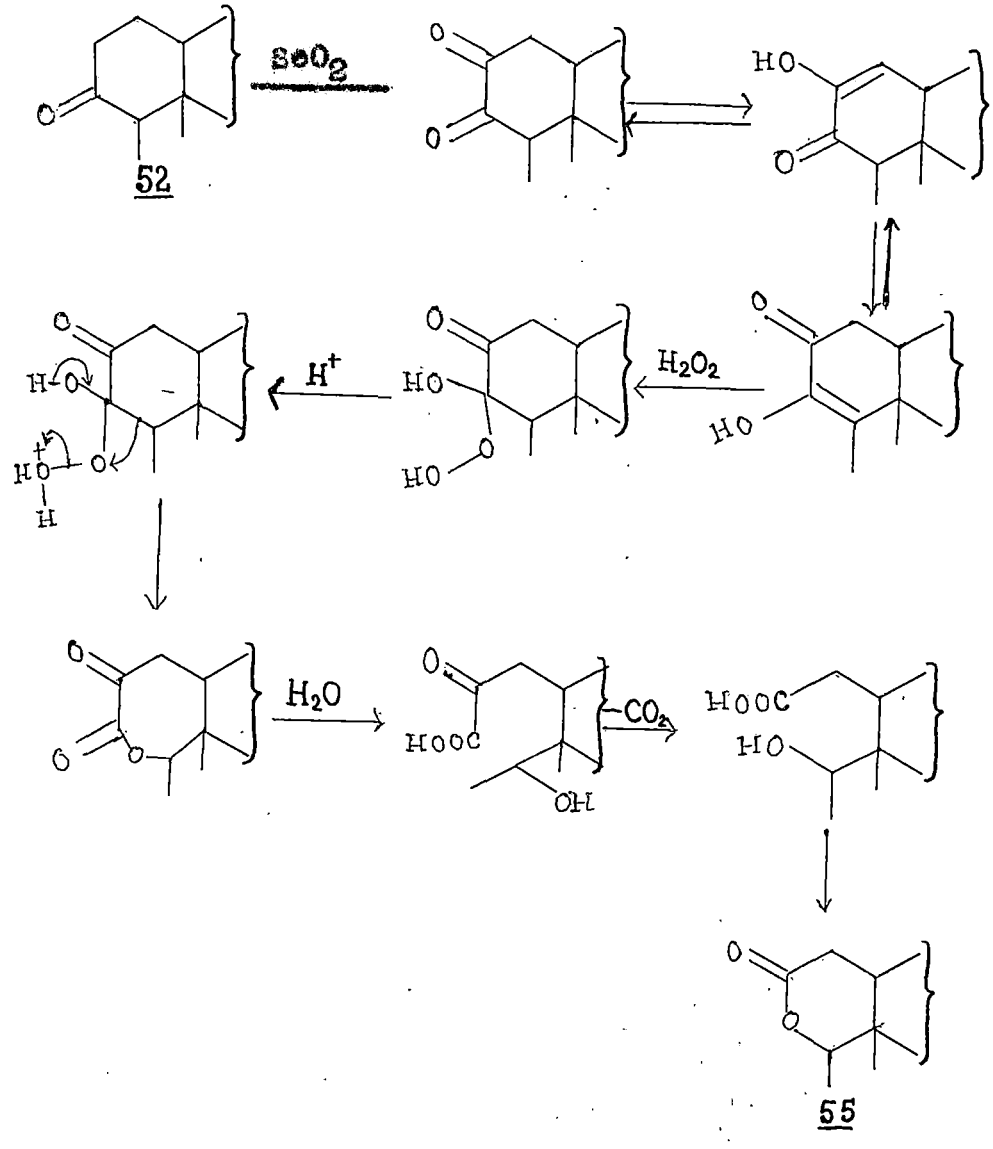
54



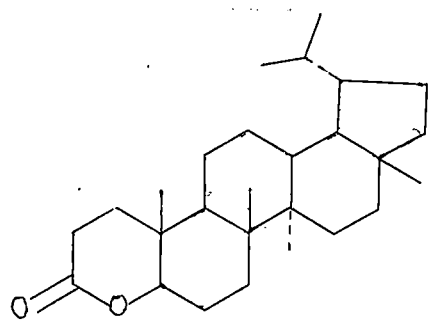
55

The products were characterised by making different derivatives and from the physical evidences. The interesting product 55 was found to be lactone. The mechanism of formation of the lactone was summerised in Scheme—4 as follows:

Scheme—4



The authors[39] also did the reaction on lupanone 56 and isolated the lactone 57.

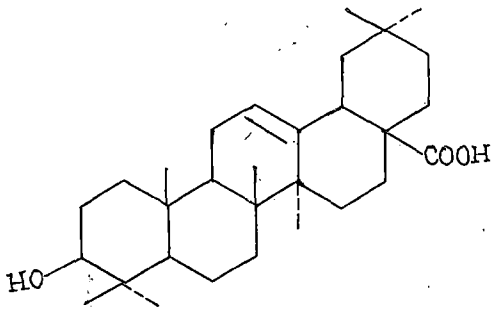


57

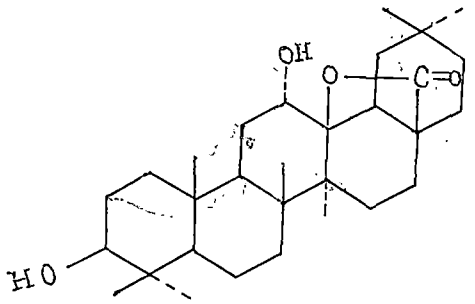
6. Photochemical oxidation on triterpenoids:

I. Kitigawa, K. Kitazawa and I. Yosioka[40] while working on biogenatic type photochemical conversion of plant observed that when oleanolic acid 58 in ethanolic solution was irradiated for 80 hours with occasional shaking afforded two lactones-one was 12α-hydroxy oleanolic lactone 59 while the other was the epoxy lactone which was assigned structure 60.

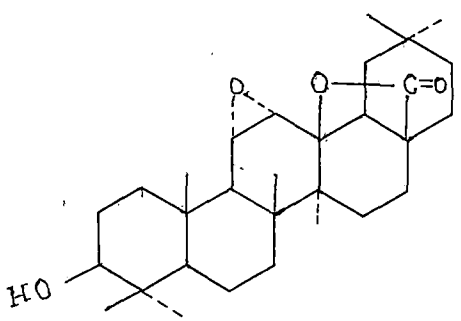
The structure of the compounds was established by the chemical and physical evidences.



58

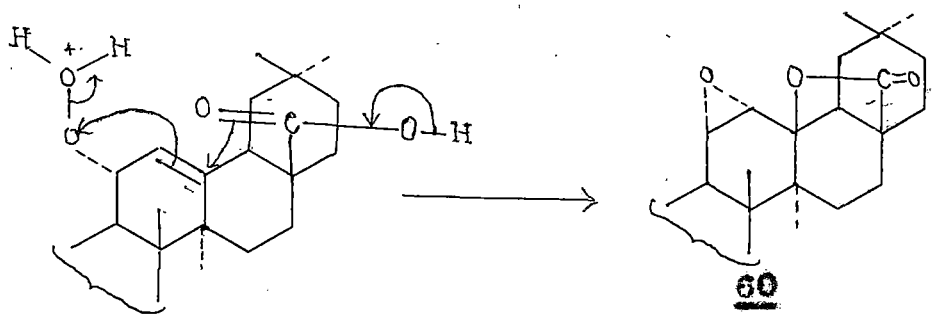


59



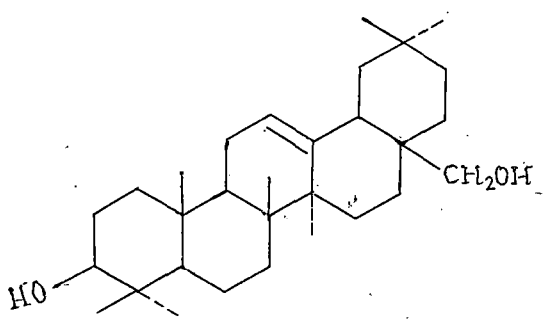
60

The mechanism of formation of the epoxy lactone was given following the Corey's[28] path as follows:

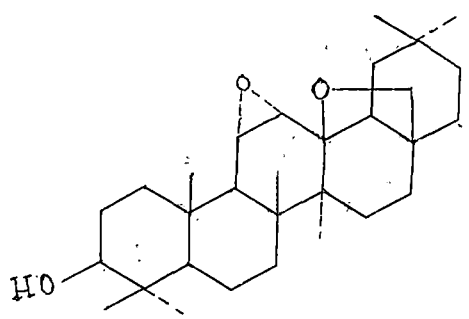


60

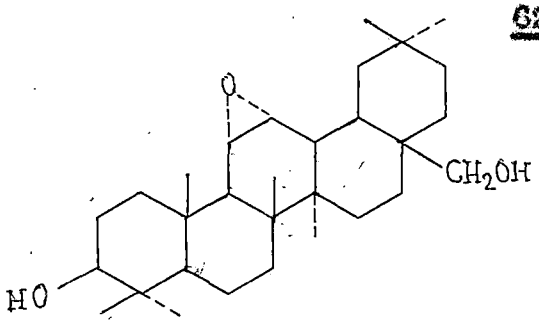
The reaction was extended in the case of erythrodiol 61 which possessed a 17β -CH_2OH function in place of 17β -COOH found in oleanolic acid 58. Here also two compounds 62 and 63 were isolated. One was an epoxy ether type of product 62 while the other product was characterised as the epoxy compound 63.



61

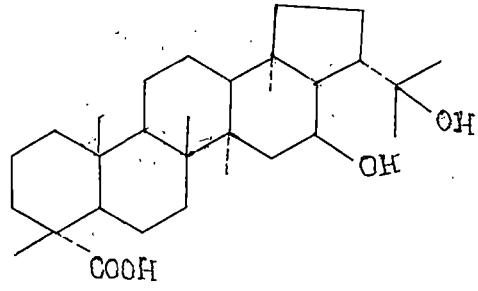


62

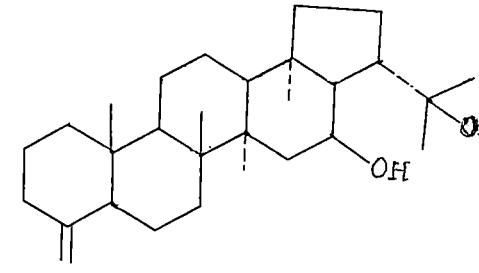


63

In a parallel examination[41] it was found that the introduction of a terminal methylene group could be done with the photooxidation method. It was observed that when leucotylic acid 64 was irradiated by mercury lamp at low pressure for 83 hours it yielded two products. The structure of the first compound was assigned as 65 where there was an introduction of terminal methylene at C—4 position which appeared as a biogenatic precursory system.

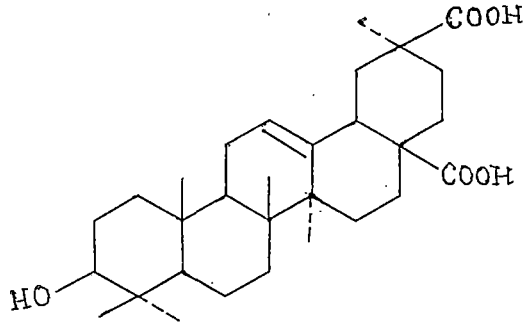


64

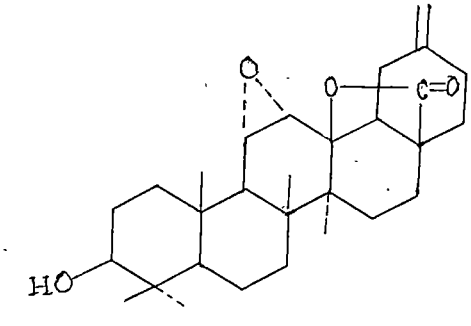


65

Taking this idea they have prepared eupteleogenin 66 from spergulagenic acid 67.



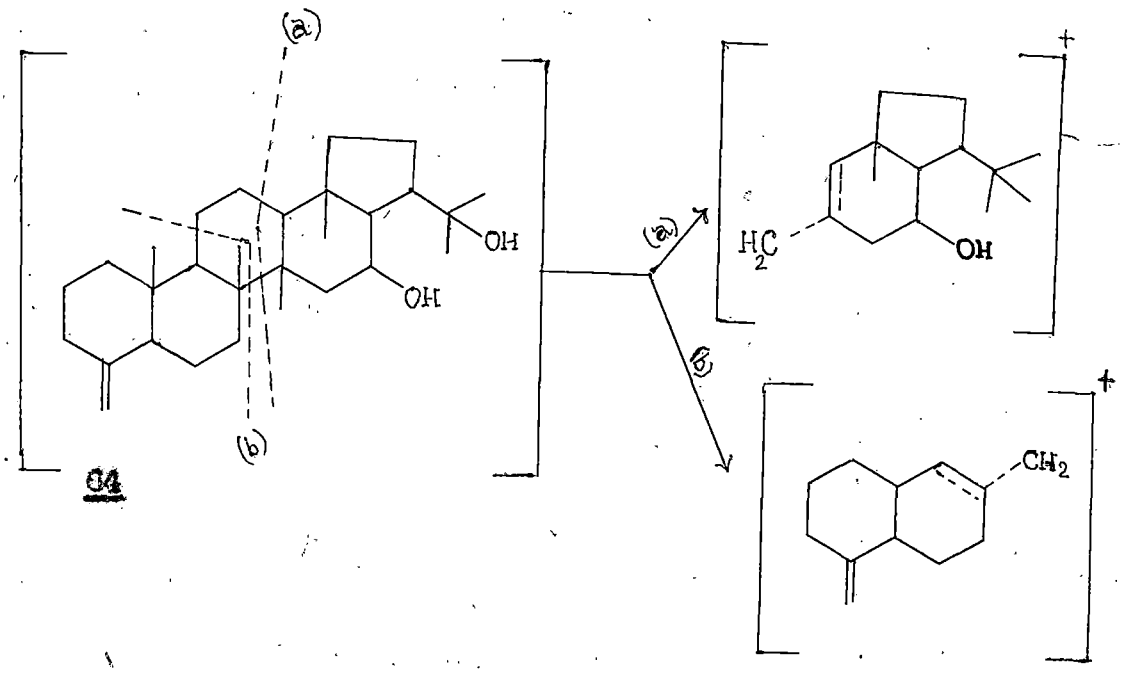
66



67

The structure of 64 was confirmed by its mass fragmentation pattern.

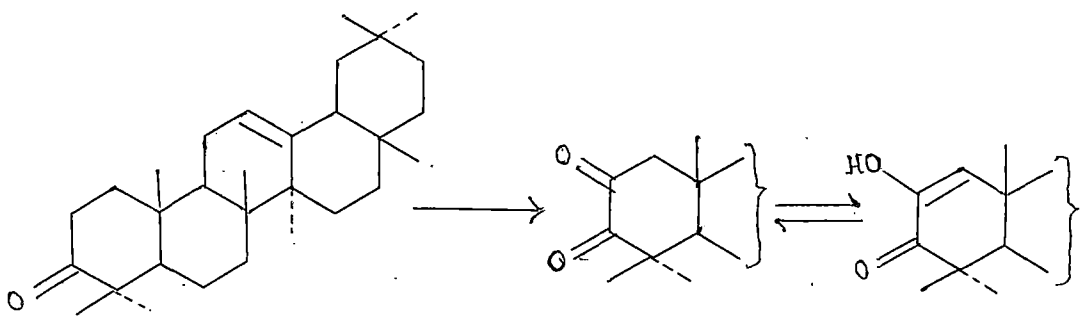
Scheme—6



7. Autoxidation on triterpenoids:

Khastgir and coworkers[42] studied autoxidation in ring A of β-amyrone. When β-amyrone 68 was oxidised by passing oxygen through its suspension in tertiary butyl alcohol containing potassium tertiary butoxide gave dioxphenol 69a and 69b. The structure of the compound was established by its physical and chemical evidences.

Scheme—7



68 69a 69b

CHAPTER—II

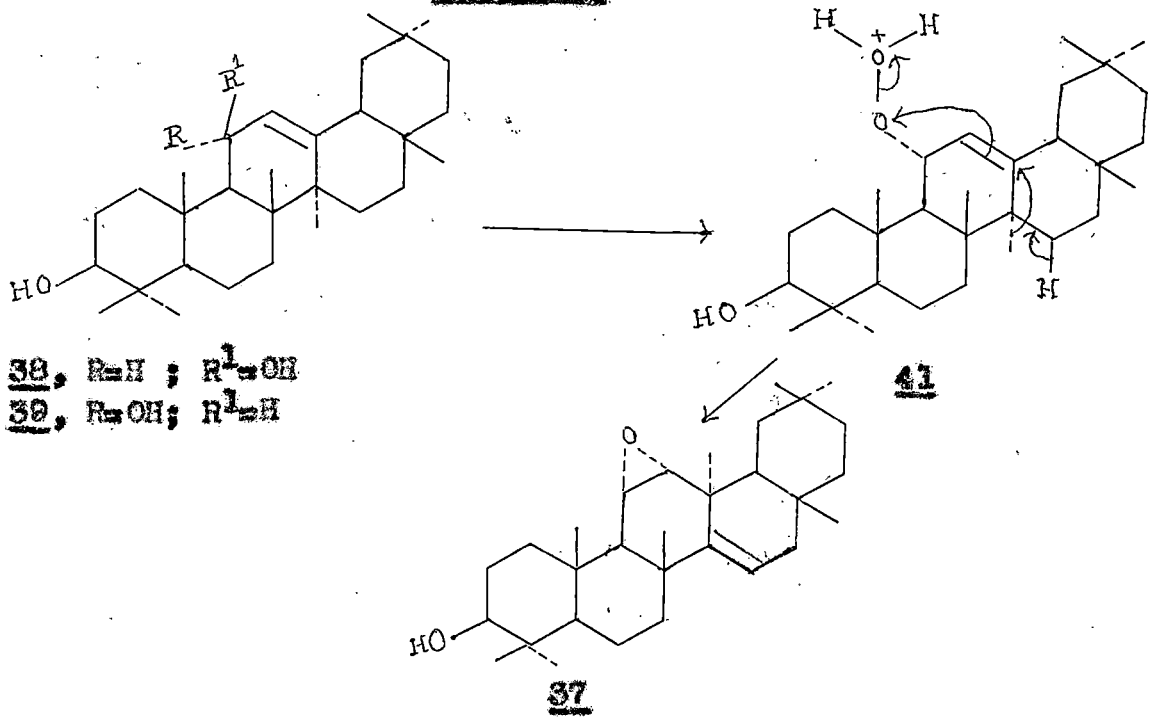
RESULTS AND DISCUSSION

SECTION—A:

Action of hydrogen peroxide on olean-12,15-dien-3,11-diol:

In order to synthesize partially some derivatives of triterpenoids like 16-hydroxy taraxerol 75 and multiflorenol derivative 74 from taraxeryl acetate we followed the methods of Corey et al[23]. They synthesized epoxy taraxerol 37, from both the olean-12-en-3β, 11β-diol 38 and olean-12-en-3β-11α-diol 40, by treatment with a mixture of hydrogen peroxide and selenious acid in t-butyl alcohol. They got the same epoxide from both the diol and not the epimeric one. This fact suggested that the formation of same epoxide 37 from the C—11 isomeric diols must proceed by C—11—O bond cleavage with the formation of the same c, 11,12,13 allylic cation which then formed the hydroperoxide 41, which can be shown in Scheme—8. The hydroperoxide 41

Scheme—8



38, R=H ; R^1=OH
39, R=OH; R^1=H

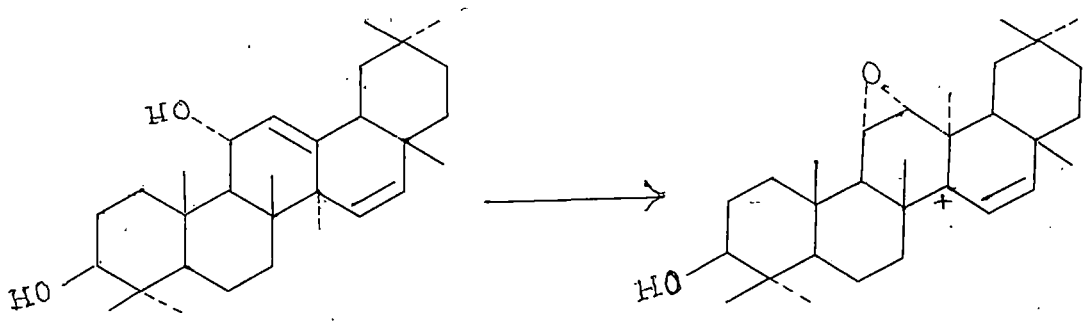
41

37

in turn undergoes O—O fission and carbon rearrangement to afford 37. They have reported that this type of rearrangement occurred in presence of para toluenesulphonic acid also.

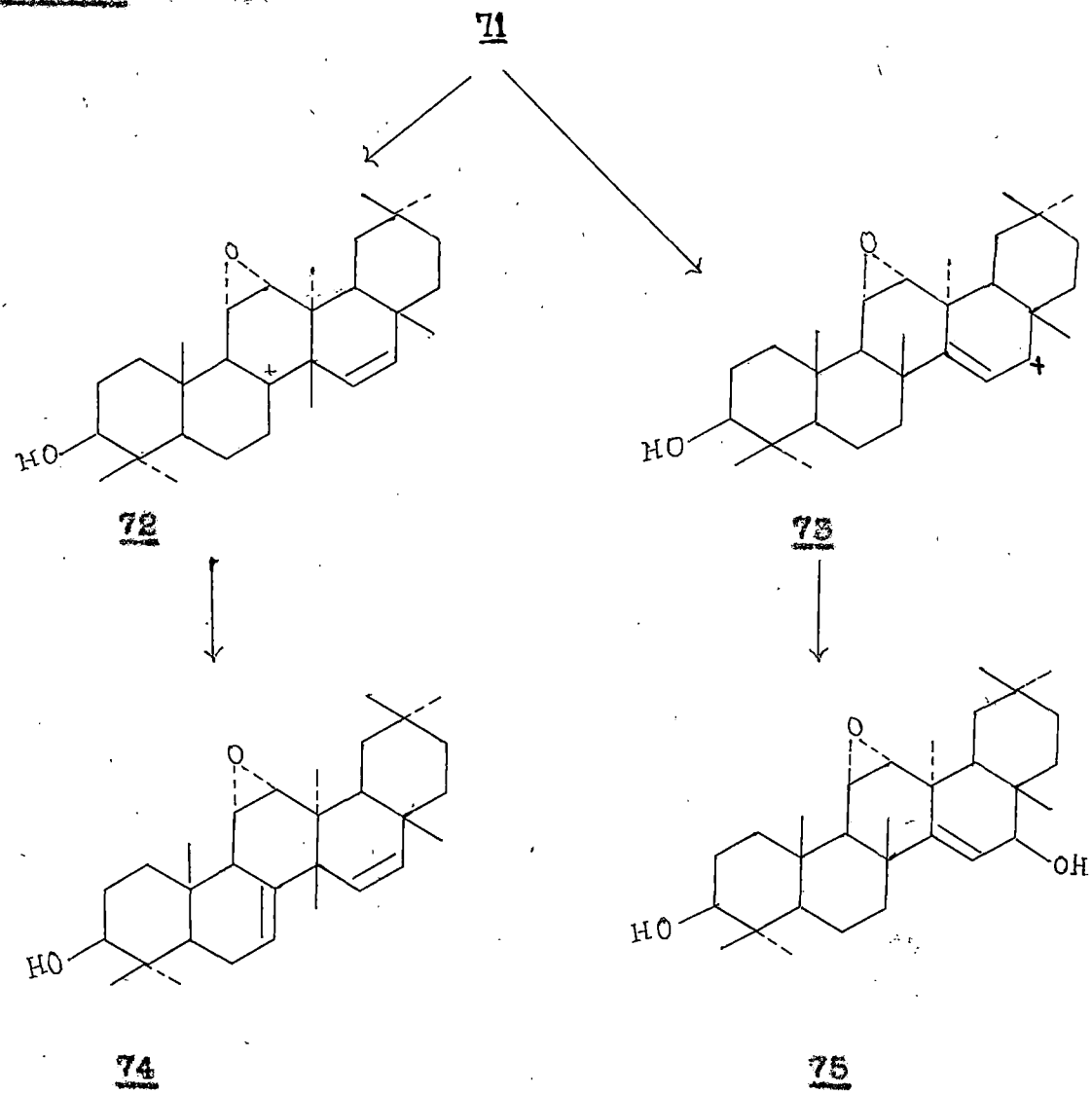
We assumed that this type of reaction could also be carried out on olean-12,15-dien-3,11-diol 70, which could be synthesized from taraxeryl acetate that will be discussed later on. The compound 70 would produce the epoxide with the allylic cation 71. The intermediate would further isomerize to produce the allylic cation 73, by the migration of C-15-16 double bond. The cation would undergo neucleophillic attack by OH^- ion to produce a 16-hydroxy-taraxerol derivative 75. In the other way the cation 71 would isomerize by the migration of C—8 methyl group to C—14 position to produce another cation 72 which was expected to elliminate a C—7 proton to give a multiflorenol derivative 74. The Scheme is given below (Scheme—9):

Scheme—9



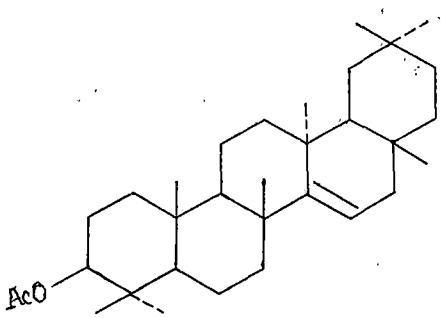
70 71

Scheme—9(contd.)

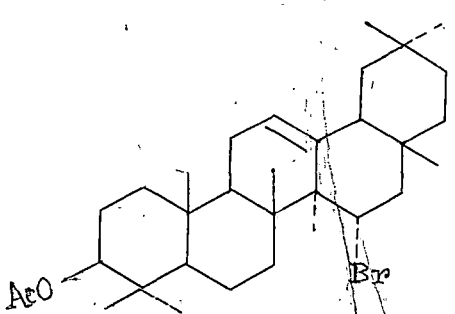


We have planned the scheme like above but when we carried out the reaction, the product which we actually got were quite different from the assumed ones, which are discussed in this section. First, the preparation of olean, 12,15-dien-3,11-diol 70, from taraxeryl acetate 76 and then the reaction of hydrogen peroxide in presence of para toluene-sulphonic acid on 70 is discussed.

When taraxeryl acetate 76 was treated with N-bromo-succinimide in presence of dimethylsulfoxide in a chloroform solution in dark for 12 hours, it furnished a solid which after crystallization gave a solid 77, $C_{32}H_{51}O_2Br$, m.p. 180—82°, $[\alpha]_D$ +47.4°. No UV absorption was observed for the compound in the region 220—350 nm. IR spectrum (Fig.1) of the compound showed two peaks at 1720 and 1250 cm^{-1} that revealed the compound contained one acetate functional group. PMR spectrum (Fig.2) of the compound showed that there was multiplet at 5.3 ppm for only one proton for $>C{=}C<_{H}$. Another multiplet centred at 4.53 ppm was due to one proton in H—C—O—COCH_3. The compound gave a multiplet at the region 4.3 ppm for one proton of H—C—Br. The singlet at 2.09 ppm was due to the three protons of —O—CO—CH_3. On the basis of IR and NMR spectrum, the structure 77 was assigned for the bromo compound. The structure



76



77

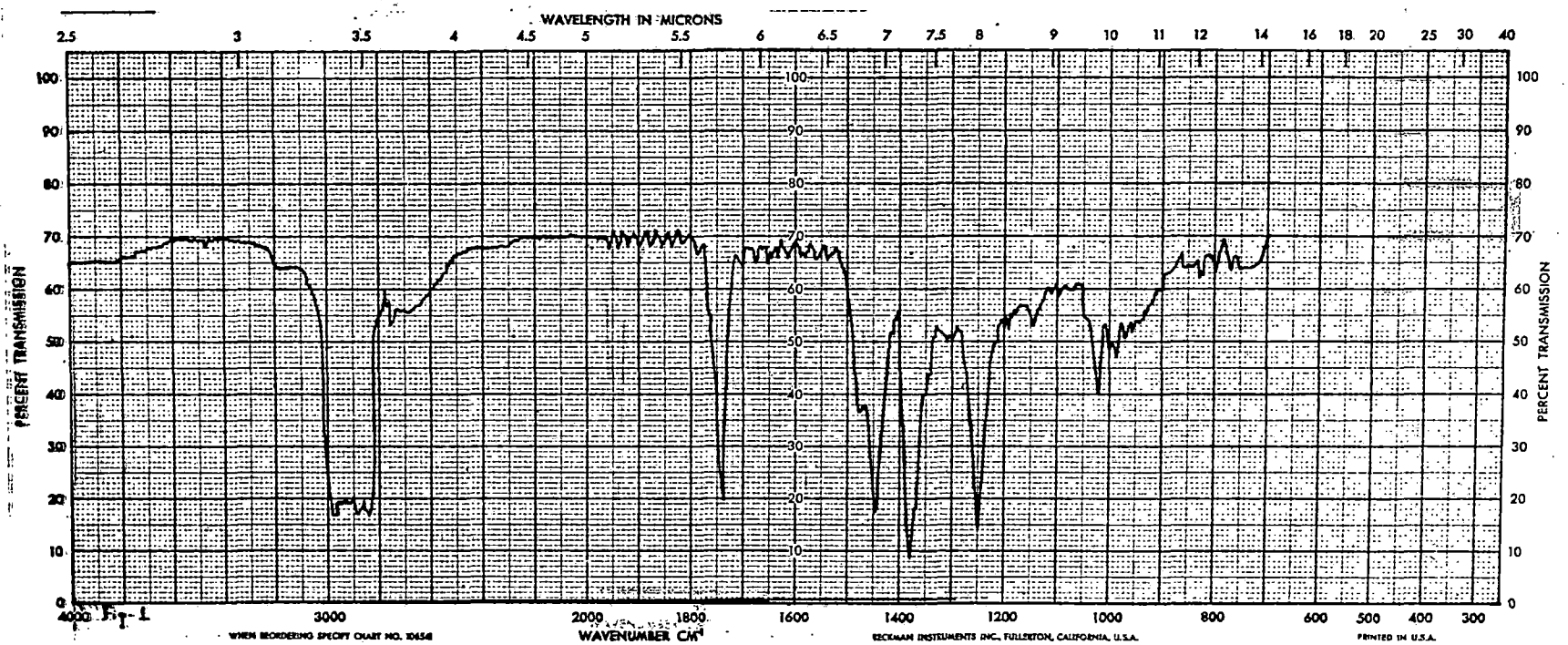


Fig-1

Fig.1: IR spectrum of 15-bromo-β-amyrin acetate 77.

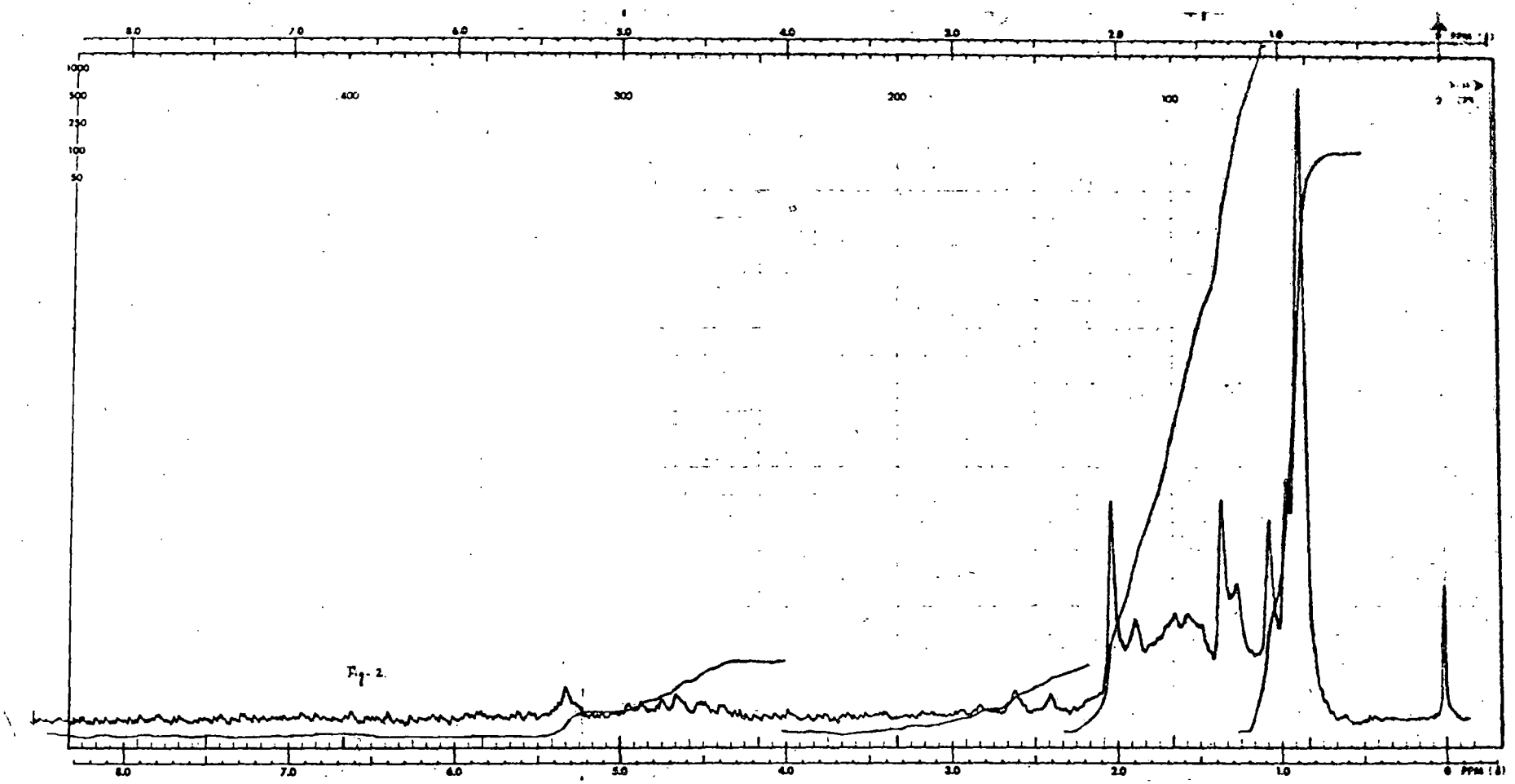
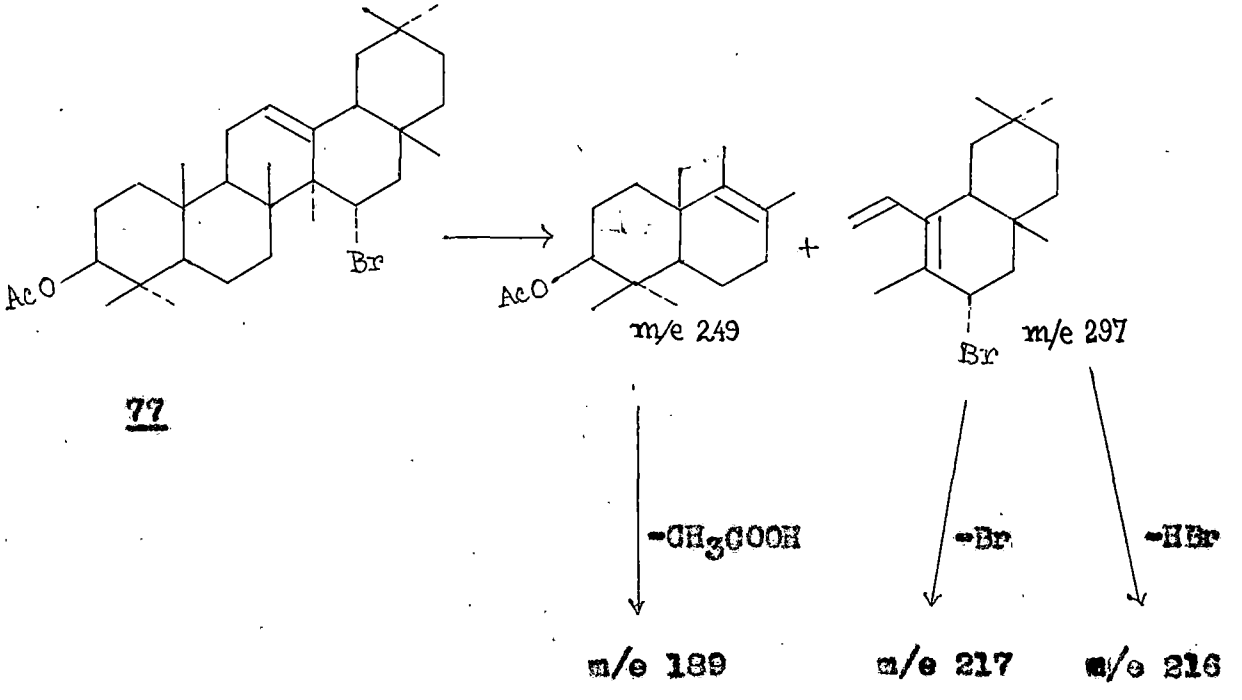


Fig.2: PMR spectrum of 15-bromo-β -amyrin acetate 77.

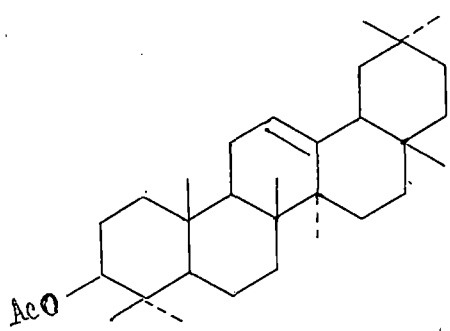
of 77 was finally confirmed by its mass fragmentation pattern. The compound showed a mass peak at m/e 548 (M^+) accompanied by other peaks at m/e 466 (M^+—HBr), 488 (M^+—AcOH), 406 (466—AcOH), 297, 249, 217, 216, 189. The fragmentation pattern may be explained if the double bond is assumed to be present at 12—13 position. The fragments at m/e 249 and 297 may be formed by Retro-Diels-Alder cleavage[43] of 12—13 double bond of structure 77 for the bromo compound. The genesis can be shown in Scheme—10:

Scheme—10

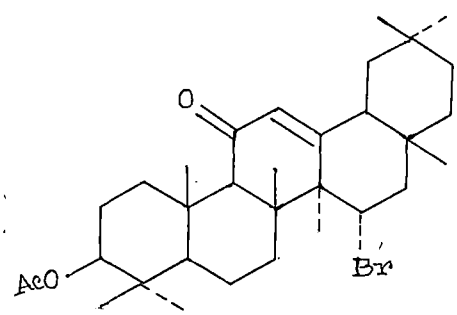


77

The structure of the compound as 77 was further established by the reaction with Zn dust in acetic acid on the compound that produced β-amyrin acetate 78, m.p. 230—31° (identical with an authentic specimen of β-amyrin acetate by mmp comparison).



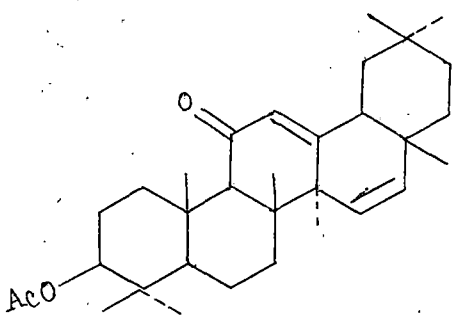
78



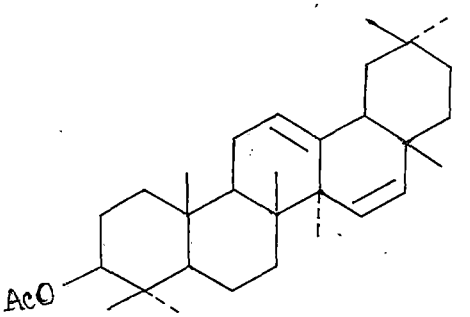
79

Oxidation of the bromo compound 77 with sodium-dichromate in presence of acetic acid furnished a gummy solid. Crystallisation of the compound from chloroform-methanol yielded 79, $C_{32}H_{49}O_3Br$, m.p. 240—41°, $[\alpha]_D$ +88°. UV absorption was found to be at the region λ max 249 nm and IR spectrum revealed that the compound had absorption at 1725, 1250 cm^{-1} (acetate) and 1680 cm^{-1} (—C=C—C=O). UV and IR spectrum confirmed the presence of an $\alpha\beta$-unsaturated carbonyl group. The compound was identified as 11-oxo-15-bromo-β-amyrenyl acetate[44] 79, when compared with an authentic specimen (mmp comparison) prepared by Khastgir and coworkers[44] from tarexeryl acetate.

Various attempts were made for dehydrobromination of the bromo ketone 79, to introduce the C—15—16 double bond. First it was tried by refluxing the compound with distilled dimethylaniline. In this case it was found that no dehydrobromination occurred and complete recovery of the starting material was obtained. As the dehydrobromination reaction was found to be not feasible by this reagent attempt was also made with s-collidine. The compound was refluxed with s-collidine for several hours and it was found that no reaction had taken place. Since the dehydrobromination could not be done even under forceful conditions it was assumed that probably some steric factors resisted dehydrobromination. As no dehydrobromination could be effected



80

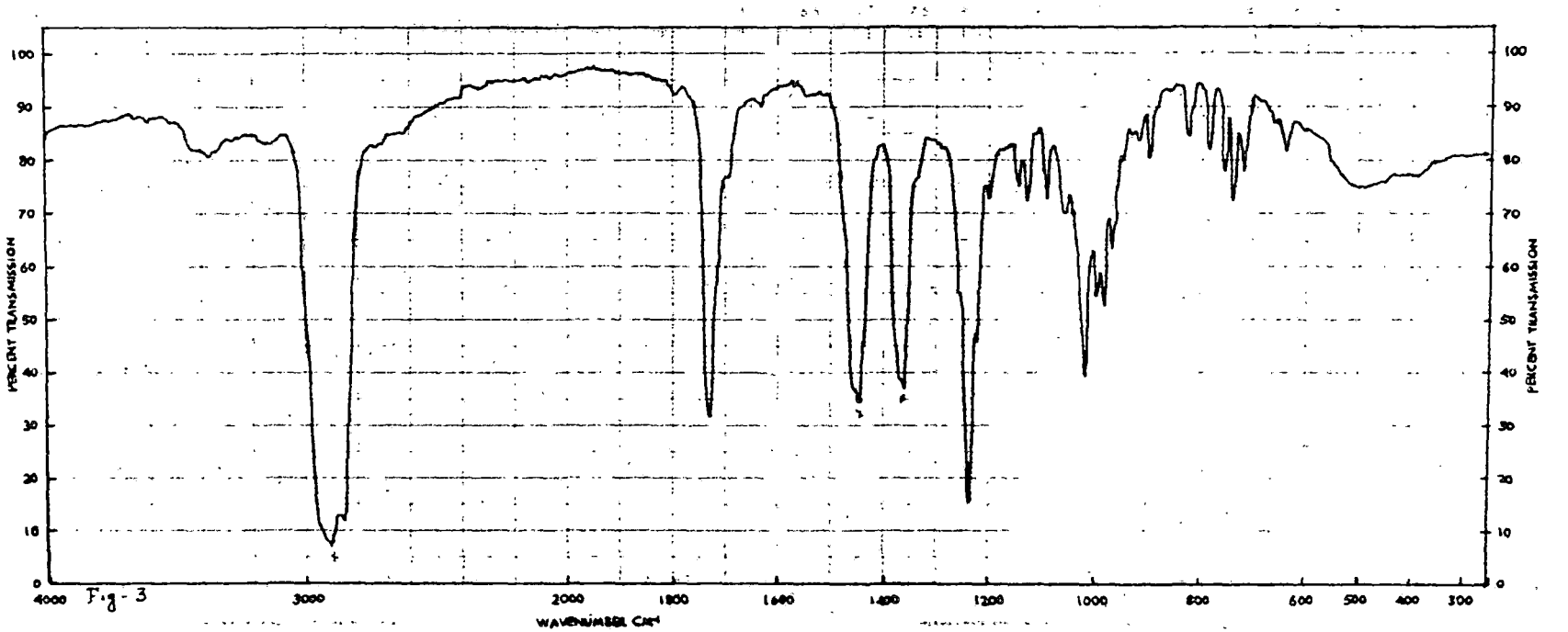


81

for the preparation of 80, a reverse route was followed. First the bromo compound 77 was dehydrobrominated and then it was oxidised to produce 80.

The bromo compound 77 was refluxed with dimethyl-aniline for a period of six hours when usual work up afforded a solid which on crystallisation from chloroform-methanol gave a product, $C_{32}H_{50}O_2$, m.p. 199—200°, $[\alpha]_D$ +42°. No UV absorption was observed for the compound in the region 220—350 nm. Beilstein test for halogen was found to be negative. IR spectrum (Fig.3) of the compound showed the presence of an acetate group at 1730 and 1240 cm^{-1}. Peaks at 750 cm^{-1} ($\overset{H}{\underset{H}{>C=C<}}$) and 820 cm^{-1} ($>C=C\overset{H}{<}$) was also observed. PMR spectrum (Fig.4) of the compound 81 showed multiplet peak in the region 5.2 to 5.6 ppm for three hydrogens of vinyl groups. At 4.5 ppm the multiplet for one hydrogen of H—C—O—$COCH_3$ was observed. One singlet at 2.08 ppm for three hydrogens of —O—CO—CH_3 was obtained. At the region 0.8—1.2 ppm peaks were observed for eight tertiary methyl groups. From the IR and NMR spectra the structure of the diene was assigned as 81.

To prepare 11-keto compound of 3β-acetoxy-olean-12,15-diene, the compound 81 was oxidised by sodium dichromate in acetic acid in refluxing condition for a period of twenty four hours. After usual work up followed by crystallisation afforded a compound in about 80% yield, identified as 3β-acetoxy-11-oxo-olean-12,15-diene 80, $C_{32}H_{48}O_3$,



F.g - 3

Fig.3: IR spectrum of olean-12,15-dien-3β-yl-acetate, 81.

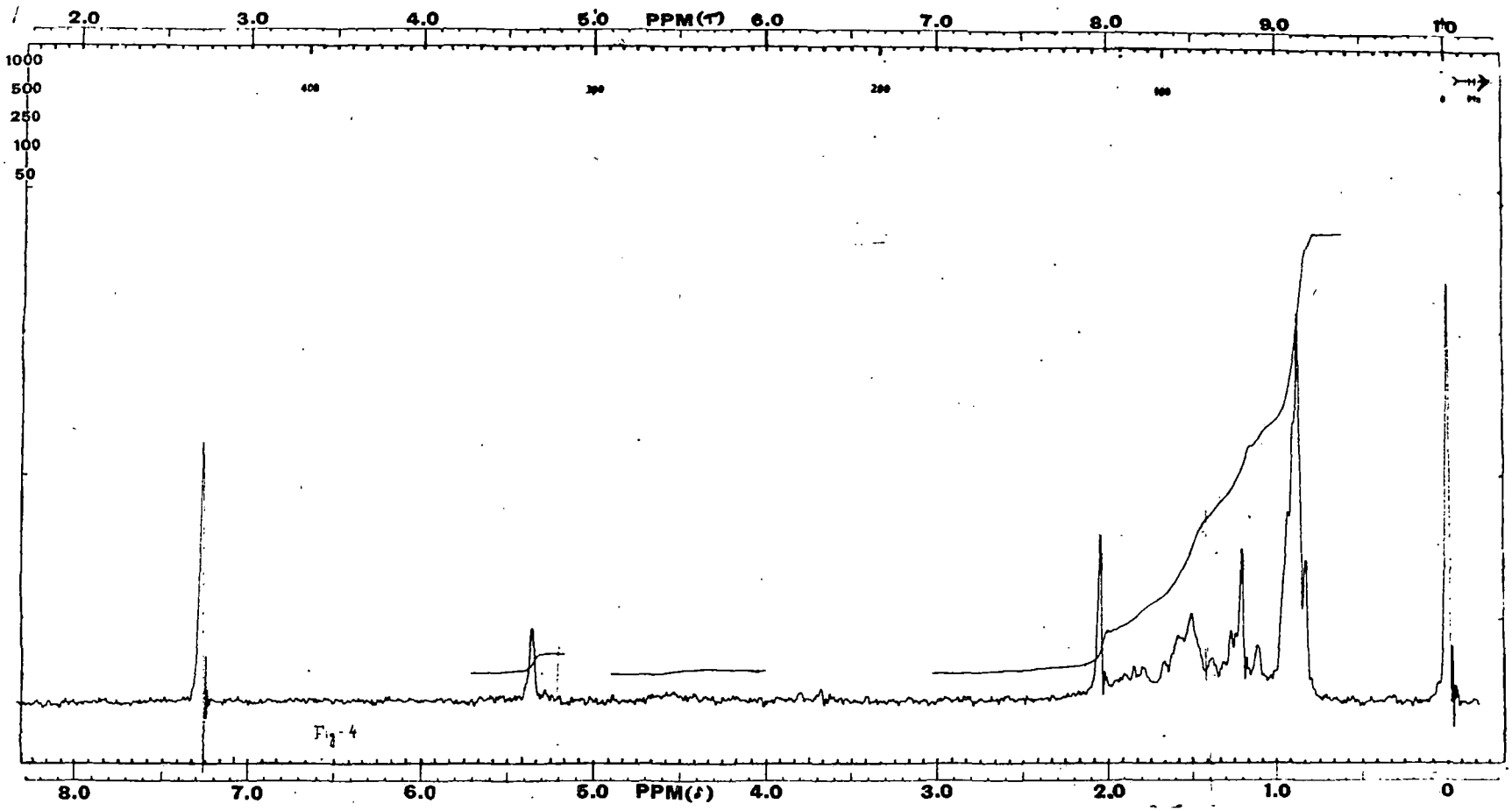
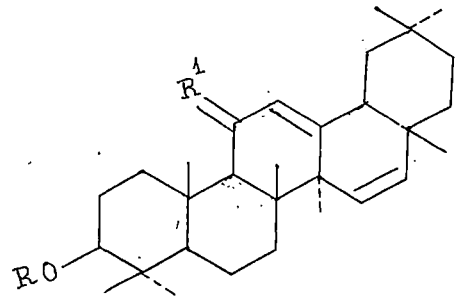
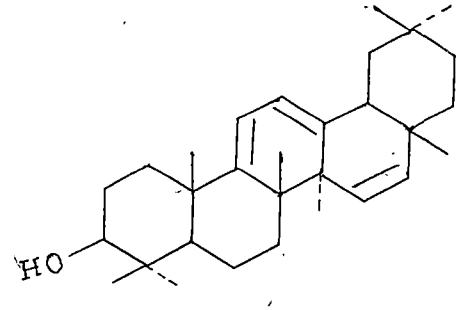


Fig.4: PMR spectrum of olean-12,15 dien-3β -yl-acetate, 81.

m.p. 243—45°, $[\alpha]_D$ +28.6°. UV absorption of the compound showed a maxima at λ_{max} 244 nm (ϵ = 11,376), which indicated the presence of an $\alpha\beta$-unsaturated ketone. IR spectrum (Fig.5) of the compound showed the presence of an acetoxy group at 1730 and 1240 cm^{-1}. The peak at 1660 cm^{-1} revealed that the compound contained an $\alpha\beta$-unsaturated ketonic functional group. Other peaks observed at 890 cm^{-1} and 750 cm^{-1} were due to the presence of —CH=CH— function. PMR spectrum (Fig.6) of the compound showed signals at 0.8 to 1.5 ppm for eight tertiary methyl groups present in the molecule. The acetoxy methyl protons appeared at 2.04 ppm. A multiplet was observed centred at 4.45 ppm for one proton of $\underline{H}—C—O—\overset{\overset{O}{\|}}{C}—CH_3$. Between 5.5—5.7 ppm peaks were obtained for three vinyl protons present in the molecule. On the basis of the above mentioned physical data the structure of the ketodiene could be assigned as 80.



80, R=AC; R^1=O
70, R=H ; R^1= 



82

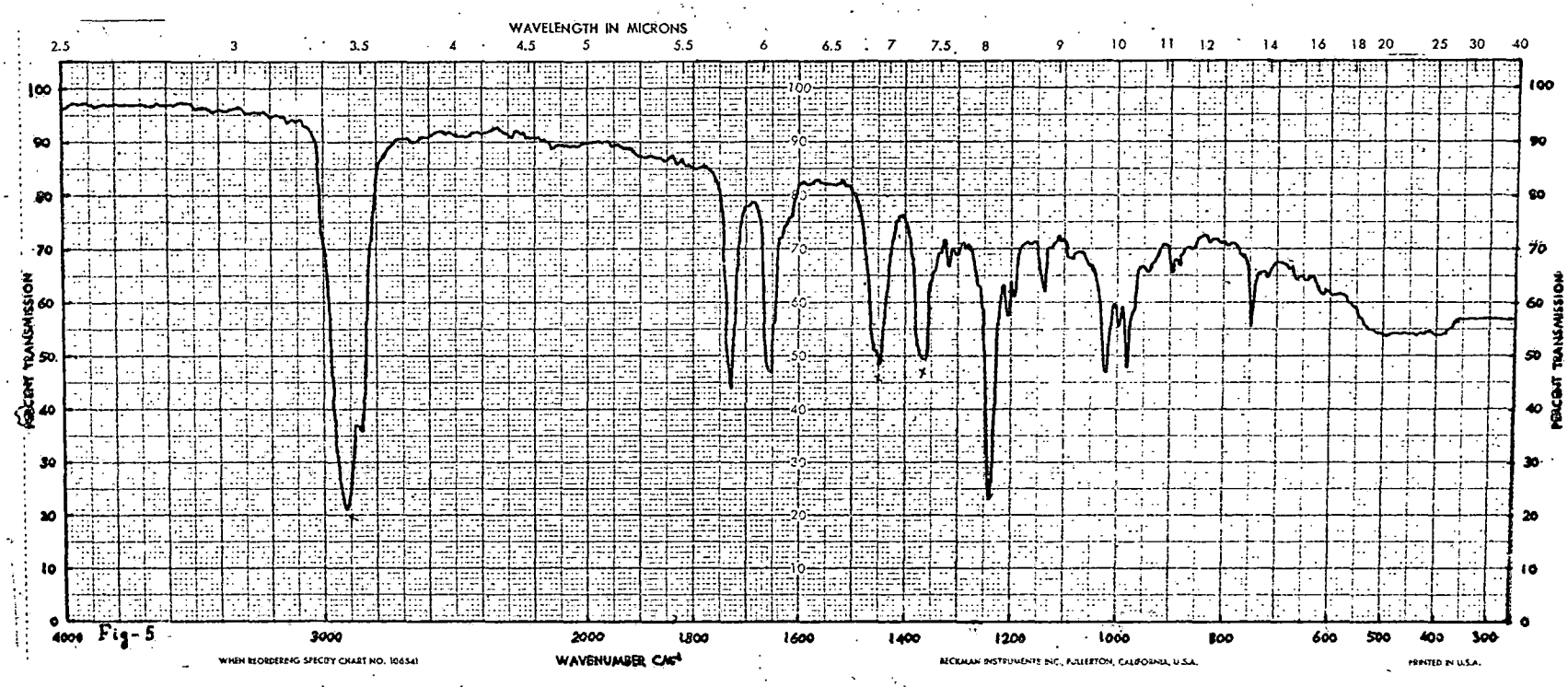


Fig.5: 3β -acetoxy-11-oxo-olean-12,15-diene, 80.

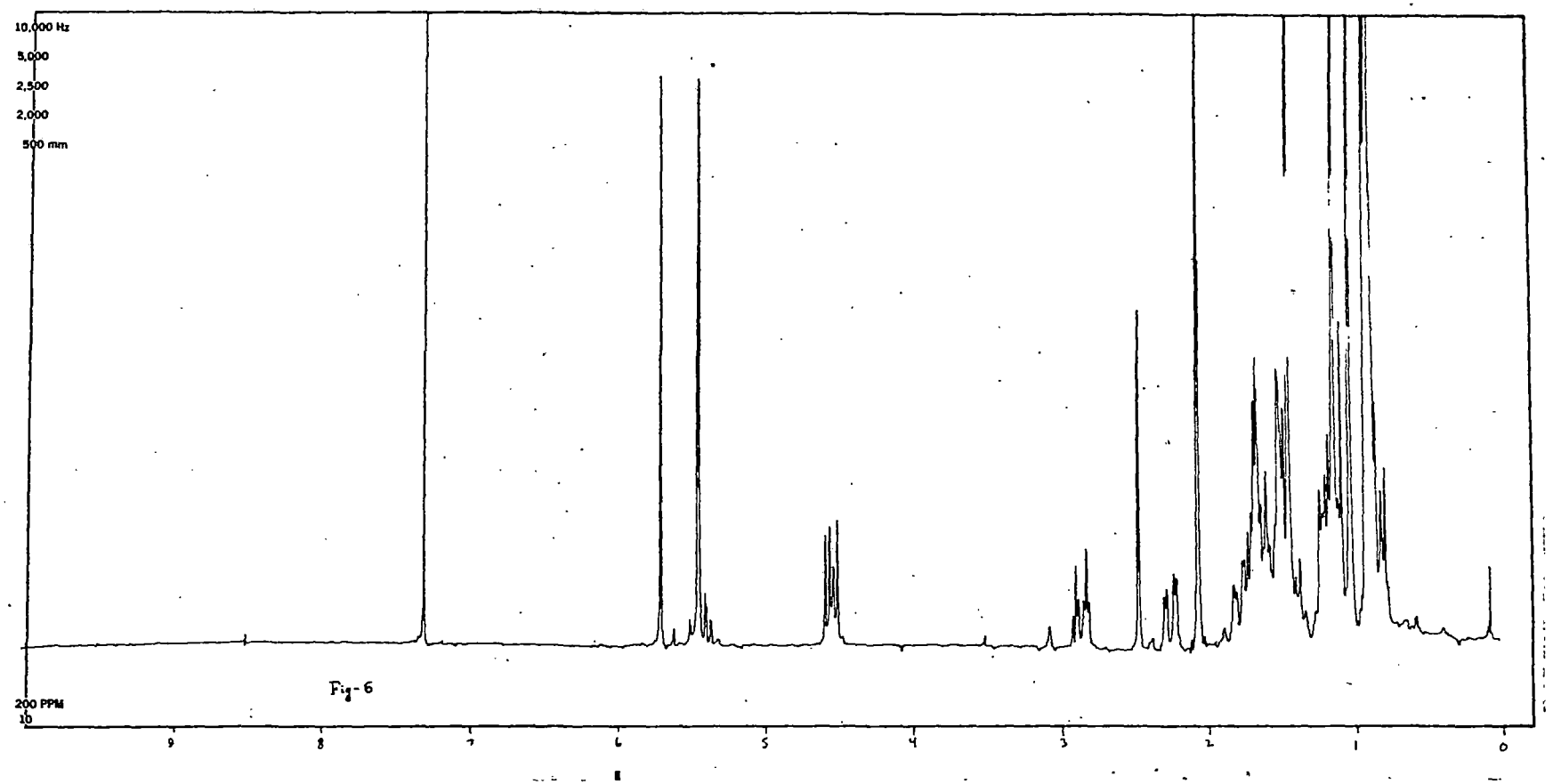
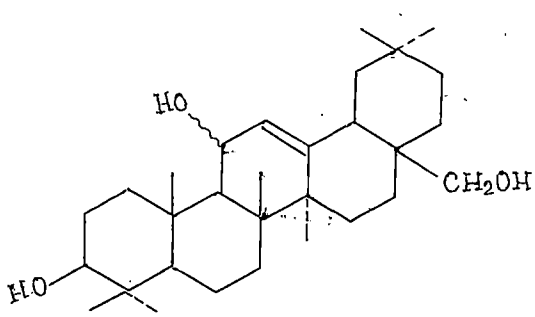
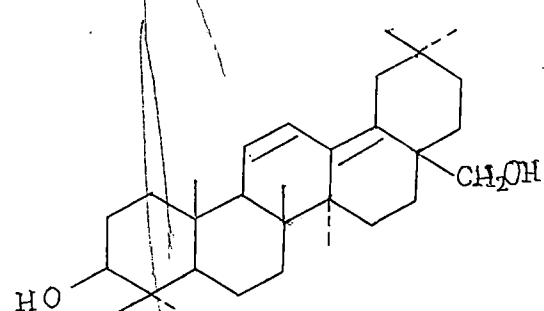


Fig.6: PMR spectrum of 3β -acetoxy-11-oxo-olean-12,15-diene, 60.

The ketodiene 80 was reduced by lithium aluminium hydride under refluxing condition for a period of six hours. The mixture was stirred at room temperature for 12 hours more. After usual work up the compound, $C_{30}H_{50}O_2$ 70, m.p. 198—200° was obtained. IR spectrum (Fig.7) of the compound showed ν max at 3460 cm^{-1} for —OH group and at 875 cm^{-1} for —CH=CH— function. It was found that if the compound was purified through column chromatography the newly introduced 11-hydroxy group underwent dehydration to form the homoannuler triene 82. The triene, $C_{30}H_{46}O_2$, m.p. 189—90° had the UV absorption at λ_{max} 276 nm (ϵ, 5260), indicating the presence of homoannuler diene system in ring C. This type of dehydration was reported earlier by I.Yosioka and coworkers[40], when 83 was heated producing the heteroannuler diene 84 (UV absorption at λ_{max} 244, 253, 263 nm).



83



84

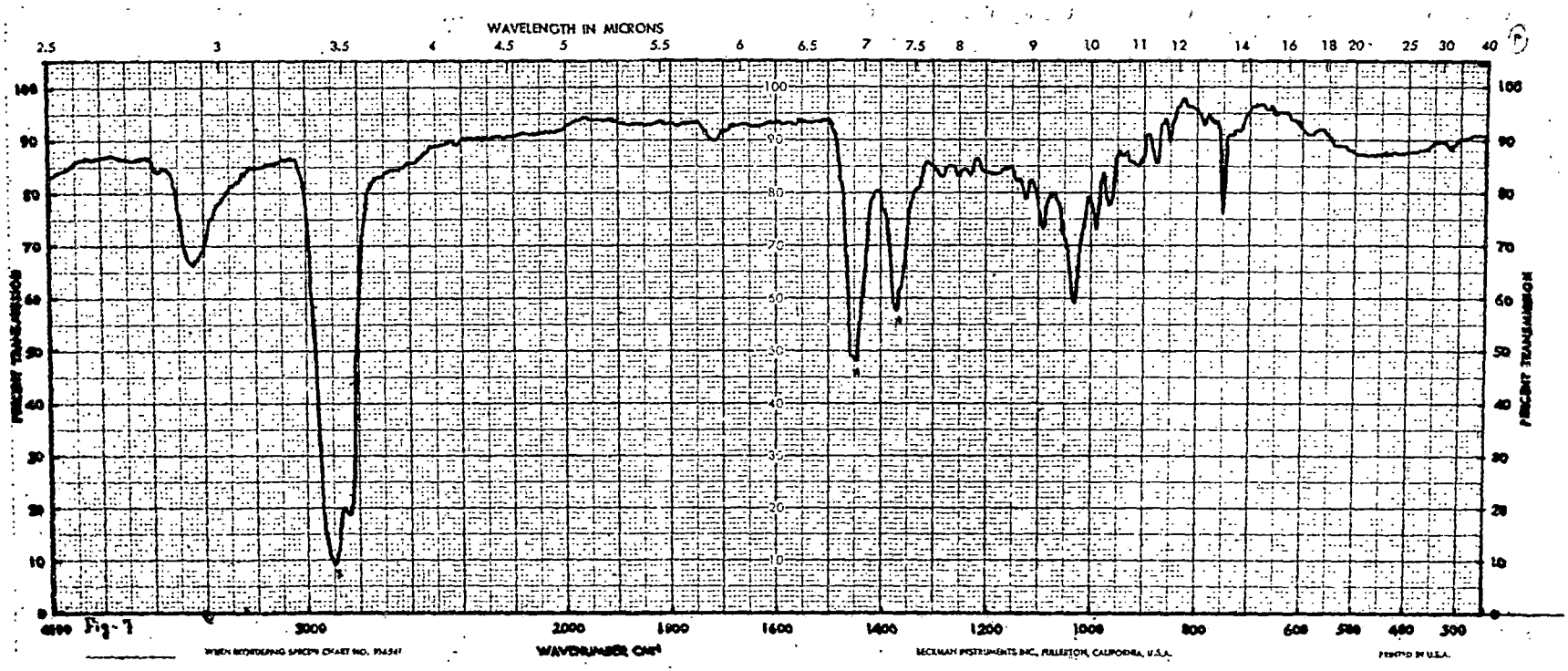


Fig-7

Fig.7: IR spectrum of olean-12,15-dien-3,11-diol, 70.

Formation of homoannuler diene in our compound is assumed for the fact, the heteroannular diene could not be produced due to the presence of a C—15,16 double bond.

So we had got the desired product 70, i.e. olean-12,15-dien-3,11-diol on which we could carry out the hydrogen peroxide reaction to get the desired products which we assumed before. The diol 70, was stirred at room temperature with a mixture of para toluene sulfonic acid, hydrogen peroxide and tertiary butyl alcohol following the procedure adopted by Covey and coworkers[23]. After stirring at room temperature for twenty four hours and usual work up the residue was chromatographed. A mixture of two compounds was eluted by benzene, TLC of which showed two distinct spots at very close range. The mixture of the two compounds were separated by fractional crystallisation. After crystallising several times the less soluble compound 85, $C_{30}H_{46}O_3$, m.p. 240—41° was obtained. No UV absorption was observed for the compound. IR spectrum (Fig.8) of the compound showed absorption in the region 890 and 820 cm^{-1} indicating the presence of HC═CH function, a peak at 3520 cm^{-1} for the hydroxyl group and another peak at 1775 cm^{-1} indicated that the compound contained a γ-lactone which was not

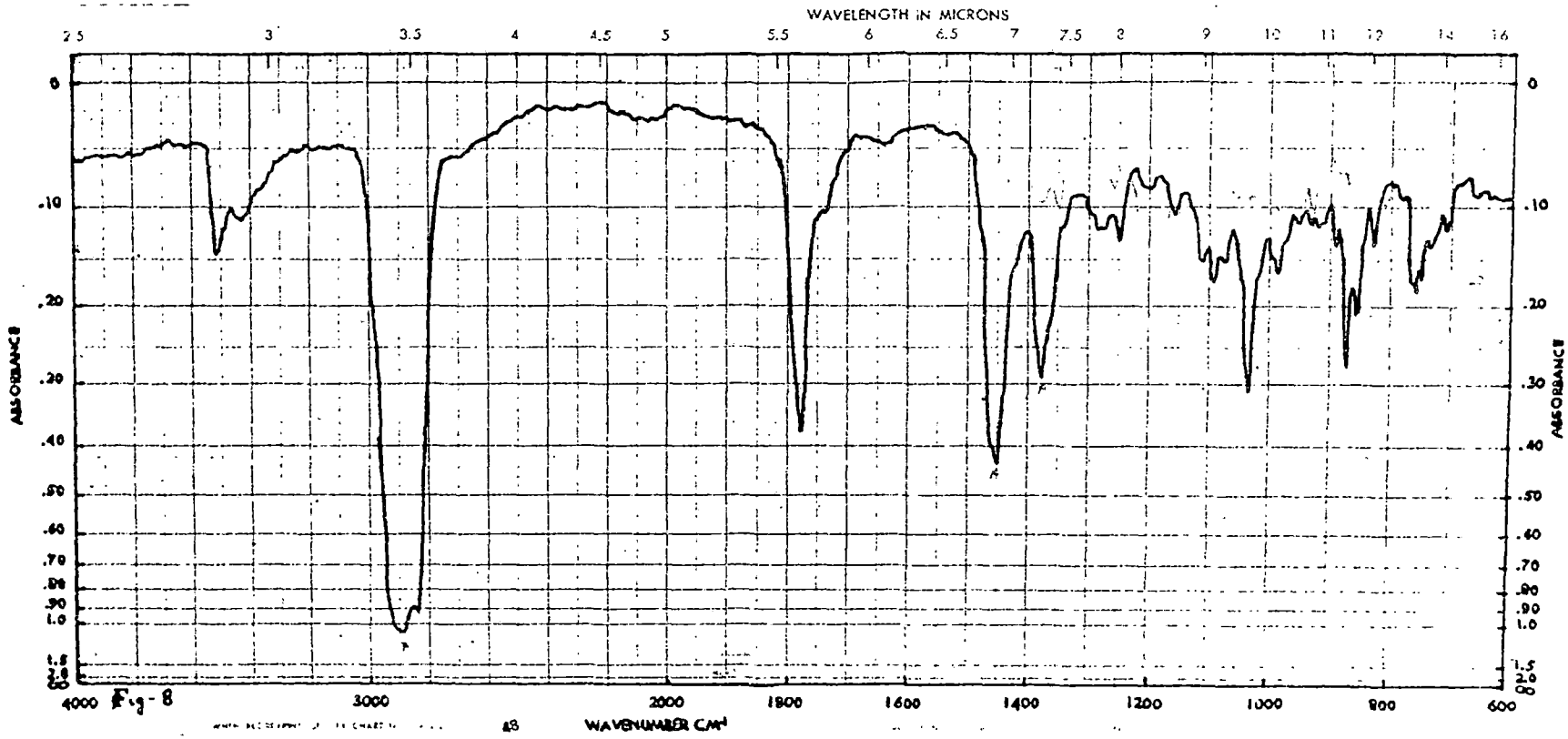
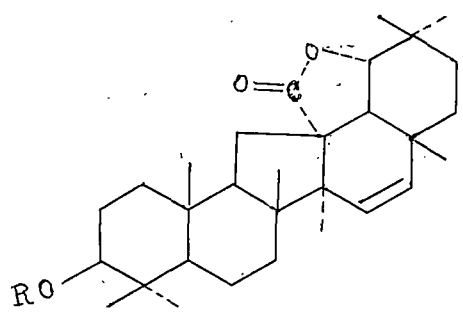


Fig-8

Fig.8: IR spectrum of the first hydroxy lactone (H_2O_2 oxidation product), 85.

originally present in the starting alcohol. The compound 85, was acetylated at room temperature with acetic anhydride and pyridine.



85, R = H
85a, R = $COCH_3$

The acetate so formed was crystallised and after crystallisation gave a compound 85a, $C_{32}H_{48}O_4$, m.p. 215—16°. IR spectrum (Fig.9) of the compound 85a showed ν_{max} at 1780 cm^{-1} for γ-lactonic functional group. Peaks at 1720 and 1250 cm^{-1} indicated the presence of an acetoxy group. Other peaks at 890, 870, 750 cm^{-1} revealed that the compound contained a —C(H)=C(H)— double bond. The formation of a lactone had thus been proved by the IR spectrum of 85 and its acetate 85a. The structure of 85 has been conclusively established by its ^1H and ^{13}C NMR spectra and mass spectral analysis of its acetate 85a.

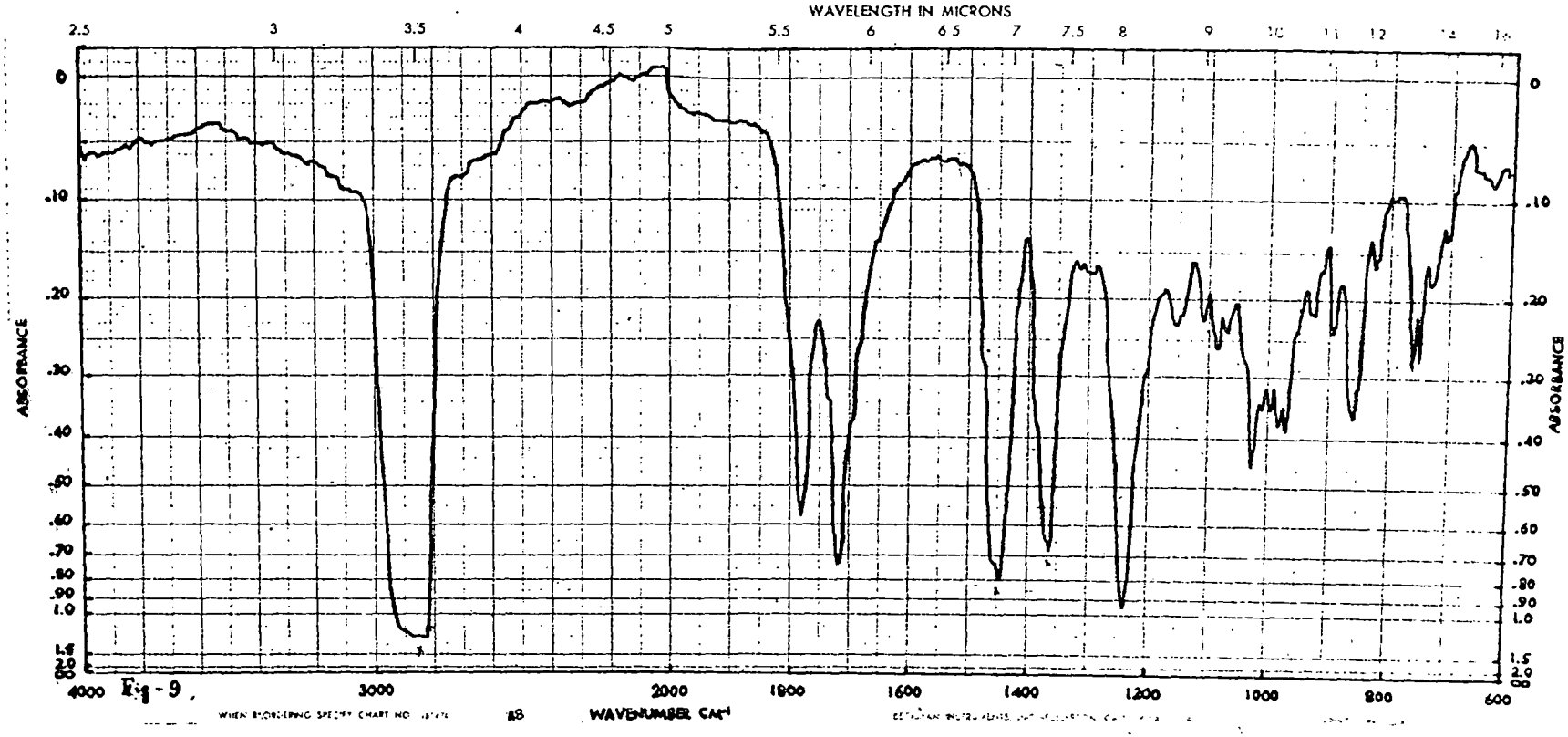


Fig-9

Fig.9: IR spectrum of the first lactone acetate, 85a.

^{1}H NMR spectrum (Fig.10) of the compound 85a showed the presence of eight tertiary methyl groups between 0.5 to 1.5 ppm. A singlet at 2.05 ppm indicated three protons of acetoxy methyl groups (—OCOCH_3). The doublet at 4.72 ppm (J=7.3 Hz) indicated the proton geminal to lactonic oxygen. The peak centred at 5.27 ppm, for cis disubstituted olefinic proton, was obtained as an AB quartet (J=10 Hz). Irradiation at 4.72 ppm (Fig.11) gave a singlet at 1.73 ppm showing the presence of a proton at 1.73 ppm to be coupled to the one at 4.72 ppm with J=7.5 Hz and weekly to other protons. Again irradiation at 1.73 ppm (Fig.12) at a fairly low level gave a sharp line at 4.72 ppm was due to the presence of a single proton adjacent to the lactonic oxygen that couples with a proton at 1.73 ppm. The multiplet at 4.5 ppm collapsed to a broard singlet (Fig.13) suggesting the proton at C—8 coupled with the protons at C—2. The absence of any peaks between 2.2 and 3.5 ppm showed that the C—α to the lactonic —CO— possessed no proton. The above observation could be explained if the lactonic —CO— group was attached to the C—13 position and the γ-lactone ring was formed with the C—19 carbon with a geminal β-H that had a single neighbouring β-axial proton at C—13 position. Thus the ^{1}H NMR and IR spectra suggested structure 85, for the hydroxy and 85a, for the corresponding acetate.

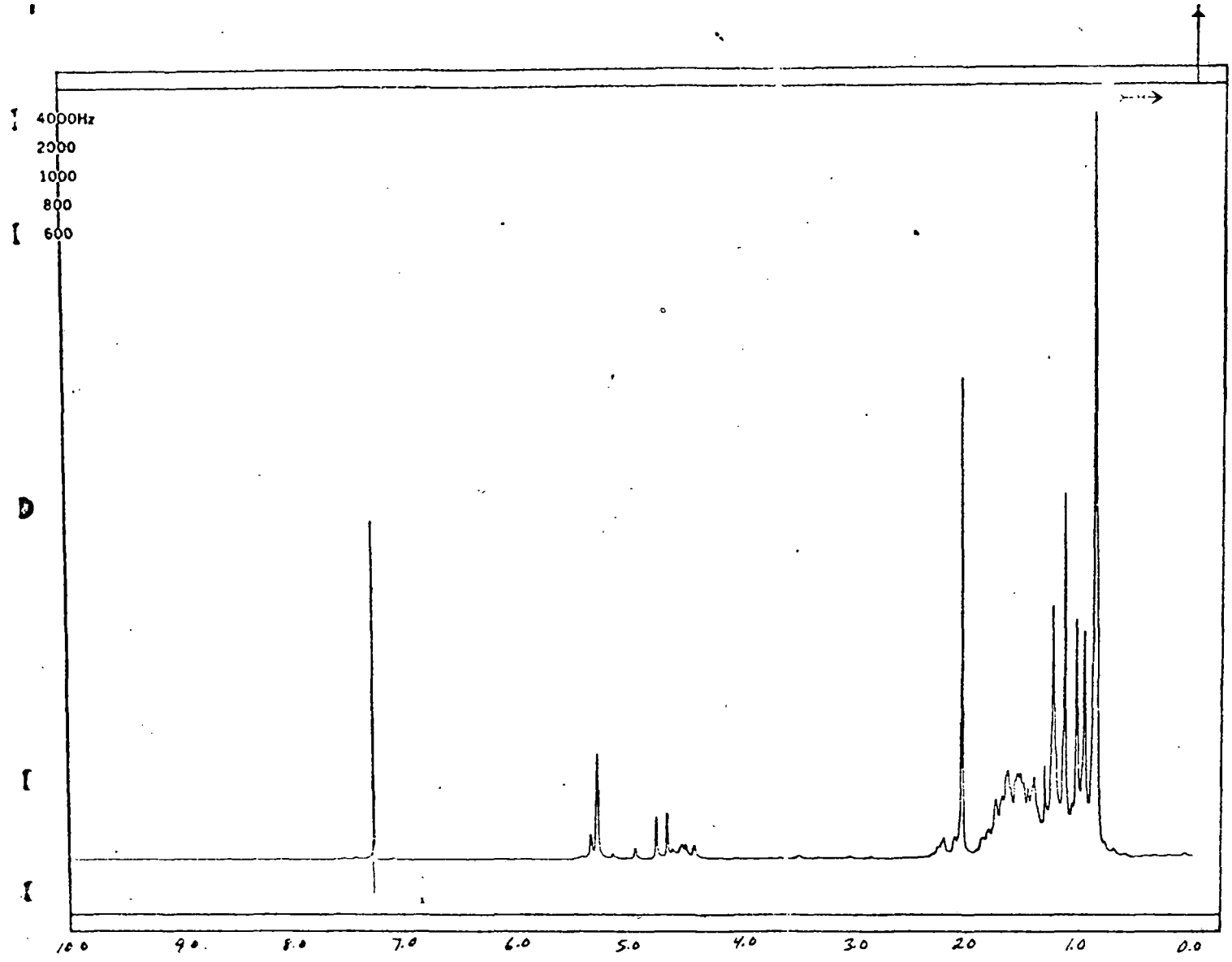


Fig.10: PMR spectrum of first lactone acetate, 85a.

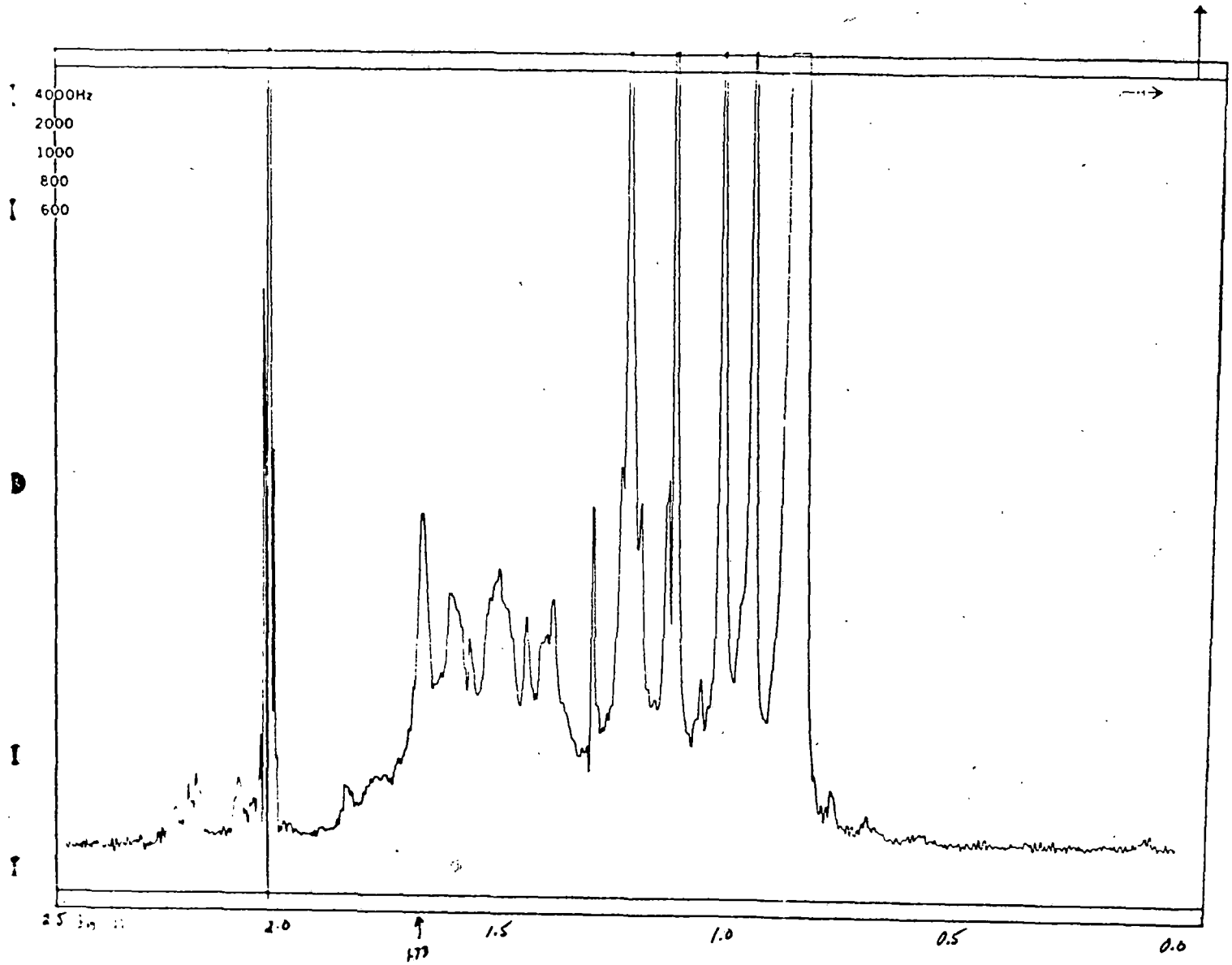


Fig.11: PMR spectrum of the first lactone acetate, 85a(irradiation at 4.72ppm)

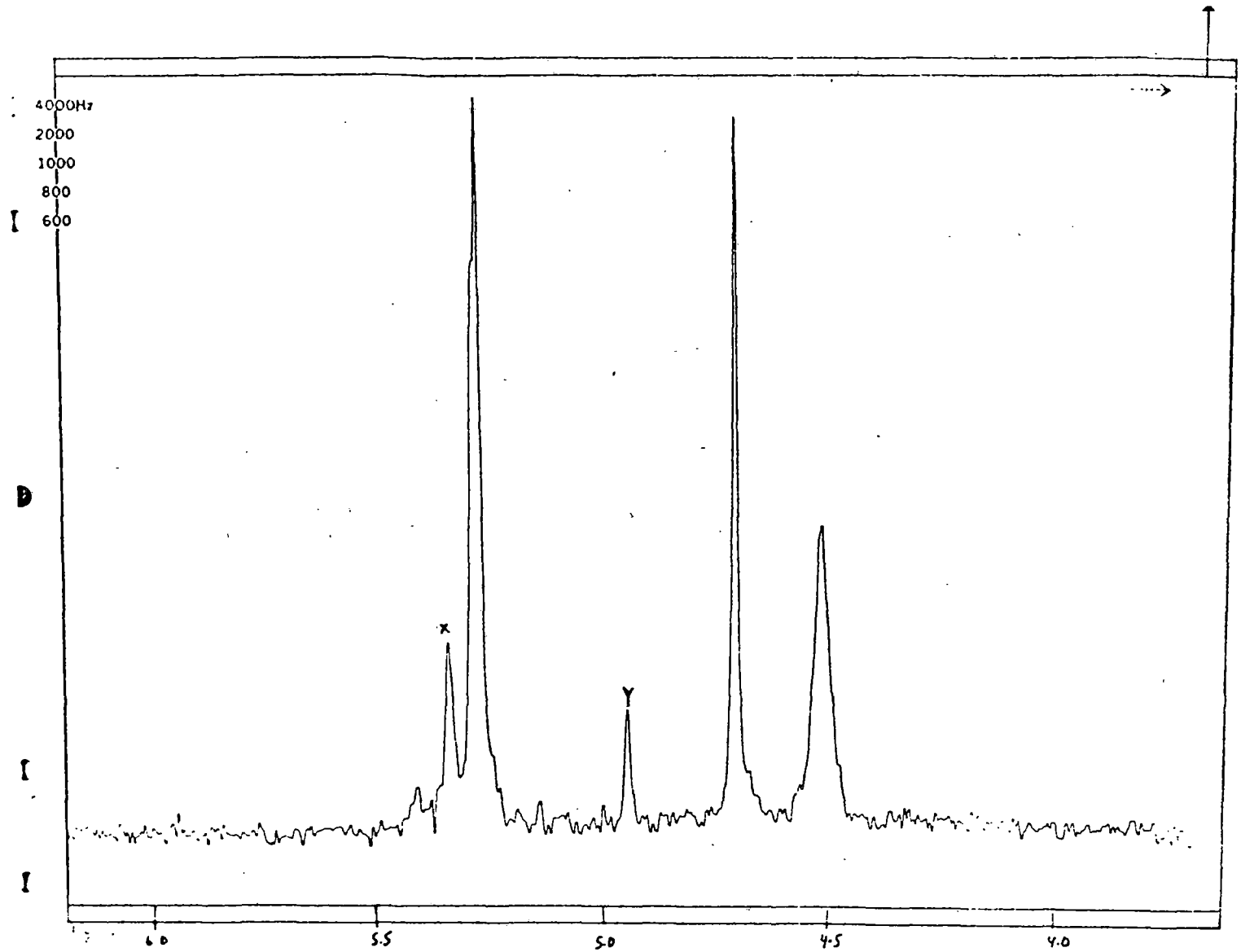


Fig.12: PMR spectrum of the first lactone acetate, 85a(irradiation at 1.73 ppm).

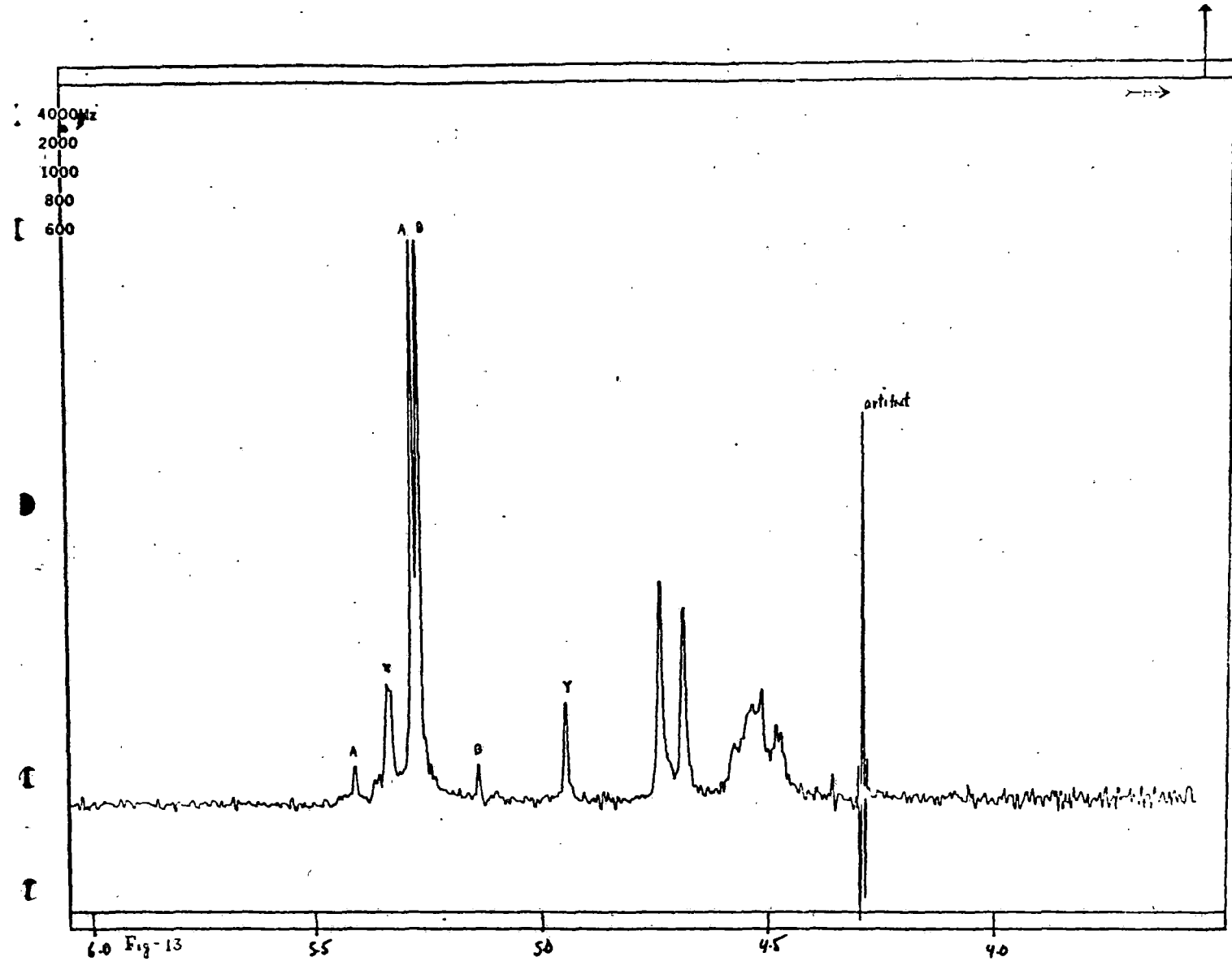
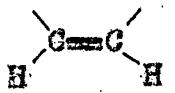


Fig.13: PMR spectrum of first lactone acetate, 85a (plot expansion).

The structure was corroborated by its ^{13}C NMR and mass spectral analysis.

^{13}C NMR spectrum (Fig.14) of the compound 85a showed that there were two singlets at 170.5 and 175.7 ppm which were due to the C—3 acetate, carbonyl and the lactonic carbonyl carbons respectively. The singlet at 175.7 ppm fairly indicated that it was either five or six membered ring lactone. The singlets at 80 and 82 ppm were doublets and were to be due to C—H groups bonded to oxygen of an ester. Thus one of the doublets was due to O—C—H group at C—3 position and the oxygen of the lactone must also contain at least one O—C—H group attached to it. The doublets at 132.75 and 130.6 ppm indicated that the double bond was counted as disubstituted olefinic bond i.e.  .

Mass spectrum (Fig.15) of the compound 85a showed the molecular ion peak at m/e 496. The other fragments obtained were at m/e 468 (M^+—CO), 452 (M^+—CO_2), 436 (M^+—AcOH), 421, 408, 392, 372, 313, 300, 269, 257, 244, 231, 217, 218, 208, 203, 191, 189, 187, 175, 171, 161, 147, 135.

Thus the first hydroxy lactone has been established as 3β-hydroxy-C-12-nor-olean-15-en-13α-carb-19α-olide 85.

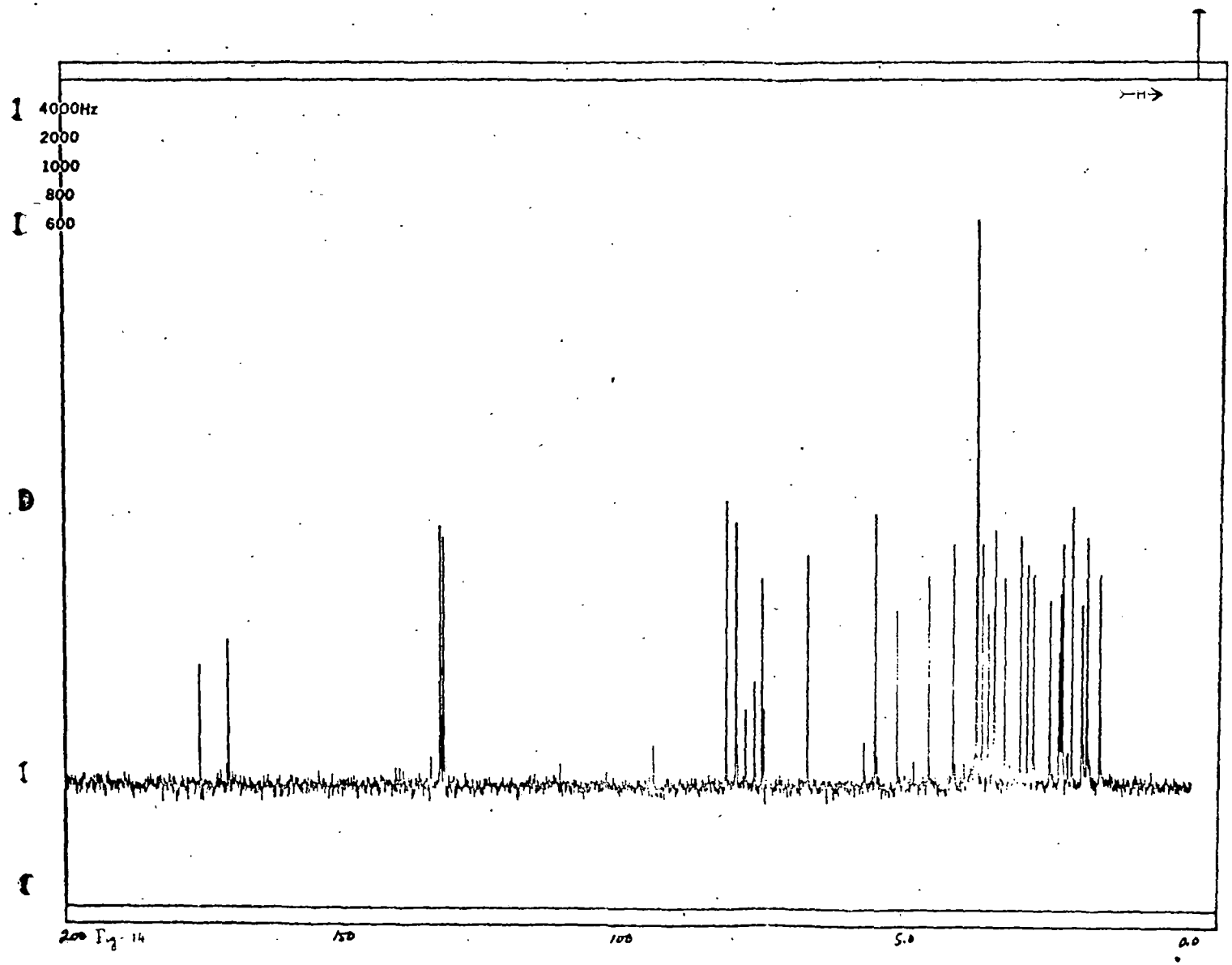


Fig.14: ^{13}C NMR spectrum of first lactone acetate, 85a.

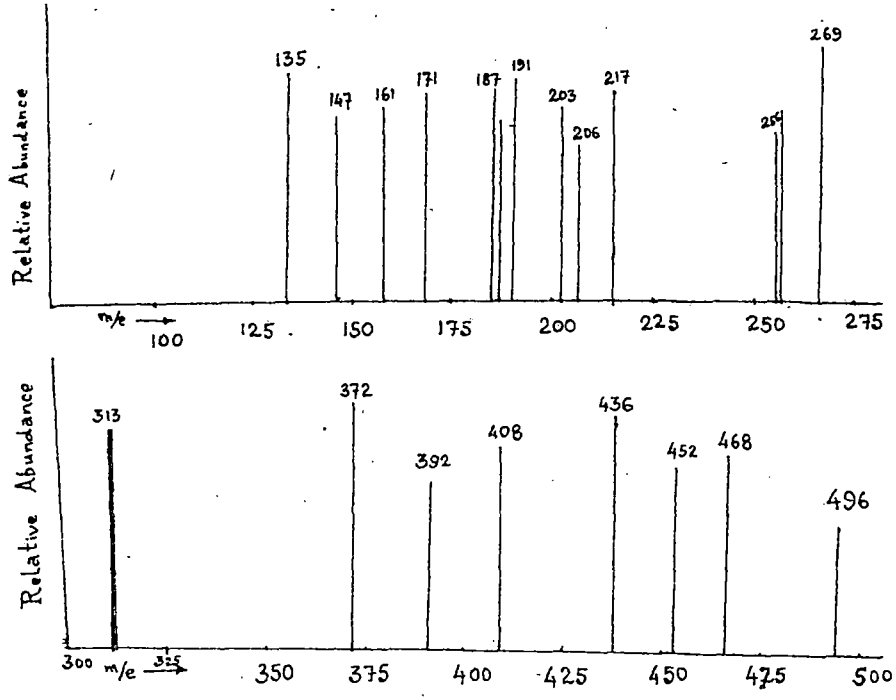


Fig 15 : Mass spectrum of the first lactone acetate, 85a.

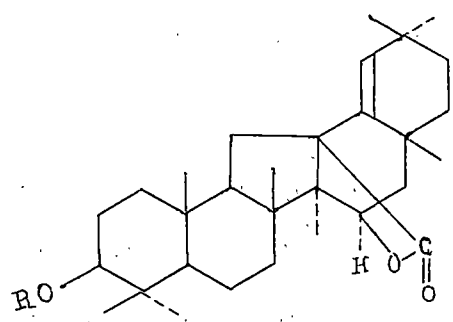
Isolation of the second compound 86:

The filtrate after repeated separation of the lactone 85, on concentration and crystallisation from chloroform-methanol mixture yielded another crystalline compound. The compound was purified by repeated crystallisation. After crystallization the compound 86, so obtained was analysed for molecular formula, $C_{30}H_{46}O_3$, m.p. 256—57°, no UV absorption was observed for the compound. IR spectrum of the compound showed peaks at 3520 cm^{-1} for hydroxyl functional group, 1780 cm^{-1} for a γ-lactone and 820 cm^{-1} for a trisubstituted double bond present in the molecule. The structure of the isomeric compound was proved by its IR, ^1H, ^{13}C NMR and mass spectral analysis of its acetate 86a, ^1H NMR spectrum of the compound 86, showed a singlet at 5.3 ppm for the vinyl proton. A multiplet at the region 4.9 ppm ($W_{\frac{1}{2}}$ = 20 Hz) was obtained due to the proton geminal to the lactonic oxygen which might have two neighbouring protons. A triplet at the region 3.23 ppm was obtained ($W_{\frac{1}{2}}$ = 20 Hz) for the proton geminal to the OH at C—3 present as an axial proton[45].

Mass spectrum of the compound 86, showed a molecular ion peak at m/e 454(M^+). Other fragments at m/e 436

(M^+-H_2O), 426 (M^+-CO), 410 (M^+-CO_2), 408, 392, 313, 269, 231, 205, 189, 187, 171 were obtained.

Acetylation of the compound 86 in presence of acetic anhydride and pyridine at room temperature was done and the product 86a, obtained after usual work up and crystallisation was analysed for $C_{32}H_{48}O_2$, m.p. 228—29°.



86, R = H
86a, R = CH_3CO

IR spectrum (Fig.16) of the compound 86a showed the presence of γ-lactone at 1780 cm^{-1} and peaks at 1730 and 1250 cm^{-1} were due to the acetate functional group present in the molecule. Another peak at 820 cm^{-1} was due to presence of a trisubstituted double bond.

^1H NMR spectrum of the compound 86a showed a multiplet at the region 4.8 ppm ($W_{\frac{1}{2}}$ = 7Hz) and found integrated

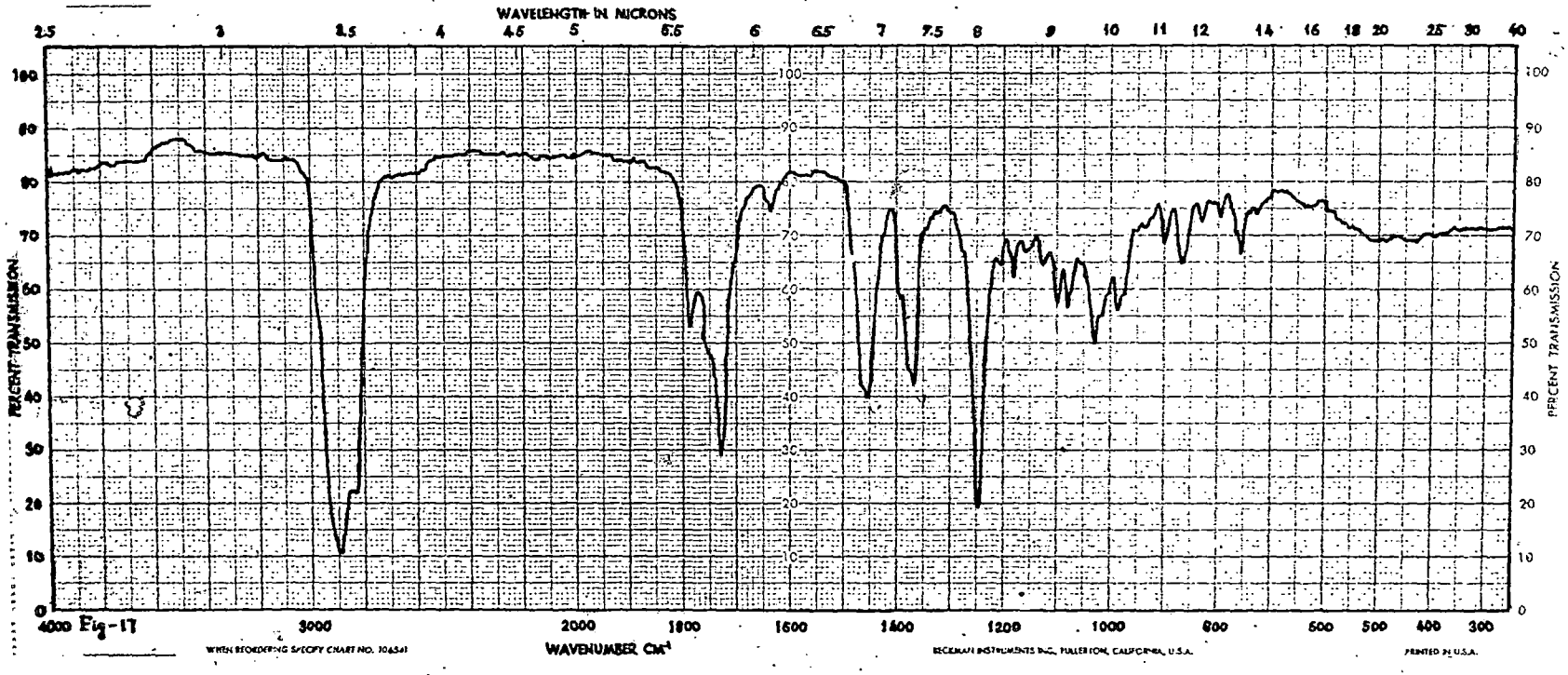
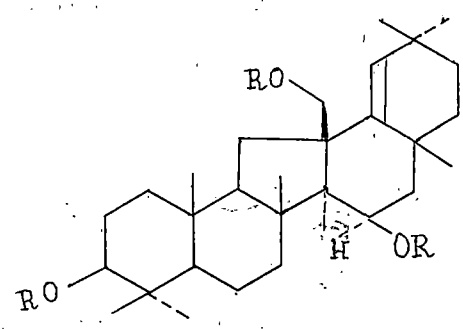


Fig.16: IR spectrum of secone lactone acetate, 86a.

for one proton was due to the fact that the proton geminal to the lactonic oxygen is equatorial which coupled with neighbouring axial and equatorial protons. The multiplet obtained in the region 4.5 ppm was due to C—3 proton. The singlet obtained at 5.3 ppm for a vinyl proton was possible only if the double bond is at C—18—19 position so that the proton at C—19 would give a singlet. The absence of any peak in the region 2.2 to 3.5 ppm showed that the lactonic —CO— had no α-H as in 85a and hence should be attached to the same carbon (C—13). The absence of A—B quartet for the cis C—15—16 olefinic protons present in 85a, indicated that this double bond must be involved in the formation of the lactone 86 and the lactyl oxygen should be attached to C—15 with an α-equatorial geminal proton that coupled with C—16 protons. The structure of 86, as well as 86a was thus proved. The mass spectrum of 86a was almost same as that of 85a.

The structure of the second lactone 86 was finally proved by the cleavage of the lactonic ring. Lithium aluminium hydride reduction of the lactone 86, yielded a solid which on crystallisation from chloroform and methanol afforded crystals of 87, that was analysed for $C_{30}H_{50}O_3$, m.p. 280—82°. The compound was found to be a triol, which

was proved by the IR, ^{1}H and ^{13}C NMR and mass spectral analysis of the acetylated product of triol i.e. 87a.



87, R = H
87a, R = CH_3CO

After acetylation of the triol 87, with acetic anhydride and pyridine at room temperature and crystallization of the product from chloroform and methanol, the acetate 87a, m.p. 190—93° was obtained. Elemental analysis showed it to be a triacetate having molecular formula $C_{36}H_{56}O_6$. IR spectrum (Fig.17) of the compound 87a, showed the broard peaks at ν_{max} 1735—40 cm^{-1} and 1250—1240 cm^{-1} that were due to the presence of acetate groups. Peak at 820 cm^{-1} showed that the compound contained a trisubstituted double bond. ^{1}H NMR spectrum (Fig.18) of the triacetate showed peaks in the region 0.88 to 1.2 ppm for eight tertiary

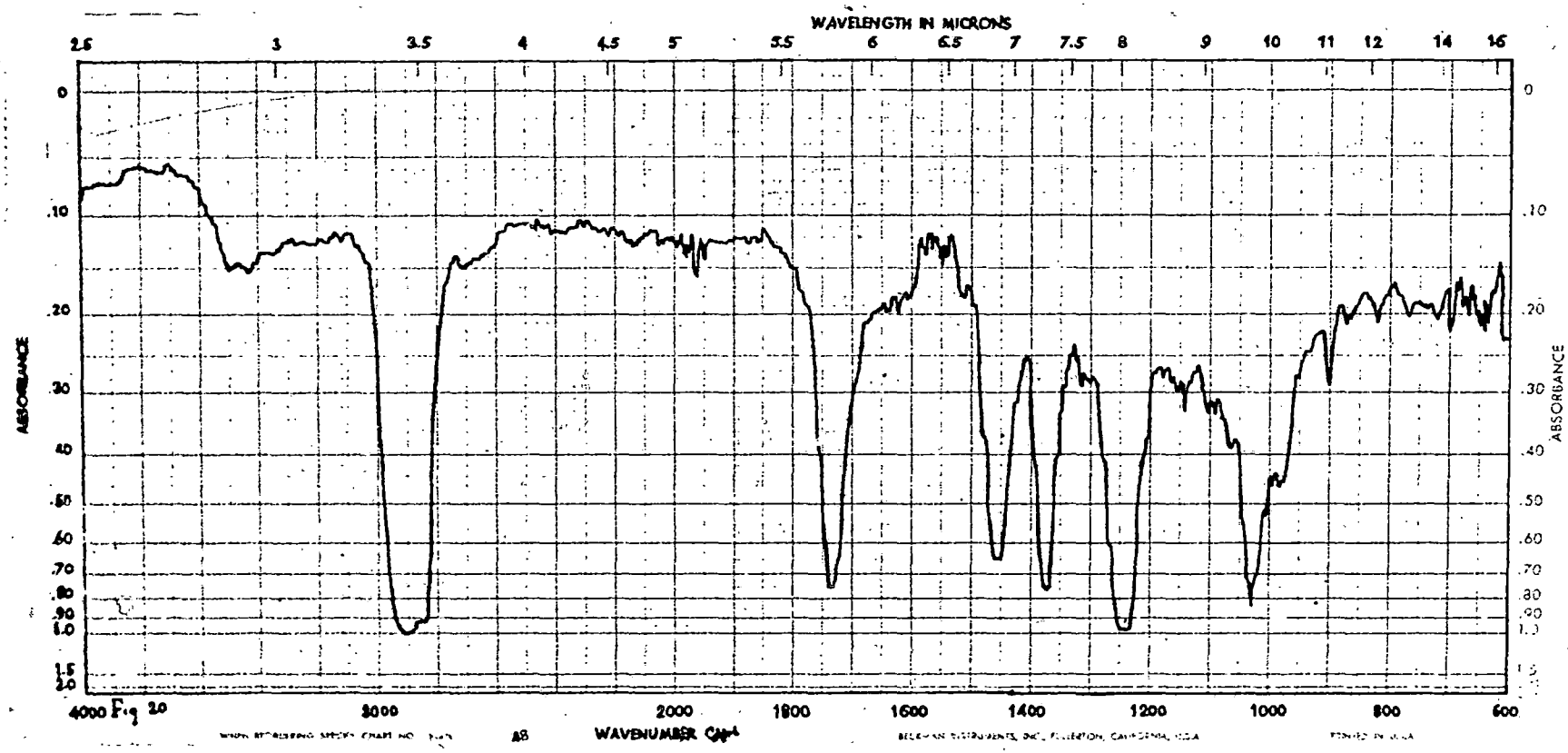


Fig.17: IR spectrum of the triacetate, 87a.

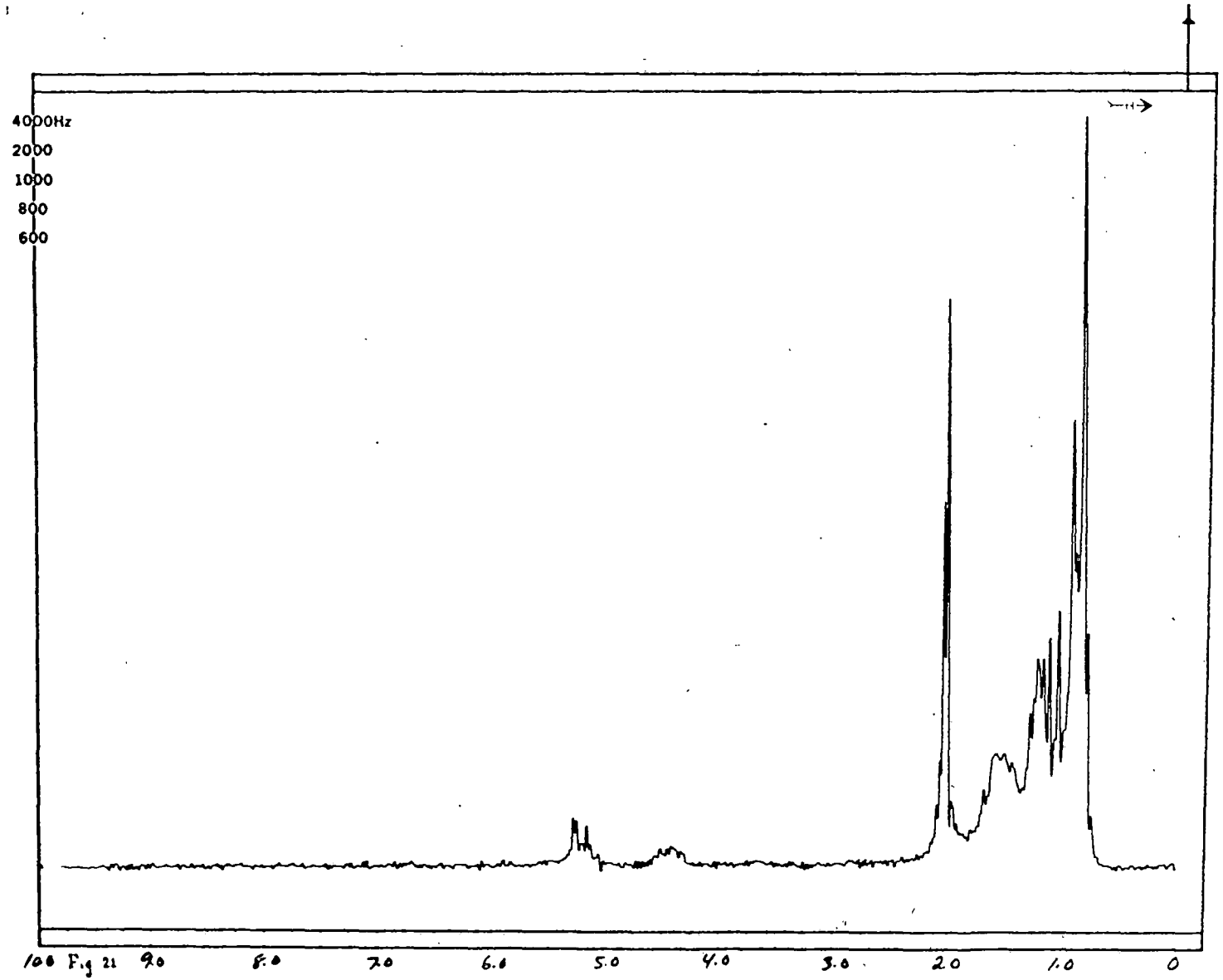


Fig.18: PMR spectrum of the triacetate, 87a.

methyl groups, the peak at the region 2.05 to 2.1 ppm showed the presence of three acetoxy methyl groups, the multiplet centred at 4.45 ppm was due to the proton at C—3 and two methylene groups geminal to acetate groups. At the region 5.3 ppm (J=8Hz), a doublet split into a multiplet might be due to the C—15 proton geminal to the acetate. The singlet at 5.3 ppm indicated the presence of a vinyl proton which had no neighbouring proton, such a vinyl proton is possible only if the double bond is situated at C—18—19 position of common pentacyclic triterpenoids. So the ^{1}H NMR spectrum of the triacetate suggested the structure as 87a. The structure of 87a was corroborated by its ^{13}C NMR and mass spectral analysis.

^{13}C NMR (Fig.19) of the compound 87a, showed that there were three singlets at 170.2 and 170 ppm which were due to three acetoxy carbonyl functions present in the molecule. The compound showed the doublets at 80.5 and 78.5 ppm due to the presence of —CH—O—CO—functional group in the molecule. The singlet at 155.5 and doublet at 122 ppm assured the presence of C=C$\diagup\!\!\diagdown_{H}$ double bond. Mass spectrum (Fig.20) of the triacetate 87a, showed a molecular ion peak (M^{+}) at m/e 584, the other prominent peaks were obtained at m/e 524

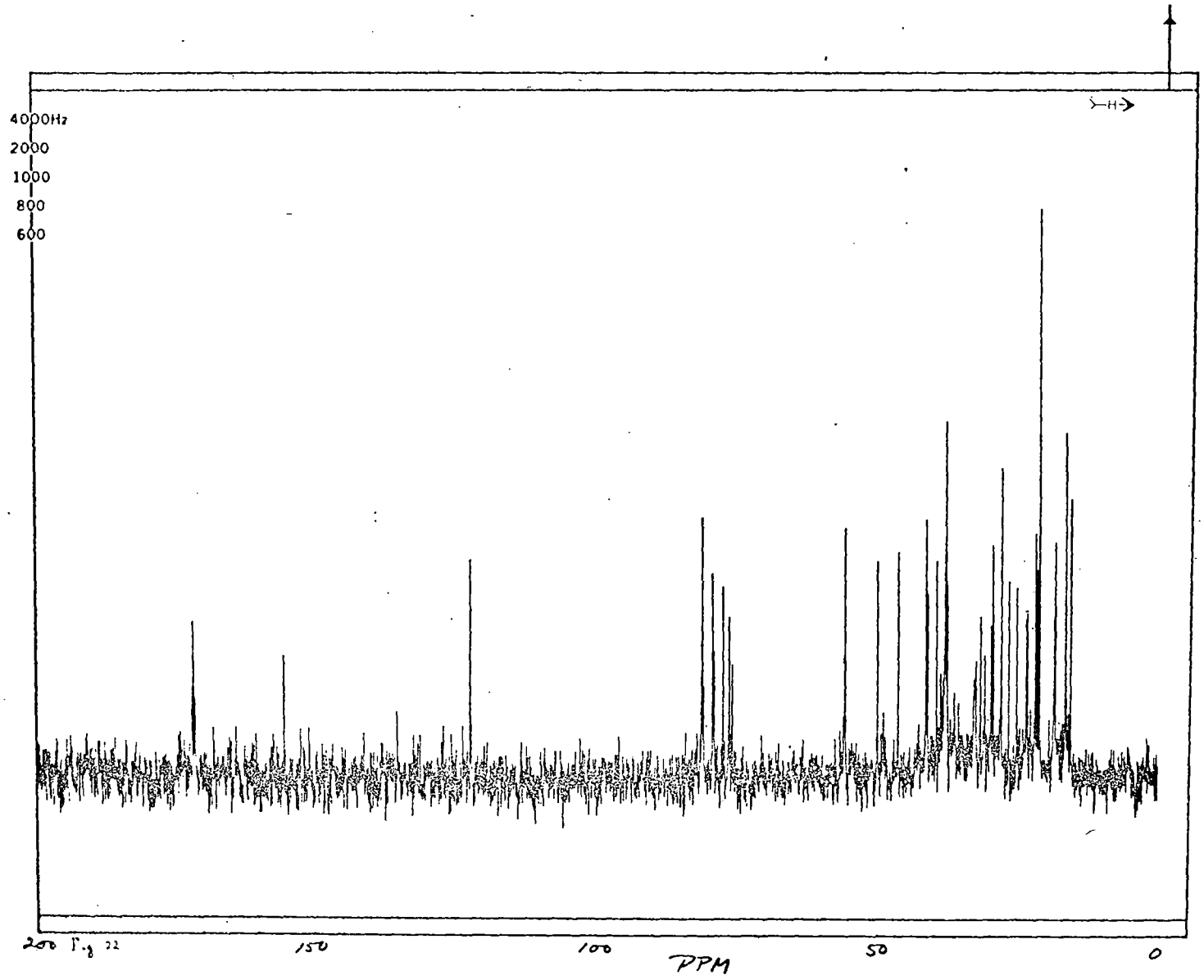


Fig.19: ^{13}C NMR spectrum of the triacetate, 87a.

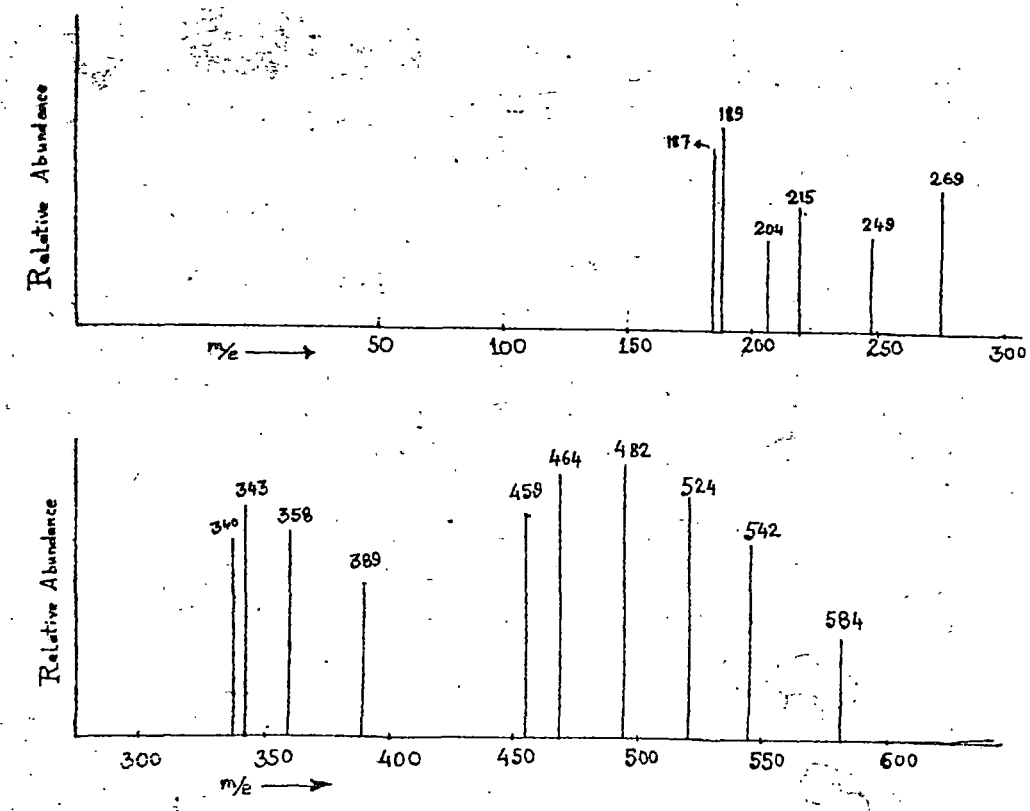


Fig 20 : Mass spectrum of the triacetate, 87a.

(M^+—ACOH), 432, 464 (M^+—2ACOH), 459, 389, 358, 343, 340, 269, 249, 215, 204, 188, 187.

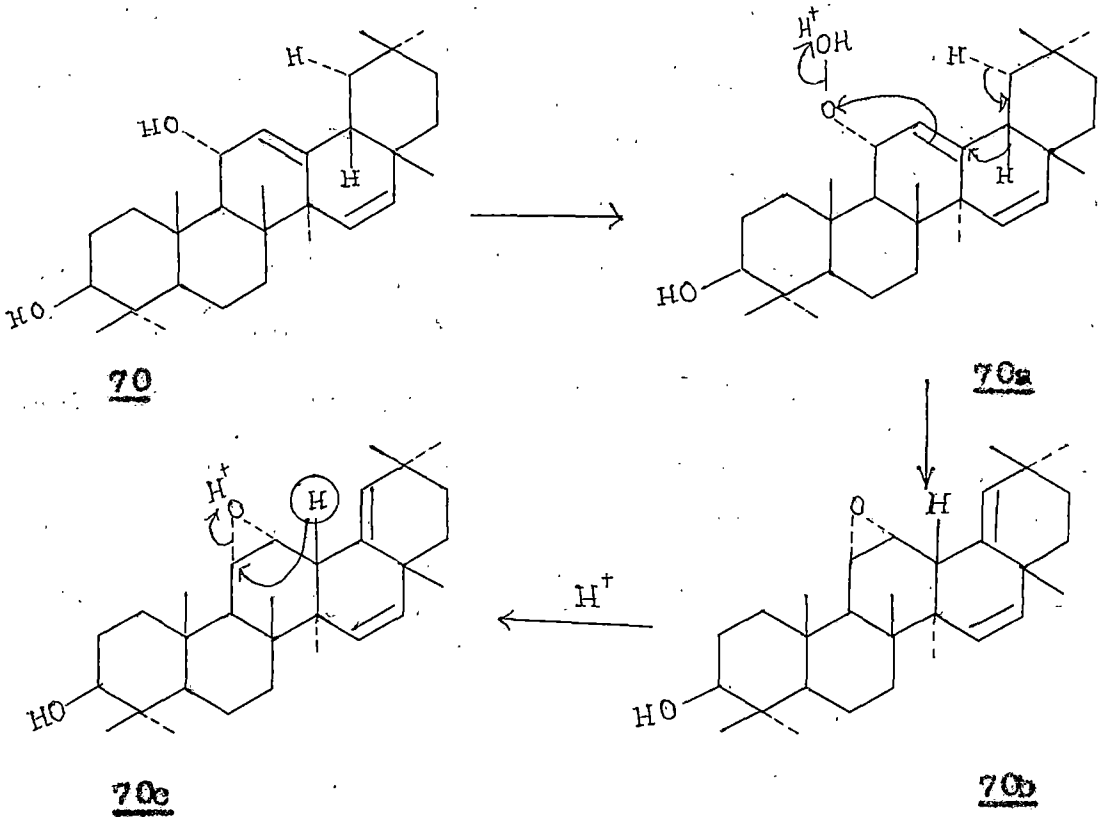
Hence from the above physical data the structure of the triacetate has been assigned as C—12-nor-olean-18-19-en-3β, 12, 15β-triacetate. This confirmed the structure of the second lactone as 86, lithium aluminium hydride reduction and acetylation of which furnished the triacetate 87a.

Mechanism of the formation of two lactones 85 and 86:

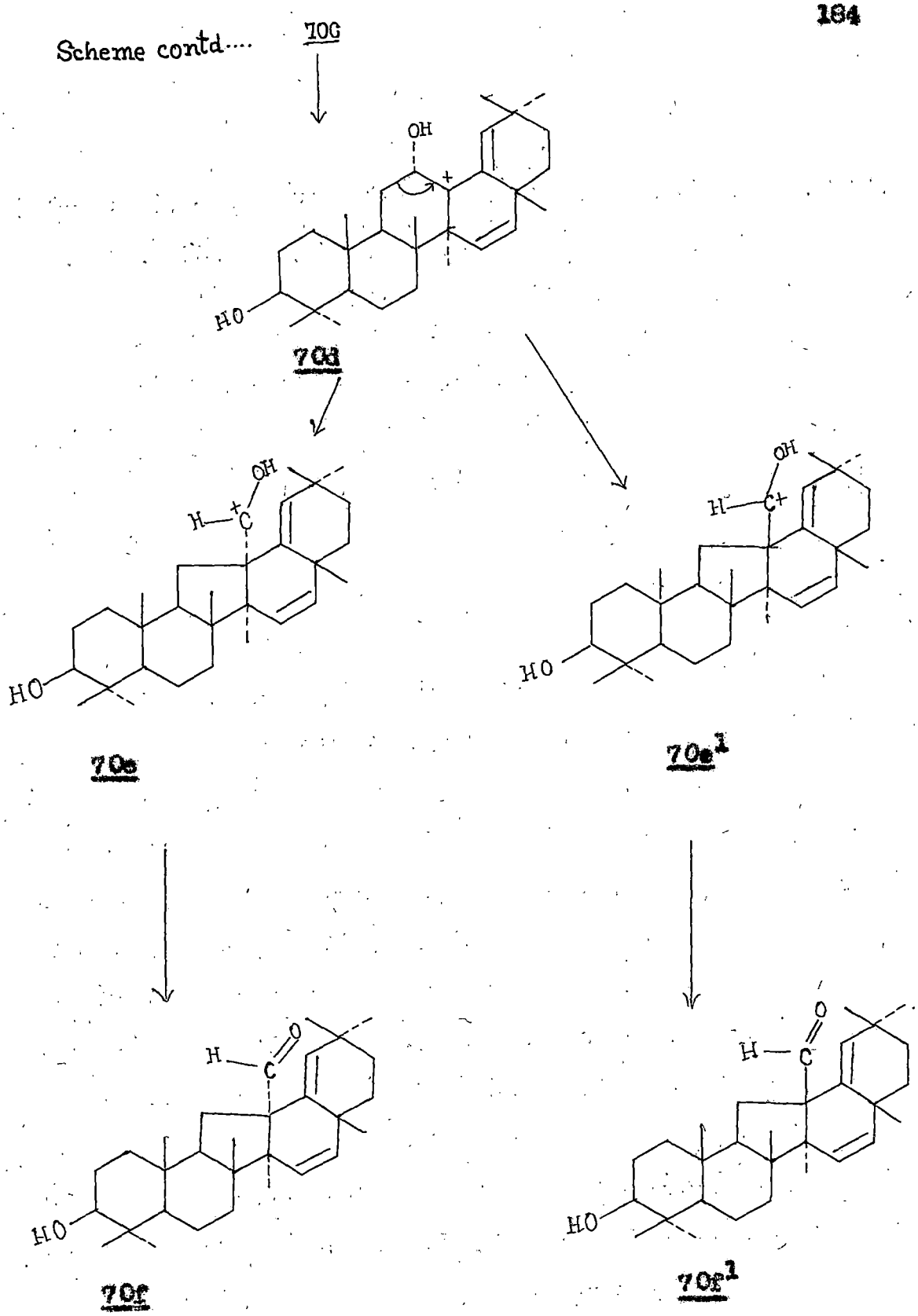
The intermediates formed in the mechanism of formation of two lactones 85 and 86, from olean-12,15-dien-3,11-diol 70, are proposed in scheme-11. Contrary to the formation of the proposed intermediates 71 and 73 (scheme—9) the double bond at C—12—13 is pushed, during the formation of the epoxide 70b from the hydroperoxide 70a towards ring E (to form a germanicol derivative) rather than to ring B or D due to the presence of double bond at C—15—16 position. Under the reaction condition the epoxide 70b is unstable due to the conformational strain caused by the double bonds at C—15—16 and C—18—19 position and hence undergoes epoxide ring opening in presence of acid forming carbonium ion 70d probably by 1,3 hydride shift from C—13 to C—11 as shown in 70c. The intermediate carbonium ion 70d undergoes ring

contraction producing the isomeric cations 70e and 70e[1], which in turn lose a proton to form isomeric aldehydes 70f and 70f[1] respectively. These aldehydes get oxidised in presence of hydrogen peroxide to the corresponding α and β carboxyllic acids at C—15 position. The α -carboxyl group in 70g being at proximity to the double bond at C—18—19, undergoes lactonisation furnishing the γ -lactone 85, whereas the intermediate 70g[1] having its carboxyl group in the β -position is at proximity to the C—15—16 double bond, lactonizes forming 86. The Dreiding model of the lactones 85 and 86, showed that the lactones are strain free, thus stabilising the molecule.

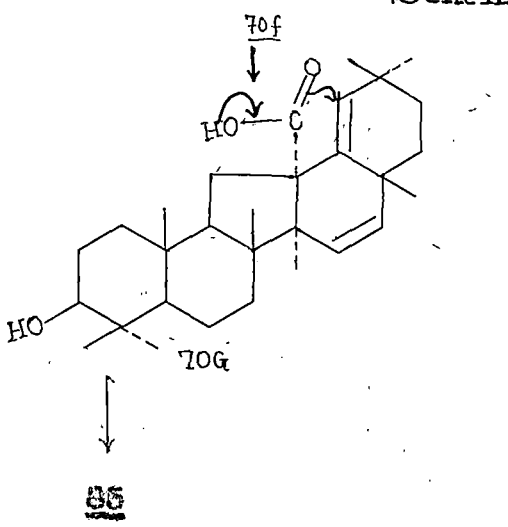
Scheme—11



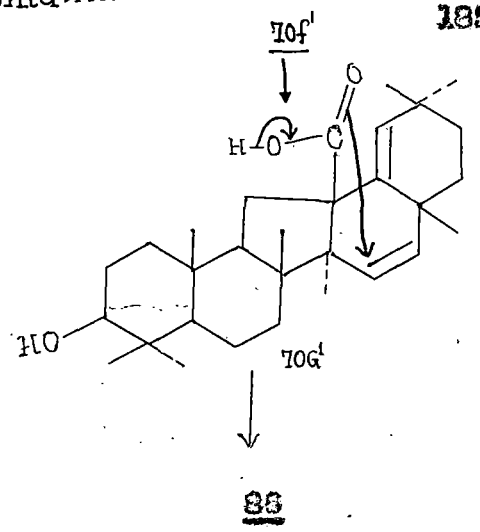
Scheme contd....



Scheme contd.....



86



88

This is perhaps the first report on the formation of a lactone with the carbon skeleton rearrangement in ring C of a triterpenoid with hydrogen peroxide in presence of p-toluene sulphonic acid which has been published by the present author[48] recently.

SECTION—B

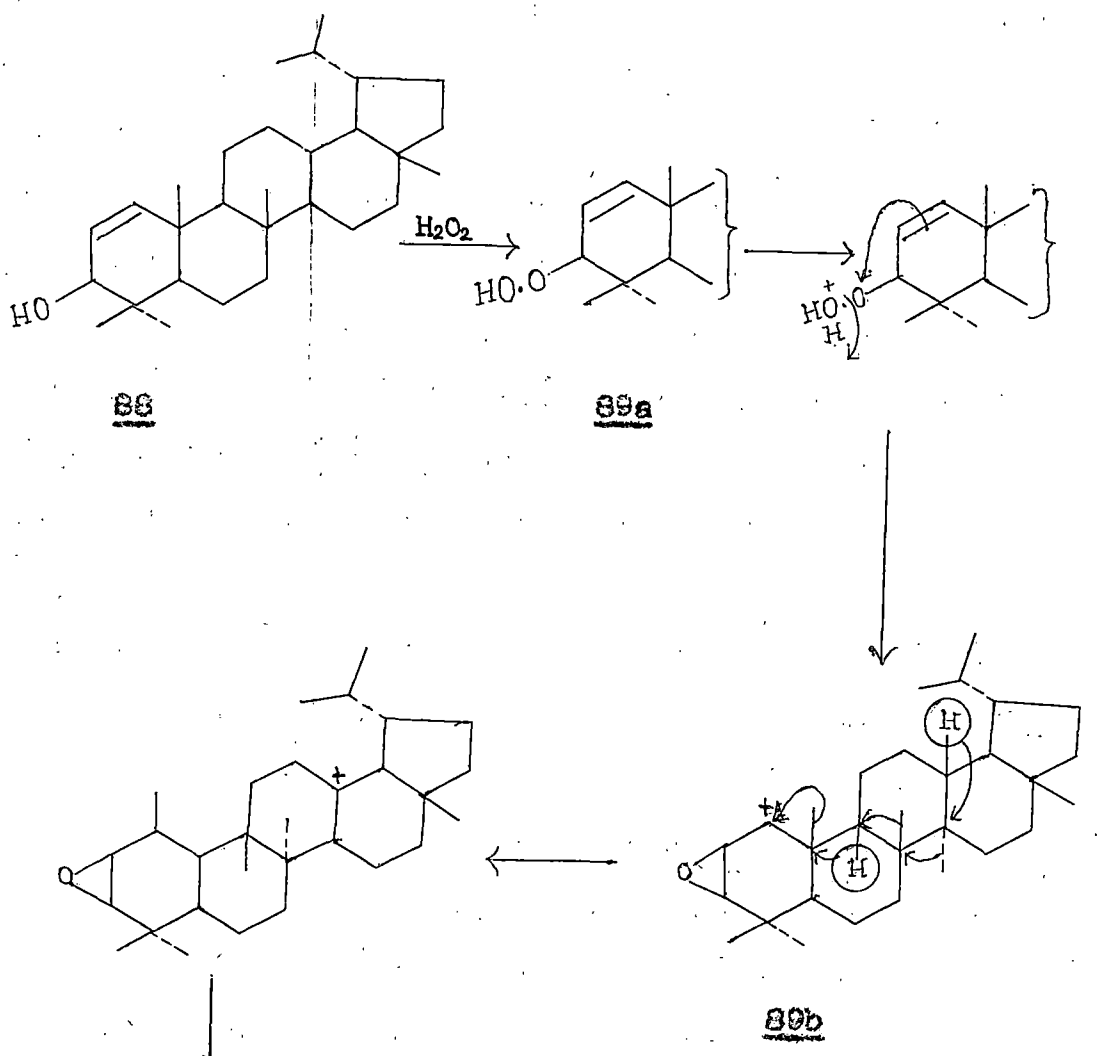
Action of hydrogen peroxide on lup-1(2)-en-3β-ol:

Introduction:

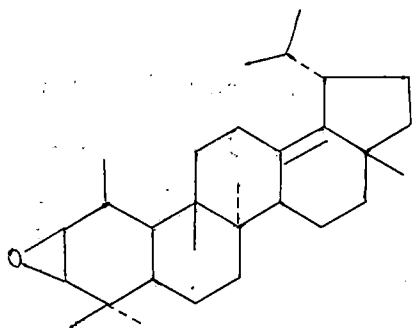
We have discussed in the section A, the action of hydrogen peroxide on olean-12,15-dien-3,11-diol 70, where the allylic alcoholic system in ring C of the triterpene furnished the interesting type of carbon rearrangement followed by lactonisation during the reaction condition. Interested by this observation, we wanted to study similar reaction on another triterpene allylic alcohol. For the model compound we selected the triterpene allylic alcoholic function in Ring A viz. lup-1(2)-en-3β-ol 88. As in the

case of olean-12,15-dien-3,11-diol if the hydroxyl group at C—3 position of lup-1,2-en-3β -ol 88 formed a hydroperoxide intermediate 89a, then it was expected to form an epoxide intermediate 89b, which could then undergo rearrangement to form 90 or 91, as shown in Scheme—12.

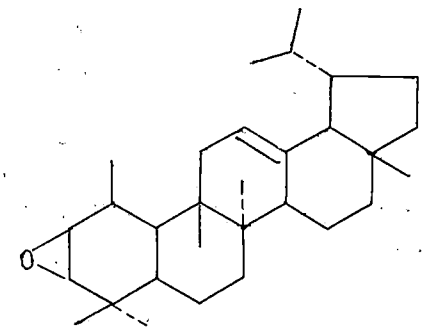
Scheme—12



Scheme contd.....



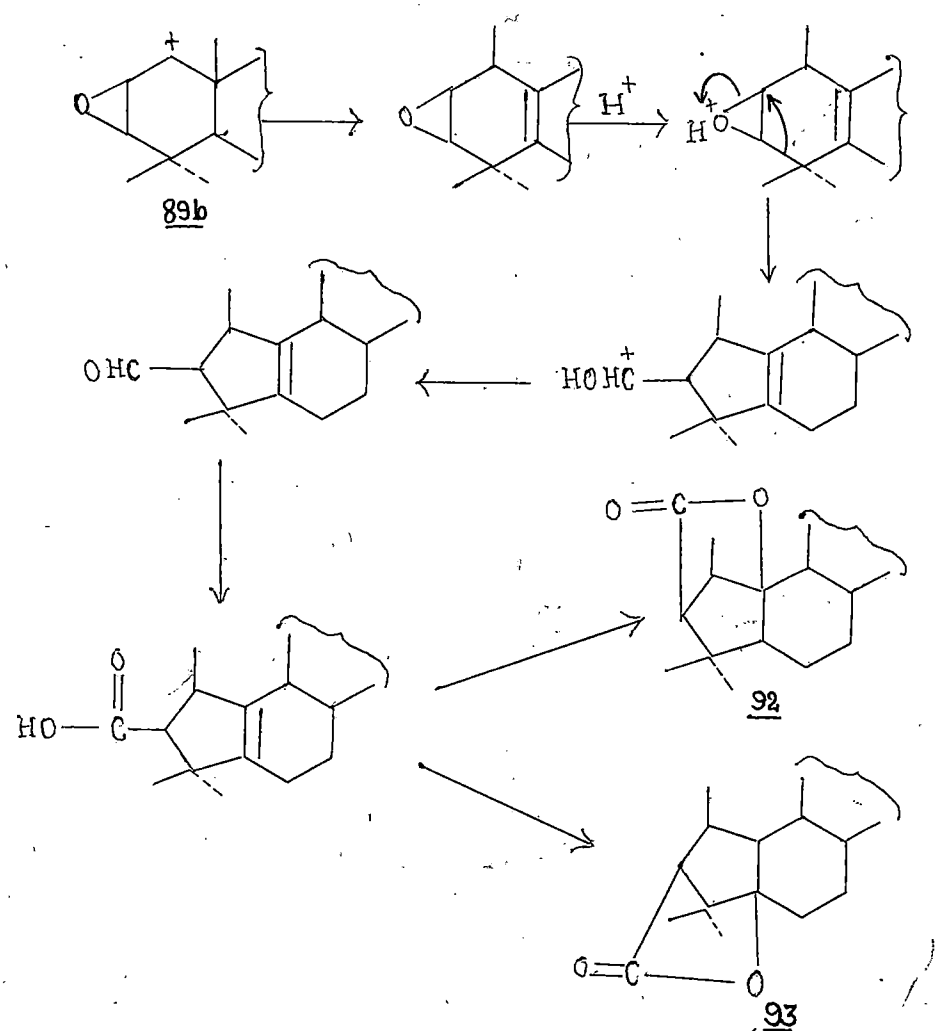
90



91

On the other hand the epoxy carbonium ion intermediate 89b may rearrange in other way to form different lactones 92 and 93 as shown in Scheme—13.

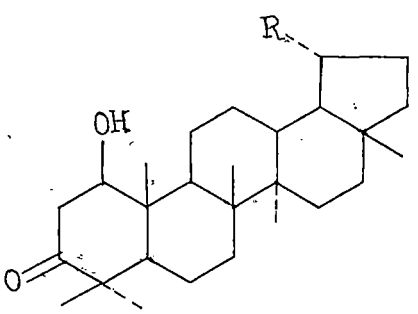
Scheme—13



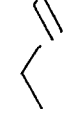
Products were expected like above (Scheme—12 & 13), but in actual practiSe the product obtained was somehow different which is discussed below:

D i s c u s s i o n:

The compound lup-1(2)-en-3β -ol 88 could not be obtained from nature. So first we had to prepare lup-1(2)-en-3β -ol 88, from a related compound which was obtained from nature. The naturally occurring compound selected was glochidonol=lup-20(29)-en-1β -ol-3-one 94, which was isolated from Glochidion-acuminatum[46]. Following the method described by Talapatra and coworkers[46], we have isolated lup-20(29)-en-1β -ol-3-one 94, from the plant Glochidion acuminatum. The glochidonol so obtained was hydrogenated



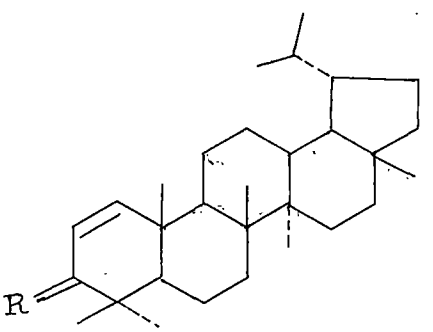
94, R = 

95, R = 

using Adam's catalyst when the isopropenyl double bond was reduced to give an amorphus solid (IR shows absence of double bond). The compound was assigned as 95.

Our aim was to prepare an allylic alcohol at ring A i.e. lup-1(2)-en-3-ol 88. So to prepare 88 from 95 first we had to dehydrate the compound 95 to produce a $\alpha-\beta$ -unsaturated ketone 96 and then reduction of 96 would give the desired product.

Dehydration of the keto alcohol 95, with acetic anhydride and pyridine by the method of Talapatra and co-workers[46], gave a solid which on chromatography followed by crystallisation yielded a compound 96, C, H analysis showed the molecular formula, $C_{30}H_{48}O$, m.p. 177—78° (lit[47] m.p. 177—179°). UV absorption of the compound showed a maxima at λ_{max} 228 nm (ϵ =18,000) which confirmed the presence of a $\alpha-\beta$ -unsaturated carbonyl functional group. IR spectrum (Fig.21) of the compound showed peaks at 1680 cm^{-1} ($\alpha-\beta$ -unsaturated ketone). The compound was identified as lup-1(2)-en-3-one, 95.



96, R = O
88, R = β-OH, α-H

The $\alpha-\beta$ -unsaturated ketone 96 so produced was reduced by lithium aluminium hydride in dry ether. After usual

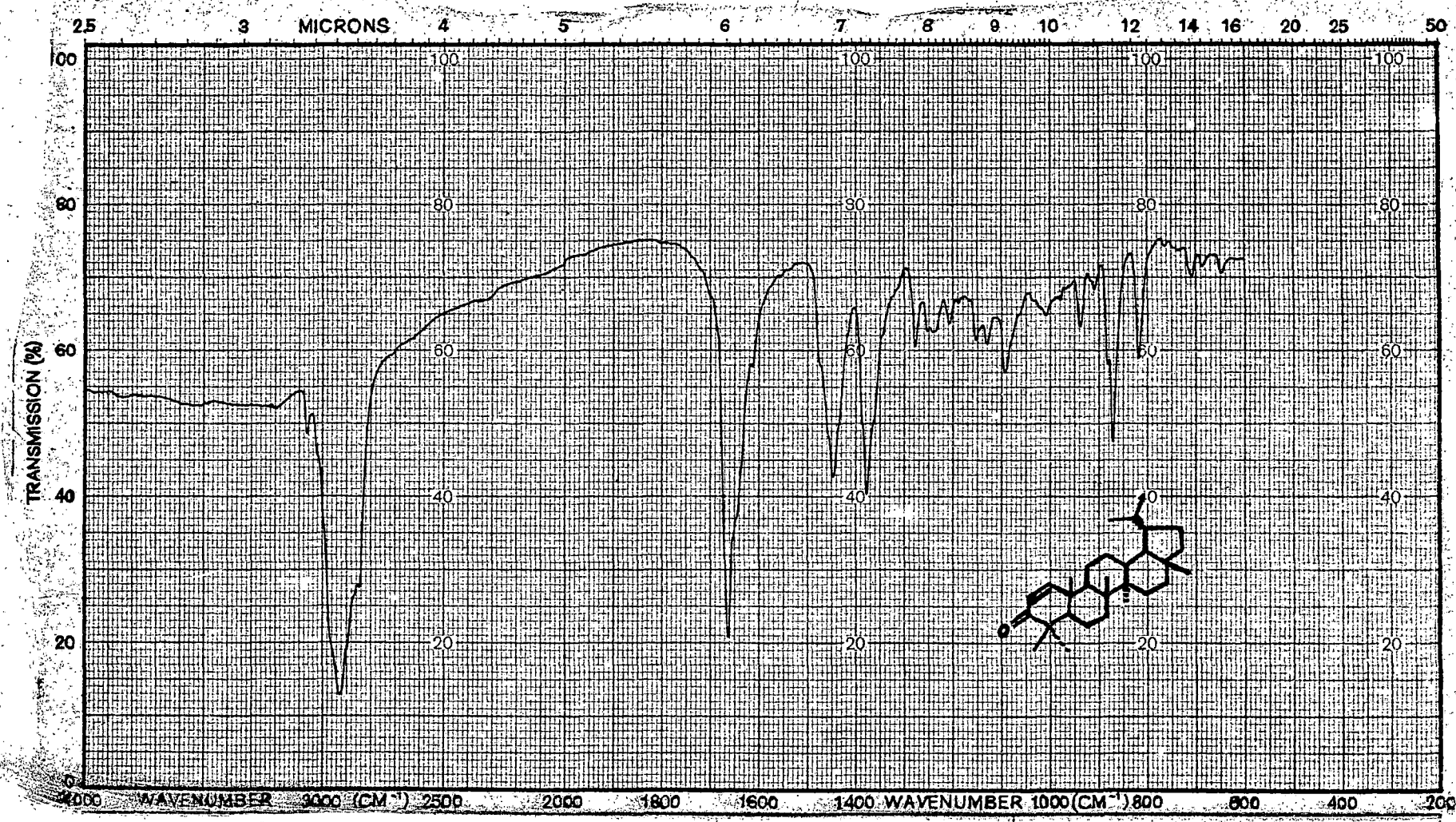


Fig.21: IR spectrum of lup-1(2)-en-3-one, 96.

work up followed by chromatography and crystallisation a compound 88, $C_{30}H_{50}O$, m.p. 314—15° was obtained. No UV absorption was recorded for the compound in the region 220—350 nm. IR spectrum (Fig.22) of the compound showed a peak at ν_{max} 3450 cm^{-1} (—OH), another peak at 880 cm^{-1} for the H—C=C—H was obtained. The ^1H NMR spectrum (Fig.23) of the compound 88 showed peaks in the region 0.7—1.01 ppm for 8-methyl groups present in the molecule. The proton attached to the C—3—OH appeared as a multiplet at 3.25 ppm with $W_{\frac{1}{2}}$ = 16Hz, indicating β-equatorial orientation of C—3 hydroxyl group. A multiplet (quartet type) centred at 4.58 ppm was due to vinyl proton at C—2 and the doublet at 4.69 was due to the vinyl proton at C—1. On the basis of above data the structure of the compound 88 was confirmed.

The allylic alcohol 88 was treated with hydrogen peroxide in presence of p-toluene sulphonic acid in tert-butyl alcohol at room temperature according to method of Corey et al[23]. After stirring for twenty four hours and then usual work up and chromatography afforded a compound 97, $C_{30}H_{50}O_3$, m.p. 280—1° was obtained. No UV absorption was observed for the compound 97. IR spectrum (Fig.24) of the compound 97 showed peaks at ν_{max} 3420 cm^{-1} (—OH) and 1695 (—COOH) cm^{-1}. The structure of 97 was established by the IR, ^1H NMR and mass spectral studies of its methyl ester 98. Esterification

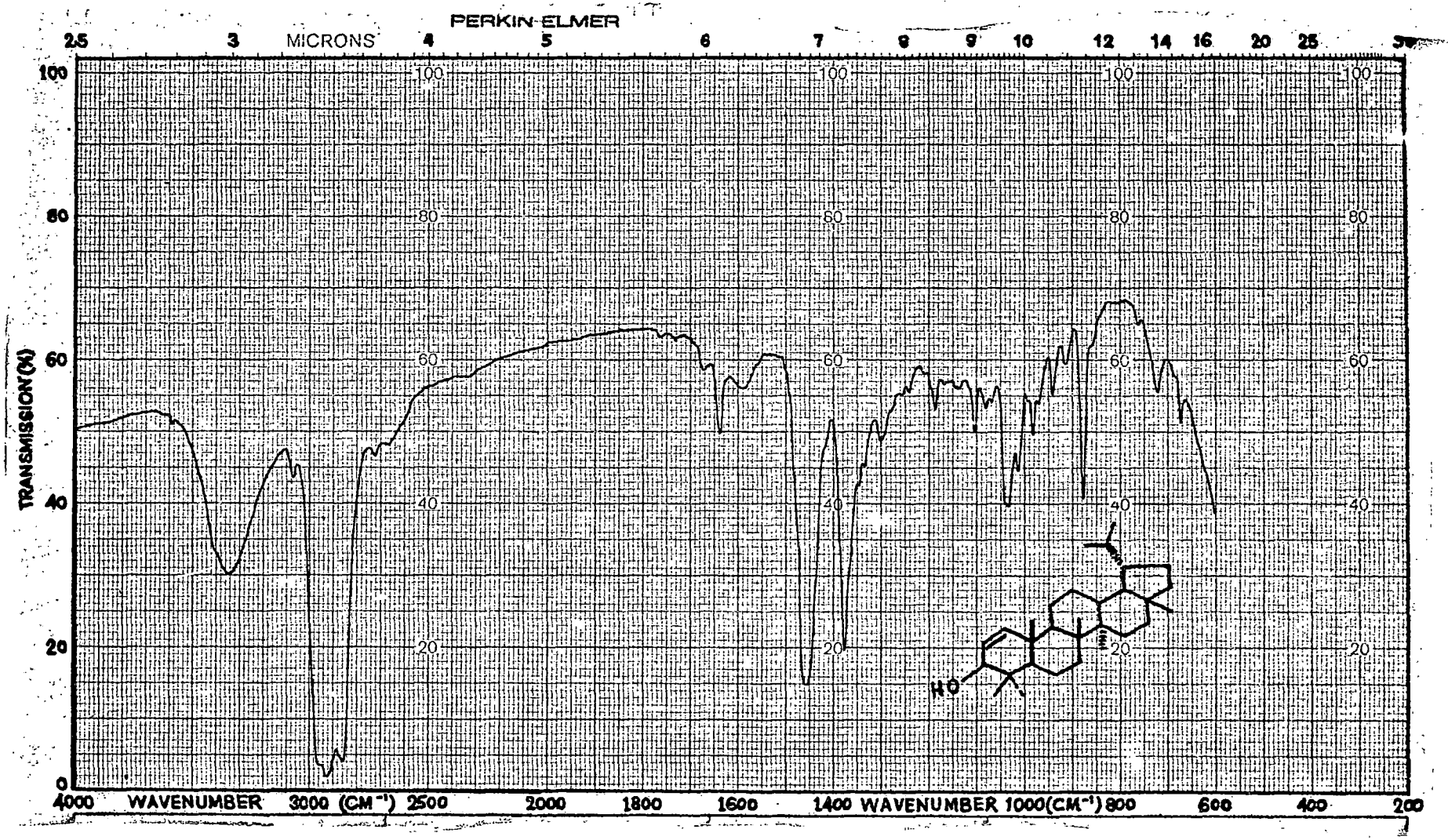


Fig.22: IR spectrum of lup-1(2)-en-3-ol, 88.

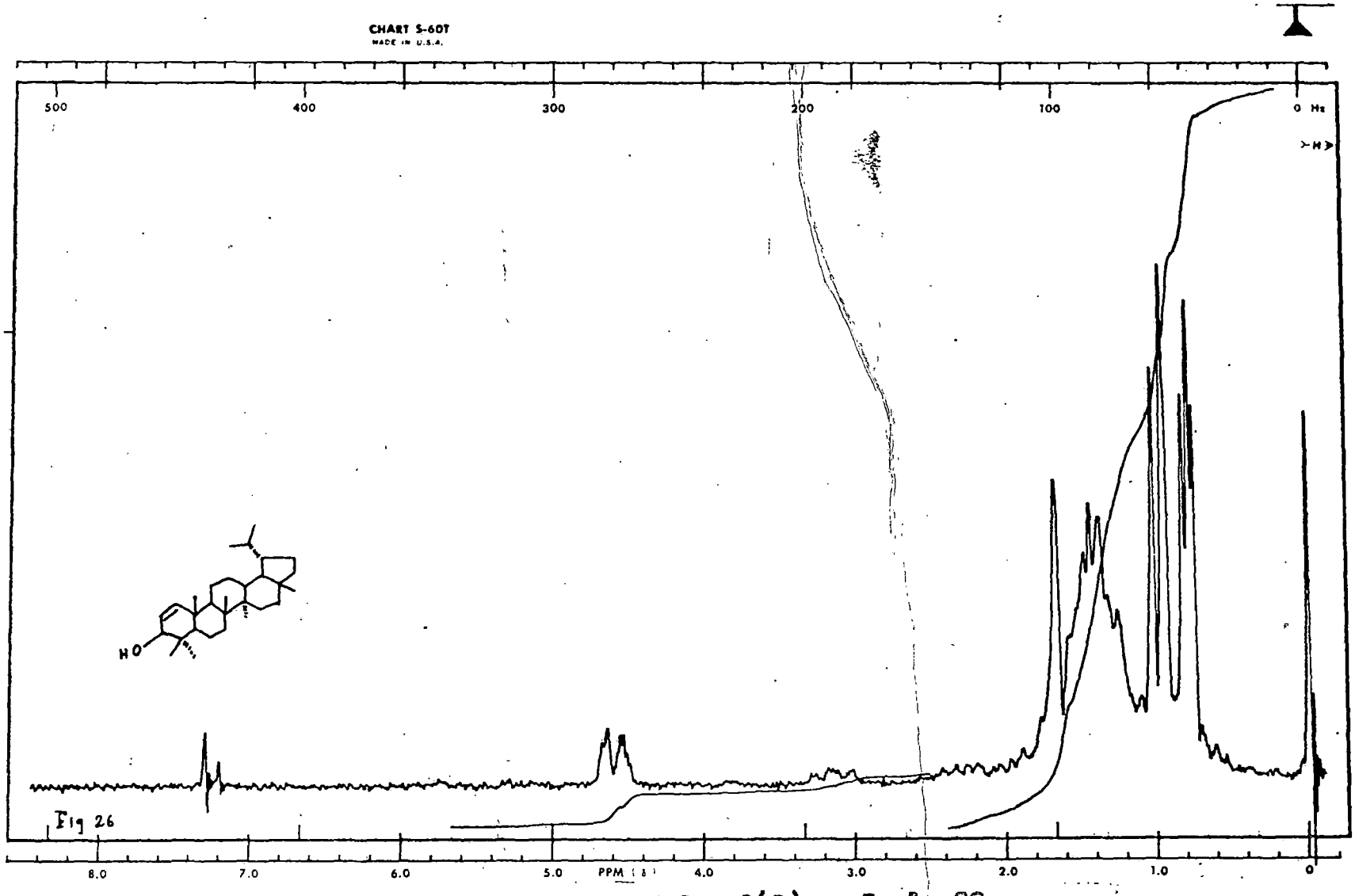


Fig.23: PMR spectrum of lup-1(2)-en-3-ol, 88.

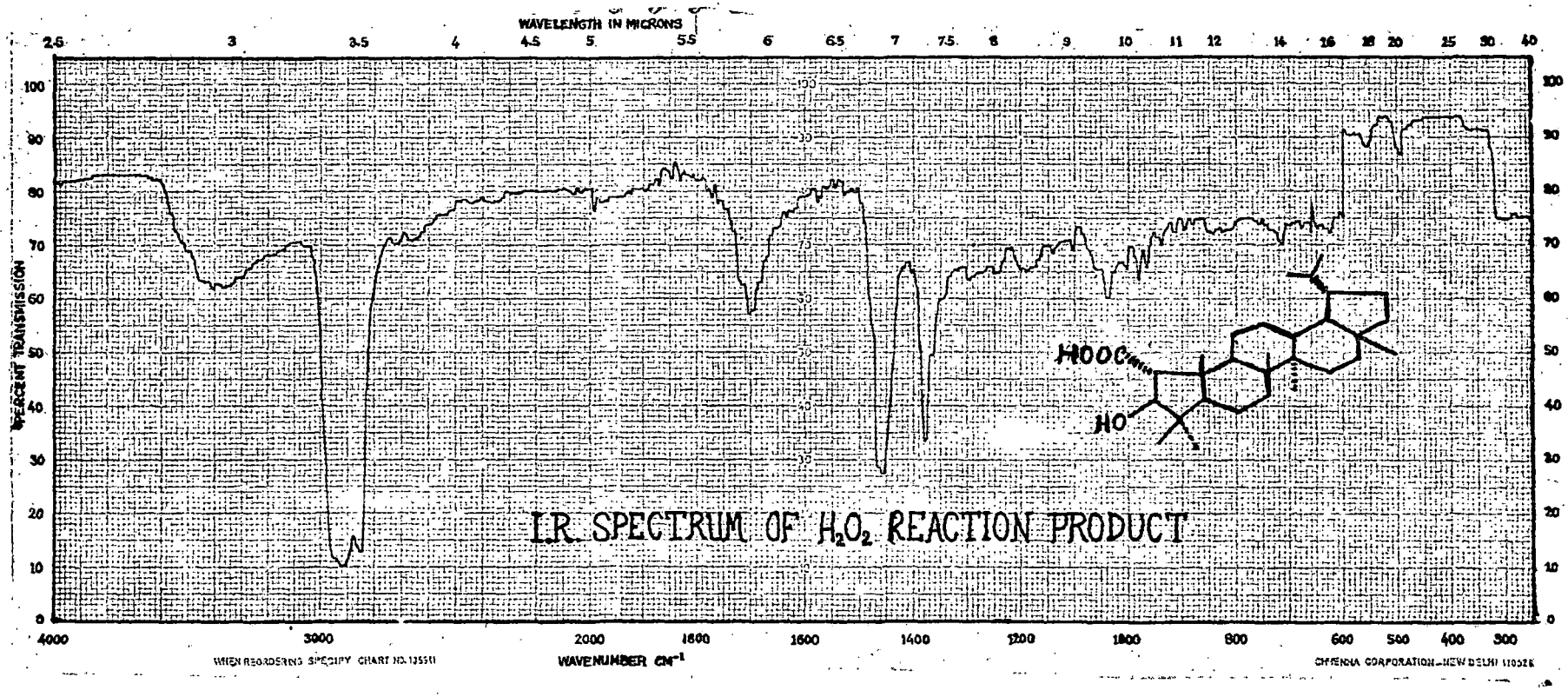
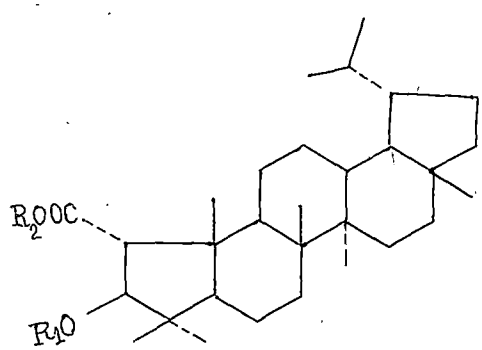


Fig.24: IR spectrum of the hydroxy acid, 57.

of the compound 97 with an ethereal solution of diazomethane afforded the corresponding methyl ester 98, $C_{31}H_{52}O_3$, of m.p. 265—66°. The structure of the methyl ester was established by its IR, ^1H NMR and mass spectral analysis. No UV absorption was observed for the compound in the region 200—350 nm. IR spectrum (Fig.25) of the compound showed peaks at ν_{max} 3380 cm^{-1} and 1735 cm^{-1} due to presence of a hydroxy and a carbomethoxy groups respectively.

^1H NMR spectrum (Fig.26) of the compound showed peaks in the region 0.8 to 1.01 ppm that was integrated for 24 protons of 8-methyl groups present in the molecule. The



97, R_1=H ; R_2=H
98, R_1=H ; R_2=CH_3
99, R_1=Ac; R_2=CH_3

peak obtained at 2.75 ppm was due to proton geminal to the carbomethoxy group (CH_3-O-CO-C<u>H</u>) which was axial from its high J-value (12 Hz) that coupled with the neighbouring proton which was α-axial, that appeared as multiplet in

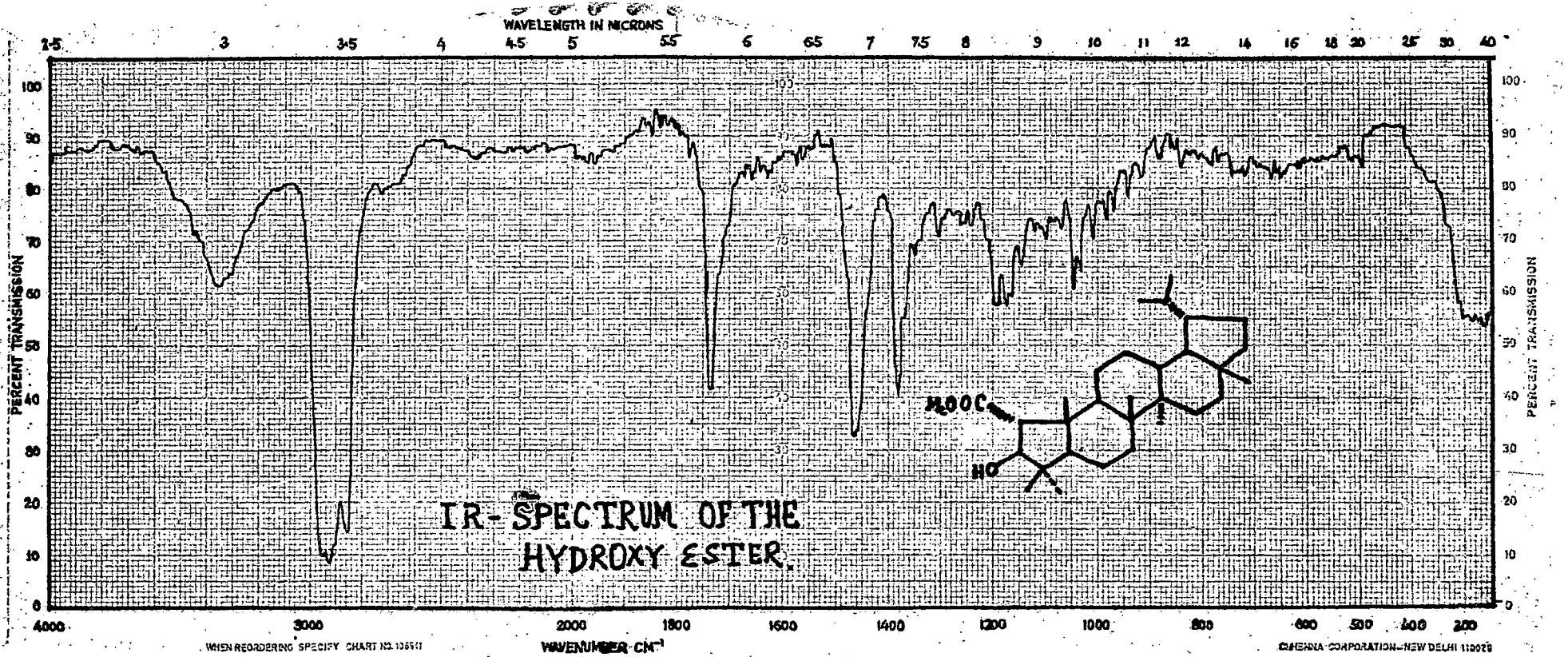


Fig.25: IR spectrum of the hydroxy ester, 98.

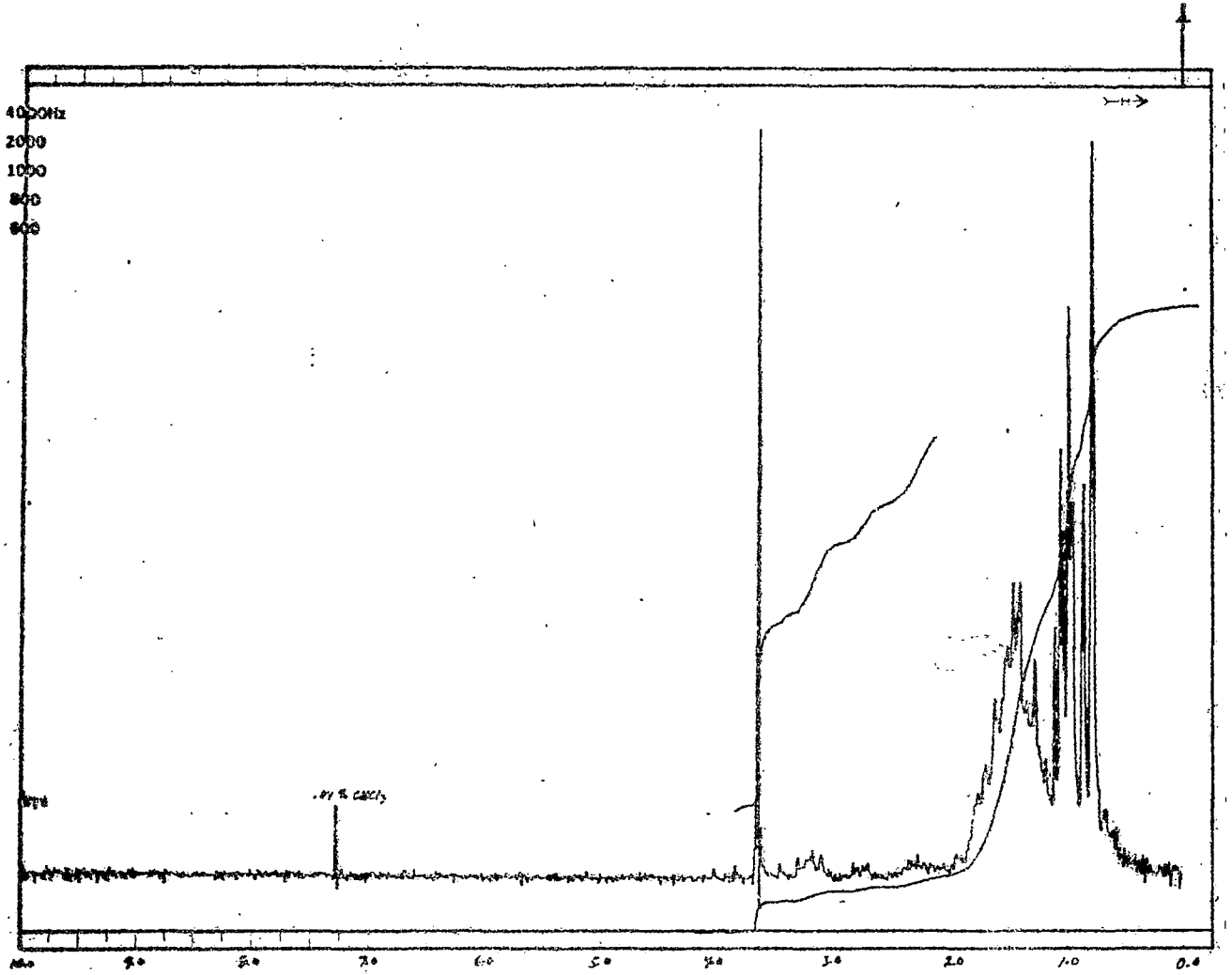
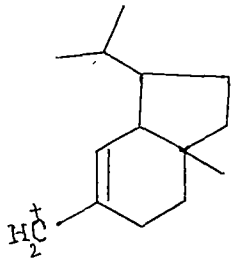


Fig.26: PMR spectrum of the hydroxy ester, 98.

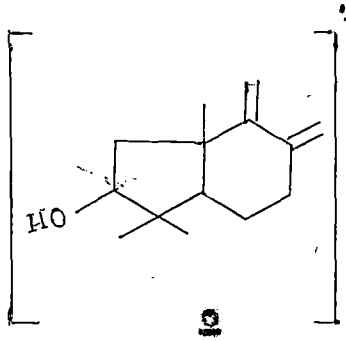
the region 3.2 ppm with the same J-value. The methyl protons in $-COOCH_3$ gave a singlet at 3.65 ppm integrated for three protons. On the basis of ^1H NMR data the structure of the hydroxy ester could be assigned as 98. The structure was finally proved by mass spectral analysis.

Mass spectrum (Fig.27) of the compound 98 showed peak at m/e 473 (MH^+) which was consistent with the molecular formula $C_{31}H_{52}O_3$, other peaks at m/e 455 (base) for MH^+-H_2O, 454 (M^+-H_2O), 439 (m/e 454—CH_3), 411(454—isopropyl), 395(454—$COOCH_3$), 251, 235, 205, 191 were observed. The fragment at m/e 191 could be characterised as the fragment of saturated lupane skeleton[43] by the species m, whereas the fragment at m/e 251 for the species g and m/e 235 by the loss of water from g; The fragment at m/e 205 could be explained by the ion fragment o without the isopropyl group.



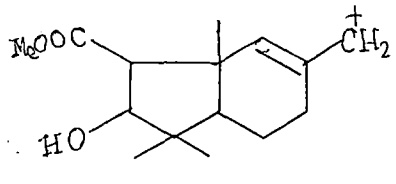
m

m/e 191



o

m/e 205



g

m/e 251

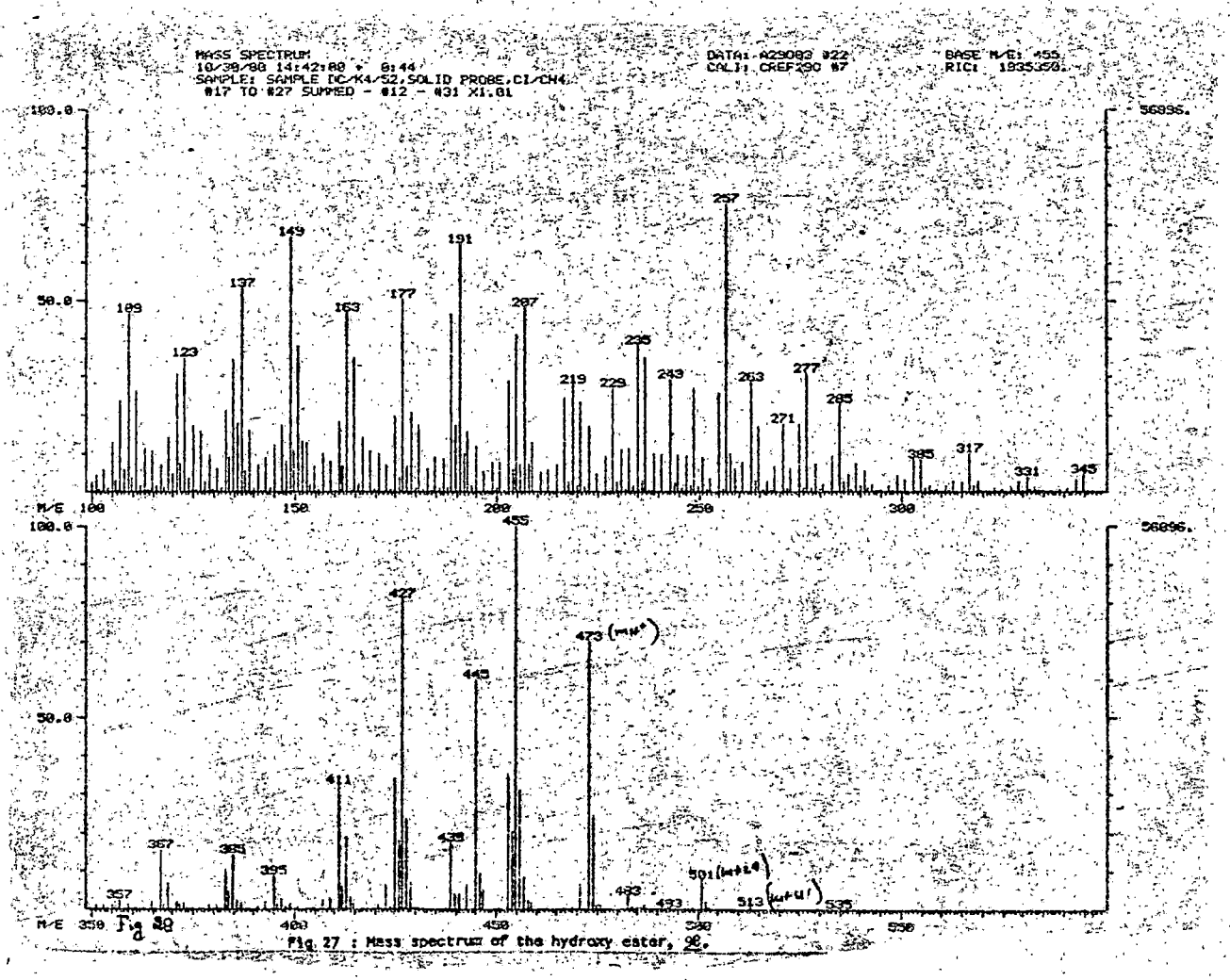


Fig 27 : Mass spectrum of the hydroxy ester, 9c.

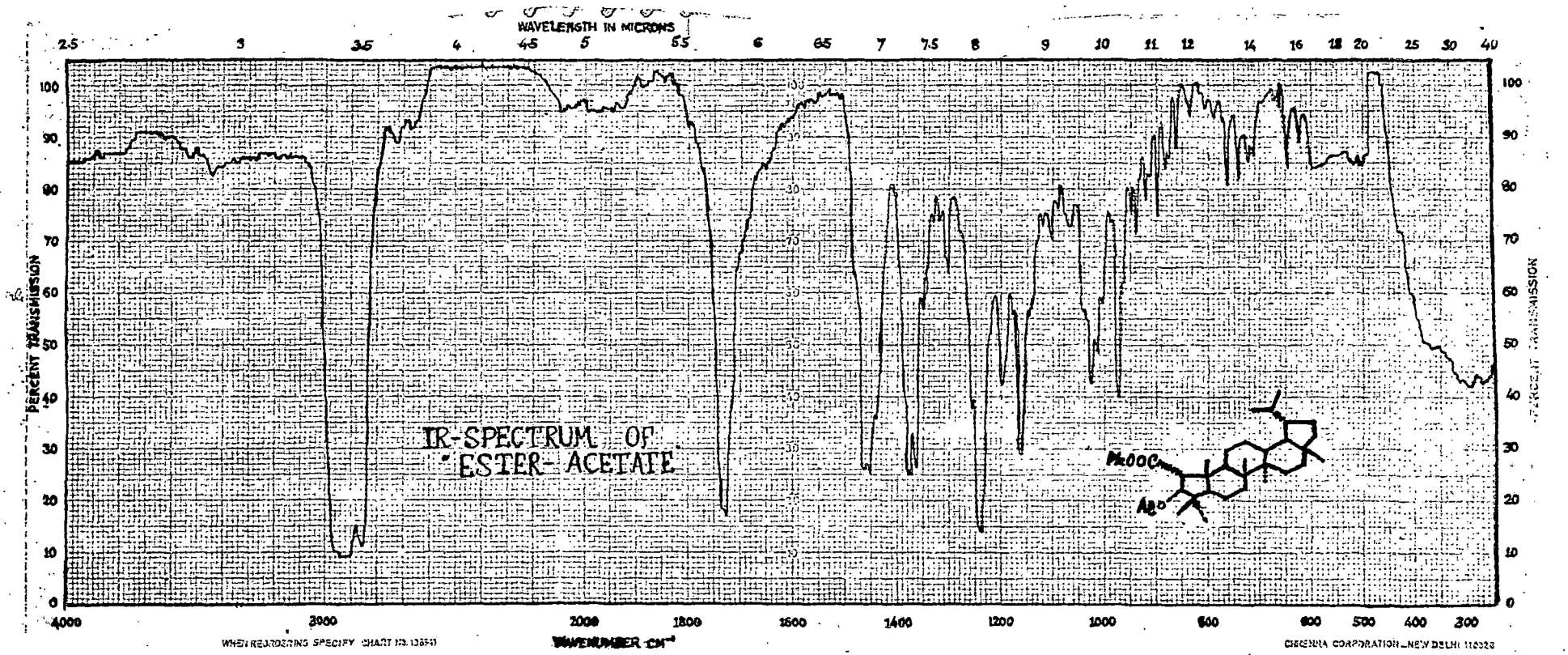


Fig.28: IR spectrum of ester acetate, 99.

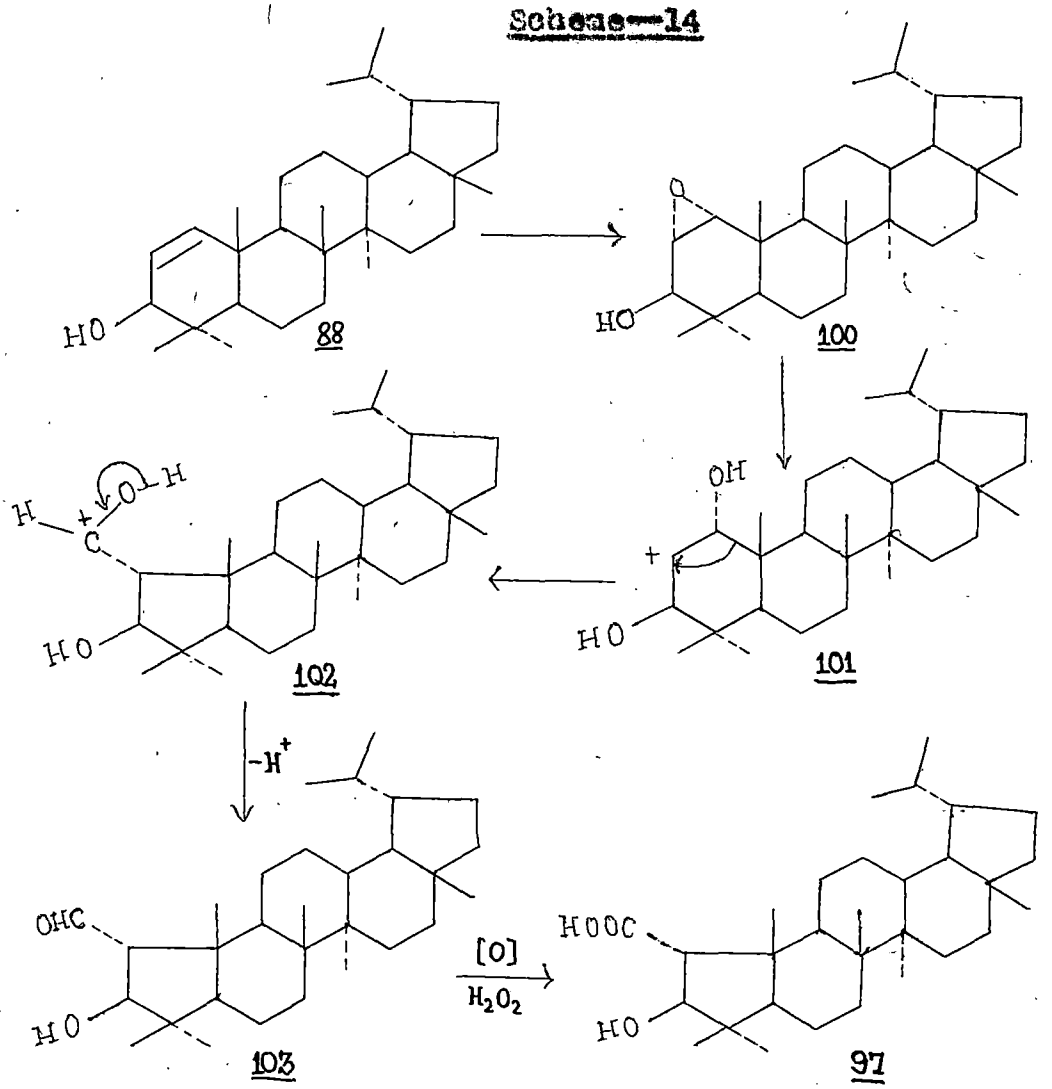
Acetylation of the hydroxy ester 98, with acetic-anhydride and pyridine at room temperature gave the ester acetate which after crystallisation from chloroform-methanol mixture afforded 99, $C_{33}H_{54}O_4$, m.p. 250—1°. IR spectrum (Fig.28) of the compound showed a broad maxima at the region ν_{max} 1700—1740 cm^{-1} which was due to presence of acetoxy and ester group both. A peak at 1240 cm^{-1} confirmed the presence of an acetoxy functional group.

Mechanism of formation of hydroxy acid 97:

In the section (A), the effect of hydrogen peroxide on olean-12,15-dien-3,11-diol 70 was discussed, where the epoxide was formed from the hydroperoxide (Scheme—11) but not directly on C—12—13 double bond. The double bond at C—12—13 position was too much sterically hindered due to the ring juncture along with different sterical effect of C—17 and C—14 CH_3 groups. The double bond was known to be very stable in ordinary reagents. But in the compound lup-1(2)-en-3-ol 38, the epoxide could be formed directly on C—1—2 double bond because the double bond was not too much sterically hindered. Govindachari et al[47] reported that the formation of epoxide on lup-1(2)-en-3-one could be done easily with alkaline hydrogen peroxide. From this idea we have schemed the mechanism as follows (Scheme—14):

First there would be a formation of 3β-hydroxy-epoxide as intermediate 100. The epoxide 100, so formed would be opened in presence of acid and carbonium ion 101 would be formed. The carbonium ion intermediate could undergo ring contraction producing the cation 102, which in turn loses one proton to form the aldehyde 103 and during reaction condition it would be oxidised to the corresponding hydroxy acid 97.

Scheme—14



CHAPTER—III

E X P E R I M E N T A L

Melting points are uncorrected. Petroleum ether used during the investigation had b.p. 60°—80°. All the optical rotations were determined in $CHCl_3$ at 1—2% solutions. The ^1H NMR spectra were recorded in Varian A—60, T—60, FT—80A and XL—100 NMR spectrometers using $CDCl_3$ containing TMS as interval reference. The IR spectra were taken in Beckman IR—20 spectrophotometer in Nujol mull. UV absorption spectra were taken in Beckman DU—2 spectrophotometer using solvent 95% ethanol. Column chromatography were done on neutral alumina deactivated with 10% aqueous acetic acid (4 ml/100 g of alumina).

SECTION—A:

Oxidation of teraxeryl acetate 76 with NBS in DMSO: Preparation of 15-bromo- β -amyrin acetate 77:

To a solution of teraxeryl acetate (1 gm) in $CHCl_3$ (50 ml) and dimethyl sulfoxide (25 ml), was added N-Bromo succinimide (1 gm) in portions and kept in dark for 14 hours. The reaction mixture was filtered and the filtrate was extracted with chloroform (200 ml), washed with water and was dried over anhydrous sodium sulphate. The solvent was

removed when a yellowish solid was obtained. The solid was dissolved in benzene (10 ml) and was poured on a column of alumina (60 gm deactivated with 3.6 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution:

Table—1

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—10	Solid (900 mg) m.p. 176°-180°
Further elution with more polar solvents did not give any solid material		

Fraction 1—10 (table—1) were combined and the mixture was crystallised from a mixture of chloroform and methanol when crystals of 15-bromo-β-amyrin acetate 77, m.p. 180—182°, $[\alpha]_D$ +47.4° were obtained. It gave a single spot on a chromatoplate (R_f = 0.8) and also showed positive Beilstein test for bromine.

Analysis report:

Found, C, 70.0%; H, 9.1%

Calculated for $C_{32}H_{51}O_2Br$ C, 70.23%; H, 9.3%

IR(nujol): ν_{max} 1720 and 1250 cm^{-1} Fig.1

NMR : Peaks at 5.3 (m, Vinyl proton), 4.30 (m, H CBr), 2.09 ($-OCOCH_3$) and 4.53 ($H-COOCH_3$) ppm. Fig.2

Mass : Peaks at m/e 189, 216, 217, 249, 297, 466 (M —HBr), 547(M^+).

Oxidation of 15-bromo- β -amyrin acetate 77: Preparation of 11-oxo-15-bromo- β -amyrin acetate 79:

To a refluxing solution of 15-bromo- β -amyrin acetate (1 gm) in benzene (40 ml) was added slowly a solution of $Na_2Cr_2O_7$ (1 gm) in glacial AcOH (30 ml) with stirring while maintaining the temp. at 70°C. After the addition was complete the stirring was continued for 24 hours maintaining the temperature at 80°—85°. At the end it was cooled and rectified spirit (5 ml) was added. The solution was concentrated to 1/3rd volume and poured into ice cold water. The ppt was filtered off, washed and crystallised

from chloroform-methanol mixture when crystals (0.75 g) having m.p. 240—241° were obtained $[\alpha]_D$ +89°.

Analysis report:

Found, C, 68.24% ; H, 8.63%

Calculated for $C_{38}H_{49}O_3Br$, C, 68.42% ; H, 8.69%

UV λ_{max} 249 nm (ϵ 11,000)

Beilstein test positive.

Attempted dehydrobromination of 11-oxo-15-bromo-β-amyrin acetate 79:

(a) With dimethyl aniline:

Compound (0.5 g) was refluxed with dimethylaniline (75 ml) for six hours. The mixture was cooled, acidified with 6N HCl and then extracted with ether and washed with water to make it acid free. The solution was dried with anhydrous sodium sulphate. Solvent was removed, the residue was then chromatographed in a column of 30 gm alumina. The chromatogram was developed with petroleum ether and the following fractions were collected:

Table—2

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—12	Solid m.p. 229°—230°
Further elution with more polar solvents did not afford any material		

Fractions 1—12 (table—2) were combined. The mass was crystallised from chloroform-methanol mixture when crystals having m.p. 240°—241° were obtained. The compound showed positive Beilstein test. Mixed m.p. with the starting material was also undepressed.

(b) With s-collidine:

Bromo compound (0.2 g) was refluxed with s-collidine (15 ml) for 18 hrs. at 180° in an oil bath. The mass was then acidified with 5% H_2SO_4, extracted with ethyl acetate. The organic layer was washed with 5% H_2SO_4 to remove last trace of collidine, washed with water to make organic layer neutral. The solvent was dried over anhydrous sodium sulphate and removed. The residue dissolved

in minimum amount of benzene was chromatographed in a column of alumina, and the following fractions were collected:

Table—3

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—10	Solid m.p. 230°—1°

Further elution with more polar solvents did not afford any solid material

Fractions 1—10 (table—3) were combined and crystallised from chloroform-methanol mixture. The crystals had the m.p. 240—241°. Mixed m.p. with the parent compound and Beilstein test indicated that dehydrobromination did not occur.

Dehydrobromination of 15-bromo-β-amyrin acetate ?? with dimethyl aniline: Preparation of olean-12,15-diene-3β-yl acetate 81:

15-Bromo-β-amyrin acetate (0.2 g) was refluxed with distilled dimethyl aniline (30 ml) for six hours. The reaction mixture was diluted with water, acidified with 6N

hydrochloric acid and was extracted with ether. The ether extract was washed with water till neutral and was dried with sodium sulphate. The solvent was removed when a solid (0.17 g) was obtained. The solid was dissolved in minimum amount of benzene and the solution was poured on a column of alumina (15 g) developed with petroleum ether. The following fractions were collected:

Table—4

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1—5	Solid (0.155 g) m.p. 195—197°

Further elution with more polar solvents did not afford any solid material

Fraction 1—5 (table—4) combined and crystallised from chloroform methanol mixture when crystals of S1, m.p. 199°—200°, $[\alpha]_D$ +42° were obtained.

Analysis report:

Found, C, 81.9% ; H, 10.8%

Calculated for $C_{32}H_{50}O_2$, C, 82.35% ; H, 10.8%

UV : No absorption in the region 220—300 nm.

IR : ν_{max} 1730, 1240 cm^{-1} (Acetate)
875 cm^{-1} (—CH = CH—)
820 cm^{-1} (trisubstituted double bond)
Fig.3

NMR : 5.2 to 5.6 ppm for three vinyl protons accounting for one trisubstituted and one disubstituted double bond. 2.03 ($OCOCH_3$), 4.5 ($\underline{H}$—O—$OCOCH_3$) ppm Fig.4

Oxidation of olean-12,15 dien-3β yl-acetate 81; preparation of 11-oxo-olean-12,15-dien-3β -acetate 80:

A solution of sodium dichromate (4 gm) in glacial acetic acid (100 ml) was added slowly during a period of one hour to a vigorously stirred solution of olean-12,15-dien-3β -acetate (4 gm) in refluxing benzene (50 ml). The mixture was refluxed for 24 hours and then cooled. Excess of dichromate was decomposed with rectified spirit (50 ml). The solution was concentrated to 1/3 rd volume and the contents poured into ice cold water. Then it was extracted

with solvent ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed. The residue (4 gm) was dissolved in minimum amount of benzene and was poured on a column of alumina (240 g), developed with petroleum ether. The following solvents were used for elution:

Table—5

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—5	Nil
Petroleum ether: Benzene (4:1)	6—10	Nil
Petroleum ether: Benzene (3:2)	11—18	Solid m.p. 238—240°

Further elution with more polar solvents did not afford any solid material

Fractions 11—18 (table—5) were combined and the mass was crystallised from chloroform-methanol mixture when needle-shaped crystals of 3β-acetoxy olean-12,15-dien-11-one 80, m.p. 243°—245° $[\alpha]_D$ +28.8° were obtained.

Analysis report:

Found, C, 79.86% ; H, 9.95%

Calculated for $C_{32}H_{48}O_3$, C, 79.95% ; H, 10.06%

UV : λ_{max} 244 mm (ϵ 11,376)

IR : ν_{max} 1730, 1240 cm^{-1} (Acetate)
1660 cm^{-1} (6 membered ring $\alpha\beta$ unsat. ketones)
890 cm^{-1} (—CH=CH—) Fig.5

NMR : 0.82—1.04 (6, t-Me protons),
2.04 (s, 3H, -OOC$\underline{H}_3$)
2.25 (dd, 1H, C-16 β -H)
2.42 (s, 1H, C, 9α-H)
2.82 (t, of d, 1H, C-1 β -H)
4.45 (t, 1H $\underline{H}$-C-O-CO-)
5.38 (s, 2H, 2 vinyl H)
5.64 (s, 1H, 1 vinyl H) ppm Fig.6

Lithium aluminium hydride reduction of 11-oxo-olean-12,15-dien-3 β yl acetate 80; preparation of olean-12,15-dien-3 11 diol 70 :

A solution of keto acetate 80 (2.5 gm) in dry benzene (30 ml) and dry ether (70 ml) was refluxed with LAH (5 gm) for six hours followed by stirring at room temperature for another twelve hours. Excess LAH was

destroyed by adding water dropwise at room temperature. The ethereal layer was separated and the aqueous layer was extracted with ether and the two ether solutions were mixed. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed and the residue (1.9 gm), of olean-12,15-dien-3,11-diol 70, m.p. 198—200° was obtained. TLC single spot.

IR : ν_{max} 3460, 890, 860, 820, 750 cm^{-1} Fig.7

Treatment of olean-12,15-dien-3,11-diol 70, with hydrogen peroxide in para toluene sulphonic acid; Isolation of two lactones 85 and 86:

To a solution of olean-12,15-dien-3,11-diol (1.9 gm) in methylene chloride (100 ml) was added a solution (85 ml) prepared by mixing para toluene sulphonic acid (3 g) and 30% H_2O_2 (5 ml) in tert-butanol (80 ml). The reaction mixture was stirred slowly for 24 hours and then poured into water. It was then extracted with methylene chloride, washed with water. The methylene chloride was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The residue (1.5 gm) was absorbed in an alumina (80 gm) column. The column was developed with petroleum ether and the following solvents were used for elution:

Table—6

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—4	Trace oil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Trace oil
Petroleum ether:benzene (2:3)	13—17	Nil
Petroleum ether:benzene (1:4)	18—20	Nil
Benzene	21—40	Solid 220°—30°

Further elution with more polar solvents did not afford any solid material

Fraction 21—40 (table—6) were mixed and found to be a mixture of two compounds. (From TLC, R_f values of 85 = .49; 86 = .46 in EtOAC). The mixture was repeatedly crystallized from chloroform-methanol when 85 (0.3 g) separated as the less soluble part. Further three times crystallization of 85 from chloroform-methanol afforded pure 85, (no increase of m.p. by further crystallisation), m.p. = 240°—41°.

Analysis report:

Found, C, 78.94% ; H, 9.89%

Calculated for $C_{30}H_{46}O_3$, C, 79.25% ; H, 10.20%

UV : No absorption in the region 220—300 nm

IR : ν_{max} 3520 cm^{-1} (free -OH group),
875 cm^{-1} (—CH=CH—),
1775 cm^{-1} (lactone) Fig.8

Acetylation of C-12-nor olean-15-en-13 α -carb⟶ 19 α -olide-3 β -ol 85; Preparation of 3 β -acetate of C—12-nor olean-15-en-13 α -carb⟶ 19 α -olide 85a:

The hydroxy lactone (0.1 g) was dissolved in dry pyridine (1 ml) and the solution was treated with acetic anhydride (2 ml). The mixture was then heated on water bath for 4 hours and then poured into ice cold water and then filtered. The residue was washed with water and dried under suction. The product was then crystallised from chloroform methanol mixture when crystals of 85a, $C_{32}H_{48}O_4$, m.p. 215°—16° were obtained.

Analysis report:

Found, C, 77.25% ; H, 9.80%

Calculated for $C_{32}H_{48}O_4$, C, 77.33% ; H, 9.74%

UV : No absorption in the region 220°—330° nm

IR : ν_{max} 1720 cm^{-1}, 1250 cm^{-1} ($OCOCH_3$)
1780 cm^{-1} (γ-lactone) Fig.9

Mass : m/e, 496 (M^+), 468 (M^+—CO),
452 (M^+—CO_2), 436 (M^+—ACOH) Fig.15

Isolation of the second lactone 86:

The more soluble fraction was crystallized twice from chloroform-methanol and the purer product was isolated from the filtrate (0.2 g), of m.p. = 253—57°, molecular formula, $C_{30}H_{46}O_3$.

Analysis report:

Found,	C, 79.45% ; H, 9.78%
Calculated for $C_{30}H_{46}O_3$,	C, 79.25% ; H, 0.20%

IR(nujol): ν_{max} 3520 cm^{-1} (free OH group),
875 cm^{-1} (—CH=CH—),
1775 cm^{-1} (γ-lactone)

Mass: m/e, 454 (M^+), 436 (M^+—H_2O), 426 (M^+—CO),
410 (M^+—CO_2).

Acetylation of lactone 86; Preparation of 3β-acetate of C-12-nor-olean-18(19)-en-13β-carb⟶15β-olide 86a:

0.1 gm of the lactone 86 was taken in 1 ml of dry pyridine and 1 ml acetic anhydride was added to it. The mixture

was kept on water bath for four hours. Then it was cooled and poured into ice cold water. The ppt. was separated out and filtered. The residue was washed with water and dried over suction. The mass was crystallized from chloroform-methanol mixture which gave the acetate 86a, m.p. 228°—229°.

Analysis report:

Found, C, 77.38% ; H, 9.94%

Calculated for $C_{32}H_{48}O_4$, C, 77.30% ; H, 9.74%

IR(nujol): 1720 cm^{-1}, 1250 cm^{-1} ($OCOCH_3$), 1780 cm^{-1} (γ-lactone) Fig.16

Mass : m/e 496 (M^+), 468 (M^+—CO), 452 (M^+—CO_2), 436 (M^+—ACOH).

Lithium aluminium hydride reduction of lactone 86:

200 mg of the lactone 86 was taken in 50 ml dry ether, 0.4 gm LAH was added. The mixture was refluxed for a period of 4 hours. Excess of LAH was decomposed with a saturated solution of sodium sulphate. The product was extracted with ether. The ethereal solution was washed with water and dried over anhydrous sodium sulphate. The solvent was distilled off and the residue when recrystallized from acetone furnished the triol 87, $C_{30}H_{52}O_3$, m.p. 280°—282° (TLC single spot).

Analysis report:

Found, C, 78.42% ; H, 10.84%

Calculated for $C_{30}H_{52}O_3$, C, 78.56% ; H, 10.99%

Acetylation of triol 87; Preparation of the triacetate 87a:

120 mg of the triol 87 was dissolved in 2.0 ml of dry pyridine. To the mixture 2.0 ml of acetic anhydride was added and the reaction mixture was kept on water bath for 12 hours. Then it was cooled and poured into ice cold water when solid separated out. It was filtered, washed with water and dried under suction. It was then crystallized from chloroform methanol to afford the triacetate 87a (0.1 g), $C_{36}H_{58}O_6$, m.p. 190°—193°. It gave single spot in TLC.

Analysis report:

Found, C, 72.96% ; H, 10.52%

Calculated for $C_{36}H_{58}O_6$, C, 73.68% ; H, 9.96%

UV : No UV absorption in the region 220—300 nm

IR : ν_{max} 1740 cm^{-1}, 1250 cm^{-1} (Acetate group)

Fig.17

NMR : 0.5 to 1.5 ppm (tertiary methyl groups) 2.05 ppm ($-OOCCH_3$); 5.25 (vinyl protons)

Fig.18

Mass : m/e 584 (M^+), 524 (M^+-AcOH), 464 (M^+-AcOH)

Fig.20

SECTION—B:

Isolation of glochidonol from Glochidon accuminatum:

10 gms of the benzene extract of Glochidon accuminatum was dissolved in minimum amount of benzene and was absorbed in a silica gel column (60 g). The column was eluted with the following solvents:

Table—7

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—4	Oil (3 g)
Petroleum ether:benzene (4:1)	5—8	Solid (0.5 g)
Petroleum ether:benzene (3:2)	9—13	Solid (1 g)
Petroleum ether:benzene (2:3)	14—20	Nil
Petroleum ether:benzene (1:4)	21—30	White solid (0.5 g)

Further elution with more polar solvents did not afford any solid material

Fractions 21—30 (table—7) were mixed and the mixture was crystallized from chloroform-methanol when crystals of m.p. 227° [Lit. m.p. 228°] was identified as Glochidonol 94; IR (nujol); ν_{max} at 3390, 1710, 1449, 1389 and 898 cm^{-1}.

Preparation of lup-3-one-1-ol, 95:

Glochidonol (900 mg), dissolved in ethyl acetate (200 ml) and acetic acid (200 ml), was stirred in presence of Adam's catalyst in an atmosphere of hydrogen till the absorption ceased. The catalyst was removed by filtration and the filtrate was washed with water till neutral. It was dried and the solvent was removed. A solid residue (850 mg) was obtained which after crystallisation from methanol furnished an amorphus solid identified as lup-3-one-1-ol 95.

Dehydration of lupane-3-one-1-ol 95 by acetic anhydride-pyridine; Preparation of lup-1(2)-en-3-one 96:

800 mg of dihydroglochidonol, 95 was taken in 8 ml dry and distilled pyridine and to the reaction mixture 8 cc of acetic anhydride was added and it was kept on water bath for 24 hours. After 24 hours the mixture was cooled and was poured into ice cold water where a solid separated out

which was filtered. The ppt. was washed several times with water and dried under suction. The mass was dissolved in minimum amount of benzene and poured into an alumina column (30 g). The column was eluted with the following solvents:

Table—8

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1—15	White solid m.p. 165°—166°

Further elution of more polar solvents did not afford any solid material

Fractions 1—15 (table—8) were combined and crystallized from chloroform-methanol mixture to afford crystals of m.p. 177—178° (lit. m.p. 177—179°).

Analysis report:

Found, C, 84.83% ; H, 11.37%

Calculated for $C_{30}H_{48}O$, C, 84.84% ; H, 11.39%

IR(nujol) : ν_{max} at 1680 cm^{-1} ($\alpha\beta$ unstd. carbonyl)

Fig.21

Lithium aluminium hydride reduction of lup-1(2)-en-3-one 96; Preparation of lup-1(2)-en-3-ol 88:

650 mg of the $\alpha\beta$-unsaturated keto compound 96 dissolved in 500 ml dry ether, 1 gm of LAH was added and the mixture was refluxed for 4 hours. The mixture was cooled and excess LAH was decomposed with saturated solution of sodium sulphate. It was then extracted with ether and washed with water. The washed ether solution was dried with anhydrous sodium sulphate. Removal of ether gave a mass which was dissolved in minimum amount of benzene and poured into a silica gel column (30 gms) developed with petroleum ether. The column was eluted with the following solvents:

Table—9

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—20	White solid m.p. 208°—209°

Further elution of more polar solvents did not afford any solid material

The fractions 9—20 (table—9) were mixed and crystallised from chloroform-methanol, when the crystals of m.p. 215° were obtained. TLC single spot.

Analysis report:

Found, C, 84.43% ; H, 11.80%

Calculated for $C_{30}H_{50}O$, C, 84.44% ; H, 11.81%

UV : No UV absorption in the region 220—300 nm

IR(nujol): λ_{max} 3450 cm^{-1} (—OH group), 880 cm^{-1} (double bond) Fig.22

NMR : 0.7—1.01 (8 methyl groups), 3.25 (m, H-C-OH), 4.58 (m, 1H vinyl proton), 4.69 (d, 1H vinyl proton) ppm. Fig.23

Treatment of lup-1(2)-en-3-ol 88, with hydrogen peroxide in acid medium:

To 0.8 gm of lup-1(2)-en-3-ol 88 in 25 ml of methylene chloride, was added a mixture of 1.5 ml of 30% H_2O_2 and 20 ml of tertiary butyl alcohol. Then 1.0 gm of p-toluene sulphonic acid was added to the reaction mixture at room temperature. The solution was stirred slowly for 24 hours and then poured into water. The mixture was extracted with methylene chloride and the methylene chloride solution was washed with water. The organic layer was dried over

anhydrous sodium sulphate and then evaporated when a solid residue (0.45 gm) was obtained. The residue was dissolved in minimum amount of benzene, poured into a column of silica gel (30 gms). The column was developed with petroleum ether and eluted with following solvents:

Table—10

Eluent	Fraction 100 ml each	Residue on evaporation
Petroleum ether	1—5	Trace oil
Petroleum ether:benzene (4:1)	6—10	Nil
Petroleum ether:benzene (3:2)	11—15	Nil
Petroleum ether:benzene (2:3)	16—20	Nil
Petroleum ether:benzene (1:4)	21—26	Nil
Benzene	27—30	Nil
Benzene:ether (4:1)	31—40	White solid m.p. 270—80°

Further elution with more polar solvents did not afford any solid material

The solid obtained in the fractions 31—40 (table—10) were mixed and the mixture was crystallised from methanol when the crystals of 97 had m.p. 280°—281°.

Analysis report:

Found, C, 78.54% ; H, 10.98%

Required for $C_{30}H_{50}O_3$, C, 78.55% ; H, 10.95%

IR(nujol): ν_{max} 3420 cm^{-1} (—OH group) 1695 cm^{-1}(—COOH group)

Fig.24

Esterification of the above hydrogen peroxide reaction product 97:

200 mg compound was dissolved in ether (30 ml), added a solution of diazomethane in ether prepared from nitrosomethyl urea (200 mg) and was kept over-night. On the following day excess of diazomethane was destroyed with acetic acid. The ethereal solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and was then dried with anhydrous sodium sulphate. Evaporation of solvent gave a solid (175 mg) which was poured into a column of silica gel (30 gm) and was eluted with following solvents:

Table—11

Eluent	Fraction 100 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—15	White solid m.p. 245°—251°

Further elution with more polar solvents did not afford any solid material

Fraction 9—15 (table—11) were mixed and it was crystallised from chloroform-methanol when the crystals of 98, having m.p. 265°—266° were obtained.

Analysis report:

Found, C, 78.77% ; H, 11.09%

Calculated for $C_{31}H_{52}O_3$, C, 78.75% ; H, 11.09%

IR(nujol): ν_{max} 1735 cm^{-1}, 3580 cm^{-1} Fig.25

NMR : δ 0.8—1.01 (for 6 methyl groups), 2.75 (H-C-$COOCH_3$), 3.2 (H-C-OH), 3.65 (-$COOCH_3$) Fig.26

Mass : m/e 473 (MH^+), 455, 454, 439, 411, 395, 251, 235, 205, 191 Fig.27

Acetylation of the above hydroxy ester 98; Preparation of ester acetate 99:

100 mg of the ester 98 was dissolved in 1 ml of pyridine. This was treated with 1 ml of acetic anhydride and the mixture was heated on a water bath for 4 hours. The reaction mixture was then cooled and poured into ice cooled water when a solid was separated out. It was filtered and was washed with water and dried under suction. The compound was crystallised from chloroform-methanol mixture when crystals of m.p. 250°—251° were obtained. TLC single spot.

Analysis report:

Found, C, 77.58% ; H, 9.85%

Calculated for $C_{33}H_{50}O_4$, C, 77.6% ; H, 9.86%

IR(nujol): ν_{max} at 1710 cm^{-1} (broad for ester & acetoxy)
1240 cm^{-1} (acetoxy) Fig.27

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PART — III

ACTION OF N-BROMOSUCCINIMIDE ON TRITERPENE ACIDS AND ESTERS

CHAPTER—I

A SHORT REVIEW OF THE ACTION OF N-BROMOSUCCINIMIDE ON TRITERPENOIDS

N-Bromosuccinimide was first prepared by Seliwanow[1] in 1893. The use of N-bromoacetamide as an allylic brominating agent was reported by Wohl and Jaschinowski[2] in 1919. Ziegler and coworkers[3] prepared eight other N-bromoimides but found them to be far less satisfactory than NBS for allylic bromination. The reaction of allylic bromination is now known as Wohl-Ziegler reaction. Many reviews have appeared on the action of N-bromosuccinimide; that by (a) Djerassi[4] and (b) Horner and Wenkelman[5] for allylic brominations and (c) Filler[6] on bromination and oxidation reactions are worth mentioning.

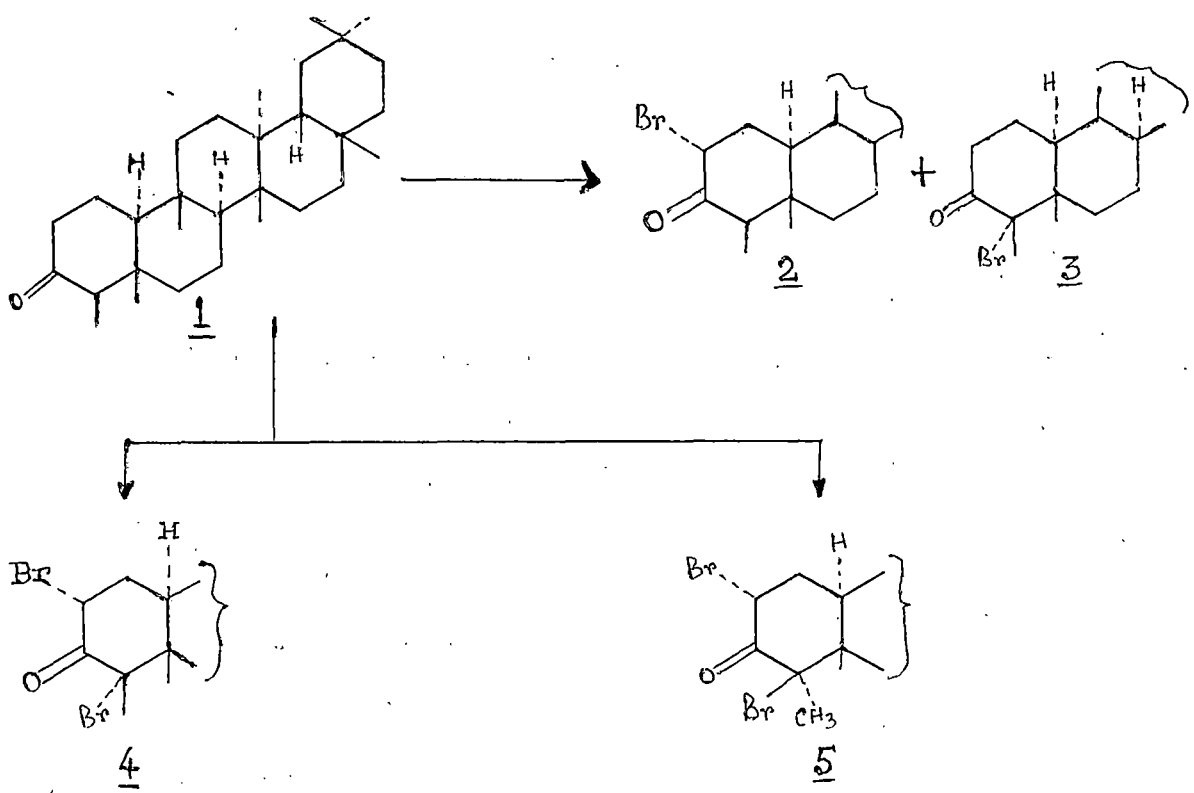
Though numerous reports on the allylic bromination on steroidal chemistry have been reported, there are very few reports on the allylic bromination on triterpenoids. A short review on the action of NBS on triterpenoids are given below:

1. Action of N-Bromosuccinimide (NBS) on friedelin and its derivatives[8]:

Corey and Ursprung[7] showed that friedelin 1 on direct monobromination gave 2 ∝ -(axial)-bromofriedelin 2

and bromination of appropriate enol benzoate gave the isomeric 4 α -axial bromofriedelin 3. They have also prepared a dibromo-friedelin 4 in presence of hydrobromic acid in chloroform solution. The UV absorption at 332 nm indicated that most probably both the bromine atoms were axially oriented and has been regarded as the 2 α , 4 α -dibromofriedelin 4. Djerassi and coworkers[9] prepared a second dibromofriedelin 5 by bromination of 2 α -bromofriedelin in acetic acid. From its UV absorption at 310.5 nm and also from ORD studies they formulated the compound as 2 α , 4 β -dibromo-friedelin 5 (Scheme—1).

Scheme—1

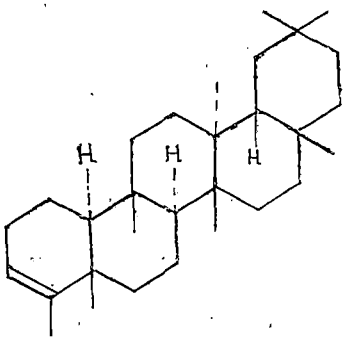


Takahasi and Ourisson[10] also prepared a dibromo-friedelin by bromination of friedelin in chloroform-acetic acid which showed an UV absorption at 320 nm. These workers did not assign any structure of this compound.

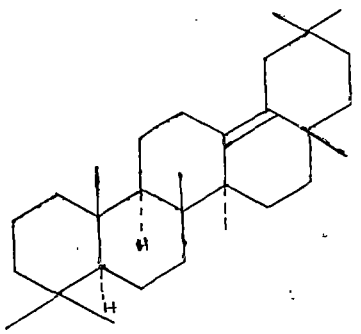
Stevenson et al[11,12] observed the action of N-bromosuccinimide on friedelin and its derivatives. Treatment of friedelin 1 in carbon tetrachloride with a molar equivalent of NBS gave 4 ∝ -bromo-friedelin 3 in good yield. By further treatment of 3 with bromine in acetic acid solution, these authors isolated 2 ∝ -bromo-friedelin 2. So in this reaction isomerisation rather than substitution occurred. As anticipated from this result, it was found that 4 ∝ -bromoketone 3, $(\alpha)_D$ 92° was unstable in chloroform-hydrobromic acid, the presumed equillibrium mixture $(\alpha)_D$ -75° being formed after 24 hours. Similar equillibration of 2 ∝ -bromo-friedelin gave the same result.

Since this route for obtaining a dibromo-friedelin was unsuccessful, the alternative method of treating 2 ∝ -bromo-friedelin 2 with NBS was examined. 2 ∝ -bromo-friedelin 2 on treatment with NBS gave unsaturated monobromo-ketone, $C_{30}H_{47}O$ Br. The later showed a positive tetranitro methane test indicating the presence of an ethylenic function; UV and IR spectra of this ketone showed that the

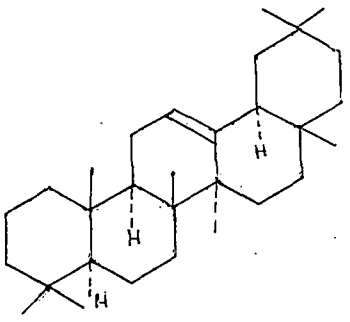
double bond was not conjugated to the carbonyl group and that the ∝-bromine atom retained an axial conformation. Since it is known that acid isomerisation of friedel-3-ene 6 afforded a mixture of olean-13,18-ene 7 and 18∝-olean-12-ene[11,12] 8, it was considered that this non-conjugated bromoketone had probably arisen by a molecular rearrangement of 2∝,4∝-dibromoketone intermediate (or derived radical or cation) with elimination of hydrobromic acid.



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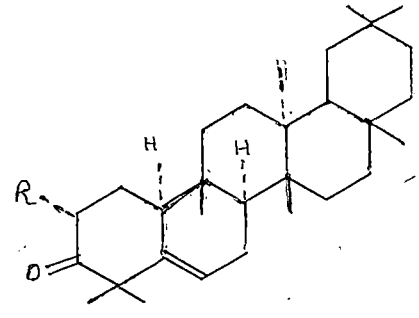


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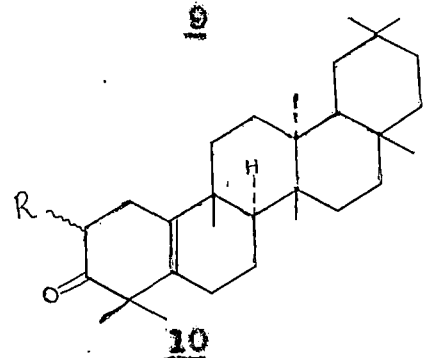


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A precedent for such a postulated rearrangement was provided by the action of silver acetate on 4 ∝ -bromo-friedelin 3 to yield a product 7 which was shown to be a mixture[13] of alnus-5-enone 9 (R = H) and alnus-5 (10)-enone, 10 (R = H). The probability that the unsaturated bromoketone derived from 2 could be represented as 2-bromo-alnus-enone 9, (R = Br) or 10 (R = Br), was excluded from the fact that the zinc-debromination product in neutral solution was different from either alnusenone 9 or 10 (R = H).



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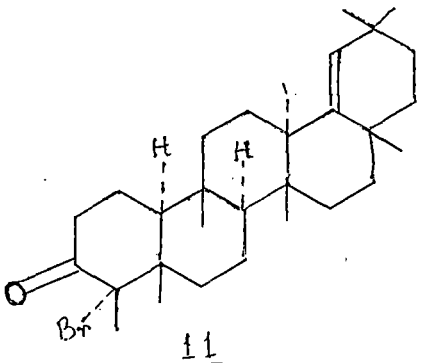


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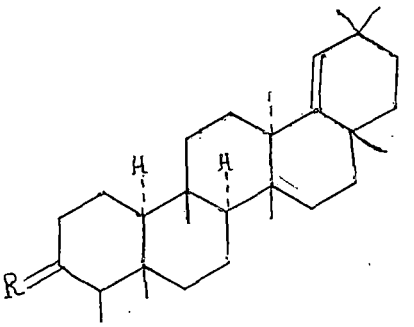
Treatment of 4 ∝ -bromo-friedelin 3 with NBS gave an isomeric non conjugated axial bromosubstituted ketone 11 $C_{30}H_{47}O$ Br, which also on debromination gave the same ketone $C_{30}H_{48}O$ 12. Reduction of 12 with lithium aluminium

hydride gave an alcohol 16 and reduction by Huang-Minlon method gave the hydrocarbon 15. From these evidences the isomeric monobromoketones obtained from 2 and 3 by the action of NBS were assigned structures 2 α -bromo-friedel-18-en-3-one 13 and 4 α -bromo-friedel-18-en-3-one 11 respectively. These assignments were corroborated by specific rotation and ORD studies.

Action of NBS on saturated hydrocarbon friedelane[8,14] 14 was also examined and they isolated an unsaturated hydrocarbon 15 identical in all respects with that obtained from Huang-Minlon reduction of 12. This further suggested that the products obtained by NBS on ketones 2 and 3 were ethylenic non conjugated ketones and hence the attack has proceeded at a site not activated by carbonyl group. The site of the double bond introduced by NBS was established by the following way. The unsaturated hydrocarbon 15, resisted catalytic hydrogenation, yielded an oxide $C_{30}H_{50}O$ with perbenzoic acid, indicating that the double bond has a degree of steric hindrance[15] comparable to the Δ^{12}-trisubstituted ethylenic linkage in β -amyrin series. The terminal UV absorption of 12, 15 and 16 indicated that the double bond was trisubstituted. The resistance of hydrogenation of 15

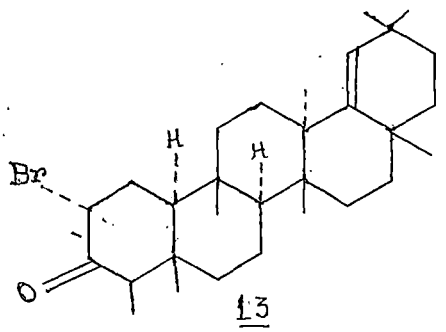


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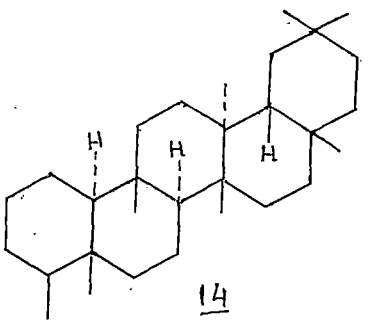


12, R = O

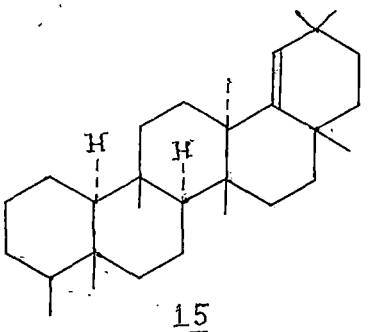
16, R =



13



14



15

further suggested that the ethylenic system was not disubstituted and the friedelin skeleton does not permit the existence of a tetra substituted double bond. NMR spectrum

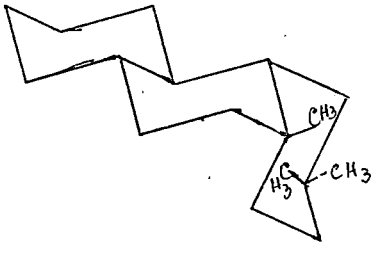
of the ketone 12 showed a singlet attributable to an olefinic proton not conjugated with the carbonyl group.

The location of the double bond introduced by the action of NBS on bromoketones 2, 3 and hydrocarbon 15 was thus restricted to position 1(10), 7 or 18. The position 1(10) and 7 were excluded by examining the dehydrobromination of 11 with silver acetate. Under these conditions they isolated a dehydrobrominated product $C_{30}H_{46}O$ which showed no UV absorption above 200 nm. Since there was no conjugation of carbonyl or ethylenic functions in this dienone, the original double bond could not have been located in ring A and B. Although there had been much work on synthetic application of allylic compounds[16], comparatively little was known with regard to the action of NBS on saturated systems. It has been established that cyclohexane[17,18] and cycloheptane[18] yield the cycloalkylbromide with NBS under certain condition and that decalin gave a tetrabromo octahydro napthalene[19] which can also be obtained from the probable intermediate 9, 10 - octalin. Ganon and coworkers[20] have also drawn attention to the fact that NBS is not a reagent of general utility for the α-bromination of saturated esters due to selective attack on t-hydrogen atom at the either sites of the molecule.

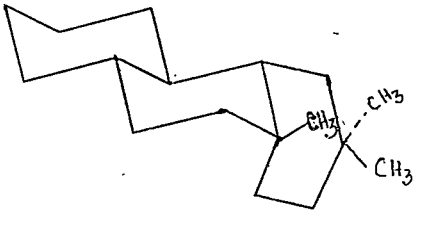
The experiments described by these workers established that in friedelin the tertiary α-hydrogen atom at

position 4 was more reactive towards NBS than the secondary hydrogen atom at position 2 but the presence of a 2 α -bromine atom effectively prevented the abstraction of a 4 α -hydrogen atom by its 1,3 diaxial blocking effect to approach a succinimide radical.

In absence of the activating C—3 carbonyl group or where there is a deactivation due to the steric influence of the neighbouring axial halogen, the most reactive hydrogen is the tertiary C—18 atom. An examination of an all chair form 17 of friedelane showed that a severe steric interaction must exist between the 13 α and 20 α -methyl groups due to its cis junction at rings D and E. This interference is removed if the terminal E ring adopted a boat configuration 18 but as a consequence an unfavourable 1:4 diaxial boat prow and stern interaction results[21]. The steric strain inherent in both conformations with cis D/E system is relieved by dissociation of the 18 β hydrogen atom and formation of ethylenic trigonal system.



17



18

2. Bromine and N-Bromosuccinimide oxidation of saturated hydrocarbon, friedelane[14]:

Olefins may be halogenated in their allylic position by a number of reagents of which NBS is by far the most common. With this reagent an initiator is needed and this is usually a peroxide. The reaction is usually quite specific at allylic position and good yields are obtained. However, when the allylic radical intermediate is unsymmetrical, then allylic shifts can take place so that a mixture of both possible products are obtained.

$$CH_3-CH_2-CH=CH_2 + NBS \longrightarrow CH_3-\underset{\displaystyle Br}{\underset{|}{CH}}-CH=CH_2$$

$$+\ CH_3-CH=CH-\underset{\displaystyle Br}{\underset{|}{CH_2}}$$

When a double bond has two different α-positions (e.g. $CH_3CH=CH-CH_2-CH_3$), then a secondary position is substituted more readily than primary. The relative reactivity of tertiary hydrogen is not clear, though many substitution at allylic tertiary position have been performed[16].

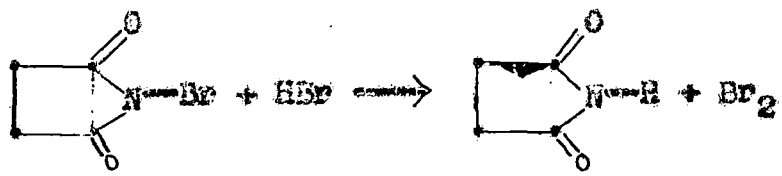
That the mechanism of an allylic bromination is of the free radical type was demonstrated by Douben and

McCoy[16] who showed that the reaction was very sensitive to free radical initiators and inhibitors and indeed did not proceed at all unless at least a trace of initiator was present. Subsequent work indicated that the species which actually abstracts hydrogen from the substrate is the bromine atom. The reaction is initiated by small amounts of bromine, once it is formed, the main propagation steps are:

1. $Br^{\bullet} + RH \longrightarrow R^{\bullet} + HBr$

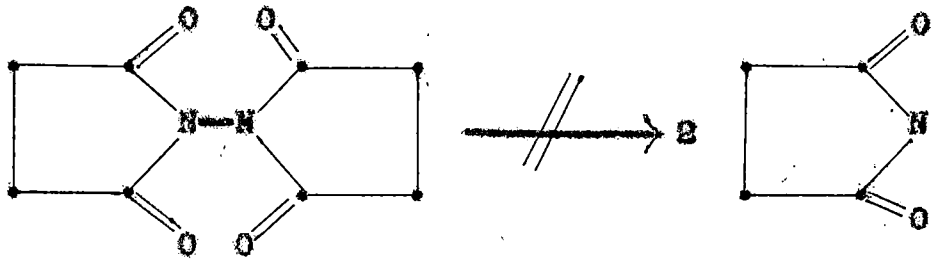
2. $R^{\bullet} + Br_2 \longrightarrow RBr + Br^{\bullet}$

The source of Br_2 is a fast ionic reaction between NBS and the HBr liberated in step 1.



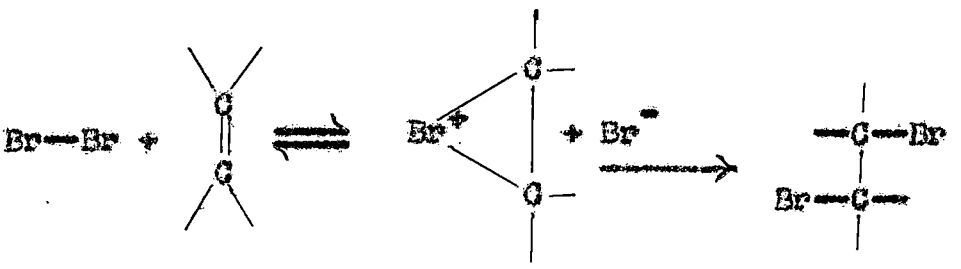
The function of NBS is therefore to provide a source of Br_2 in a low-steady state concentration and to use up the HBr liberated in step-1[22,23]. It was previously believed that the abstracting species was the succinimide radical 18a but there is now much evidence that this species is not involved in the reaction and is probably not even formed. The main evidence is that NBS and Br_2 show similar

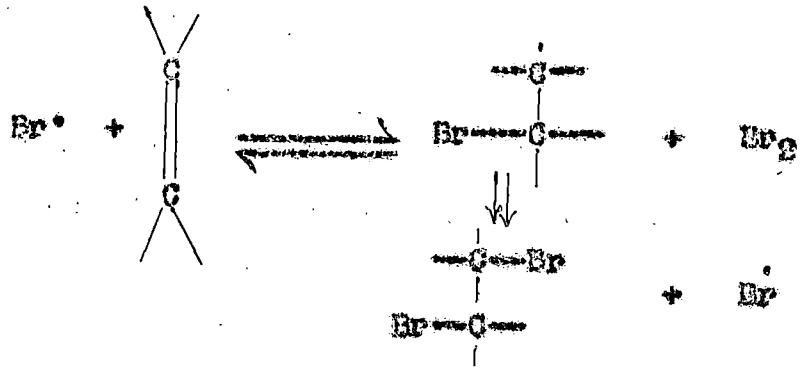
selectivity[24]; that the various N-bromosuccinimide also show similar selectivity, which would not be the case if a



18a

different species was abstracting in each case[25] and that 18a has proved itself to be a much less stable species that was originally thought, since its dimer shows no tendency to dissociate[26]. The later observation casts doubt of the ability of NBS to dissociate, The fact that the reacting species Br_2 does not add to double bond, either by an ionic or by a free radical mechanism can be explained in the following way. Apparently the concentration is too low, in bromination of a double bond, only one atom of an attacking bromine molecule becomes attached to the substrate, whether the addition is electrophilic or free radical:





The other bromine comes from another bromine molecule. If the concentration is sufficiently low, there will not be a high probability that the proper species will be in the vicinity once the intermediate forms and the equillibrium will lie to the left. This slows the rate of addition so that the allylic substitution can successfully complete. If this is true, then it should be possible to brominate an olefin in the allylic position without competition from addition, even in the absence of NBS or a similar compound, if a very low concentration of bromine is used and if the HBr is removed as it is formed, so that it is not available to complete the addition step. This has been demonstrated by McGrath et al[27].

Stevenson et al[8,24] reported that the saturated hydrocarbon friedelane, 14 was oxidised by NBS to the olefin, friedel-18-ene, 15. In order to demonstrate that the function of NBS was to provide molecular bromine, they have

compared the action of bromine on 14 in carbon tetrachloride solution. A solution of bromine in carbon tetrachloride added to friedelane was decolorized and the reaction was worked up in the usual way[8a], yielded friedel-18-ene 15 in comparative yield. This demonstrated that in this oxidation, the intermediacy of the succinimide radical was unessential. No unchanged friedelane was recovered by chromatographic examination. They however, isolated an unstable bromofriedelane which readily yielded friedel-18-ene and was consequently considered by them to be an 18-bromo-friedelane. The discrepancies and poor reproducibility reported[8a] in the bromination of 3-ketone, friedelin by NBS, particularly in the formation of di and tribromo derivatives at C—2 and/or C—4 may be attributed to accompanying halogenation at C—18.

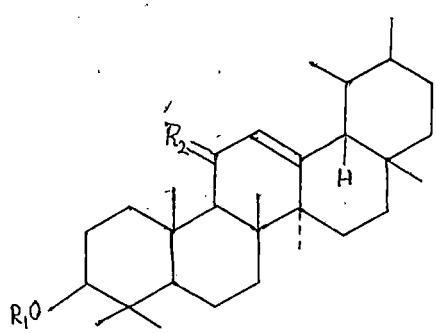
3. Allylic oxidation by N-bromosuccinimide[28]:

Corsano et al[29] reported formation of 3β-acetoxy-urs-12-en-11-one 20 in 60% yield by treatment of α-amyrin-acetate 19 with NBS in a aqueous dioxan solution.

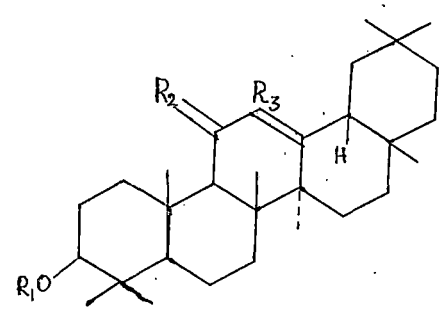
Finucane et al[28] obtained 3β-acetoxy-olean-12-en-11-one 24 from β-amyrin acetate 23 under the same conditions[28] in yields that fluctuated between 20 to 60% together with a number of by-products. They also claimed that when the reaction

mixture was irradiated with visible light, $\alpha\beta$-unsaturated ketones were formed in near quantitative yields from a number of trisubstituted olefins containing an allylic methylene group. Pinuccna et al[28] treated β-amyrin acetate 23 with NBS in dioxan in a typical ambient - light experiment as described by Corsano et al[29]. They separated the products by chromatography over silica gel column and isolated starting material (Ca 50%), 3 β-acetoxy-olean-12-en-11-one 24 (Ca 40%), bromo compounds (Ca 8%) and 3 β-acetoxy olean-12-en-11α-ol 25 (Ca 2%). Oxidation of the later 25 with CrO_3 in acetone afforded 3 β-acetoxy-olean-12-en-11-one 24.

In another experiment the products were isolated by chromatography over alumina, the compounds isolated were β-amyrin acetate 23 (Ca 35%), 3 β-acetoxy-olean-12-en-11-one 24 (Ca 40%), bromo compounds (Ca 10%) and polar materials (Ca 10%). The polar fraction, on elution with methanol was acetylated and on rechromatography gave 11α-methoxy-olean-12-en-3β-yl acetate 26 together with smaller amounts of 11α-ol 25 and oleana-9(11), 12 dien-3β-yl acetate 39 and a trace of 3β, 11α-diacetate[30] 27. The methoxy acetate 26 with p-toluene sulfonic acid in acetic anhydride afforded 3β-acetoxy olean-11(12),13(18)-diene 41 in quantitative yield.

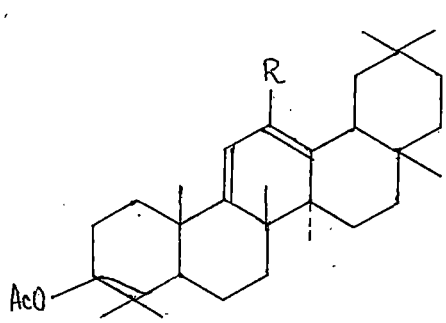


19, R_1=Ac; R_2=H
20, R_1=Ac; R_2=O

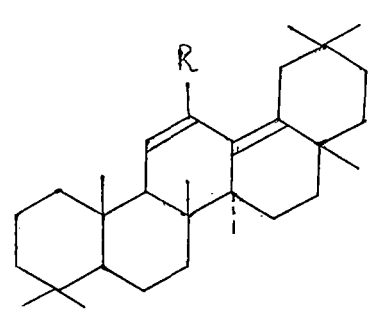


23, R_1=Ac; R_2=H, R_3=H
24, R_1=Ac; R_2=O, R_3=H
25, R_1=Ac; R_2= α-OH; R_3=H
26, R_1=Ac; R_2= α-OMe; R_3=H
27, R_1=Ac; R_2= α-OMe; R_3=OAc
28, R_1=Ac; R_2= α-OH, R_3=Br
29, R_1=Ac; R_2=O, R_3=Br
31 R_1=Ac; R_2=H_2, R_3=Br

The 11α-configuration of the methoxy group was assigned on the basis of the magnitude of the 9α-H, 11-H coupling constant 9Hz.



39, R = H
40, R = Br



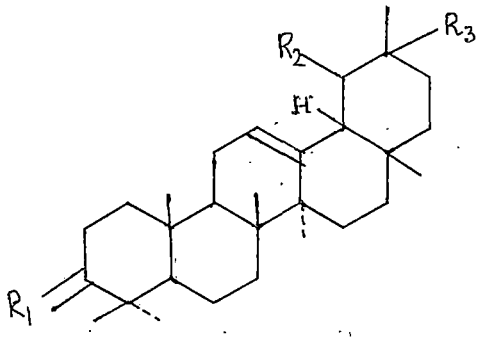
41, R = H
42, R = Br

The fraction containing the bromo compound was resolved by chromatography over alumina and fractional crystallisation into two components. The major product was a diol monoacetate ($C_{32}H_{51}BrO_3$). Although free hydroxyl group was secondary (τ 5—6, $\underline{H}$—COH), the monoacetate resisted further acetylation. The compound gave a yellow colour with tetranitromethane and showed an absorption in UV, but no olefinic proton or $\underline{H}$CBr signal in its NMR Spectrum. This data suggested that the bromo compound was 3 β -acetoxy-12-bromo-olean-12-en-11-ol $\underline{28}$. The latter resisted attempted dehydrobromination although prolonged treatment with lithium chloride in hot DMF resulted in dehydration with formation of 12-bromo-olean-9(11),12-dien-3 β -yl acetate $\underline{40}$. Acid catalysed dehydration of 12-bromo-11-ol, $\underline{28}$, also yielded the homoannular diene $\underline{40}$ and not hetero annular diene $\underline{42}$. In the absence of 12-bromine atom, the heteroannular diene $\underline{41}$ was the sole product. Model of $\underline{42}$ showed a severe non-bonding interaction between the bromine atom and 1β proton, irrespective of the conformation of ring B. This interaction was absent in $\underline{40}$. Mild oxidation of $\underline{28}$ gave 3 β -acetoxy-12-bromo-olean-12-en-11-one $\underline{30}$. This confirmed the presence of 11-hydroxy group in $\underline{28}$. The large negative difference on molecular rotation (-266°) between the bromo alcohol $\underline{28}$ and 12-bromo-olean-12-en-3β-yl-acetate $\underline{31}$ suggested the 11α -configuration for the

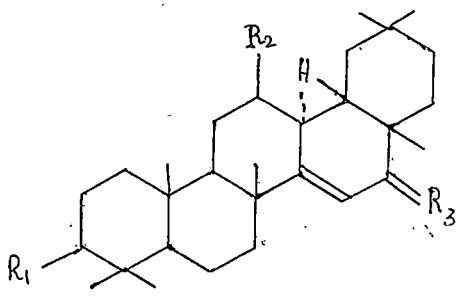
hydroxyl group, which was supported by the magnitude of 9α-H, 11-H coupling constant (7.8 Hz) for the 11α-ol.

The minor component of the mixture of bromo compounds was an $\alpha\beta$-unsaturated ketone ($C_{32}H_{49}O_3Br$) 43, λ_{max} 244 nm. Dehydrobromination of 43 yielded a dienone 49, in which the new double bond was not conjugated. It could not be brought into conjugation by treatment with base. NMR of 49 showed the presence of an isolated double bond and this double bond was disubstituted (τ 4.14, 2H, m). The structures 43 and 49 were consistent with the observed spectroscopic properties and were also mechanistically acceptable. Thus the initial α-face attack on β-amyrinacetate 23 at C—12 would lead to a carborium ion 48. Elimination of a proton from C—12, followed by allylic hydroxylation would then lead to 28. Alternatively, migration of 14α-methyl group to C—13, elimination of a proton from C—15, and subsequent allylic oxidation would give the 12α-bromo-16-one 43.

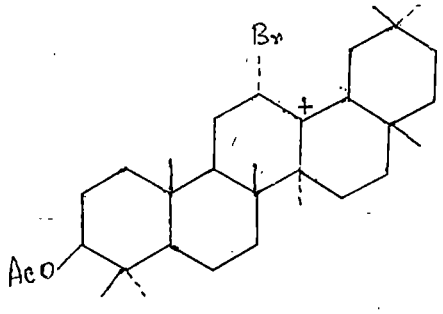
In the NMR spectrum of the bromoketone 43, HCBr signal overlapped the 3α-H multiplet. However in the spectrum of 12α-bromo taraxer-14-ene-16-one 45 which was obtained from 35 in a reaction similar to that described for β-amyrin acetate, the HCBr signal was a triplet (J, 8.1 Hz).



35, $R_1=H_2$, $R_2=H$, $R_3=Me$
36, $R_1=H_2$, $R_2=Me$, $R_3=H$



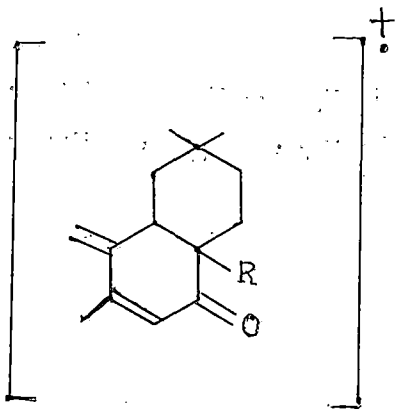
43, $R_1=OAC$, $R_2=Br$, $R_3=O$
44, $R_1=OAC$, $R_2=H$, $R_3=H_2$
45, $R_1=H$, $R_2=Br$, $R_3=O$
46, $R_1=OAC$, $R_2=H$, $R_3=O$
47, $R_1=OAC$, $R_2=H$, $R_3=\beta$-OH, α-H



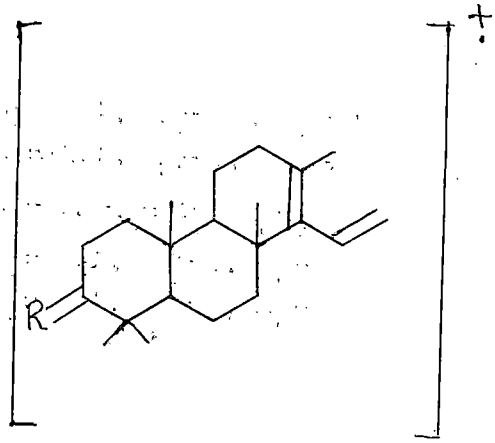
48

The result was compatible with the proposed 12 β -H configuration in the ring C - boat of tarazerene skeleton when the effect of substituent on the vicinal coupling constant was considered[32].

The most characteristic features of the mass spectra of taraxer-14-ene are the D/E ring fragments 32 and the retro - Diels Alder fragment[33] 33. The presence of an 11,12-double bond in dehydrobromination product 49 was expected to prevent formation of 32. The mass spectrum of the dienone 49 showed no fragment corresponding to D/E ring

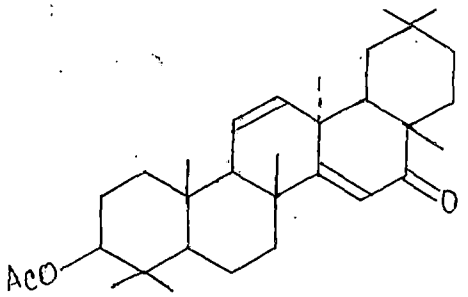


32

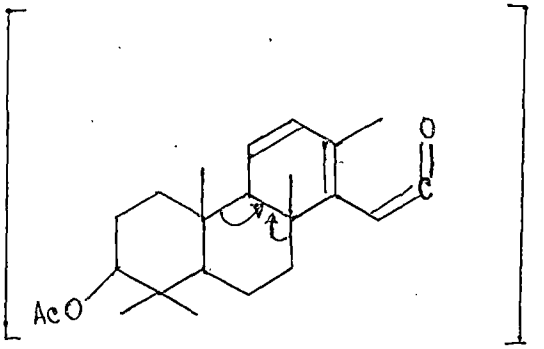


33

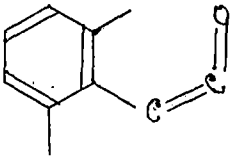
species and only two abundant ions viz m/e 356 ($C_{22}H_{32}O_3$), 21 and 148 were prominent. High resolution measurements confirmed the constitution of m/e 356 fragment and showed that the species of m/e 148, of equal abundance was $C_{10}H_{10}O$ to which the aromatic ketone structure 22, was ascribed



49

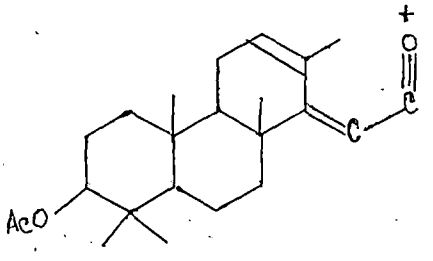


21



22

In the spectrum of the bromoketone 43 a D/E ring fragment was absent and the expected species 29 was the most abundant ion.



29

Thomson et al[28] also carried out oxidation of taraxeryl acetate 44 by the method of Corsano et al[29] and obtained two major products to which they assigned 16-oxotaraxeryl acetate 46 (Ca 30%) and 16 β -hydroxy taraxeryl acetate 47 (Ca 30%). Treatment of 47 with chromic acid in acetone gave the unsaturated ketone 48. They suggested a 16- β -configuration for the hydroxyl group from the study of the Dreiding model. The 15-H signal in the NMR spectrum of taraxeryl acetate 44 appeared as a quartet (J_1=4, J_2=8Hz) whereas in 47 as a doublet (J=4Hz). A Dreiding model of tarax-14-ene showed 15-H, 16-H dihedral angles to be Ca 90° (16 α -H) and Ca 30° (16 β -H), thus the expected vicinial coupling constants are very small and Ca 8Hz respectively. The increased magnitude of the smaller coupling constant may be attributed to efficient overlap of the Π - orbital with the axial 16 α -H bond. The appearance of the 17 β - methyl signal as a doublet (J 1Hz) in the NMR spectrum was also taken as the confirmation for the α -axial configuration for the 16-H atom.

These authors reported that when solutions of α or β -amyrin acetate i.e. 19 or 23 in aqueous dioxan containing NBS were irradiated with visible light the corresponding 11-ketones 20 or 24 were formed in essential quantitative yield. Similar high yields of α β -unsaturated ketones

were claimed from olean-12-ene 35, Urs-12-ene 36, and tarax-eryl acetate 44.

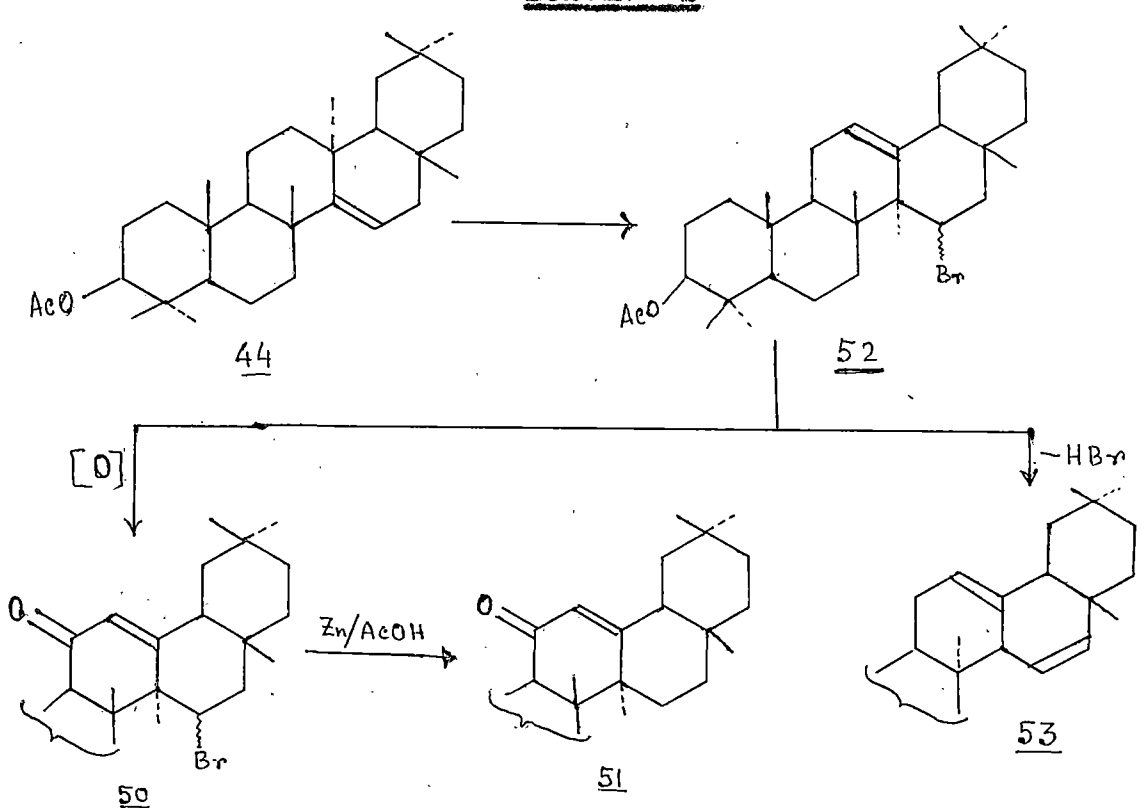
Khastgir et al[34] reported that tarexeryl acetate 44 on treatment with N-bromosuccinimide in aqueous dioxan according to the method of Finucane and Thomson[28], and observed that the products obtained was quite different from those recorded by Finucane and Thomson[28]. They found that the product was a mixture of three compounds which was separated by column chromatography.

The first compound was found to be of m.p. 238—240° having molecular formula $C_{32}H_{49}O_3Br$ was identified as 15 bromo amyrenonyl acetate 50. The structure was proved by different reactions on the compound. When 50 was treated with Zn dust in acetic acid and compound isolated gave negative test for halogen was found to be identical with an authentic sample of β-amyrenonyl acetate[35] 51. The compound was also prepared from β-amyrin acetate by CrO_3 - AcOH oxidation. Khastgir et al[34] tried to prepare the 15 bromo compound 52 from a known species to confirm the structure 50. Taraxeryl acetate 44 on treatment with NBS according to the procedure by Dalton and Jones[36] obtained a solid of molecular formula $C_{32}H_{51}O_2Br$, m.p. 180—182°. The compound did not show any UV absorption between 220—300 nm. IR spectrum of

the compound showed peaks at 1720 cm^{-1} and 1250 cm^{-1} ($-O-CO-CH_3$). NMR spectrum showed multiplet at 5.3 (vinyl proton), 4.30 ($-\underline{C}H-Br$) in addition to peaks at 2.09 ($-OCO\underline{CH}_3$) and 4.53 ($-\underline{C}H-OCOCH_3$) ppm. The mass fragmentation pattern also confirmed the structure of the 15-bromo compound 52. This on oxidation with CrO_3-AcOH gave 50, m.p. 238—240° identical with the product obtained from NBS - aq. dioxan reaction of teraxeryl acetate.

Dehydrobromination of the compound 52 on treatment with potassium acetate and acetic acid at 130° for 4 hours gave a product $C_{32}H_{50}O_2$ m.p. 199°—200°. The same compound was also obtained when 52 was refluxed with dimethyl aniline for six hours and structure 53 was proposed for it (Scheme—2).

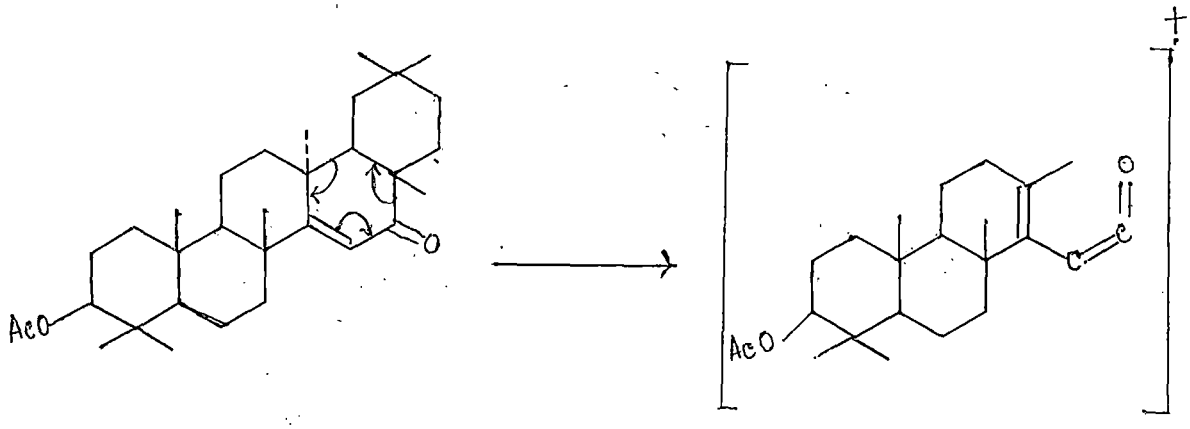
Scheme—2



44 52 50 51 53

The second compound, having molecular formula $C_{32}H_{50}O_3$, m.p. 280—2°, UV λ_{max} 248 nm and IR peaks at 1730, 1630, 1250 cm^{-1} indicating the presence of an unsaturated ketone, was assigned as 16-oxo-taraxeryl acetate from NMR and mass spectral analysis. It showed a mass peak at M^+ 482. Moreover, an abundant base peak at 358 was observed. The appearance of this peak may be explained by the genesis given in Scheme—3. NMR spectrum of the compound showed a

Scheme—3

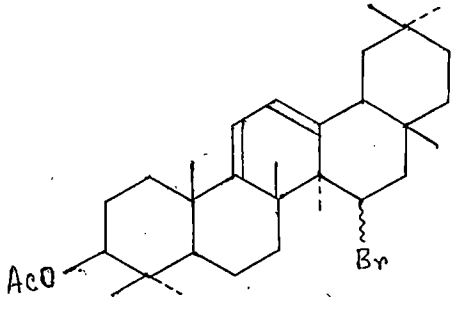


46 m/e 358

peak at 5.85 ppm for one vinyl proton. This low field peak for the vinyl proton may be ascribed to the presence of the electronegative oxygen at C—16. Other peaks were at 2.10 (-$OCOCH_3$) and 4.5 (-CH—$OCOCH_3$) ppm. This compound 46 was widely different from the sample of 16-oxo taraxeryl acetate,

m.p. 251—52° described by Finucane and Thomson[28]. All physical and chemical evidences proved that the compound 46 m.p. 280—82° had the correct structure as 16-oxo-taraxeryl acetate 46.

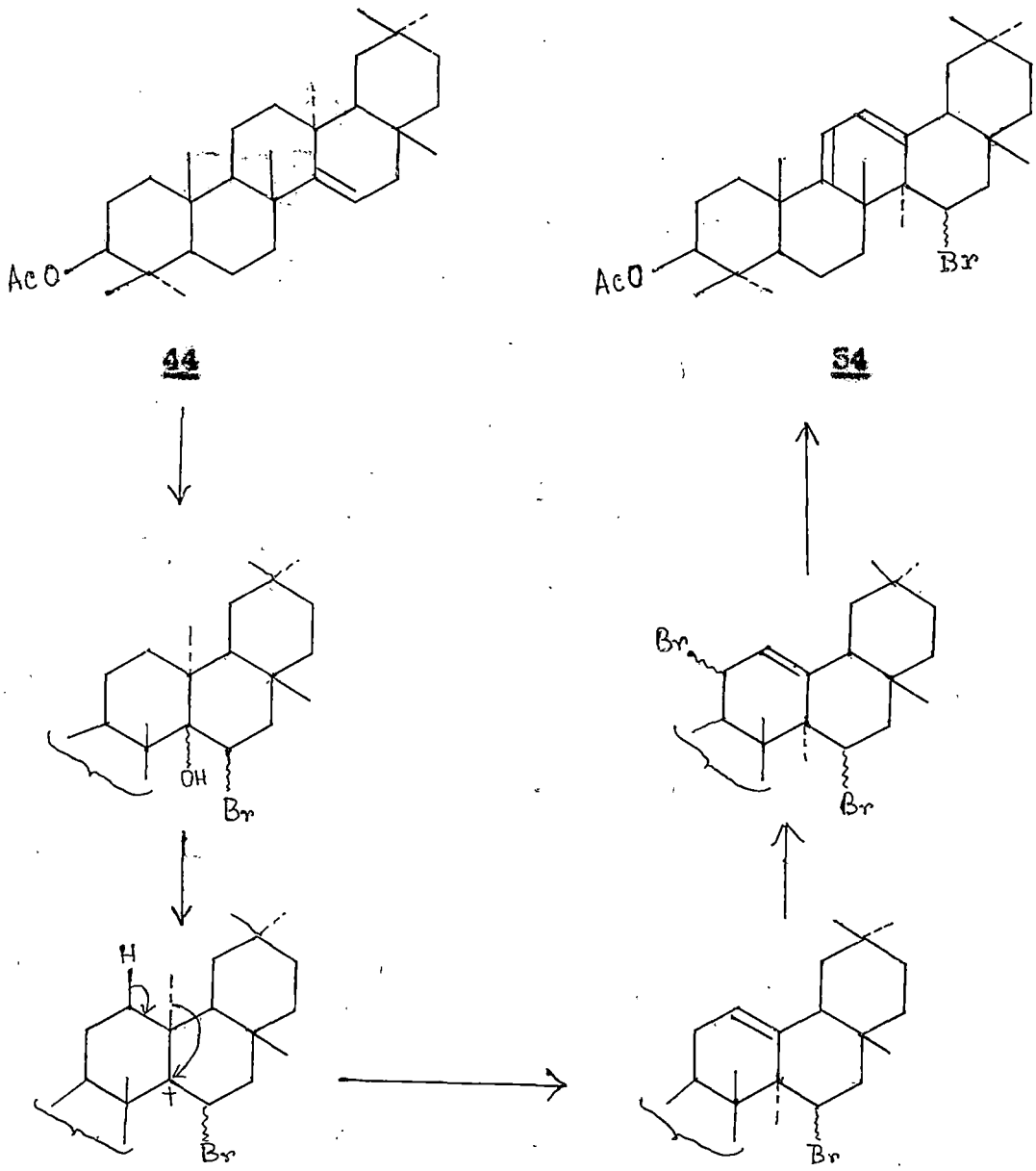
The third compound 54 having molecular formula $C_{32}H_{49}O_2Br$, m.p. 176—78° showed UV maxima at 276 nm(ε6000) indicating the presence of homoannular diene. NMR spectrum of the compound showed signals at 5.34 and 5.85 ppm for one proton each attribute to the protons in a homoannular diene system in which both the double bonds are trisubstituted. In addition to this the spectrum showed a sharp signal at 2.08 (-OCOCH_3) multiplets at 4.65 (-CH-O-COCH_3) and 4.12 (-CHBr) ppm. On the basis of this evidence the compound was assigned structure 54.



54

The probable mechanism for the formation of **54** from **44** was suggested as shown below (Scheme—4):

Scheme—4



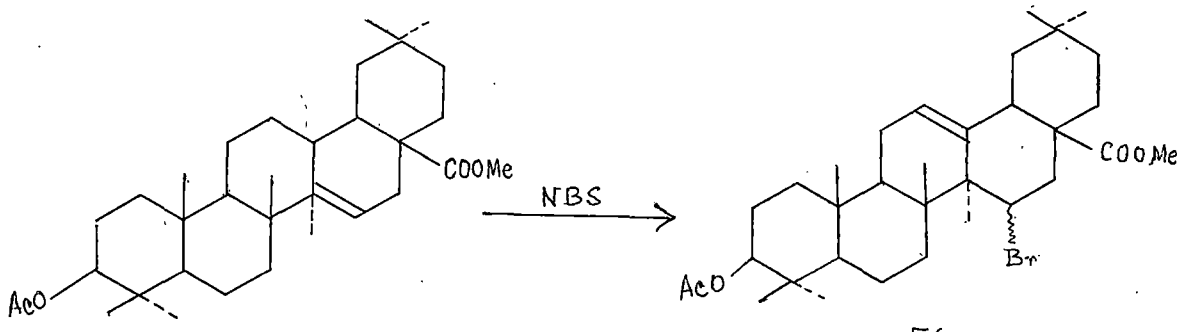
44 **54**

CHAPTER—II

RESULTS AND DISCUSSION

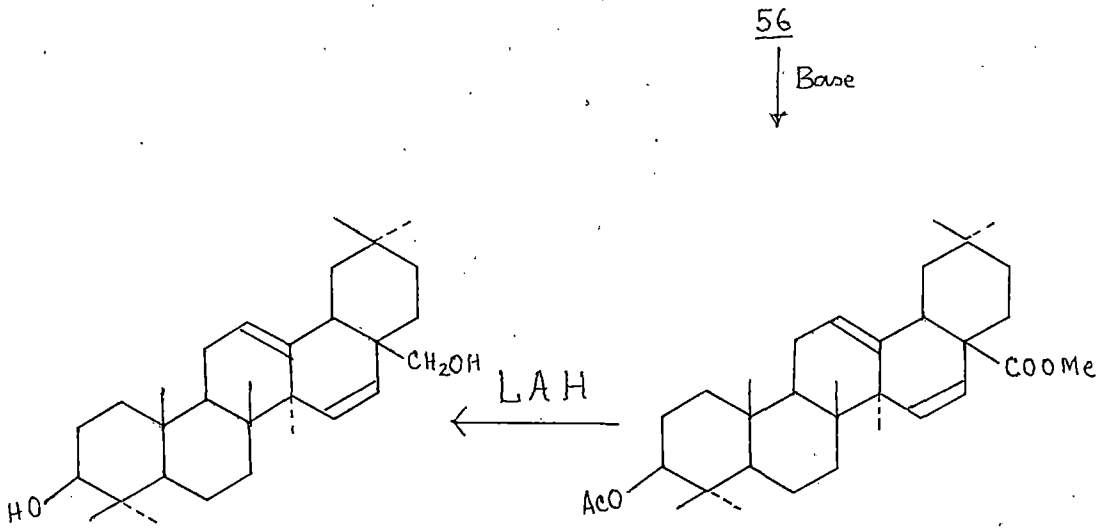
In connection with the partial synthesis of some triterpenoids the preparation of 15-bromo acetyl methyl oleanolate 56 was required. It was found that when taraxeryl acetate was treated with N-bromosuccinimide in presence of dimethyl sulfoxide[36] produced 15-bromo- β -amyrin acetate in good yield. Interested by this result we planned to prepare 15-bromo acetyl methyl oleanolate 56 from acetyl methyl aleuritolate[37] 55 by treatment with N-bromosuccinimide in presence of dimethyl sulfoxide. The bromo compound so formed would be dehydrobrominated by means of dimethyl aniline to produce 3-acetoxy methyl oleanolate-11(12), 15(16)-diene 57. This would produce aegiceradiol[38] 58 by means of lithium aluminium hydride reduction. The scheme which we had planned initially is given below (Scheme—5).

Scheme—5



55 56

Scheme contd.....



58 57

(i) N-bromosuccinimide treatment on acetyl methyl aleuritolate in presence of dimethyl sulfoxide:

For the preparation of 15-bromo acetyl methyl oleanolate, acetyl methyl aleuritolate was taken in chloroform and dimethyl sulfoxide was mixed. To the resulting solution N-bromo-succinimide was added in portions with constant shaking. After keeping the reaction mixture in dark for twelve hours and then usual work up yielded a gummy mass. The mass after column chromatography and crystallisation afforded a colourless crystal 60, $C_{32}H_{49}O_4Br$, m.p. 280°—82°, $[\alpha]_D$ +19.51°. The compound showed positive test for halogen but developed no colouration with TNM showing the absence of any double bond in the compound. IR spectrum (Fig.1) of the compound revealed that the compound possessed a γ -lactone as indicated by the presence of a peak at

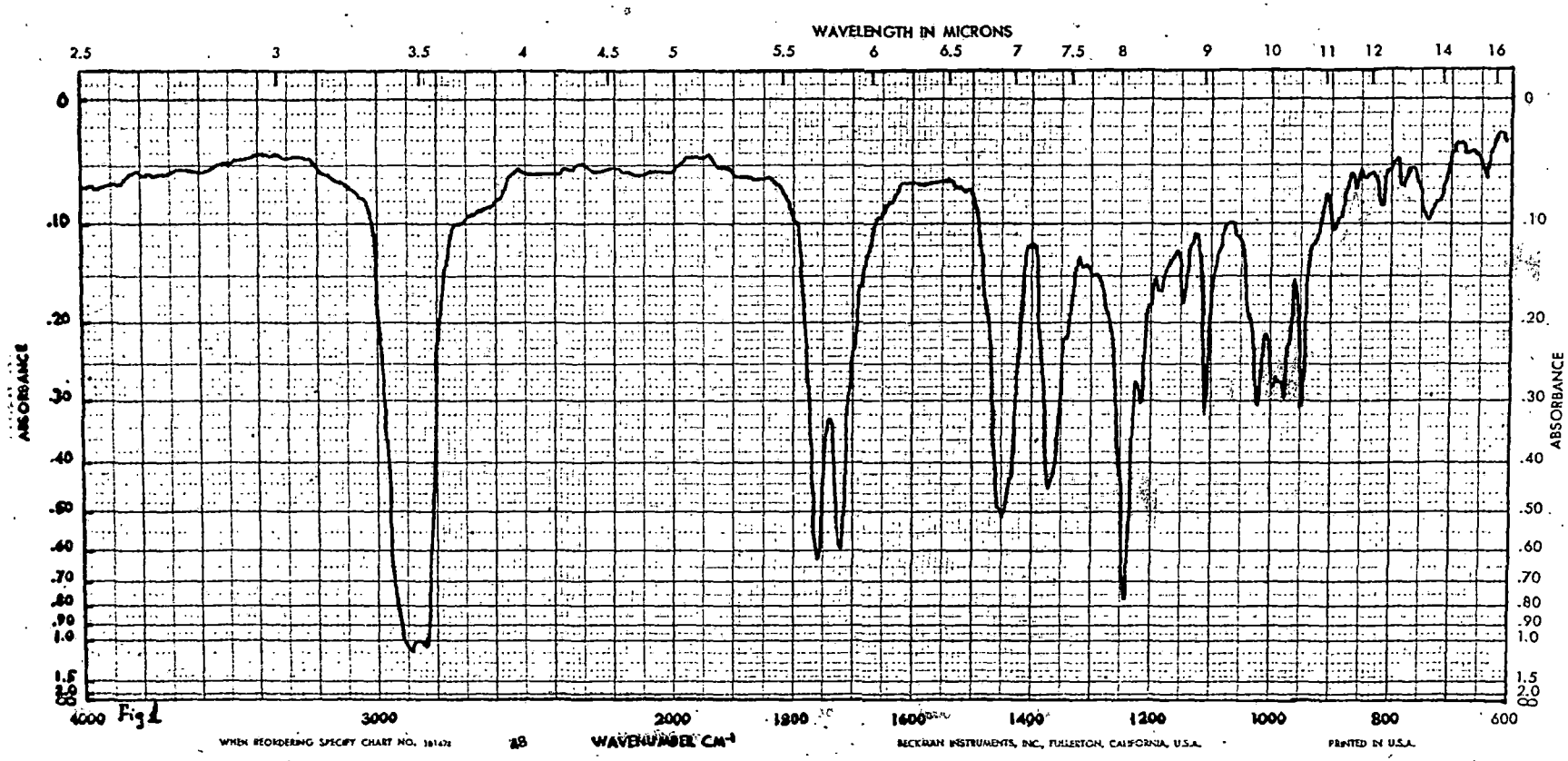


Fig.1: IR spectrum of 15-bromo oleanolic lactone acetate 60, obtained from acetyl methyl aleuritolate.

1760 cm^{-1}, other peaks at 1720 cm^{-1} and 1240 cm^{-1} were due to acetate functional group. The compound showed no UV absorption in the region 200—350 nm. PMR spectrum (Fig.2) of the compound indicated the absence of any vinyl and methoxy carbonyl proton originally present in the original compound 55. So, it was first assumed that during the reaction condition the methoxy carbonyl group was hydrolysed and lactonisation occurred on the double bond. In order to prove the assumption we treated acetyl methyl betulanate with NBS in dimethyl sulfoxide under the same condition when the starting methyl ester was recovered suggesting that no hydrolysis occurred on NBS treatment.

The reaction of N-bromosuccinimide in dimethyl sulfoxide was repeated with acetyl aleuritolic acid 59 and the same γ-lactone was obtained which was confirmed by the m.m.p. and IR comparison.

To establish the structure of the bromo lactone the following reactions were carried on the compound 60.

Treatment of the bromolactone 60 with dimethyl aniline:

The bromolactone 60 was refluxed (4 hrs) with freshly distilled dimethyl aniline for dehydrobromination and after usual work up a compound was obtained which on crystallization from chloroform-methanol mixture, furnished a solid

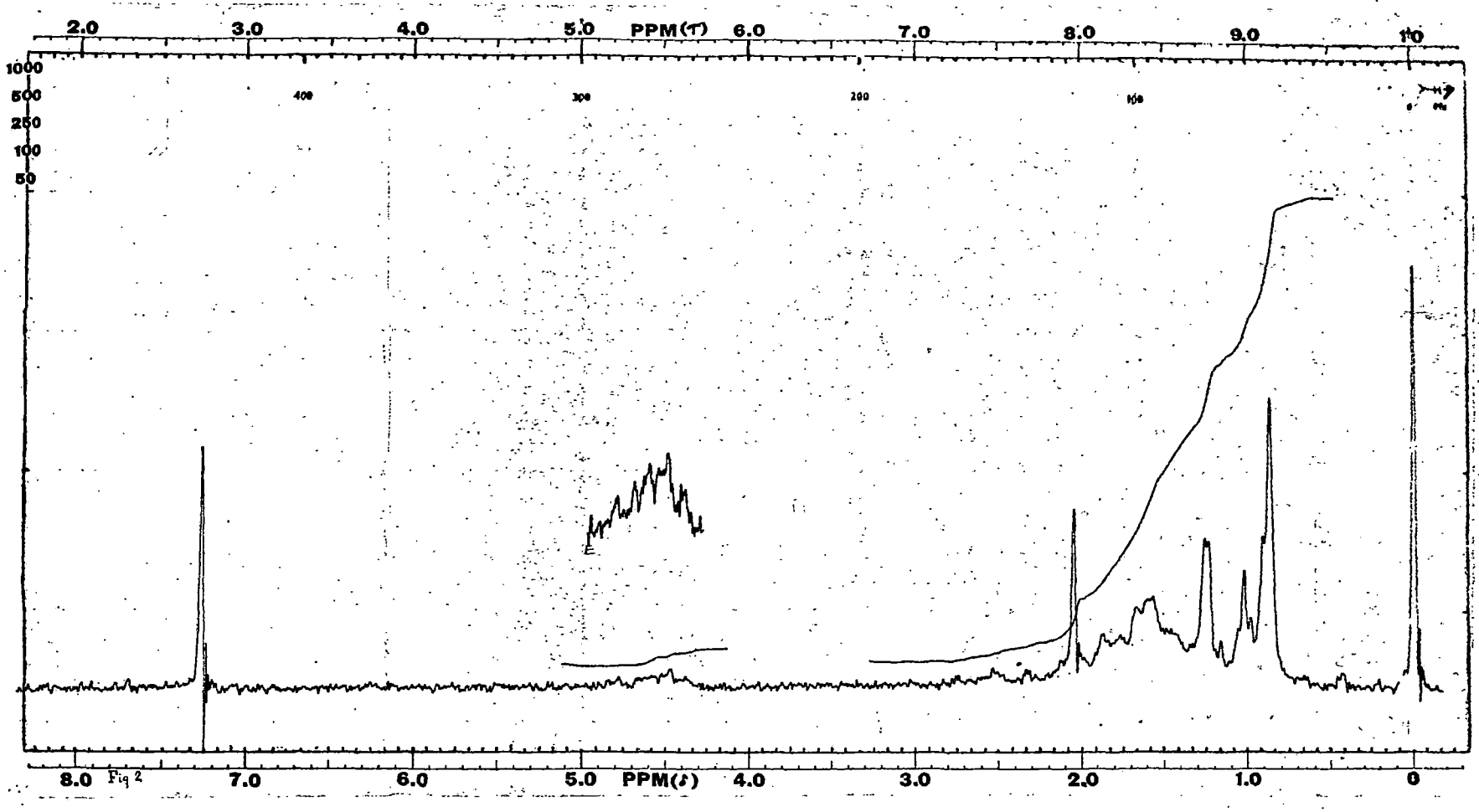


Fig.2: PMR spectrum of 15-bromo-oleanolic lactone acetate 60.

61, $C_{32}H_{48}O_4$, m.p. 308—10°. The compound gave negative Beilstein test for bromine, no UV absorption was observed in the region 220—350 nm; IR (nujol) spectrum (Fig.3) showed the presence of γ-lactone at 1770 cm^{-1}, the acetate group ν_{max} 1730, 1250 cm^{-1} and the disubstituted double bond at 900 cm^{-1}. In the PMR spectrum (Fig.4) of 61, the signals at 0.8 to 1.3 ppm showed the presence of seven tertiary methyl groups, the peak at 2.04 ppm was due to acetoxy methyl protons and the multiplet at 4.5 ppm was due to axial proton geminal to the C—3—acetyl group. Appearance of AB quartet at 5.5 ppm with coupling constant 10 Hz indicated the presence of the grouping —C—C(H)=C(H)—C— .

The unsaturated lactone so obtained was hydrogenated in a mixture of ethyl acetate and acetic acid solution using Adam's catalyst in Parr' Hydrogenation apparatus under pressure of 40 PSI. After usual work up the solid obtained was chromatographed and crystallised from chloroform and methanol mixture. The crystallised product so obtained had m.p. 293—94°, $[\alpha]_D$ +19°, was found to be identical with an authentic sample of oleanolic lactone acetate[39] 62 (by m.m.p. and IR comparison). The authentic sample of oleanolic lactone was prepared by the method of Barton et al[39] from acetyl oleanolic acid. The formation of oleanolic lactone acetate clearly

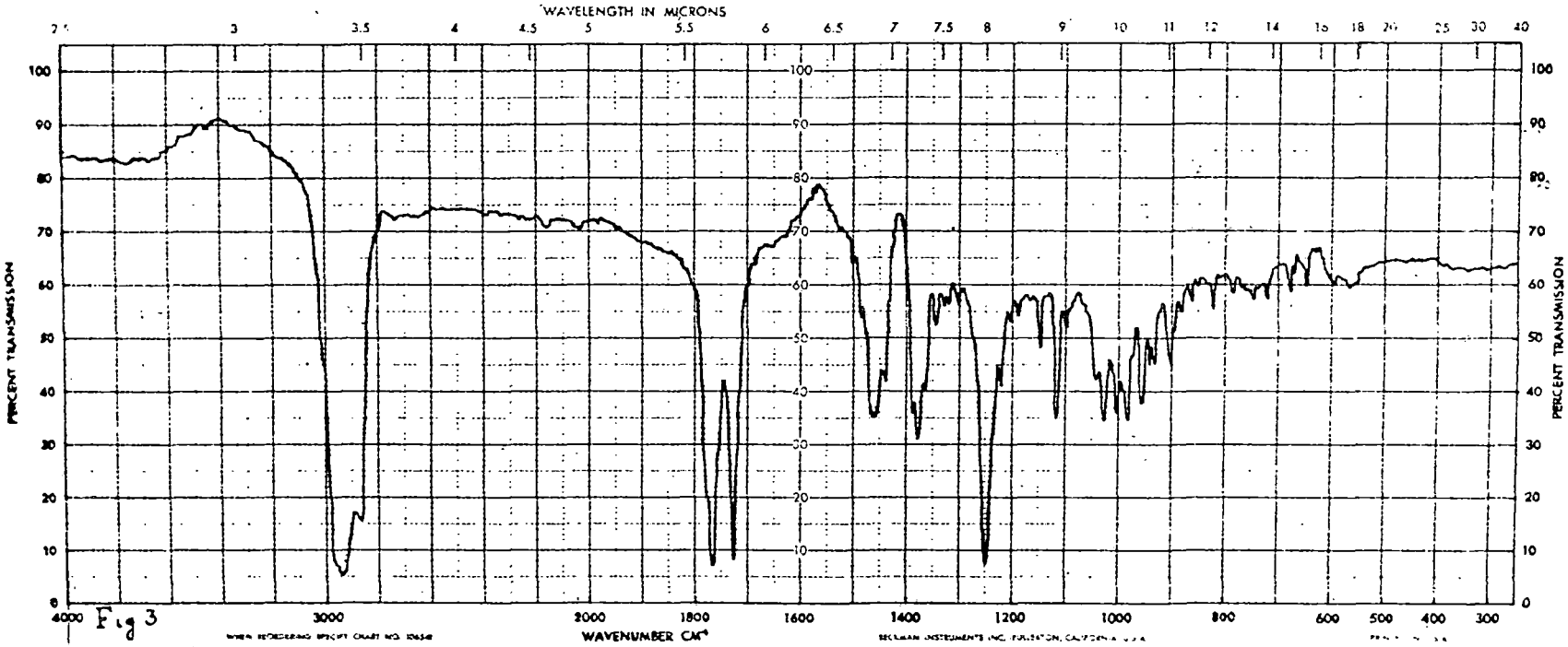


Fig.3: IR spectrum of 15,16-dehydro oleanolic lactone acetate, 61.

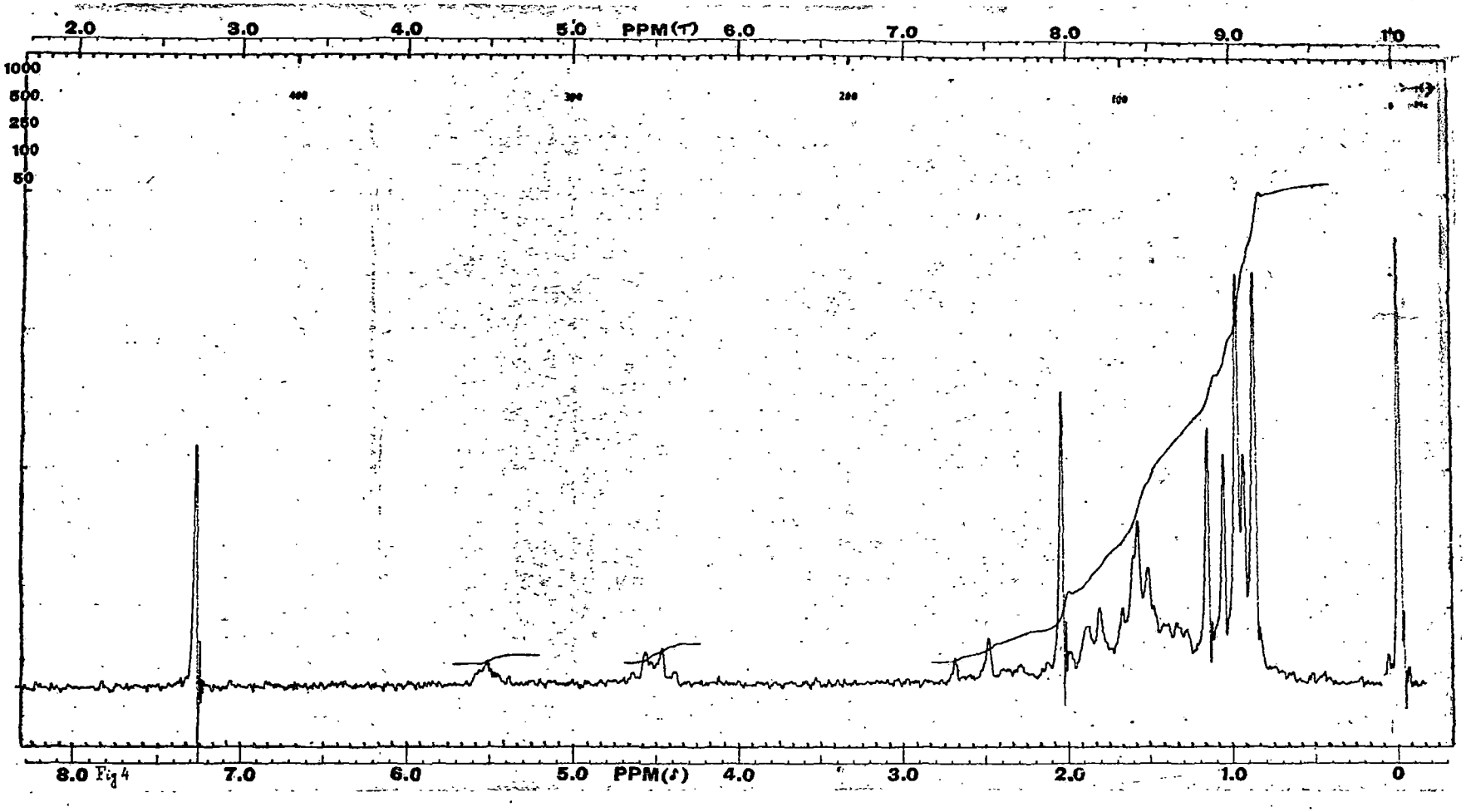
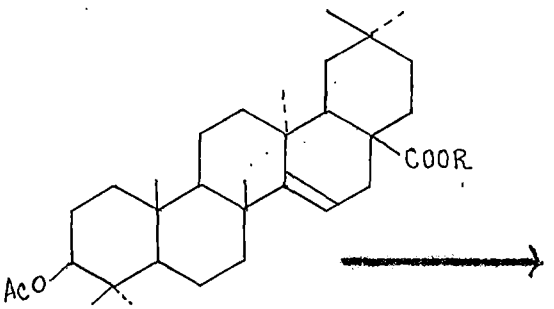


Fig.4: PMR spectrum of 15,16-dehydro oleanolic lactone acetate, 61.

illustrates that the lactyl oxygen is attached to C—13 carbon atom and is β oriented. The whole reaction can be shown in Scheme—6.

Scheme—6



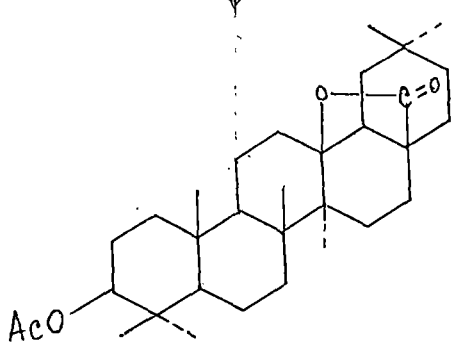
55, R = Me
59, R = H

→ Bromolactone 60

60 —Dimethyl Aniline→ Dehydrobrominated γ lactone with a double bond

61

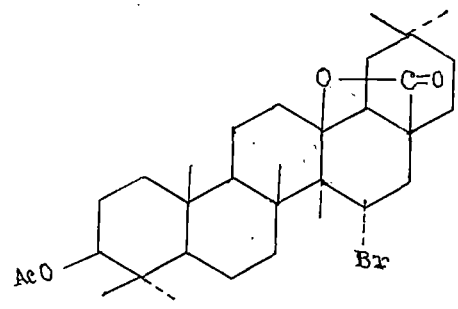
↓ H_2, under pressure



62

So the position of attachment of the lactonic functional group in the bromo lactone 60 was clear. But the position of bromine atom was not clear. To ascertain the position of bromine atom in the bromolactone, lithium aluminium hydride reduction on the lactone 61 was done which furnished a compound, $C_{30}H_{48}O_2$, m.p. 185—86° that was identical with an authentic specimen of aegiceradiol[38] 58 (mmp and IR comparison). The formation of aegiceradiol which contains one of the double bonds at C—15—16 position clearly indicates the position of bromine atom either at C—15 or C— 16 position. The position of the bromine atom should be at C—15 position since the double bond in the original acetyl methyl aleuritolate was at C—14—15 position. So entrance of bromine atom should be at C—15 position as in the case of taraxeryl acetate which gave the 15 bromo β -amyrin acetate 44 with NBS[36]. In the lithium aluminium hydride reduction the lactone ring was affected with the formation of a tertiary hydroxyl group at C—13 which undergoes dehydration producing C—12—13 double bond and $-CH_2OH$ group at C—17 position.

So the structure of the bromolactone can be assigned as 60.



60

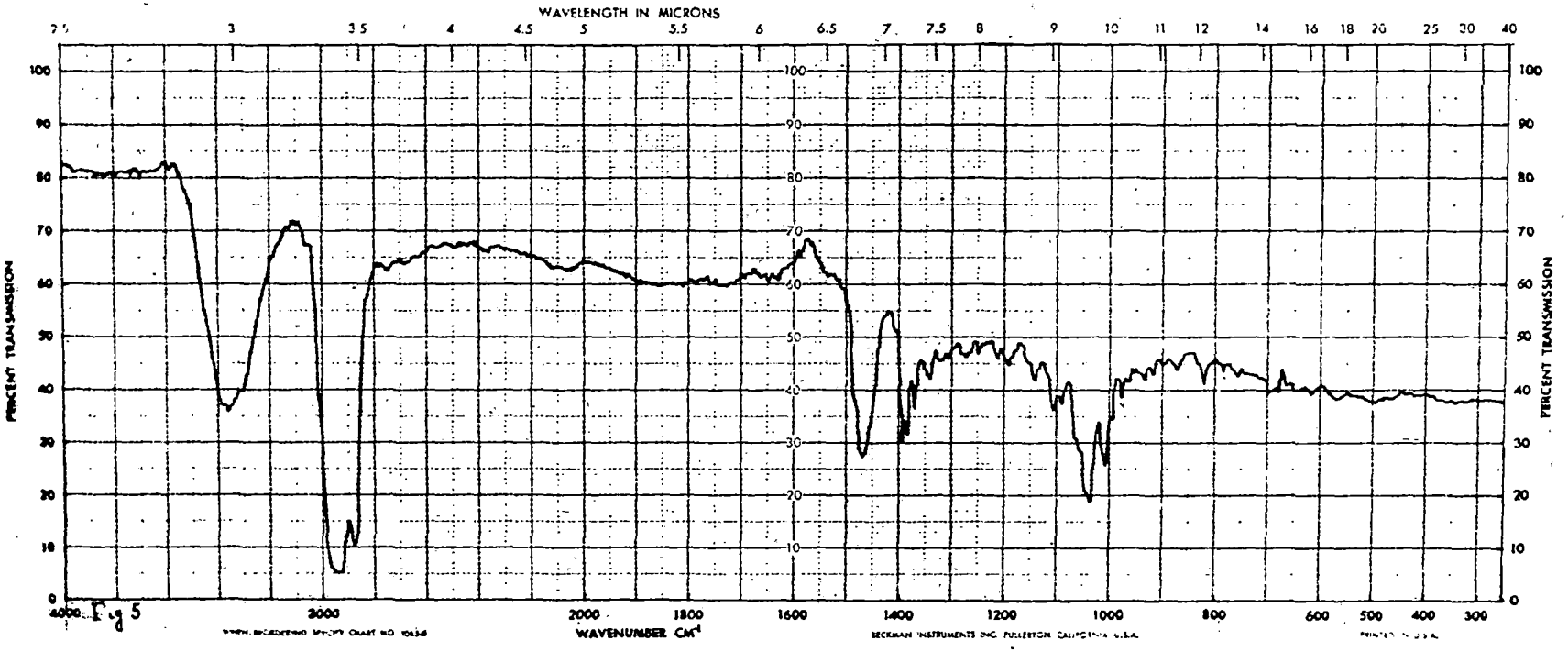
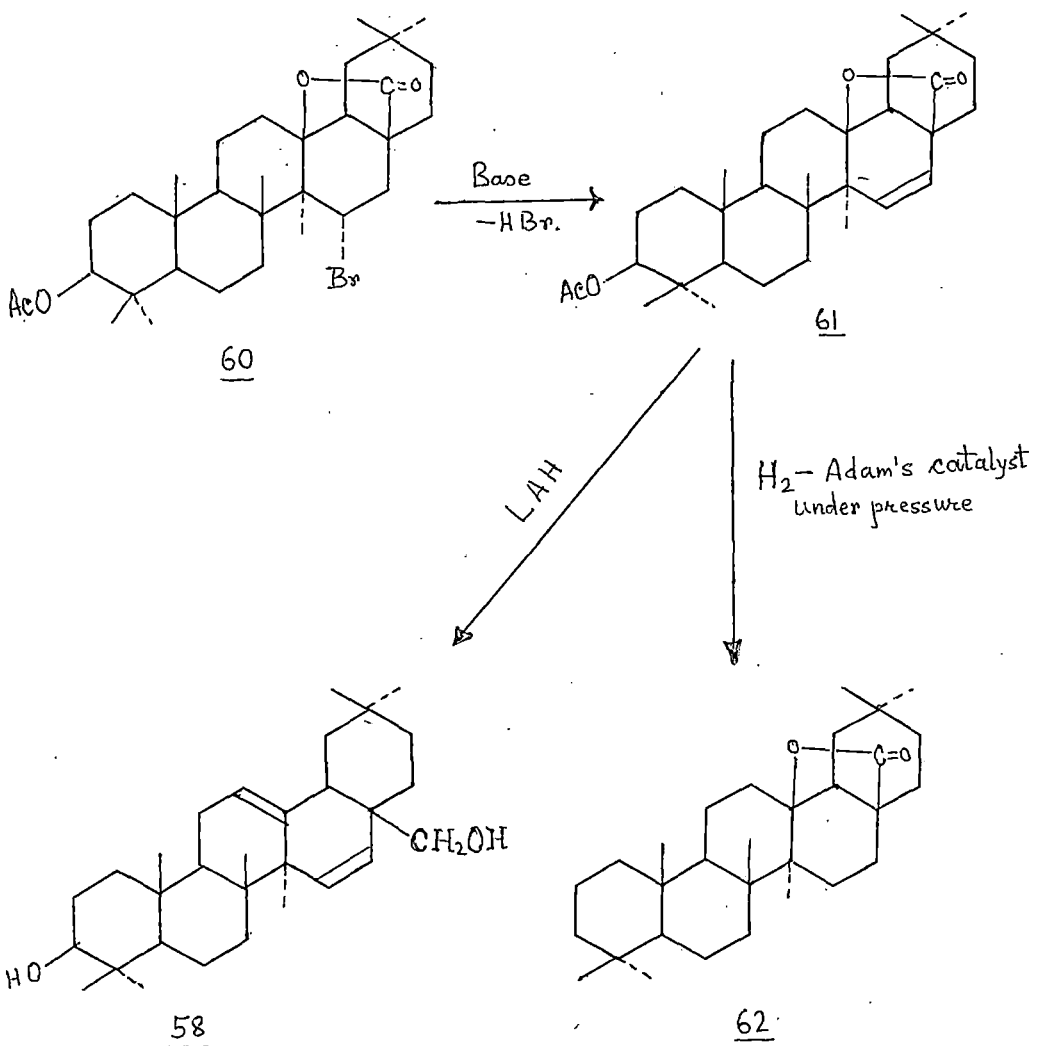


Fig.5: IR spectrum of aegeceradiol, 58.

Thus, the above sequence of reactions on the bromoketone 60 can be represented in the following way (Scheme—7):

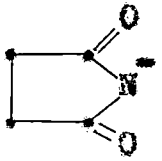
Scheme—7

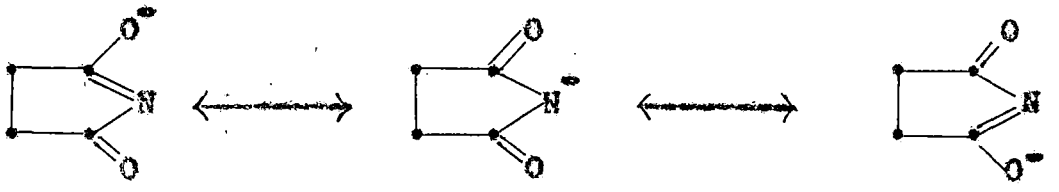


60 61 58 62

A study of the Dreiding model of the lactone 60 showed the bromine atom at C—15 position should be oriented ∝-equatorial. This stearochemistry was confirmed by the PMR spectral data. The proton centred at 4.3 ppm (d,d) with coupling constants 14Hz (Ja, a) and 3Hz (Ja, e) was due to the axial proton geminal to the bromine which must have an axial and equatorial protons on the vicinal carbon. This confirmed the presence of bromine atom at C—15 equatorialy oriented.

Mechanism of formation of the bromolactone 60:

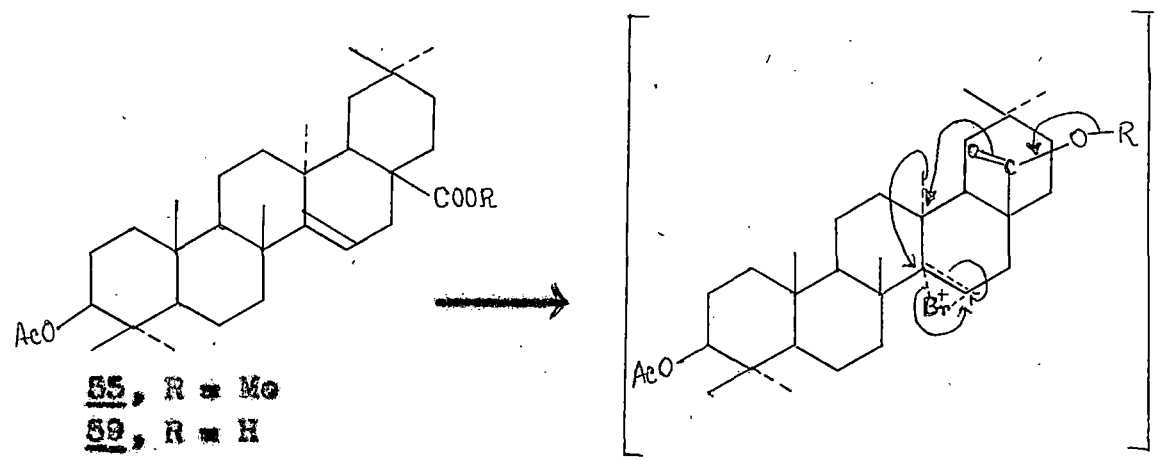
It is well known that dimethyl sulfoxide is a very polar solvent and it is expected that it probably dissociates to  anion and Br^+ cation; the anion being stable due to two other resonating structures.



The bromonium ion thus produced, attacks the olefinic double bond at C—14—15 position forming a three membered charged intermediate which immediately rearranges

to the stable form by the opening of the ring system with concerted migration of C—13 methyl to C—14 position and attack of the C=O⟶ II bond to the vacant C—13 position forming the lactyl bond with loss of CH_3 or H as cation from the COOH group. The mechanism can be shown in Scheme—8.

Scheme—8



55, R = Me
59, R = H

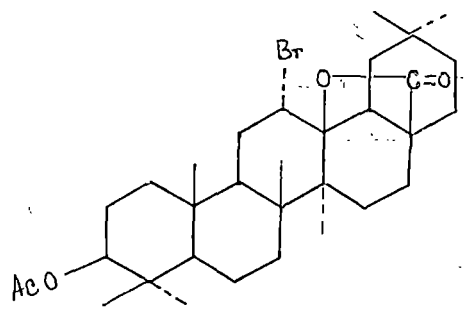
Since bromine atom is bulky it assumes to enter in the equatorial position so as to have minimum steric interactions.

So treatment of NBS in presence of dimethyl sulfoxide on triterpene acid or ester produced bromolactone. As no systematic work was reported on the action of N-bromosuccinimide in presence of dimethyl sulfoxide we were interested whether the reaction could be applied to other triterpene

acids or esters as well. For this purpose the reaction was performed on two other triterpenoids, one belonging to oleanane skeleton viz. acetyl methyl oleanolate and the other to the lupane skeleton viz. acetyl methyl betulenate.

(11) Treatment of N-bromosuccinimide in presence of dimethyl sulfoxide on acetyl methyl oleanolate 63:

Acetyl methyl oleanolate 63 was taken in chloroform and dimethyl sulfoxide was added. To the resulting solution N-bromosuccinimide was added in portions as mentioned in the case of acetyl methyl aleuritolate. After usual work up and chromatography followed by crystallisation a product was isolated 65, $C_{32}H_{49}O_4Br$, m.p. 215—16°, IR(nujol) spectrum (Fig.6) showed a peak at 1770 cm^{-1} indicating the presence of γ -lactone and other peaks at 1725 cm^{-1} and 1240 cm^{-1} were due to acetate functional group. It gave positive Beilstein test for bromine. The compound 65 was found to be identical with 3 β -acetyl-12 α -bromo olean-28→ 13 olide[40], (m.m.p. and IR comparison) prepared by the treatment of 3 β -acetyl oleanolic acid 64 with bromine in acetic acid. So the structure of the bromolactone can be assigned as 65.



65

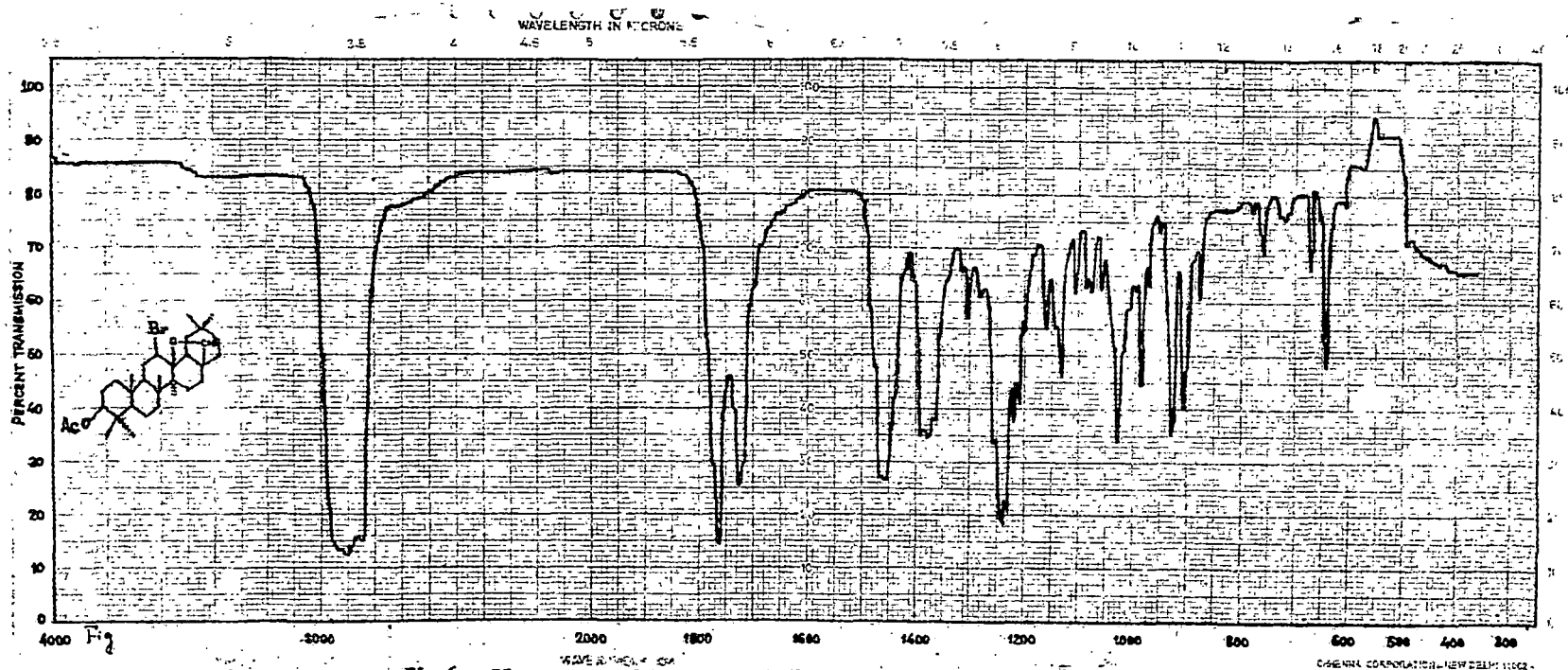


Fig 6 : IR spectrum of bromolactone 65, obtained from acetyl methyl oleanolate.

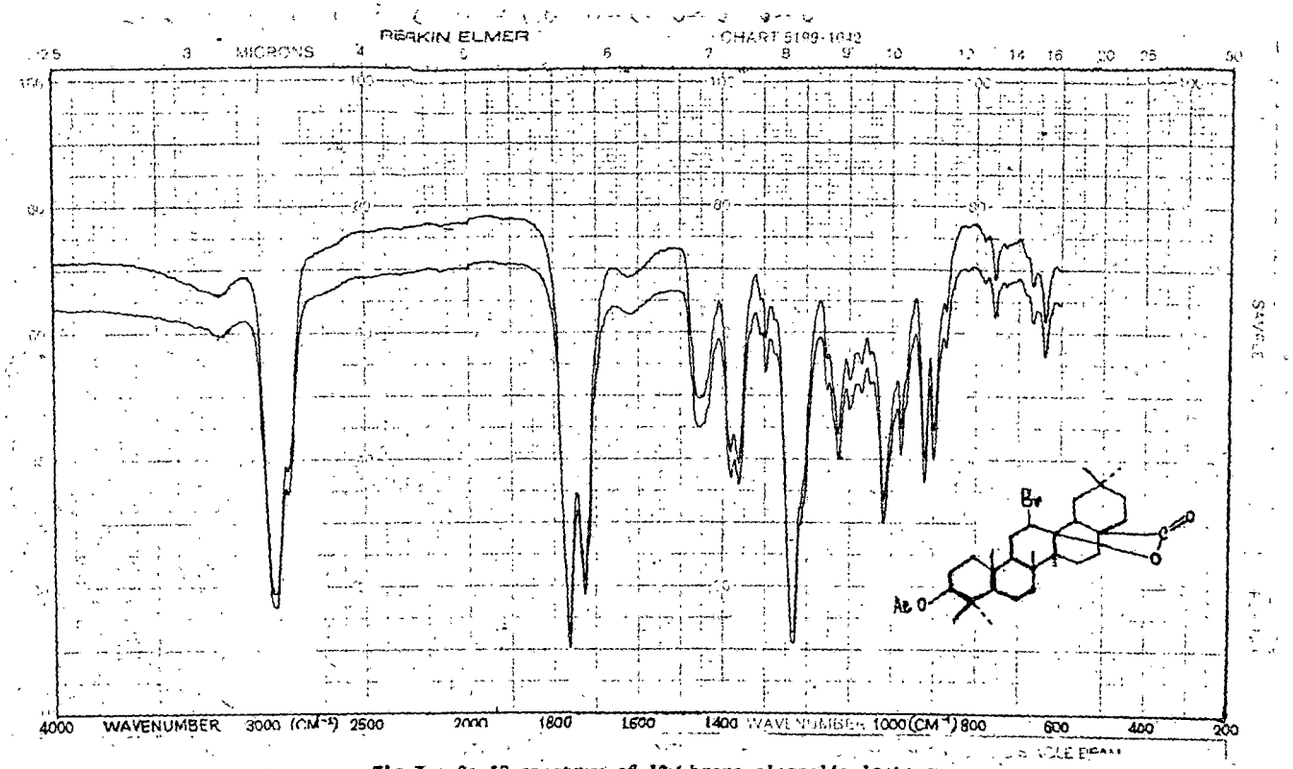
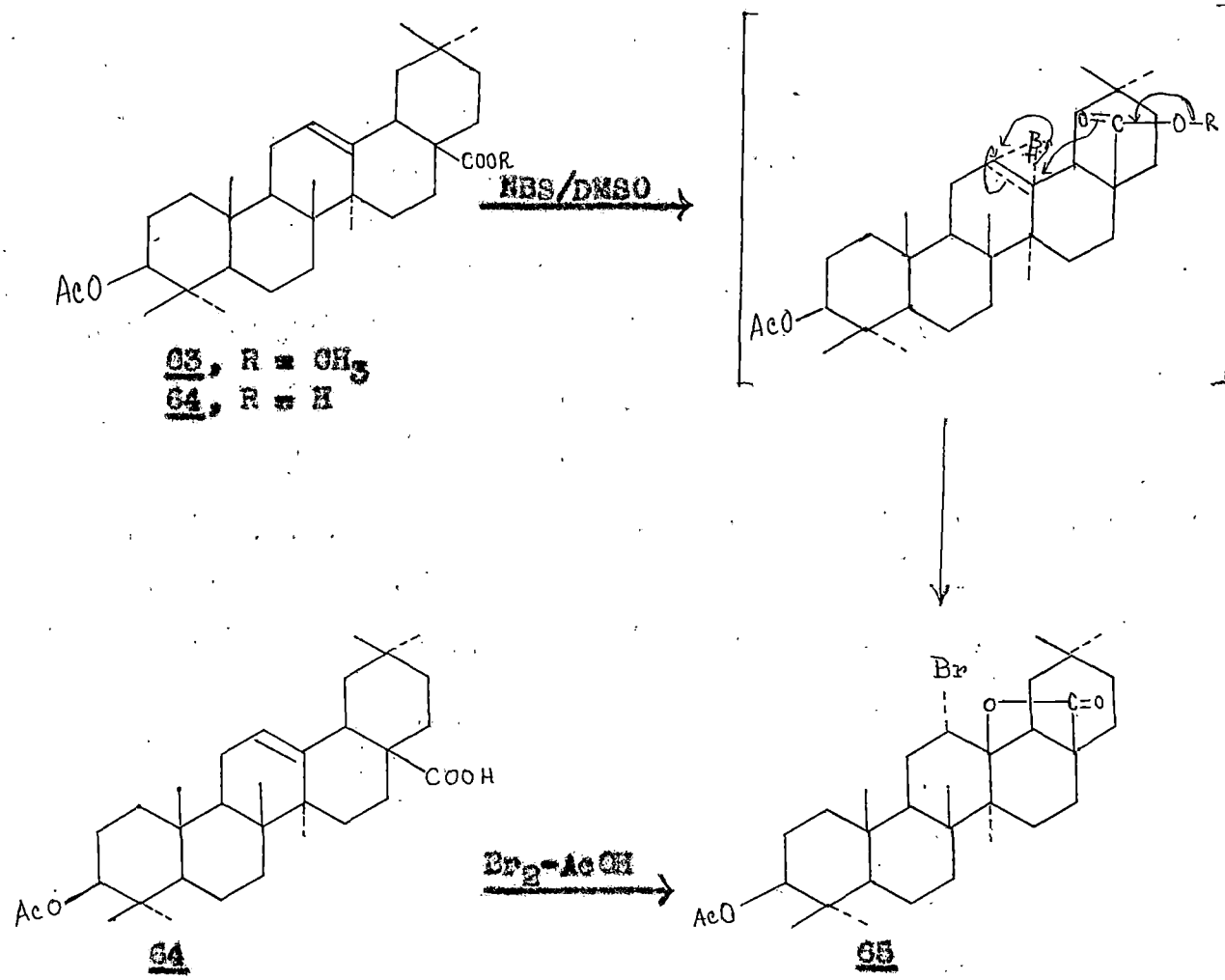


Fig 7 : Co-IR spectrum of 12α-bromo-oleanolic lactone acetate 65, with authentic specimen.

The mechanism (Scheme—9) of formation of 65 can be anticipated as given in case of lactonisation of acetyl methyl aleuritolate, mentioned earlier.

It was found that 3β-acetyl oleanolic acid 64 also formed the same bromolactone 65 on treatment with N-bromo-succinimide in dimethylsulfoxide (by m.m.p. and IR comparison).

Scheme—9



63, R = CH_3
64, R = H

64

65

The reaction of N-bromosuccinimide in presence of dimethylsulfoxide was carried out on a third compound 3β -acetyl methyl betulenate[41] 66. In 3β -acetyl methyl betulenate 66 the double bond is in the form of isopropenyl and the ring D of the triterpene ester is five membered.

(iii) Treatment of NBS in presence of dimethylsulfoxide on methyl 3β -acetyl betulenate[41] 66:

3β -acetyl methyl betulenate 66 was taken in distilled chloroform and dimethyl sulfoxide was added to it. To the resulting solution N-bromosuccinimide was added in portions as was done in earlier cases. Following the same procedure and work up a mass was isolated which on chromatography on a silica gel column afforded three different bromo compounds.

The first compound which was obtained from the column on elution with petroleum (4):benzene (1) was crystallised form $CHCl_3$—MeOH. The crystallised product gave positive test for bromine. Analysis showed the compound had the molecular formula $C_{33}H_{51}O_4Br$ m.p. 235—36°, $[\alpha]_D$ +42.55°. IR spectrum (Fig.8) showed peaks at 1735 cm^{-1} (—COOCH$_3$) 1725 cm^{-1} (—OCOCH$_3$), 1260 cm^{-1} (—CH$_2$Br), 1660, 875 cm^{-1} (=CH$_2$).

PMR spectrum of the compound showed 5-tertiary methyl groups in the region 0.87—1.02 ppm, three hydrogens of —OCOCH$_3$ group was found to be at 2.06 (singlet), two

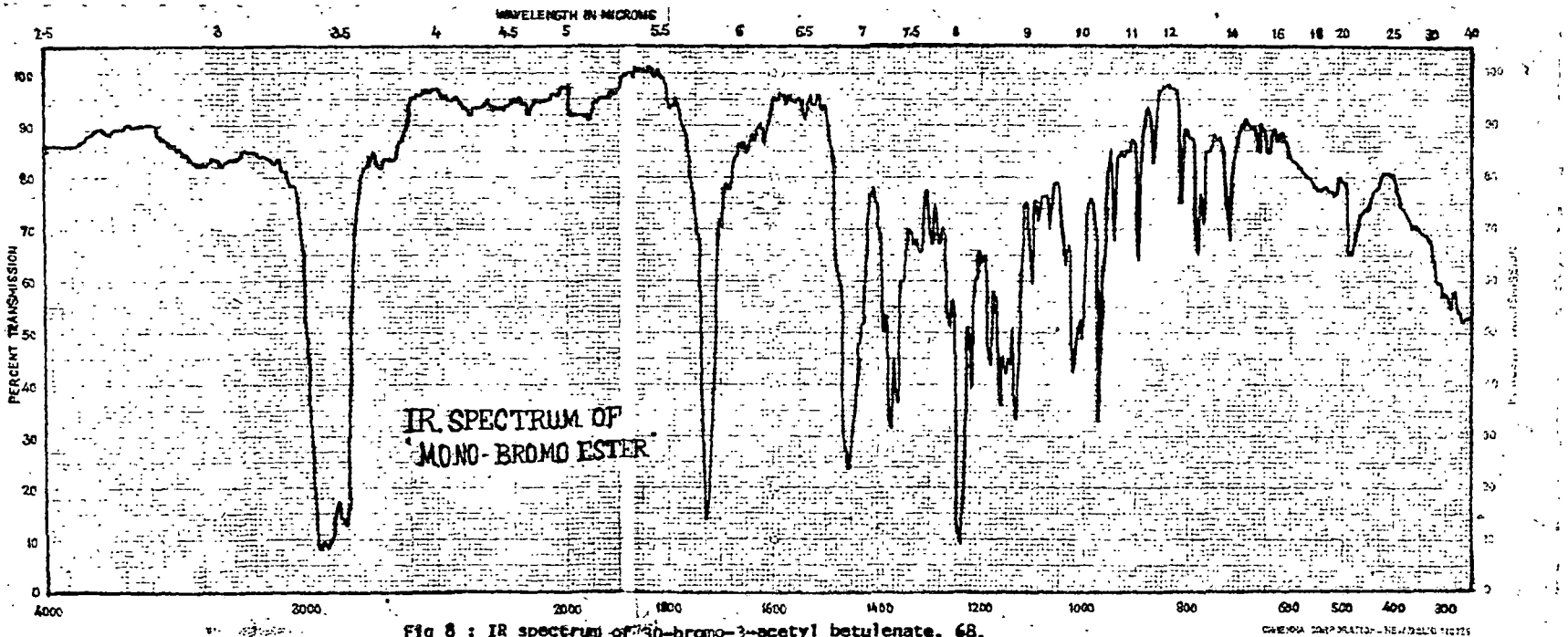
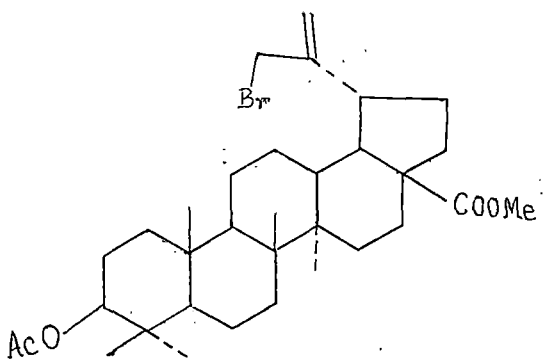


Fig 8 : IR spectrum of 30-bromo-3-acetyl betulenate, 68.

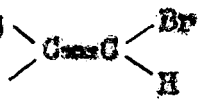
hydrogens as singlet at 3.9 ppm for $-CH_2Br$. At 5.1 ppm two hydrogens were obtained as multiplet due to presence of $=CH_2$. The proton geminal to the 3-acetyl group appeared as a multiplet at 4.5 ppm. From these spectral data the structure of the brome compound has been assigned structure 68 as methyl 30 brome-3 β -acetyl betulenate.

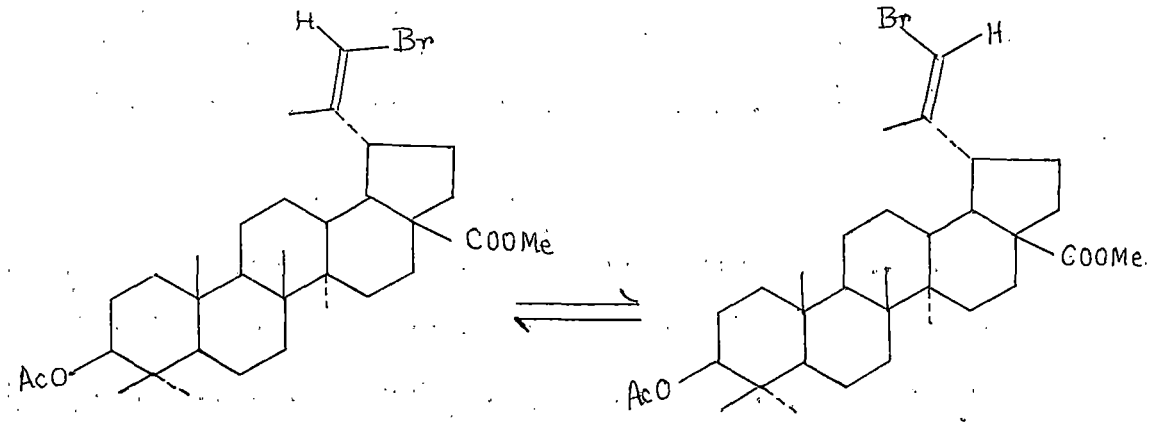


68

The structure has been confirmed by debrominating the compound 68 by treatment with Zn dust and acetic acid and the product that was isolated was found to be identical with methyl 3 β -acetyl betulenate 66 (m.m.p. 200—210).

Further elution of the column with petroleum (4): benzene (1) furnished a second brome compound. This on crystallisation from chloroform-methanol mixture was analysed for $C_{33}H_{51}O_4Br$, m.p. 228—30°, $[\alpha]_D$ +36°. IR spectrum showed

the presence of ester group at 1735 cm^{-1}, acetate carbonyl at 1725, 1240 cm^{-1} and methylene double bond at 1640 cm^{-1} and 900 cm^{-1}. The compound gave positive Beilstein test for bromine. The structure of the compound was derived from the ^1H NMR (Fig.9) spectrum. The peaks in the region 0.78—1.0 ppm indicated the presence of five tertiary methyl groups, the two singlets at 1.7 and 1.78 ppm that was integrated for 3 protons suggested the presence of a methyl group on a double bond, the singlets at 2.05 and 3.7 ppm that were integrated for three protons each showed the existence of acetoxy methyl and carbomethoxyl groups in the compound. The multiplet at 4.48 ppm was due to the proton geminal to the C—3 acetyl group. The presence of singlet at 5.76 and 5.97 ppm integrated for one proton suggested the cis trans isomerism of  group. Hence the structure 68a and 68b is suggested for this bromo compound.



68a 68b

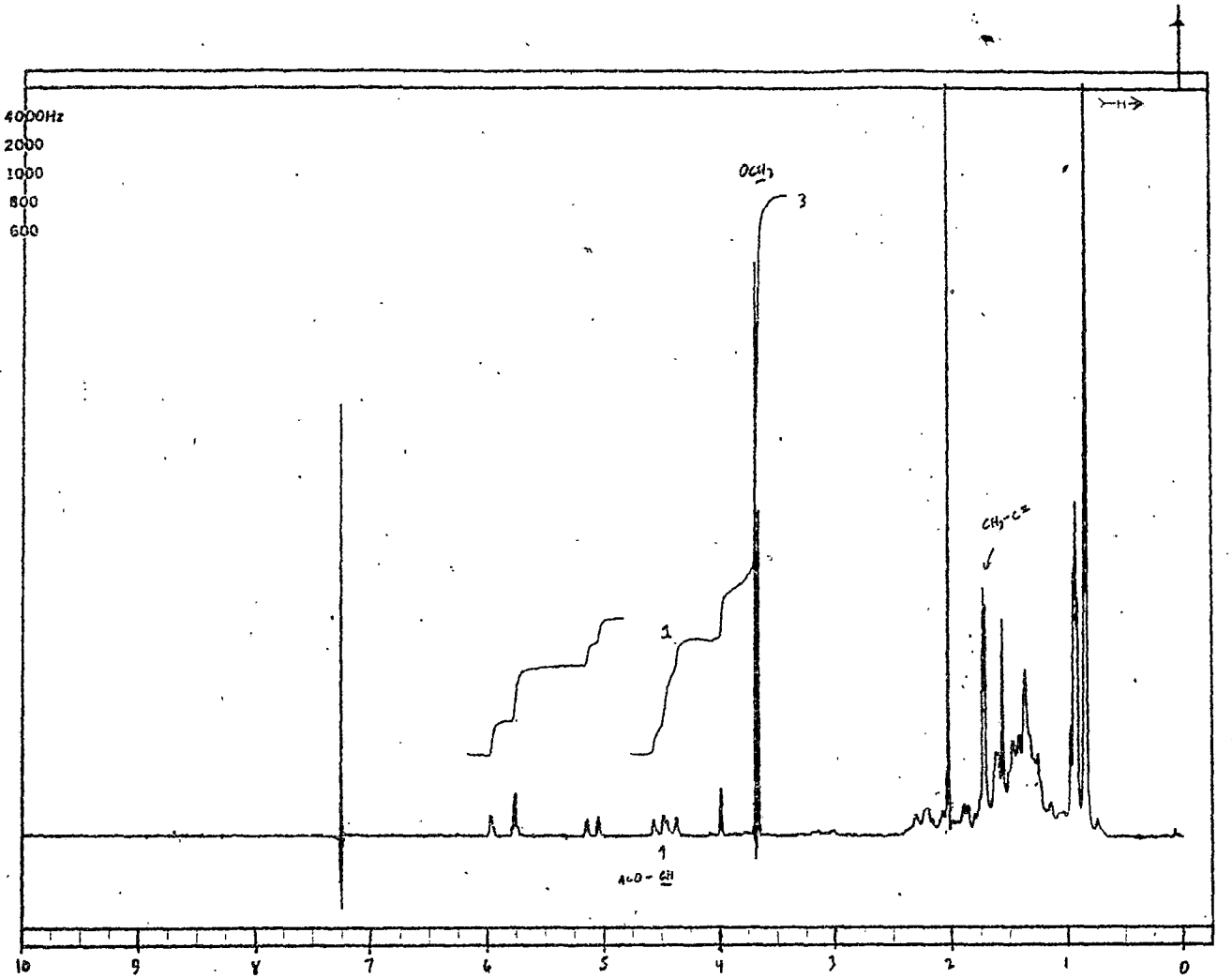


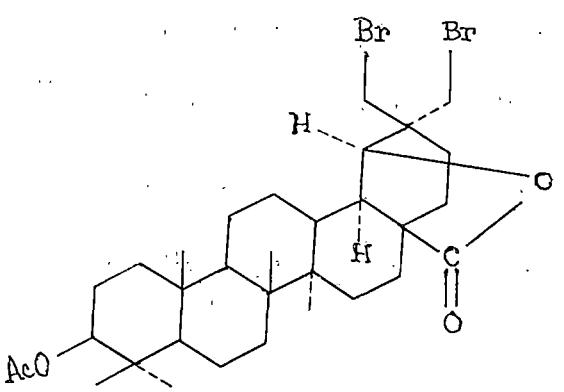
Fig.9: PMR spectrum of 68a ⇌ 68b.

Besides these the presence of peak at 3.9 for CHBr and doublet at 5.1 ppm for $=CH_2$ indicated that there is still some 68 present as impurity.

With the increase of polarity of the solvent petroleum ether (2):benzene (3), the third compound was obtained from the column which was crystallised from a mixture of chloroform-methanol. After crystallisation the compound obtained, gave positive Beilstein test for halogen that indicated the existence of bromine in the compound. The compound was analysed for $C_{32}H_{48}O_4Br_2$, m.p. 303—4°, $[\alpha]_D$ + 47°, showed a negative cotton (ϵ = -0.99) effect in the CD at 218 nm.

IR spectrum (Fig.10) of the compound showed peaks at ν_{max} 1780 cm^{-1} (γ-lactone), 1720, 1240 cm^{-1} (—OCOCH$_3$), 1260 cm^{-1} (—CH_2Br),

The structure of the dibromolactone was proposed to be 69 on the basis of IR, NMR and mass spectral studies.



69

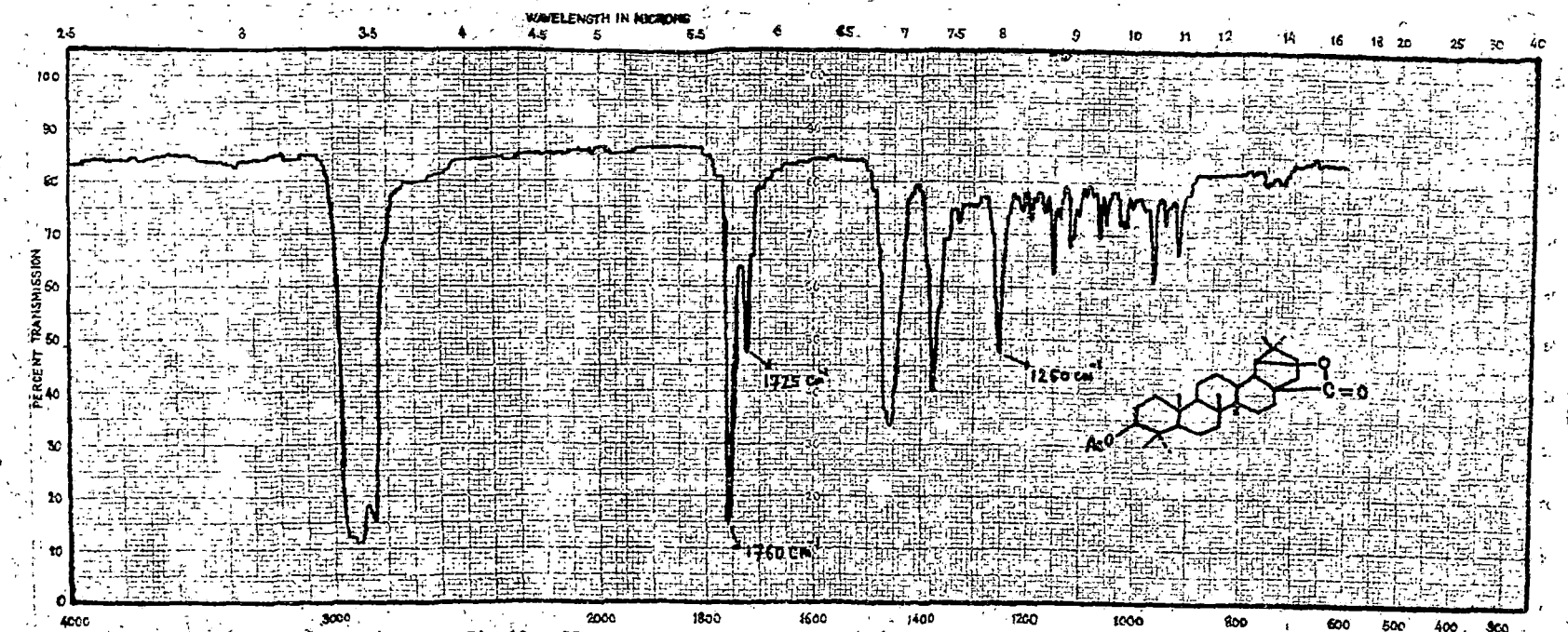


Fig 10 : IR spectrum of dibromolactone 69, obtained from acetyl methyl betulenate.

PMR spectrum (Fig.11) of the compound showed the presence of 5 tertiary methyl groups in the region 0.87 to 0.92 ppm. The singlet at 2.03 ppm integrated for three protons was due to the acetoxy methyl group. Between the region 3.44 to 3.78 ppm there were four peaks. The multiplets centred at 3.44 and 3.65 ppm showed the presence of one proton each. The doublets at 3.73 and 3.78 ppm also showed the presence of one proton each. These peaks were due to 4 hydrogens of C—28—$\underline{H}_2$Br and C_{30}—$\underline{H}_2$Br. The methylene protons present at C_{29} and C_{30} were non equivalent and their position was fixed due to hindered rotation of these groups containing the bulky and highly electronegative bromine atoms which were at the farthest position. The coupling constant was found to be 11 Hz.

At the region 4.34 ppm one peak which was found to be singlet containing only one hydrogen was assumed for the $\underline{H}$—C—OCO i.e. the C—19 proton adjacent to the lactonic oxygen. This value of the PMR spectrum suggested that this proton had either no neighbouring proton or the conformation was such as to have no coupling with the neighbouring proton. A study of the Dreiding model of the compound 69 showed that the dihydral angle between the C—18 and C—19 protons was almost 90° to make it strain free model if the C—18 proton was α-oriented. So the C—19 proton should also be α-oriented.

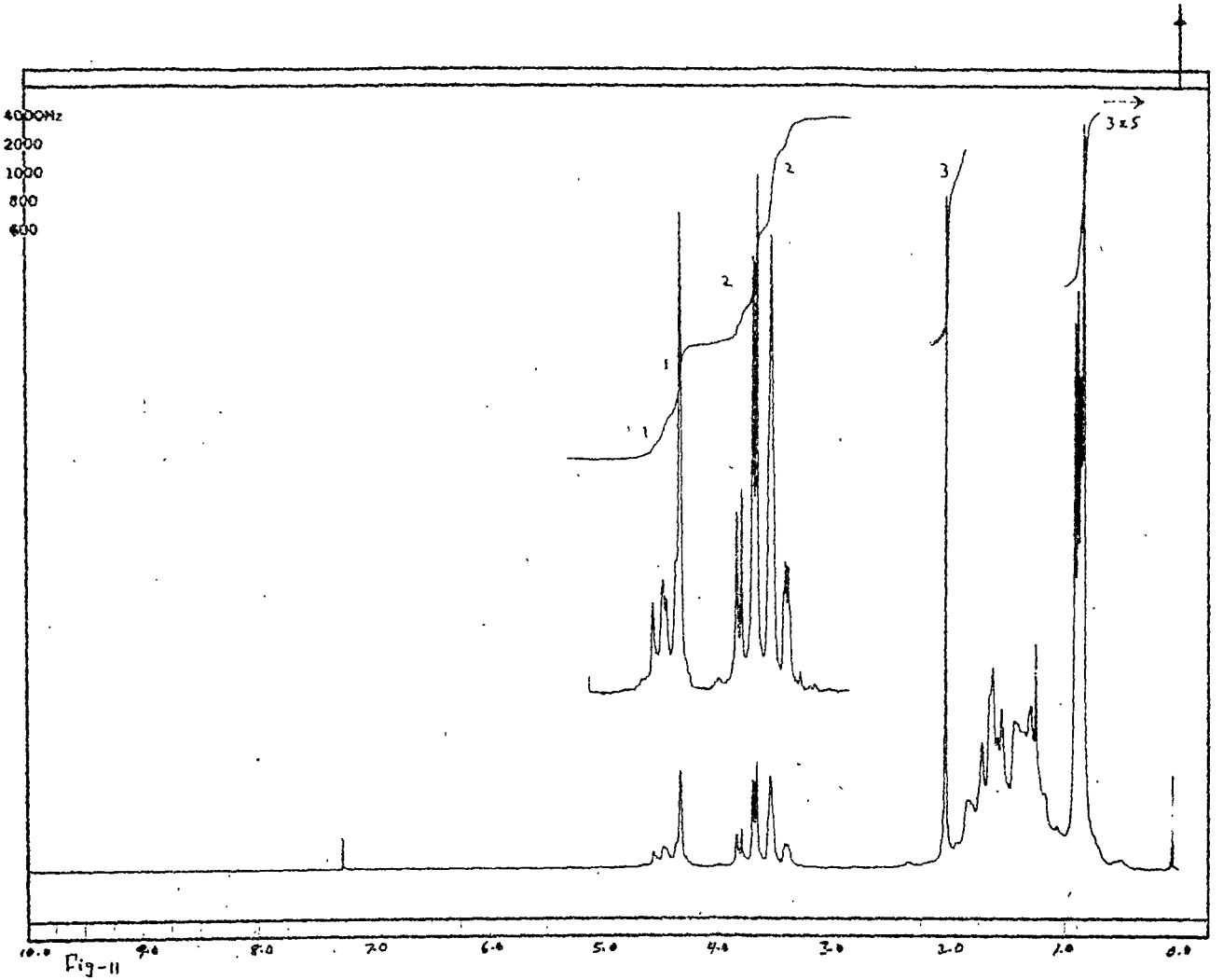


Fig.11: PMR spectrum of dibromo lactone 69.

The peak centred at 4.5 ppm which was found to be a multiplet for only one proton was due to the proton of H—C—O—$COCH_3$.

^{13}C NMR spectrum (Fig.12) of the compound 69 showed a peak at 178.48 ppm which was singlet revealed the presence of a lactonic carbonyl group in a five membered ring. The peak at 171 ppm which was also a singlet indicated the presence of an acetoxy carbonyl group, doublets at 80.77 and 60.95 ppm were due to C—H groups bonded to acetoxy and lactyl oxygen atoms.

Mass spectrum of the compound 69:

Mass peaks (Fig.13) of the compound 69 i.e. 3β-acetyl-29,30-dibromo-18α-olean-28→19β-olide is given in the following way. In the following explanation M_1H represents the protonated molecular mass with the two bromine atoms of isotopic mass 81. M_2H represents the mass with the isotopic bromine 79 and 81 and M_3H for the isotopic mass 79. The molecular ion peaks at 659 (M_1H^+, 10%), 657 (M_2H^+, 19%) and 655 (M_3H^+, 10%) were obtained. The peaks at 599, 597 and 595 might be due to loss of acetic acid from the molecular ion peaks (M_1H^+—CH_3COOH, M_2H^+—CH_3COOH, M_3H^+—CH_3COOH respectively). The peak at 503 might be due to 599—$CH_3{}^{81}Br$ and 597—$CH_3{}^{79}Br$ whose abundance was found to be 5%. The peak

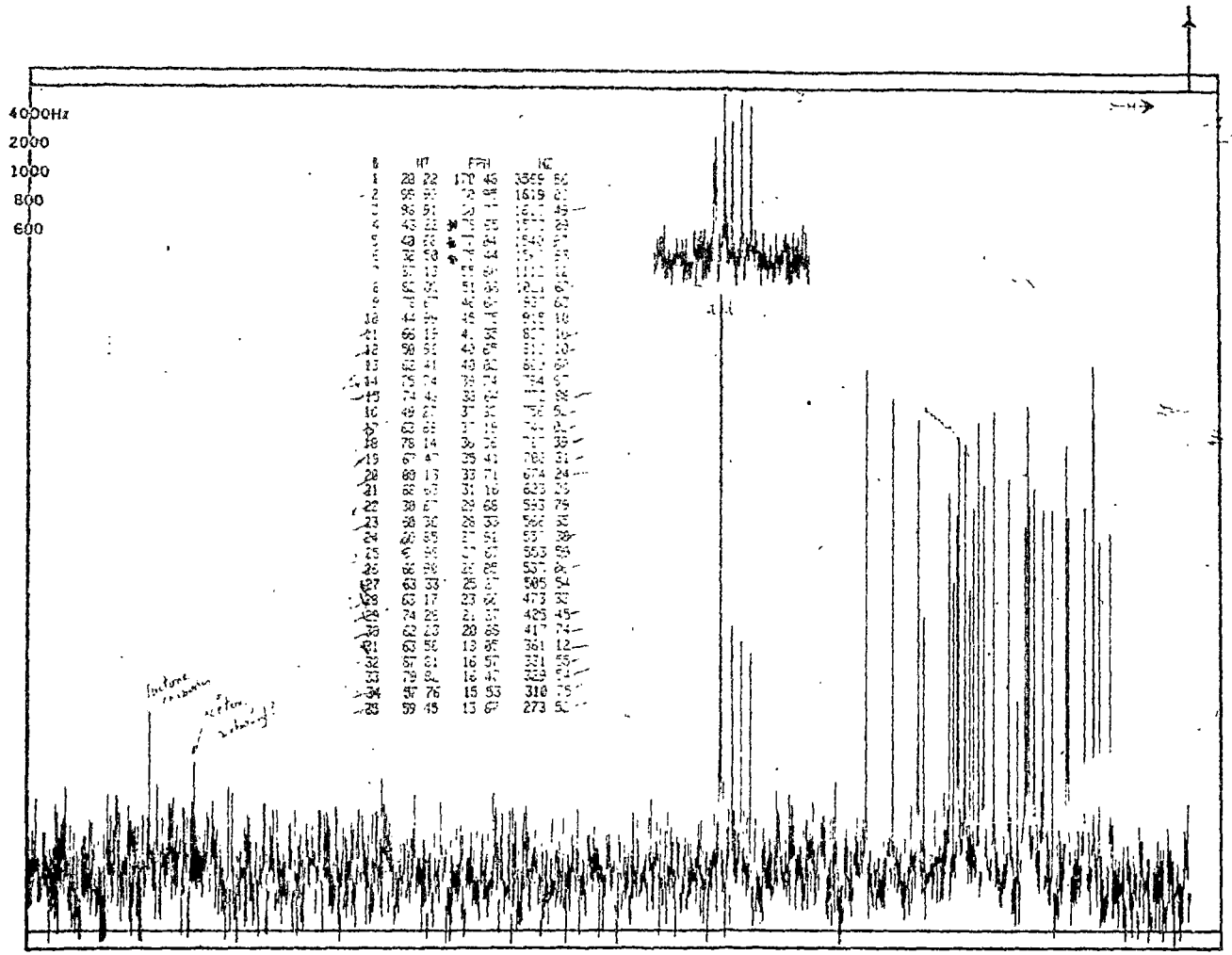


Fig.12: ^{13}C NMR spectrum of dibromolactone 69.

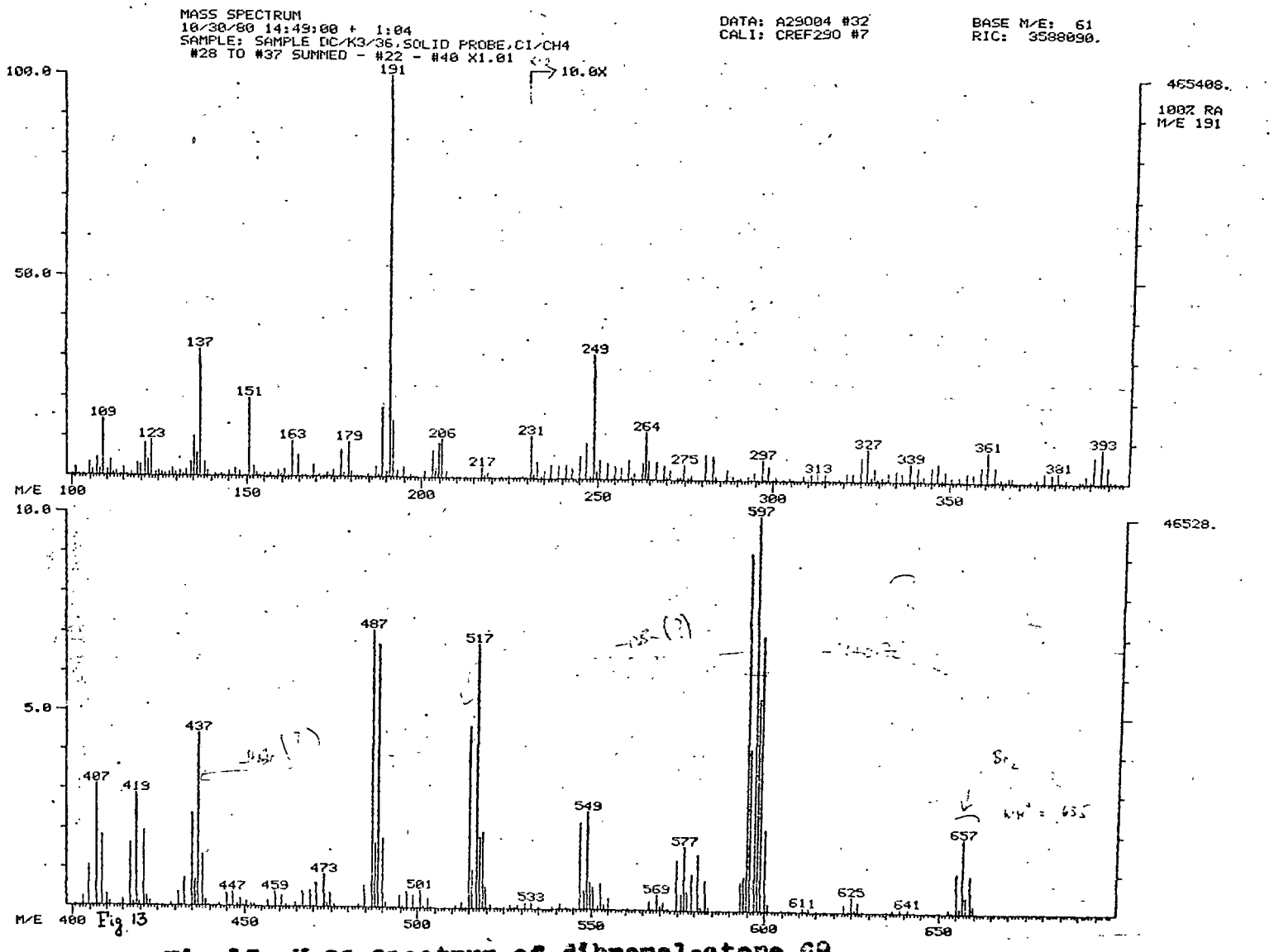


Fig.13: Mass spectrum of dibromolactone 69.

at 501 was assumed for 597—$CH_3{}^{81}Br$ and 595—$CH_3{}^{79}Br$ also of same abundance (5%). The fragment 407 would be for 503—$CH_3{}^{81}Br$ or 501—$CH_3{}^{79}Br$ whose abundancy was 31%. The peak at 517 might be due to 599—$H^{81}Br$ and 597—$H^{79}Br$. The other fragments were 515 (597—$H^{81}Br$ and 595—$H^{79}Br$), 489 (517—CO), 487 (515—CO), 457 (MH—Br_2), 435 (517—$H^{81}Br$ and 515—$H^{79}Br$), 407 (503—$CH_3{}^{81}Br$ and 501—$CH_3{}^{79}Br$ or 457—$H^{79}Br$ and 439—$H^{81}Br$), 249, 191 (base), 189.

The structure of the dibromolactone 69 was finally proved by the following reactions. The dibromolactone was attempted for dehydrobromination by refluxing with dimethyl aniline. It was found that the compound resisted dehydrobromination and the starting material was recovered. This was one supporting evidence of the presence of bromine aton at C—29 and C—30 positions.

Debromination reactions on the dibromolactone 69 was also carried out with the help of Raney Nickel. This reaction furnished a compound 70, $C_{32}H_{50}O_4$, m.p. $\rangle$ 360°, $[\alpha]_D$ +59° was found to be identical with an authentic specimen of 3β-acetyl-oleanan-28→19β olide[42] (m.m.p. and IR comparison). The authentic specimen of 70 was prepared from acetyl betulinic acid. From these observation the structure of dibromolactone can be assigned as 3β-acetyl 29,30 dibromo-18α-H-oleanan-28→19β olide 69.

Treatment of 3β-acetyl betulenic acid[41] 67 with N-bromosuccinimide and dimethyl sulfoxide:

3β-acetyl betulenic acid 67 was taken in chloroform and dimethyl sulfoxide was added. To the resulting solution N-bromosuccinimide was added in portions as in the previous cases. After usual work up the mixture was separated as acid and neutral parts by treatment with alkali. Chromatography followed by crystallisation of the neutral part furnished a compound, $C_{32}H_{48}O_4Br_2$, m.p. 305—4°, was found to be identical (m.m.p. and CO—IR) with 69 i.e. the dibromolactone.

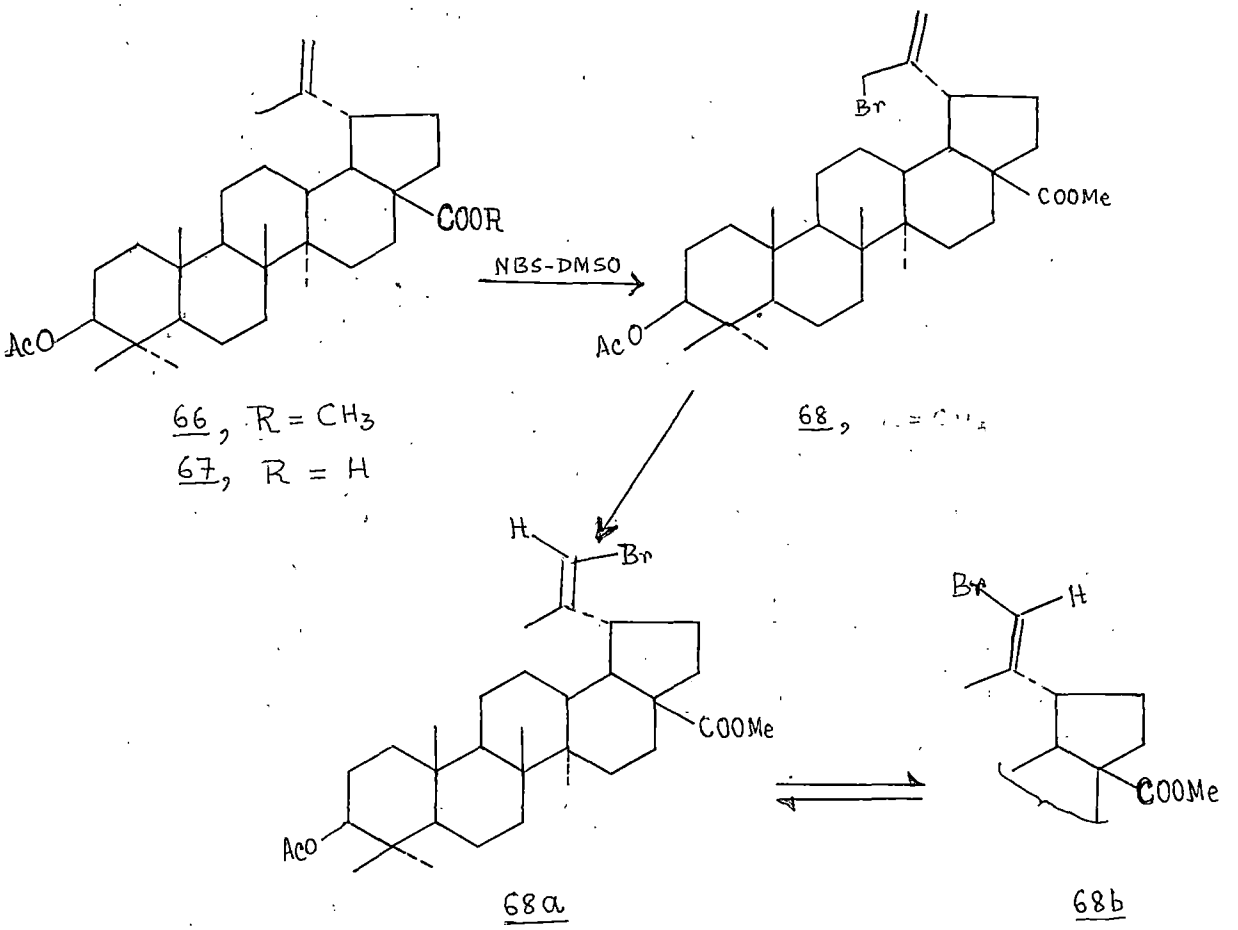
The alkali soluble part was acidified, extracted with ether and esterified with diazomethane. The product so obtained was chromatographed which on elution with solvent petroleum ether(4):benzene(1) furnished two compounds. The first compound was found identical with 68 and the second compound was identified as 68a $\rightleftharpoons$ 68b as discussed in the previous case.

Mechanism of bromination along with lactonisation:

When 66 or 67 was treated with N-bromosuccinimide in presence of dimethyl sulfoxide, first the allylic brominated product 68, was formed. This allylic brominated product might undergo isomerisation to furnish the isomeric cis-trans

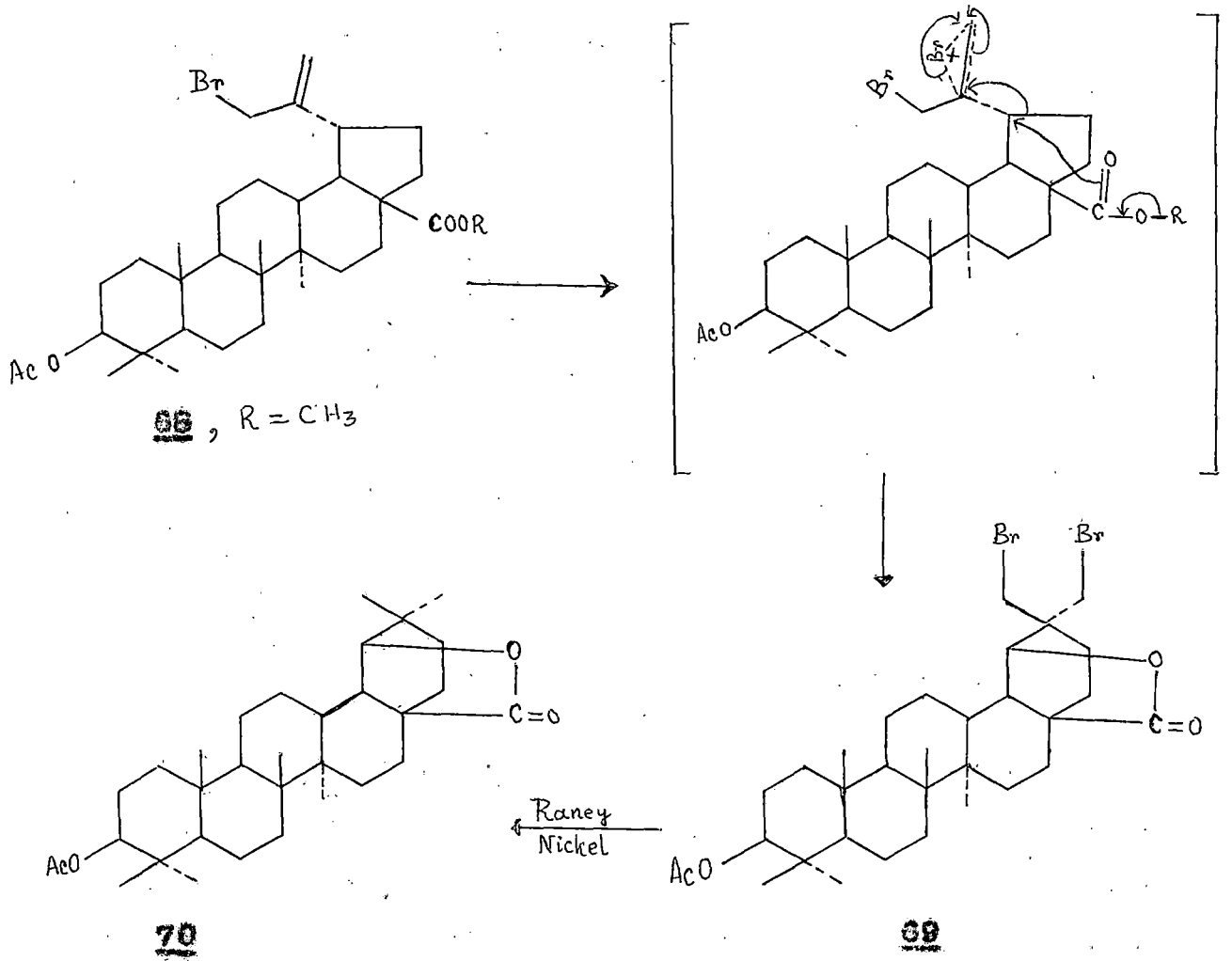
29-bromo compounds 68a ⇌ 68b. Further attack of the bromonium ion on the C—20—30 double bond of 68 caused simultaneously ring expansion and lactonisation. This fact was supported by the experiment with allylic brominated product 68. The isolated compound 68 was treated with N-bromosuccinimide in presence of dimethyl sulfoxide and the same bromolactone 69 was obtained. Again the recovery of starting material in the reaction of 3 β -acetyl methyl betulanate 71 with NBS suggested that the attack of bromonium ion on the double bond initiated the lactonisation (Scheme—10).

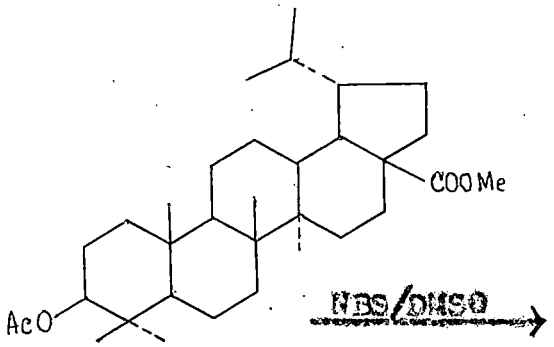
Scheme—10



68a 68b

The allylic brominated product 68 in the presence of NBS would be attacked by the bromonium ion on the olefinic bond at C—20—30 position forming a three membered charged intermediate which immediately rearranged to the stable form by opening of ring system with concerted migration of bond C-19-21 to C-20-21 position and attack of C=O→π bond to the vacant C-19 position forming the lactyl bond with loss of CH_3 or H as cation from the COOR group.





No reaction

71

The above findings show that the esters can be lactonized with NBS in DMSO as their corresponding acids if there is a double bond placed at an appropriate position in the compound. This is perhaps the first finding where the ester group is directly involved in lactone formation without being hydrolysed.

CHAPTER—III

E X P E R I M E N T A L

All melting points are uncorrected. The petroleum used throughout the experiment had the b.p. 60°—80°. Brockmann alumina (S.Merck) deactivated with 5% of 10% AcOH or silica gel (B.D.H.) was used for column chromatography. TLC plates were coated with Silica gel G (acc. to Stahl) having the thickness of about 0.2 mm and the spots located by exposing to iodine vapour. All the optical rotations were determined in chloroform solution. IR Spectra were recorded as nujol mulls and also in KBr disk on a Beckman IR—20 and Perkin Elmer Spectrophotometer respectively. NMR spectra were recorded on Varian A-60 or T-60 or EM-360 and FT-80A (1_H and 13_C) NMR instruments using $CDCl_3$ as solvent containing TMS as internal standard. Mass spectra were recorded by solid probe Cl/CH_4 method.

Treatment of methyl 3 β -acetylalcuritolate 55 with NBS: Formation of 3 β -acetyl olean-15-α -brom-28→13 β -olide 60:

200 mg of methyl 3 β -acetylalcuritolate dissolved in 12 ml dry and distilled $CHCl_3$ was mixed with freshly distilled 6 ml of dimethyl sulfoxide. The reaction mixture was cooled and 200 mg of NBS was added and kept in dark for

12 hours. The reaction mixture was then filtered and the filtrate was extracted with chloroform. The organic layer was washed several times with water and dried over anhydrous Na_2SO_4 . After distilling off chloroform, a gummy residue was obtained. The residue was dissolved in minimum amount of benzene and the solution was chromatographed on a column of alumina (25 gm alumina deactivated with 1 ml 10% acetic acid). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—1

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—7	Nil
Petroleum ether:benzene (3:2)	8—12	Solid m.p. 260°—270°

Further elution with more polar solvents did not give any solid material

Fraction 8—12 (table—1) were combined and the mixture was crystallised from a mixture of chloroform-methanol to give the product 60, having m.p. 280—82°.

Analysis report:

Found, C, 67.8% ; H, 8.8%

Required for $C_{32}H_{49}O_4Br$ C, 67.1% ; H, 8.6%

UV : No absorption above 220 nm.

IR(nujol): ν_{max} 1730 cm^{-1}, 1250 cm^{-1} (Acetate)
1760 cm^{-1} (γ- lactone) Fig.1

PMR : 0.8 to 1.4 ppm (7 tert. CH_3), 2.04 ppm (s, 3H, O-COC$\underline{H}_3$), 4.3 ppm (dd, 1H, C $\underline{H}$Br, J_{aa} = 14 Hz and J_{ae} = 3Hz), 4.8 ppm (m, 1H, $\underline{H}$-C-O-COCH$_3$) Fig.2

Beilstein test for halogen was positive.

TNM test - Negative.

Treatment of 3 β -acetyl aleuritolic acid 59 with NBS:

150 mg of the acid dissolved in 10 ml chloroform was mixed with 5 ml of dimethyl sulfoxide. Then 150 mg NBS was added to the reaction mixture and it was kept in dark for 24 hours. The chloroform solution was washed several times with water and the organic layer was dried over anhydrous sodium sulphate. After evaporation of chloroform the residue was chromatographed over a column of 10 gms of silica gel. The solid was dissolved in minimum amount of benzene and was

poured on the column. The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—2

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1—3	Nil
Petroleum ether:benzene (4:1)	4—7	Nil
Petroleum ether:benzene (3:2)	8—10	Nil
Petroleum ether:benzene (2:3)	11—20	Solid

Further elution with more polar solvents did not afford any solid material

Fractions 11—20 (table—2) were combined and the solid was crystallised from chloroform-methanol mixture. The crystal of m.p. 280—82° was identical with 60 obtained from the previous experiment (m.m.p. 00—1R). Beilstein test - Positive. TNM test - Negative.

Dimethyl aniline treatment on 3β -acetyl olean-15α -bromo-28 ⟶ 13β olide 60: Preparation of 3β -acetyloleen-15,16-en-28 ⟶ 13β olide 61:

150 mg of the bromo lactone 60 was refluxed for 4 hr with freshly distilled 30 ml dimethylaniline. The

mixture was poured into water followed by acidification with 6N HCl. The mass was then extracted with ether. The ether solution was washed with water till neutral and dried over anhydrous sodium sulphate. The solvent was removed and the residue (130 mg) was dissolved in minimum amount of benzene and was then chromatographed in a column of 50 gm deactivated alumina. The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—3

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—16	Solid m.p.298°—300°

Further elution with more polar solvents did not afford any solid material

Fractions 9—16 (table—3) were combined and the solid was crystallised from chloroform-methanol mixture. The crystals of the dehydrobromo compound had the m.p. 308—10°.

Analysis report:

Found, C, 77.1% ; H, 9.7%

Calculated for $C_{32}H_{48}O_4$ C, 77.4%; H, 9.7%

UV : No UV absorption above 220 nm

IR(nujol): ν_{max} 1730 cm^{-1}, 1250 cm^{-1} (acetate), 1770 cm^{-1} (lactone), 900 cm^{-1} (cis-disubstituted olefin)

Fig-3

PMR : 0.8 to 1.3 ppm (7 - tert CH_3), 2.04 (s, 3H-O $COC\underline{H}_3$), 5.5 (AB quartet $-\overset{|}{C}-\underline{C}\underline{H}=\underline{C}\underline{H}-\overset{|}{C}-$, JAB = 10 Hz).

4.5 (m, 1H, $\underline{H}-\overset{|}{C}-OCOCH_3$)

Fig.4

Beilstein test - Negative

TNM test - Positive

Hydrogenation of 3β -acetyl olean-15,16 en-28⟶ 13β olide 61:

Preparation of 3β -acetyl-olean-28⟶ 13β -olide 62:

A mixture of the unsaturatedlactone 61 (200 mg), dissolved in 50 ml ethyl acetate and 50 ml of acetic acid was stirredin presence of Adam's catalyst (25 mg) under hydrogen atmosphere in a Pars' hydrogenation apparatus under pressure (40 p.s.i.) till the absorption ceased. The catalyst was removed by filtration and the filtrate was washed with water till neutral. The solvent was dried andremoved under reduced pressure. A solidresidue (180 mg) was obtainedwhich after

crystallisation from chloroform-methanol furnished a solid m.p. 293—94°, $[\alpha]_D$+19° (m.m.p. and CO—IR identical with an authentic sample of 3β -acetyl olean-28⟶13β -olide).

IR : ν_{max} at 1780 cm^{-1} (γ -lactone), 1720, 1240 cm^{-1} (acetate)

Analysis report:

Found,	C, 77.0% ; H, 10.1%
Calculated for $C_{32}H_{50}O_4$	C, 77.1% ; H, 10.1%

TNM test - Negative.

Preparation of authentic 3β -acetyl olean-28⟶13β olide 62:

200 mg pure oleanolic acid was taken in 25 ml chloroform, To the solution a stream of gaseous hydrogen chloride was passed at room temperature. Then the reaction mixture was poured into water. The product was separated in acid and neutral fraction in the usual way. After separation the neutral fraction was heated with 2 ml acetic anhydride and 2 cc pyridine and it was kept over-night. After usual work up followed by crystallisation a product was obtained m.p. 293—94°, $[\alpha]_D$ +19° [Lit. m.p. 293—95°].

Analysis report:

Found, C, 77.2% ; H, 10.2%

Calculated for $C_{32}H_{50}O_4$, C, 77.1% ; H, 10.1%

IR(nujol): ν_{max} 1770 cm^{-1} (γ -lactone), 1720, 1240 cm^{-1} (acetate)

TNM test - Negative.

Lithium aluminium hydride reduction of 3β-acetyl-olean-15, 16-en-28⟶13β-olide 61; Formation of sogloeradiol 58:

50 mg of the lactone was taken in dry ether (100 ml). The solution was refluxed with 100 mg of lithium aluminium hydride for 4 hours. The mixture was cooled, lithium aluminium hydride was decomposed with a saturated solution of Na_2SO_4 and extracted with ether. The ethereal solution was washed with water and then dried over anhydrous sodium sulphate. The residue m.p. 225—28° was dissolved in minimum amount of benzene and was chromatographed over a column of alumina. The chromatogram was developed with petroleum ether and eluted with the following solvents:

Table—4

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil

Table—4 (contd.)

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether;benzene (2;3)	13—16	Nil
Benzene	17—20	Nil
Benzene:ether (4:1)	21—30	Solid m.p.230—231°

Further elution with more polar solvent did not afford any solid material

The compound from flask 21—30 (table—4) were mixed and crystallised from chloroform-methanol mixture. The crystallised product, m.p. 235—36°, was found to be identical with authentic specimen of sapogeradiol (m.m.p. and IR comparison).

Analysis report:

Found, C, 81.7% ; H, 10.9%

Calculated for $C_{30}H_{48}O_2$, C, 81.6% ; H, 11.0%

IR(nujol): ν_{max} at 3360 cm^{-1} (hydroxyl group)

Fig.5

Treatment of 3β -acetyl oleanolate 63 with NBS in DMSO; Formation of 3β -acetyl olean-12α-bromo-28—→ 13β-olide 65:

200 mg of 3β -acetyl oleanolate dissolved in 10 ml of chloroform was mixed with freshly distilled 5 ml of dimethyl

sulphoxide. To the reaction mixture 200 mg NBS was added. The reaction mixture was kept in dark for 24 hr. The chloroform solution was then washed with water and then dried over anhydrous sodium sulphate. The solvent was removed. After removal of solvent an oily residue (180 mg) was obtained which was chromatographed over a column of alumina (25 g alumina deactivated with 1 ml 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—5

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—14	Solid m.p. 200—209°

Further elution with more polar solvents did not afford any solid material

Fractions 9—14 (table—5) were mixed and the mixture was crystallised from chloroform-methanol, m.p. of the crystallised product was found to be 215°—16°. The compound was found to be identical (m.m.p. and CO—IR) with an authentic sample of 12α-bromo-3β-acetyl olean-28⟶ 13-olide.

Analysis report:

Found, C, 67.2% ; H, 8.6%

Required for $C_{32}H_{49}O_4Br$, C, 67.1% ; H, 8.6%

IR : ν_{max} 1770 cm^{-1}, 1180, 1160 cm^{-1} (γ-lactone)
1725 cm^{-1}, 1240 cm^{-1} (acetate) Fig.6

Beilstein test - Positive

TNM test - Negative

Treatment of 3β-acetyl oleanolic acid 64 with NBS; Formation of bromo lactone 65:

200 mg of 3β-acetyl oleanolic acid, dissolved in 10 ml of chloroform, was mixed with 5 ml of dimethyl sulfoxide. To the reaction mixture 200 mg of NBS was added and it was kept in dark for 24 hr. The mixture was diluted with water and extracted with chloroform. The organic layer was washed with water and then the solvent was distilled off. The residue obtained was extracted with ether, stirred with aq. sodium hydroxide (5%) and separated into alkaline and neutral extracts. The alkali extract on acidification and extraction with ether did not furnish any solid residue indicating the absence of any acidic component in the reaction mixture. The neutral ether layer was concentrated after drying over anhydrous sodium sulphate, and afforded a residue (150 mg) which was homogeneous in TLC (single spot). It was crystallised from chloroform-methanol mixture. The m.p. of

the crystallised product was 215°—17° and found identical with 3 β -acetyl-12 α -bromo-oleanan-28⟶ 13β-olide (m.m.p. and CO—IR).

Treatment of methyl 3 β -acetyl betulenate 66 with NBS; Formation of (i) methyl 3 β -acetyl-30-bromobetulenate 68 (ii) a mixture of isomeric bromo compounds 68a ⇌ 68b and (iii) 3 β -acetyl-29-30-dibromo oleanan-18 α-H,28⟶ 19β -olide 69.

400 mg of 3β -acetyl betulenate dissolved in 20 ml chloroform was mixed with 10 ml dimethyl sulphoxide. To the reaction mixture 800 mg NBS was added in lots of 100 mg each and the solution was kept in dark for 24 hr. The mixture was diluted with water. The organic layer was washed with water and dried over anhydrous sodium sulphate and after removal of solvent a residue (0.35 g) was obtained. It was dissolved in minimum amount of benzene and chromatographed over a column of silica gel (400 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents;

Table—6

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Solid m.p. 215—220°
Petroleum ether:benzene (4:1)	10—12	Solid
Petroleum ether:benzene (3:2)	14—16	Nil
Petroleum ether:benzene (2:3)	17—21	Solid m.p. 270°

Further elution of more polar solvents did not afford any solid material

The fractions 5—8 (table—6) were mixed and crystallised from chloroform-methanol. The m.p. of the crystallised product 68 was 235°—36° $[\alpha]_D$ +42.55°.

Analysis report:

Found, C, 65.2%; H, 8.6%, Br, 13.5%

Calculated for $C_{33}H_{51}O_4Br$, C, 65.3%; H, 8.6%, Br, 13.5%

IR(nujol): ν_{max} 1735 cm^{-1} ($-OCOCH_3$), 1725, 1240 ($-OCOCH_3$), 1260 ($-CH_2Br$), 1660, 876 cm^{-1} ($=CH_2$) Fig.8

NMR : 0.87—1.02 (15H, 5 tert-CH_3), 2.03 (s, 3H, $-OCOCH_3$) 4.9 (m, 2H, $=CH_2$), 4.5 (m, 1H, CH-O-$COCH_3$) ppm.

The fractions 10—12 (table—6) were combined and crystallised from $CHCl_3$—MeOH, m.p. 228—30°, $[\alpha]_D$ +50°.

IR(nujol): 1735 ($COOCH_3$), 1725, 1240 ($O-COCH_3$) 1265, 900 cm^{-1}.

PMR : 0.78—1.0 ppm ($C-t-CH_3$) , 1.7, 1.78 (2s, 3H, $C=C-CH_3$), 2.05 (s, 3H, $OCOCH_3$), 3.7 (s, 3H, $COOCH_3$), 3.9 (s, CH_2, Br), 4.48 (m, 1H, $H-C-O-COCH_3$), 5.1 (d, $C=CH_2$), 5.76 and 5.97 (2s, cis trans $C=C<^{Br}_{H}$) Fig.9

The compound was identified as 68a $\rightleftharpoons$ 68b

Compound obtained in the petroleum ether:benzene: 2:3 elution was white solid which was crystallised from $CHCl_3$—MeOH to give the dibromolactone, 69 m.p. 303°—304°, $[\alpha]_D$ +47.22°; CD (n-hexane) 218 nm, (ϵ -0.99).

Analysis report:

Found, C, 58.5% ; H, 7.3%

$C_{32}H_{48}O_4Br$ requires, C, 58.6% ; H, 7.4%

IR(nujol): ν_{max} at 1780 cm^{-1} (γ-lactone), 1720, 1240($-OCOCH_3$), 1260 cm^{-1} ($-CH_2Br$) Fig.10

PMR : 0.87—0.92 (15H, 5 tert CH_3), 2.03 (s, 3H,$-OCOCH_3$), 3.44 (m), 3.55 (m); 3.73 (d) and 3.73 (d) (4H, $C-29-H_2Br$ and $C_{30}-H_2Br$, 4.34 (s, 1H, $HC-OO$) and 4.5 (m, 1H, $H-C-OCOCH_3$) ppm Fig.11

CMR : 178.48 (s, lactone -O-C=O), 171 (s, -O-$\underline{C}$O-CH_3), 80.77 (d, $H\underline{C}$-O-C=O), 80.95 (d, $H\underline{C}$-O-C=O) ppm

Fig.12

Mass peaks: m/e at 659, 657, 655, 599, 597, 595, 503, 501, 407, 517, 515, 489, 487, 437, 435, 249, 191 (base), 189

Fig.13

Treatment of 3β-acetyl betulinic acid 67 with N-bromosuccinimide in presence of dimethyl sulfoxide:

To a solution of the acid 67 (0.2 gm) in chloroform (10 ml) containing DMSO (5 ml), N-bromosuccinimide (0.2 g) was added and the reaction mixture was kept in dark for 24 hours. The mixture was diluted with water and then extracted with chloroform. The organic layer was washed with water and then dried over anhydrous sodium sulfate. After removal of solvent a gummy mass was obtained which was chromatographed over a column of silica gel (30 gm). The column was eluted with the following solvents:

Table—7

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil

Table—7 (contd.)

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether:benzene (2:3)	13—20	White solid m.p. 290—300°
Petroleum ether:benzene (4:1)	21—25	Nil
Benzene	26—30	Nil
Benzene:ether (4:1)	31—35	Solid

Further elution with more polar solvent did not afford any solid material

The fractions obtained from 15—20 (table—7) were combined and crystallised from chloroform-methanol and the product m.p. 303—4° was identified as 3β -acetyl 29,30-dibromo olean-18α-H, 28⟶ 19β -olide 68 (compared with the authentic sample). Fractions obtained from benzene:ether (4:1) polarity was identified as its ester. Esterification of the compound by ether solution of diazomethane furnished the compound 68 i.e. 3β -acetyl-30-bromo betulonate (compared with an authentic specimen).

Attempted dehydrobromination of 3β-acetyl-29,30-dibromo-18α-H oleanan-28⟶ 19β olide, 69 by dimethyl aniline:

150 mg of the dibromolactone was refluxed for 4 hours with freshly distilled dimethyl aniline (30 ml). The mixture was poured into water, acidified with 6N HCl and extracted

with ether. The ether layer was washed with water till neutral and dried over anhydrous sodium sulphate. The solvent was removed and the compound obtained was found to be identical with the starting material.

Debromination of 3 β -acetyl-30-bromo betulenate 68; Preparation of 3 β -acetyl-betulenate 66:

200 mg of the compound was dissolved in 40 cc glacial acetic acid. 40 mg of Zn dust was added to the mixture and it was refluxed for 8 hours. After work up in the usual way it afforded a solid which on crystallisation from chloroform-methanol gave crystals, m.p. 201—2°, identified as 3β -acetyl betulinate (m.m.p. and IR comparison). Beilstein test — negative.

Debromation of lactone 69; Preparation of 3 β -acetyl oleanan-28—→ 19β olide 70:

200 mg of the lactone 69 was dissolved in 50 ml of rectified spirit and then 5 gm of Raney Nickel was added to the reaction mixture and the reaction mixture was refluxed for 8 hours. The mixture was filtered and the filtrate on concentration furnished 140 mg of 3 β -acetyl-olean-28—→ 19β -olide m.p. $>$ 360°, $[\alpha]_D$ +59°. It was compared with an authentic specimen of 3 β -acetyl oleanan-28—→ 19β olide (m.m.p. and IR comparison).

Preparation of authentic 3β -acetyl oleanan 28⟶ 19β olide 70:

Acetyl betulenic acid (1 g) was dissolved in benzene (10 cc) and a mixture of acetic acid (60 cc) and sulphuric acid (18 g, d, 1.84) was added with shaking. After two days at 20°, the reaction mixture was diluted with water and taken in benzene. The benzene layer was washed several times with water till neutral and dried over anhydrous sodium sulphate. Removal of solvent gave a mass which was taken in solvent ether. The ether layer was washed with 5% cold sodium hydroxide solution and the washings were discarded. The ether layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a mass which was crystallized from chloroform-methanol mixture. The crystals of m.p. $>$ 350° (lit. m.p. $>$ 360°) were identified as 3β -acetyl-oleanan-13α-H-28⟶ 19β -olide.

Analysis report:

Found,	C, 77.03% ; H, 10.06%
Calculated for $C_{32}H_{50}O_4$	C, 77.1% ; H, 10.04%

IR(nujol): ν_{max} at 1765 cm^{-1} (γ -lactone)
1735 and 1240 cm^{-1} (acetate).

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PART — IV

CHEMICAL INVESTIGATION OF THE ROOT OF *LEUCAS ASPERA* SPRENG

CHAPTER—I

I N T R O D U C T I O N

Leucas Aspera[1] which is called "Chota-halkusa" in hindi belongs to Labiatae family. The plant is fragnent and used as a Pot-herb. It is eaten in times of scarcity. It is commonly used as an antipyretic in villages in S. India. The juice of the leaves is used as an external application for psoriasis, chronic skin eruptions and painful swellings. In North Bengal flowers are given with honey for coughs and colds in children. An alcoholic extract of leaves shows[2] anti-bacterial activity against Micro Coccus pygens, Var. aureus and Escherichia coli.

Leucas Aspera which is commonly known as "Dandakalas" in North Bengal is abundantly available in the barren cultivated land. The plant generally grows in the barren lands during December to April. It is found that the married folks of "Rajbangshies" - the local inhabitans of North Bengal region use the root of this plant as oral contraceptive. Moreover, the flowers have also been found to be used for treatment of cold. In order to explore whether there is any active principle present in the plant that could behave as oral contraceptive, we attempted to isolate the different constituents present in the plant.

Aditya Chowdhuri and his coworkers[3] have reported the presence of oleanolic acid, ursolic acid and β-sitosterol from the petroleum extract of the whole plant of Leucas Aspera. Since the local people take the root of the plant as contraceptive we thought that the active principle must be present in the root of the plant. Part IV of the desertion containe chemical investigation of the root extract of Leucas Aspera Sprong.

CHAPTER--II

Morphological features of the plants of Labiatae family, Leucas and Leucas Aspera spreng:

Labiatae[4] is a family of 2600 species, which are chiefly of north temperate region.

Morphological features: Members of this family are herbs, rarely shrubs, usually load with oil glands.

Stem usually 4-gonous, Leaves opposite or whorled, stipules. O.flowers irregular, solitary 2-nate or fascicled and axillary or in centrifugal spicate cymes which by their union or pairs form false whorles. Calyx persistent, subregular, 4-5 cleft or 2-lipped. Corolla monopetalous, hypgynous; limb 4-5 lobbed or 2-lipped, lobes imbricate in bud. Stamen inserted in the corolla tube, 4-didynamous, or the 2 upper imperfect. Anther - cells connate or separate or confluent. Disc prominent, Ovary free, 2 of 2-celled carpels; style simple, inserted between the lobes, stigma usually 2-fid; Ovules one in each cell, erect anatropous. Fruit of 4 dry or rarely fleshly 1-seeded lobes (nutlets) at the base of the calyx. Seeds small, erect, albumen spering or O.radicle inferior.

Leucas[4] is one of the genera of Labiatae family. It is wooley or villous rarely galorate herbs on under-shrubs.

Whorles axillary, usually distant, Calyx 10-nerved striate; month equal or oblique, equally or unequally 8-10 toothed. Corolla-tube included, annulate or not within; upper lib erect, concave crown villous, lower spreading, 3-fid, midlob very large. Stames 4, ascending; anthers conniving, cells divaricate at length confluent. Style sublate, posterior lobe obsolate. Nutlets ovoid, triquetrous, obtux - species 50, Asiatic and African.

Leucas Aspera sprengl,4:

Annual, erect or diffuse, stem stout, hispid or scabrid, leaves 1-3 inch, linier or oblong obtuse entire or crenate, whorles large terminal and axillary, bract, long linier and filiform. Calyx 1/3 - 2/3 inch. tubular carved smooth below green and ribbed and scabrid above contractal, above the nutlets, month small glabrous very oblique shortly irregularly toothed. Flowers small, white in dense terminal and axillary whorels, nutlets small, oblong smooth brown.

CHAPTER—III

Investigation on the root of Leucas Aspera sprong

SECTION—A : Extraction:

Dried and powdered root of Leucas Aspera (5 kg) was extracted in a Soxhlet apparatus directly with ethyl alcohol for 24 hours.

SECTION—B : Isolation of stigmasterol and a new triterpene dihydroxy lactone:

(1) The alcoholic extract was concentrated under reduced pressure to about 500 cc and it was hydrolysed directly with 7% H_2SO_4 by boiling under reflux for 10 hours. It was extracted with ether and neutral fractions were separated. The neutral fraction of ether layer was concentrated which gave an oily residue (5 gms). This was chromatographed over deactivated alumina and the following fractions were collected:

Table—1

Fraction	Eluent	Residue on evaporation
1	Petroleum ether:benzene (2:3)	White solid m.p. 140—50°
2	Benzene:ether (4:1)	White solid m.p. 270—90°

Examination of fraction 1:

Fraction 1 was crystallised from chloroform-methanol mixture and the crystal obtained had the m.p. 154—56°, $[\alpha]_D$ -50°. IR spectrum showed peak at 3430 cm^{-1} for hydroxyl group. The compound was analysed for $C_{29}H_{48}O$. On acetylation with acetic anhydride and pyridine, furnished an acetate, $C_{31}H_{50}O_2$, m.p. 137—9°, $[\alpha]_D$ -34° $[M^+$ 454(Fig.1)], IR spectrum showed peaks at 1720 and 1240 cm^{-1} for acetate groups, which was identified as stigmasteryl acetate, when compared with an authentic specimen (m.m.p. and CO—IR). Thus the fraction 1 contained the compound stigmasterol which was confirmed by comparing with authentic sample (m.m.p. CO—IR, CO—TLC).

Examination of fraction 2:

(ii) Purification and characterisation of the new dihydroxy lactone - leucolactone, $C_{30}H_{48}O_4$:

Fraction 2 was combined and crystallised from chloroform-methanol mixture when shinning crystals (500 mg) of m.p. 310° were obtained. Elemental analysis by Rast's method showed the molecular formula of the compound as $C_{30}H_{48}O_4$ which was supported by the mass spectrum that showed the molecular ion peak at m/e 472. The compound showed no UV absorption in the region 200—350 nm. IR spectrum (Fig.2) of the compound in

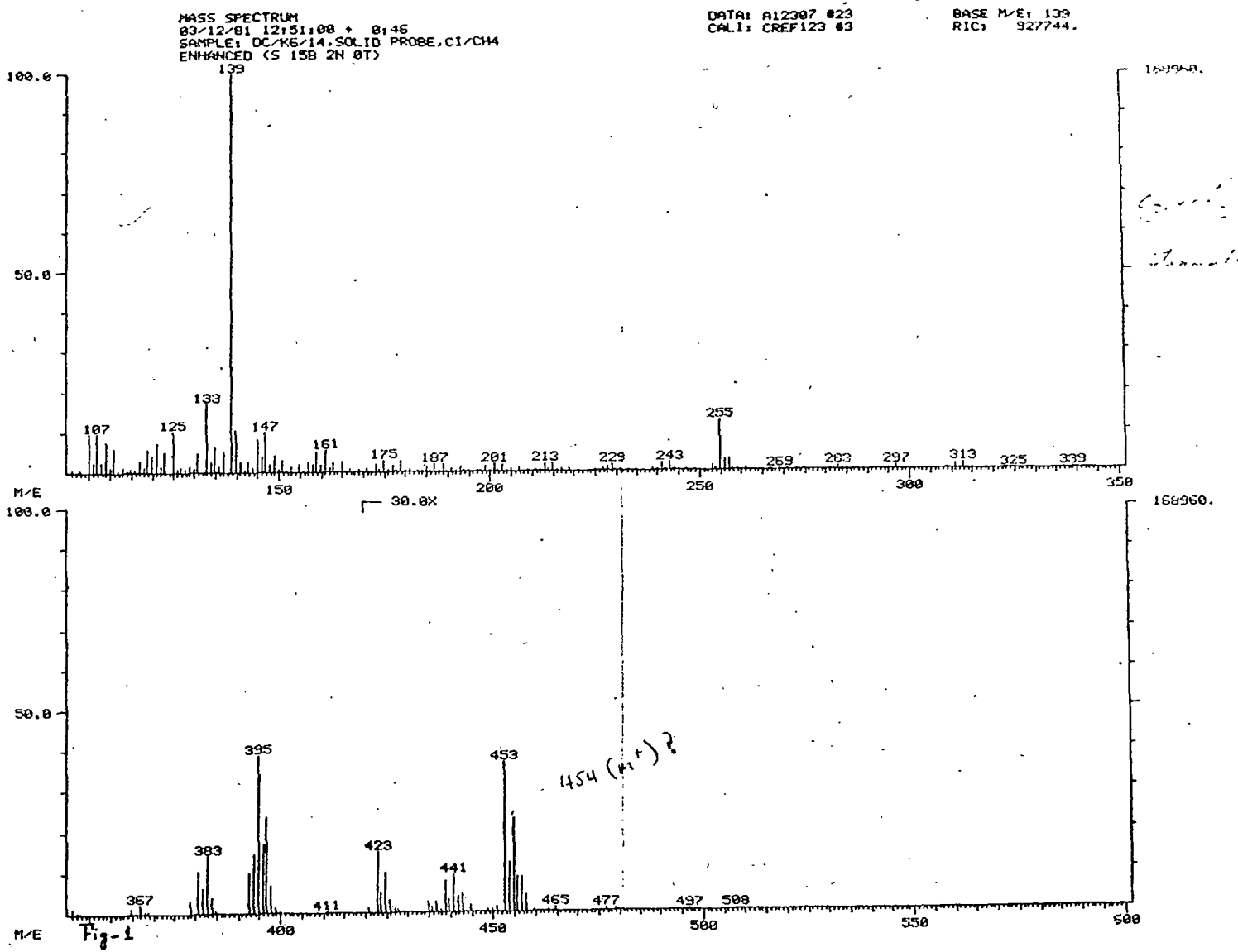


Fig.1: Mass spectrum of stigmasterol.

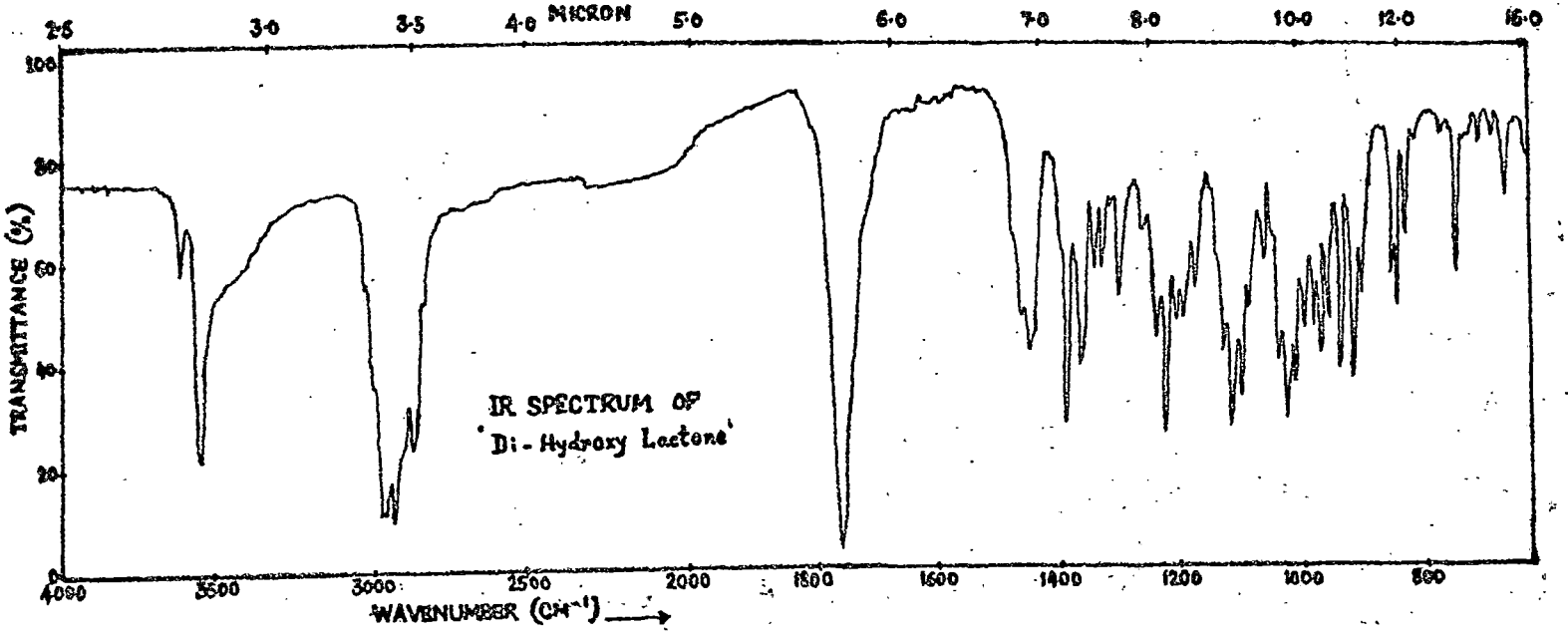


Fig.2: IR spectrum of leucolactone 1.

KBr disc showed the presence of two hydroxyl group at 3607 and 3540 cm^{-1} as two sharp peaks indicating that there is no hydrogen bonding and probably all the three functional groups are situated quite apart from each other. The peak at 1750 cm^{-1} indicated the presence of a γ -lactone ring. Peaks at 1380 and 1355 cm^{-1} indicated the presence of gem dimethyl group. The compound showed positive Libermann - Burtchard test for triterpenoids. It showed the absence of olefinic double bond as indicated by negative TNM test. The compound was designated as leucolactone 1.

(iii) Preparation of acetyl derivative of leucolactone 1—leucolactone diacetate 2, $C_{34}H_{52}O_6$:

200 mg of the hydroxy lactone 1 was acetylated with acetic anhydride and pyridine by keeping over water bath for four hours. Usual work up and chromatography over deactivated alumina furnished a compound of m.p. 270—80°, which on repeated crystallisation from chloroform-methanol mixture furnished needle shaped crystalline solid, m.p. 284—85°. Elemental analysis and mass spectrum showed the molecular formula of the acetate as $C_{34}H_{52}O_6$ (M^+ 556). IR spectrum (Fig.3) showed the presence of two acetate carbonyl streaching at ν_{max} 1728 and 1740 cm^{-1}, 1218 and 1235 cm^{-1}. The lactone carbonyl appeared at 1768 cm^{-1}, the gem dimethyl appeared at 1359 and 1379 cm^{-1}.

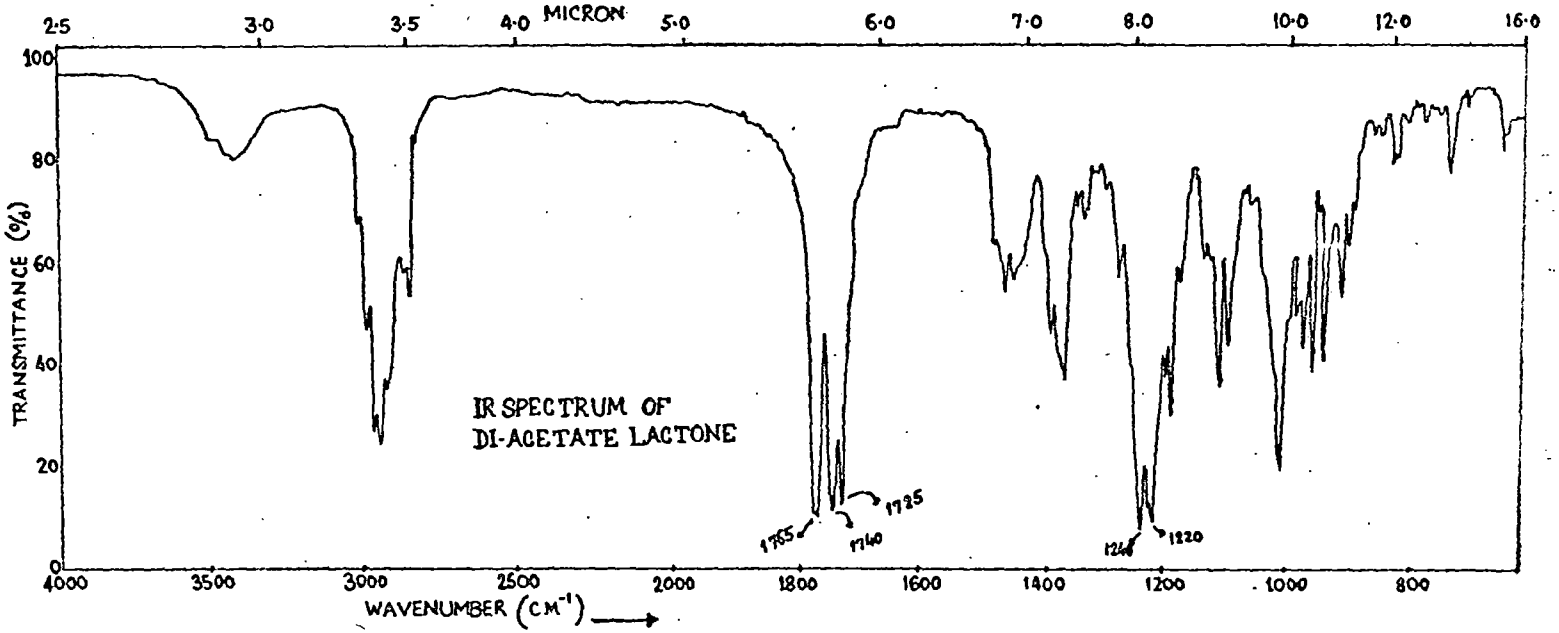


Fig.3: IR spectrum of leucolactone acetate 2.

The compound did not show any peak in the region 700—900 cm^{-1} indicating absence of double bond.

(iv) Oxidation of leucolactone 1 — diketoleucolactone 3:

100 mg of the compound 1 was oxidised by chromium trioxide in presence of pyridine by stirring for about 12 hours. Usual work up and chromatography over desactivated alumina furnished a compound of m.p. 310—20°, which on repeated crystallisation from chloroform-methanol mixture furnished needle shaped crystalline solid m.p. 323—24°. Elemental analysis and mass spectrum showed the molecular formula of the diketolactone 3 as $C_{30}H_{44}O_4$ (M^+ 468). IR spectrum (Fig.4) showed peak at 1760 cm^{-1} (γ -lactone), broad peak in the region 1705—1715 cm^{-1} for the carbonyl functional groups present in the molecule.

From the foregoing data it may be said that the compound 1 contains two hydroxyl groups which are acetylable, one lactone functional group which is probably a γ -lactone, thus accounting for all the four oxygen atoms of the molecule, $C_{30}H_{48}O_4$. The negative TNM test and absence of absorption peaks in the region 700—900 cm^{-1}, 1600—1640 cm^{-1} definitely showed the absence of olefinic double bond. From the molecular formula it may thus be inferred that the compound 1 is probably a saturated pentacyclic triterpenoid. Literature survey of dihydroxy triterpenoid indicated that the compound could be

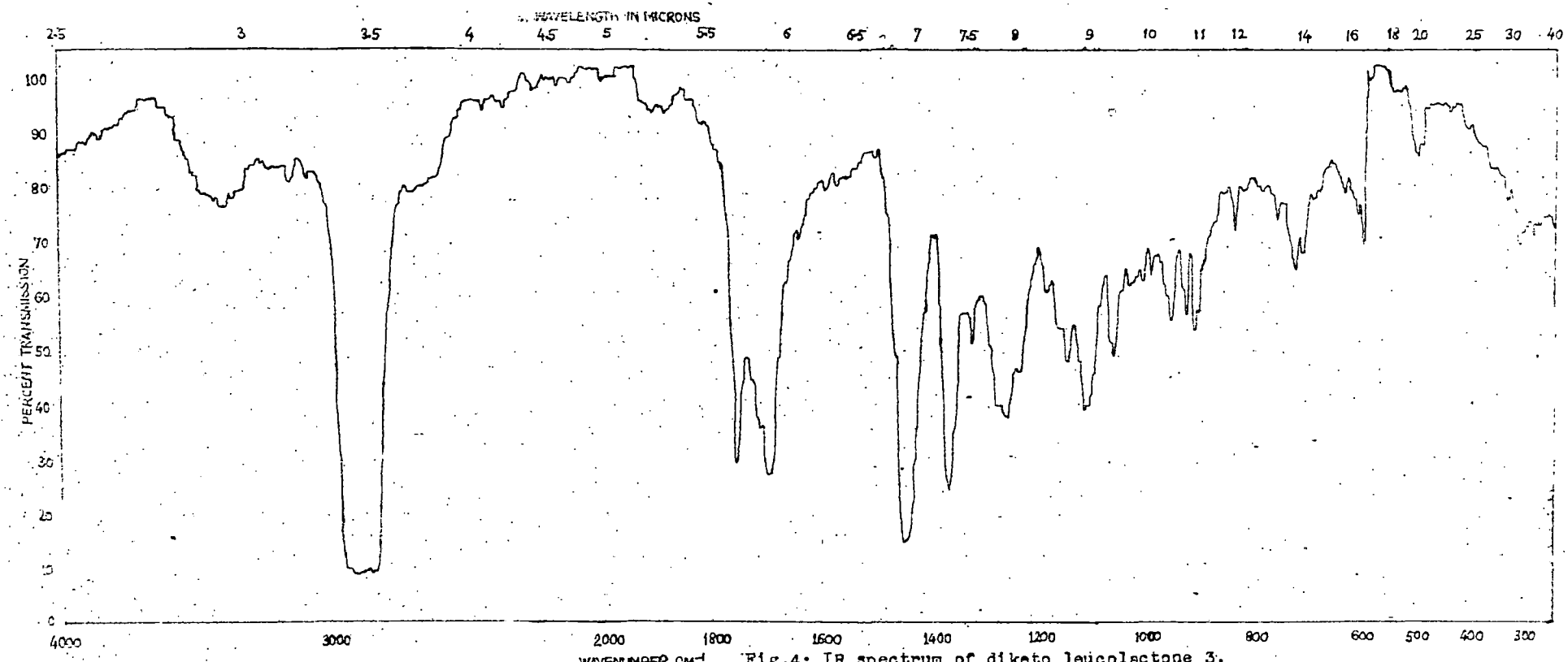


Fig.4: IR spectrum of diketo leucolactone, 3.

a new one. Hence we have carried a thorough physical analysis of the compound 1 and its derivatives which are discussed below:

SECTION—C:

(i) PMR spectral data of leucolactone 1:

^1H NMR spectrum of the compound 1 showed peaks at 0.85 ppm for 1-tert-methyl group, at 0.92 ppm for three tertiary methyl protons, at 0.95, 1.18 and 1.28 ppm for one tertiary methyl each as singlet. The multiplets at 2.6 ppm was integrated for one proton. The peak centred at 3.18 ppm with width at half height 8 Hz indicated the presence of axial hydroxyl group and the broad peak centred at 3.85 ppm with width at half height 18 Hz indicated the presence of equatorial hydroxyl group.

(ii) NMR spectral analysis of acetyl derivative of leucolactone 1:

^1H NMR spectrum (Fig.5) of the acetate of leulactone 1 showed a singlet at 0.85 ppm which was integrated for protons of four tertiary methyl groups, the peak at 0.92 ppm was integrated for three protons of a tertiary methyl group. The singlets at 1.18 ppm and 1.28 ppm integrated for six protons showing the presence of two tertiary methyl groups.

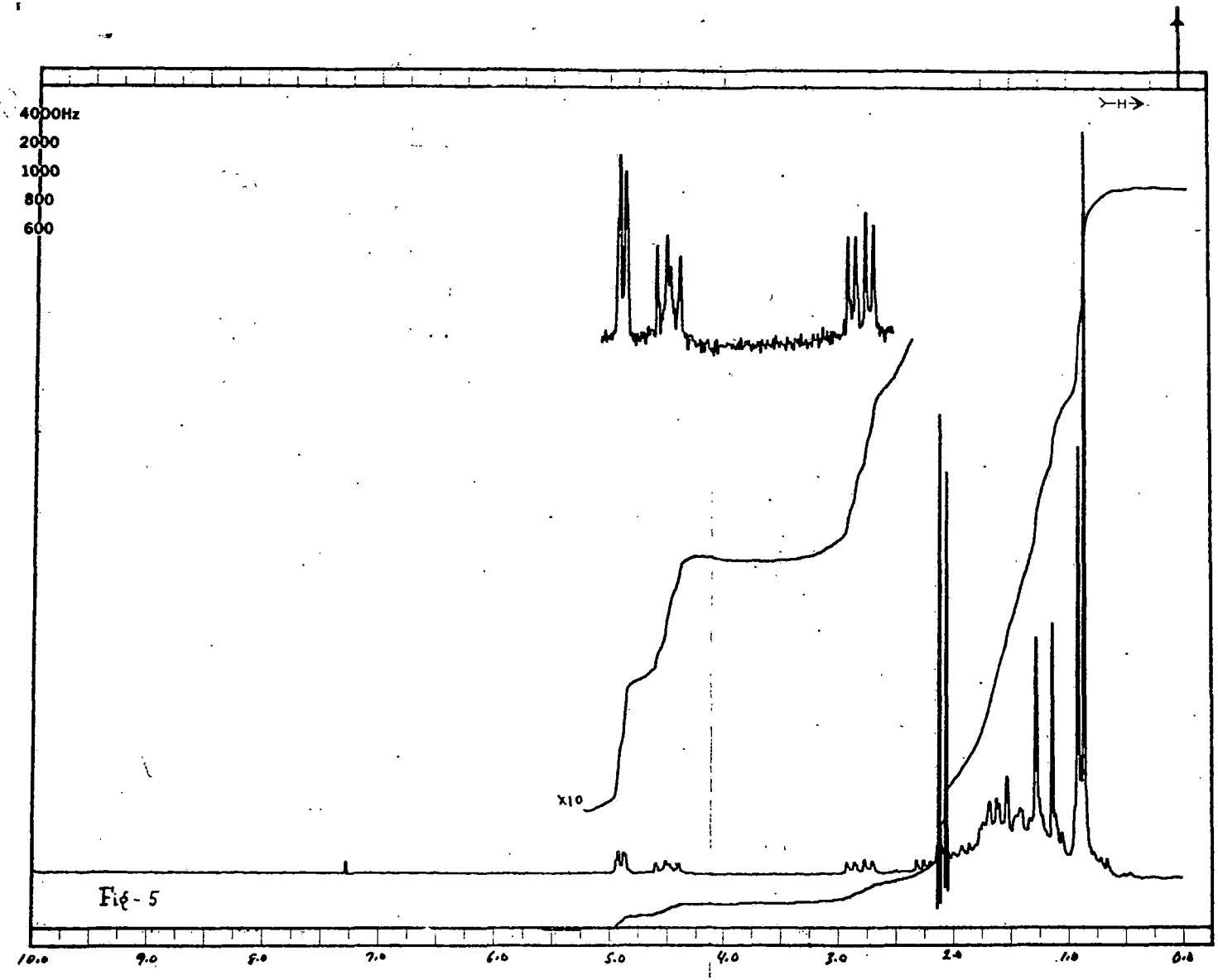


Fig.5: PMR spectrum of leucolactone acetate 2.

The singlets at 2.05 and 2.12 ppm clearly indicated the presence of two acetoxy methyl groups. The presence of two doublets centred at 2.8 ppm with J value about 8 Hz, which was integrable for one proton showed that the lactone carbonyl has an α-proton which had two neighbouring protons that coupled to cause splitting of the peak due to the α-proton. The low coupling constant of 8 Hz indicated that this α-proton must be equatorially oriented and hence the lactone carbonyl should be axially oriented. The peak centred at 4.45 ppm was a triplet that had the J value of 18 Hz. The high coupling constant shows that this proton is axially oriented and is attached to carbon bearing the acetate group which must be equatorially oriented. The last absorption was centred at 4.9 ppm that appeared as a doublet with J value of 8 Hz. This indicated that the proton attached to carbon bearing the second acetoxy group must be equatorially oriented, thus the acetoxy group must be axially oriented. The coupling as a doublet showed that this proton had either only one α-neighbouring proton or it might have two neighbouring protons, the coupling of one of which must be negligible. Decoupling experiments of these multiplets between the range 2.5—5.0 ppm showed that none of these protons were spin coupled with each other showing that carbonyl carbon and the two hydroxyl groups are quite far from each other.

^{13}C NMR spectrum (Fig.6) of the acetate lactone 2 showed the presence of 34 carbon atoms in the range between 15 ppm to 180 ppm. Off resonance CW-decoupled experiments (Fig.7) showed the presence of 9-CH_3 groups, 10(—CH_2—) groups, 5 (—C—H) groups and 10 (—C—) accounting for the 34 carbon atoms and 52 protons of the acetate, thus confirming the molecular formula as $C_{34}H_{52}O_6$. Again in ^{13}C NMR spectrum (Fig.8) of the acetate lactone 2 revealed that there are three singlets at 176.09, 170.85 and 169.79 ppm for the carbonyl carbon. The one that appeared at 176.09 ppm is probably due to the lactone carbonyl which is either 5 or 7 membered ring. The other two being due to acetate carbonyl groups. The singlet that appeared at 90.2 ppm must be due to the carbon containing the lactyl oxygen atom. The doublet at 80.53 and 71.64 ppm must be due to the carbon bearing the acetyl groups. The two acetyl methyl carbons appeared as quartet at 21.4 ppm.

Before considering the structure of the compound 1 i.e. the leucolactone, we may write the various pentacyclic triterpenoids belonging to different carbon skeletons having 8-methyl groups at various positions.

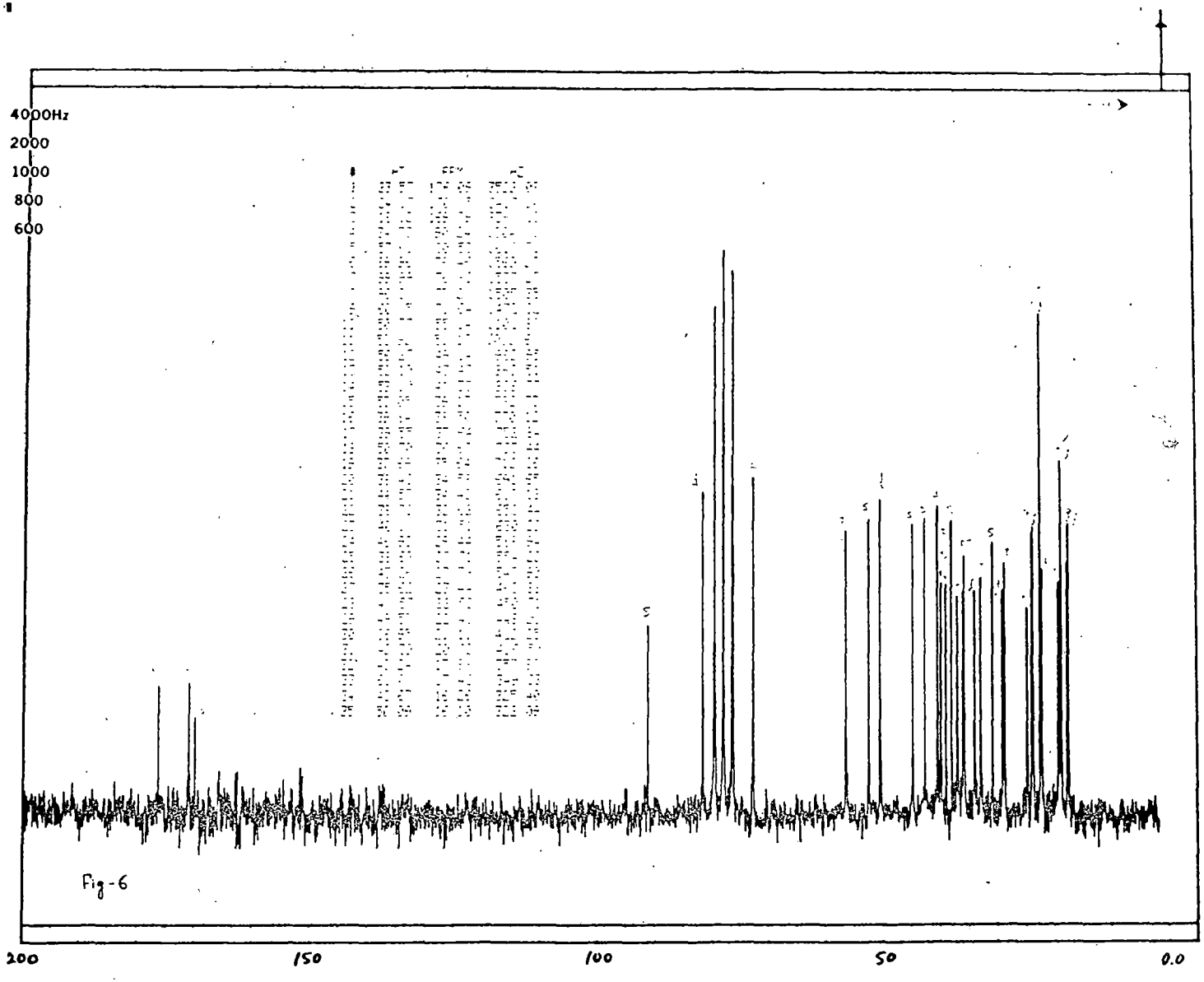


Fig.6: ^{13}C NMR spectrum of leucolactone acetate 2.

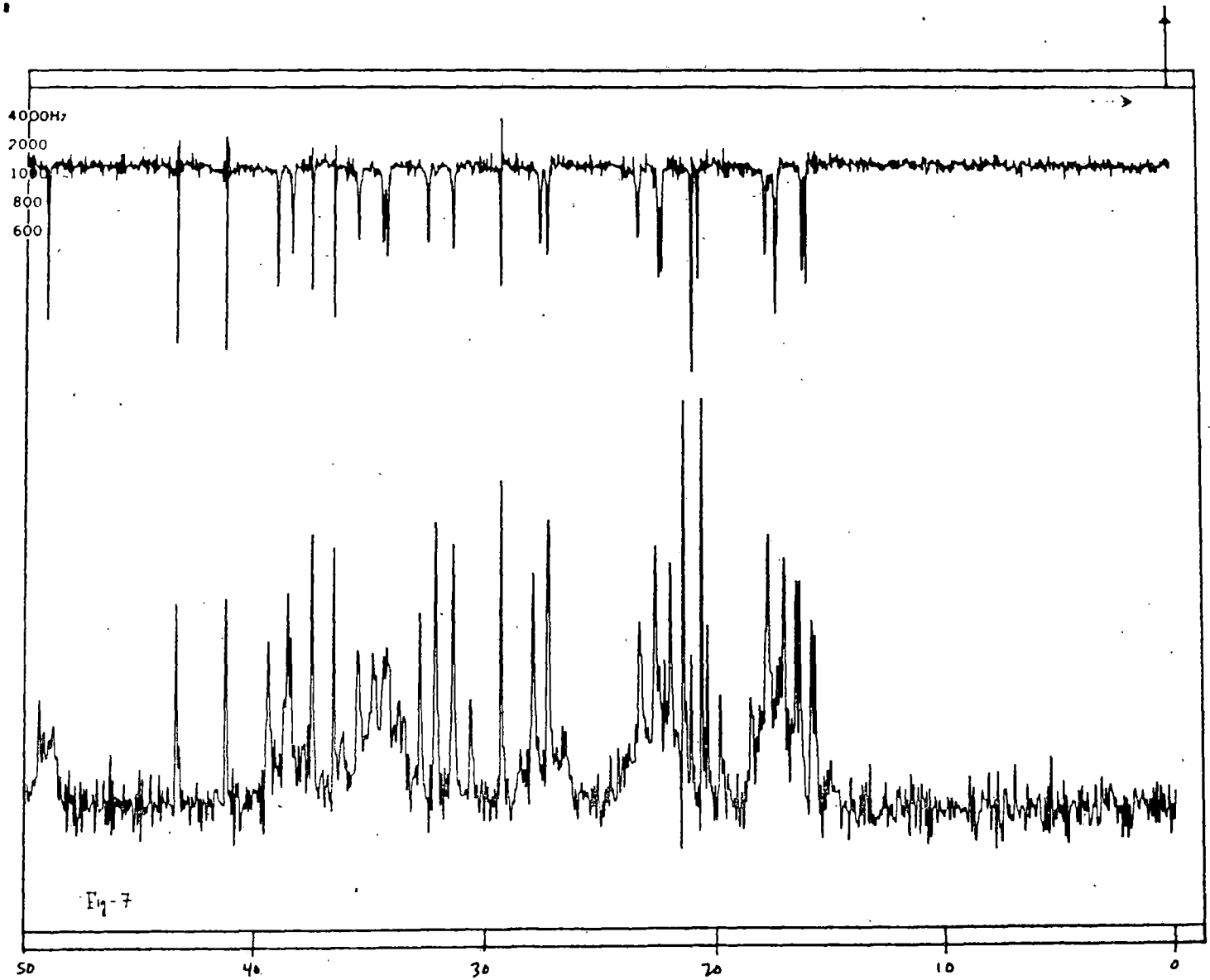


Fig.7: ^{13}C NMR spectrum of leucolactone acetate 2,(off resonance cw decoupled).

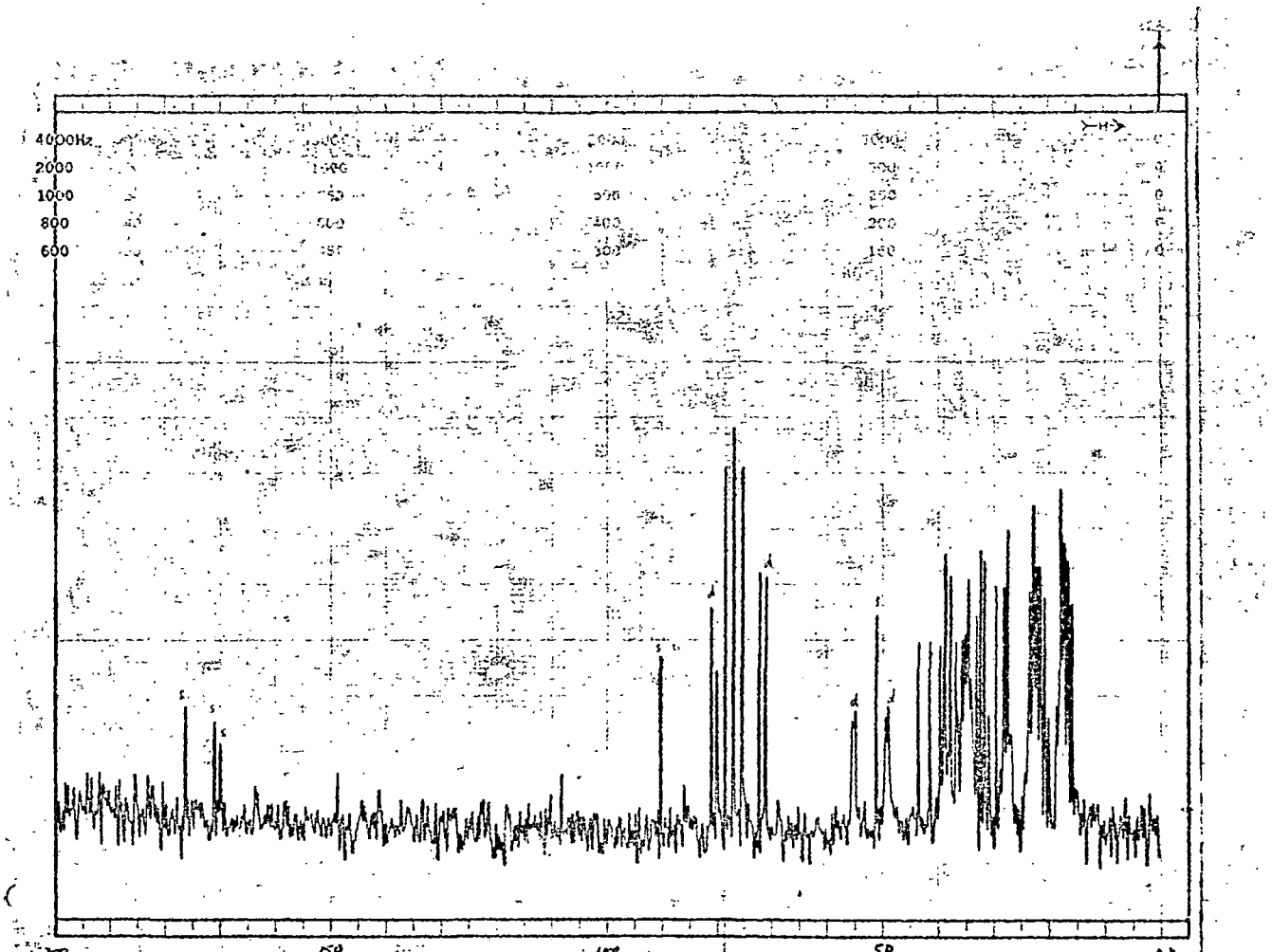
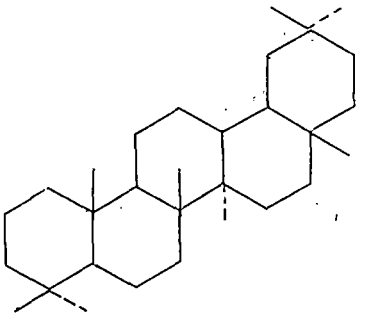
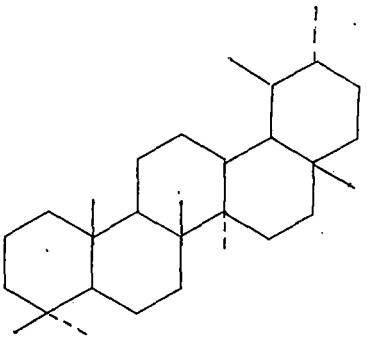


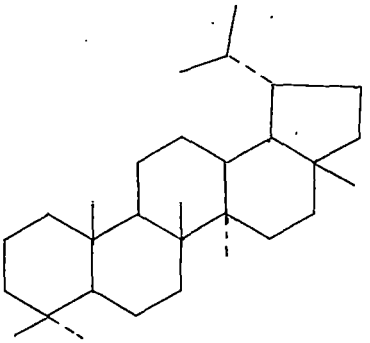
Fig 8 : ^{13}C NMR spectrum of leucolactone acetate, Δ (off resonance CW decoupled)



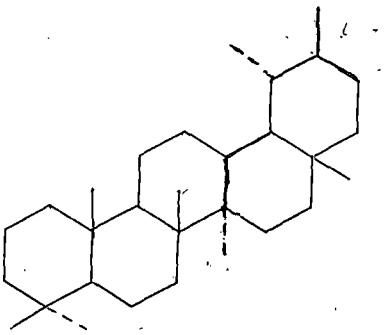
Oleanane



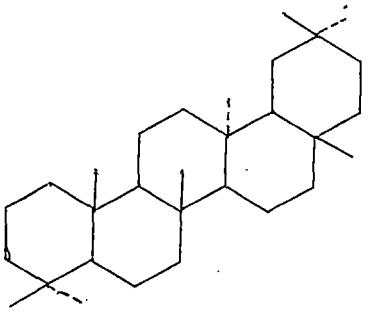
Ursane



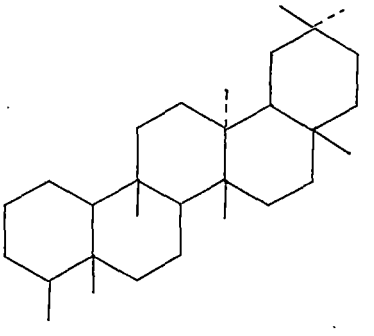
Lupane



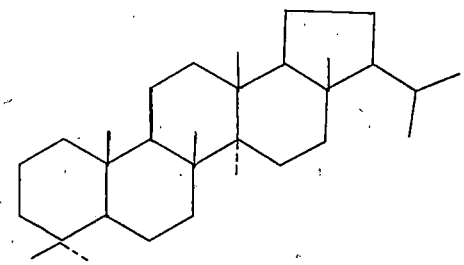
Taraxastane



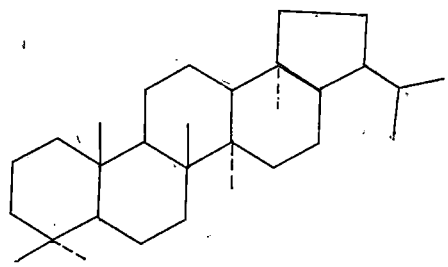
Taraxerene



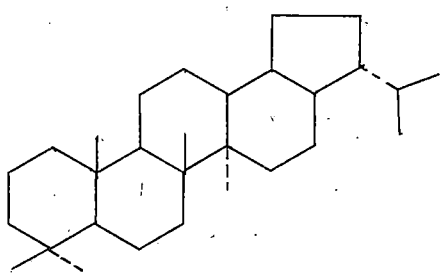
Friedelane



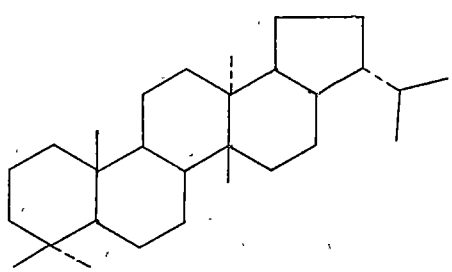
Arborane



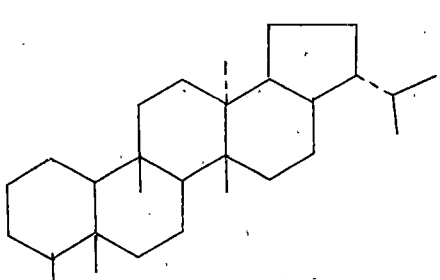
Hopane



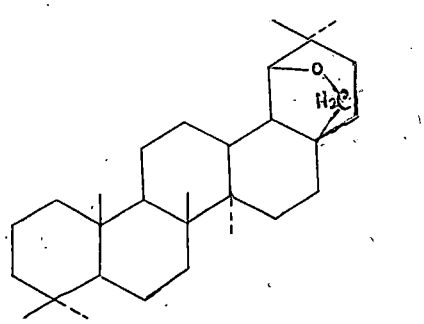
Isohopane



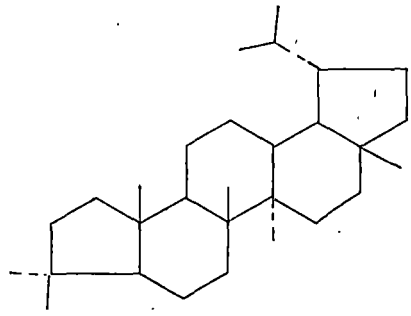
Fernane



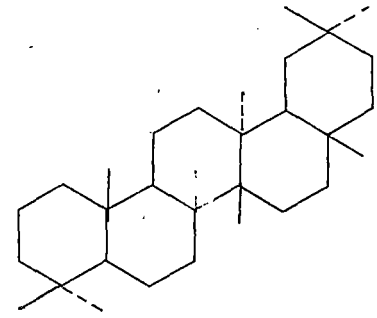
Filliocne



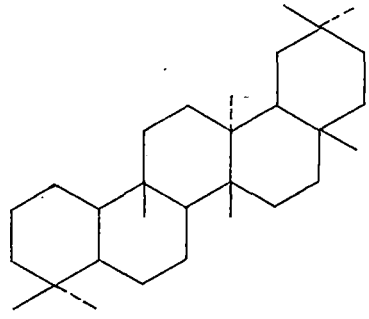
Allobetulin



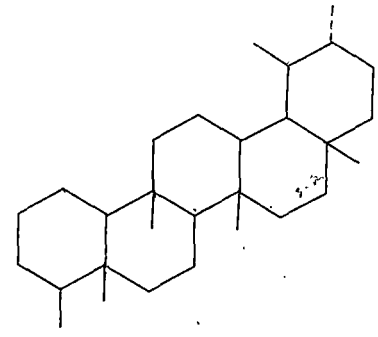
Ceanothane



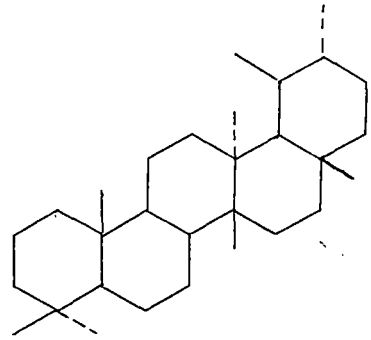
Multiflorane



Glutane

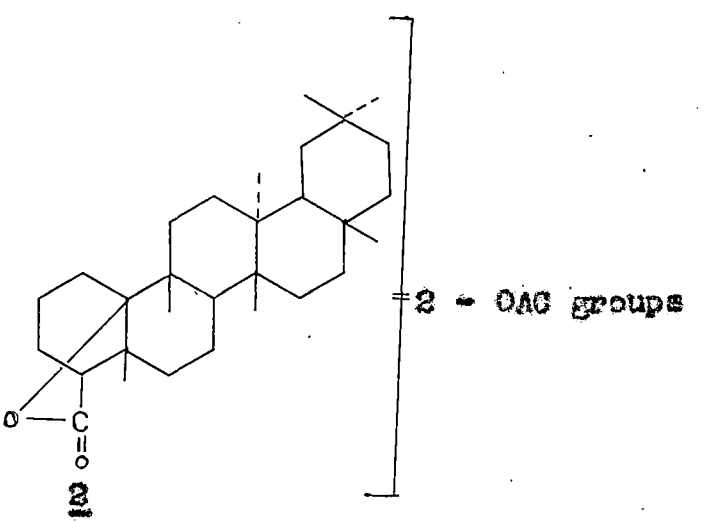


Eupacane



Baurane

Of all the above skeletons of the pentacyclic triterpenoids if we assume that the lactone carbonyl is the C—23 carbon of friedelaneskeleton with the lactyl oxygen being attached to the C—10 carbon atom then we would be able to explain partially the ^{1}H NMR and ^{13}C NMR spectral data mentioned above. So the probable partial structure of leucolactone acetate can be written as 2.



2

Since the proton α -to the lactone carbonyl group is having two α -protons that coupled with each other it indicates that there is no oxygen function at the usual C—3 position. Hence the two hydroxyl groups may be present at any other secondary carbon atoms except the position C—3.

(111) NMR spectral analysis of diketoleucolactone 3:

XL—300 PMR spectroscopy of the ketolactone 3 (Fig.9) showed the presence of seven tertiary methyl groups

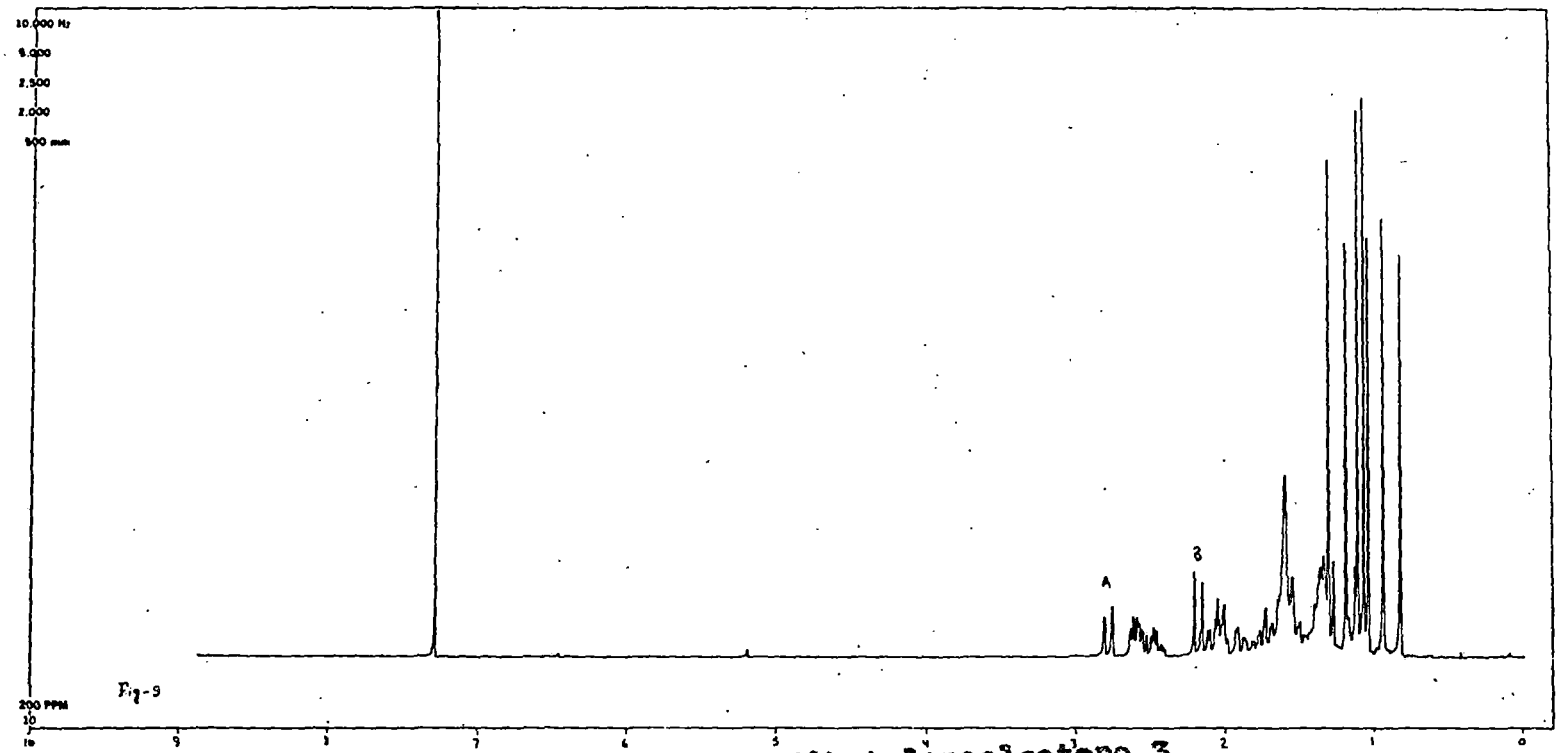


Fig.9: PMR spectrum of diketoleucolactone 3.

at 0.82, 0.93, 1.02, 1.06, 1.10, 1.18 and 1.30 ppm, it showed a series of multiplets between 2.0 to 3.0 ppm, accounting for five protons situated at the α-carbon atoms of the carbonyl carbons. It was already shown that the lactone carbonyl has only one α-proton so the two ketonic carbonyl groups are far apart from each other. The protons that appeared as doublets at 2.18 and 2.78 ppm are coupled with a gem coupling that are separated by 0.6 ppm. One appeared to be shifted upfield and the other downfield presumably by the anisotropic effect of the adjacent carbonyl. These protons have no other proton neighbour showing that the carbonyl functional group can not be present at C—7 position. Otherwise if the oxygen function would have occupied C—7 position it would have 3 α-protons which is not possible in this case. Again it cannot be at C—6 position, as the proton at C—8 would couple with α-protons at C—7 position. The proton at 2.78 ppm shows a coupling to the methyl at 1.18 ppm with a very small J-value (Fig.11) of 0.6 Hz which has been confirmed by decoupling experiments to the methyl at 1.18 ppm as shown in the Fig.10 where the irradiation at 1.18 ppm gave singlets at 2.18 and 2.78 ppm. Therefore, this $-CH_2-$ group can not be at C—1 or C—21 positions because a gem-dimethyl peak would be broadened by CH_3-CH_3 coupling and would not show the coupling observed and 1.18 ppm. This eliminates the presence of oxygen function at C—2 or C—22 positions.

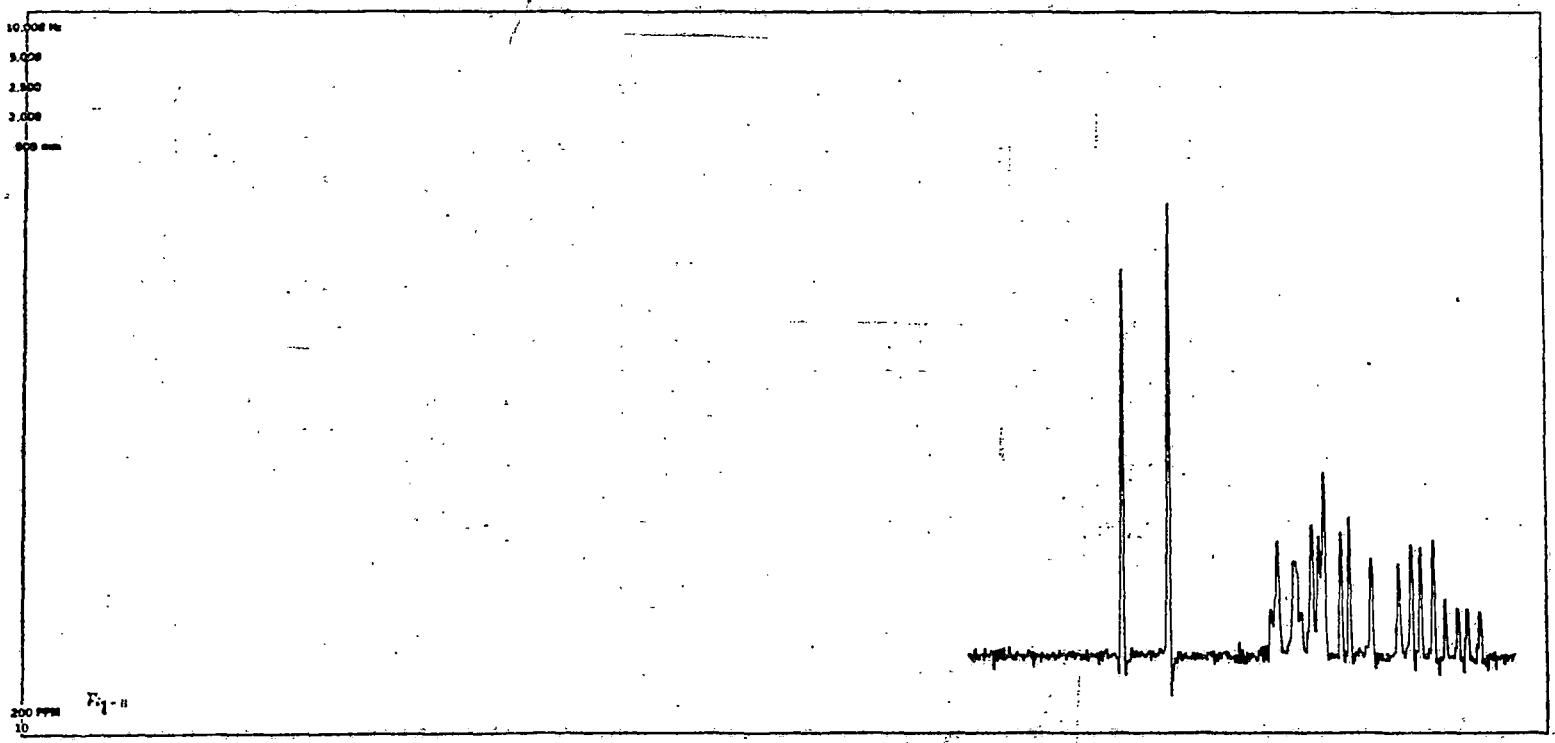


Fig.10: PMR spectrum of diketo lebcolactone 3 (decoupled methyl at 1.18 ppm.

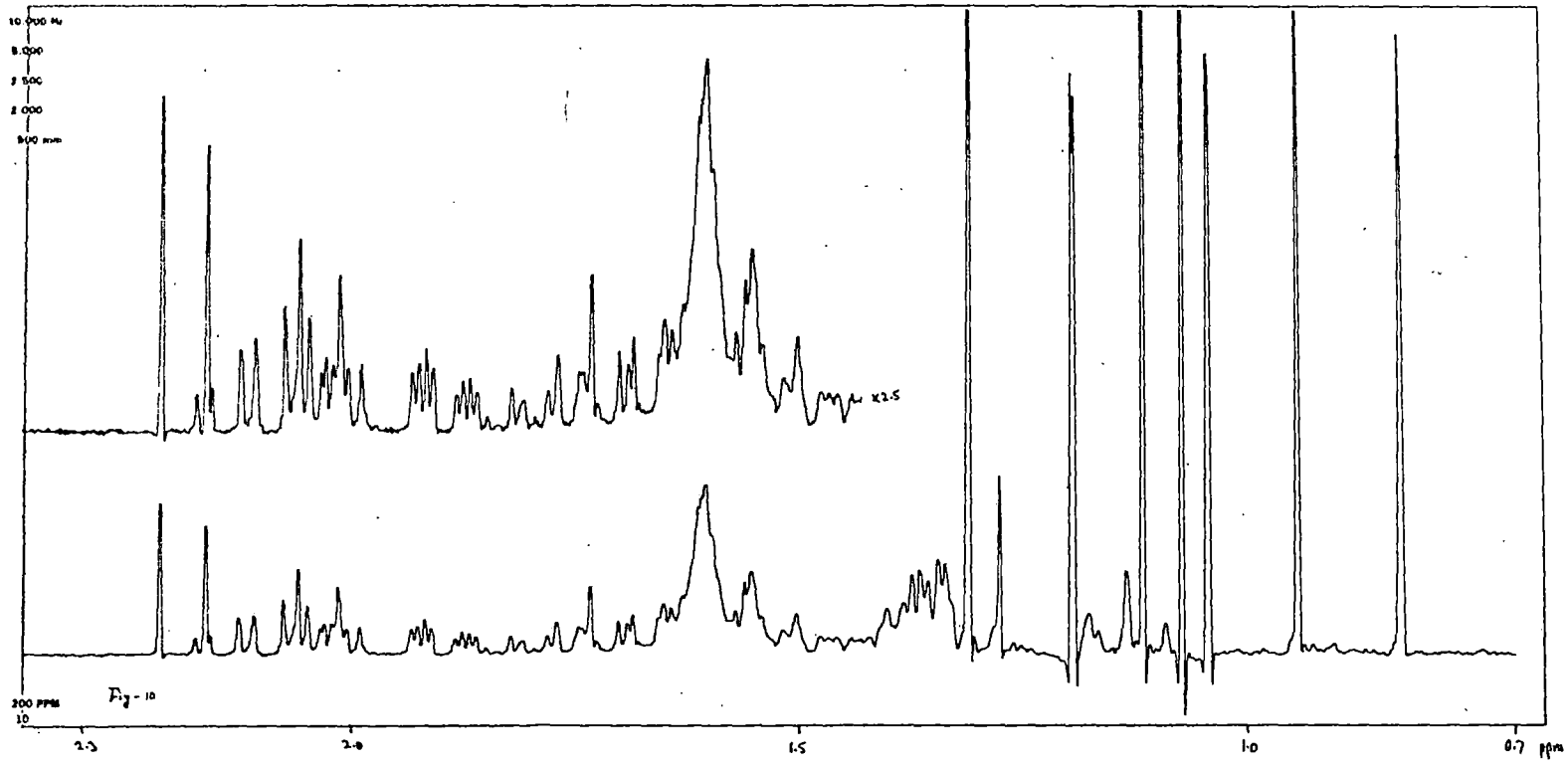


Fig.11: PMR spectrum of diketo - lactone 3 (plot expansion)

We have mentioned that the doublet that appeared at 4.9 ppm of the acetate lactone (Fig.5), would be due to the presence of one neighbouring proton α - to the methine carbon containing the acetate group. Had therebeen only one α-proton, then we could very easily place one of the two hydroxyl groups at C—19 position of the friedelaneskeleton but the presence of two α-protons in each of the carbonyl groups of the ketone established that the oxygen function is not present at C—19 position. Had the carbonyl group been present in C—19 position we would observe a singlet for one proton due to the C—18 proton which is not observed in the NMR spectrum of the diketolactone. From the above derivations the ketone functions or the hydroxy function must occupy any two of the carbon sides at C-1, C-11, C-12, C-15, C-16 and C-21 positions. The multiplets of eight lines between 2.4 and 2.5 ppm (Fig.12) showed that these protons i.e. one of the $-CH_2-$ protons next to one of the ketones has two vicinal neighbours, such a situation is possible only if one of the ketone functions is located at C—1 positions, the α-protons at C—2 would then couple with protons at C—3 positions. Thus we may fix one of the oxygen functions at C—1 position. Hence the second oxygen function may occupy any one of the positions at C-11, C-12, C-15, C-16 or C-21.

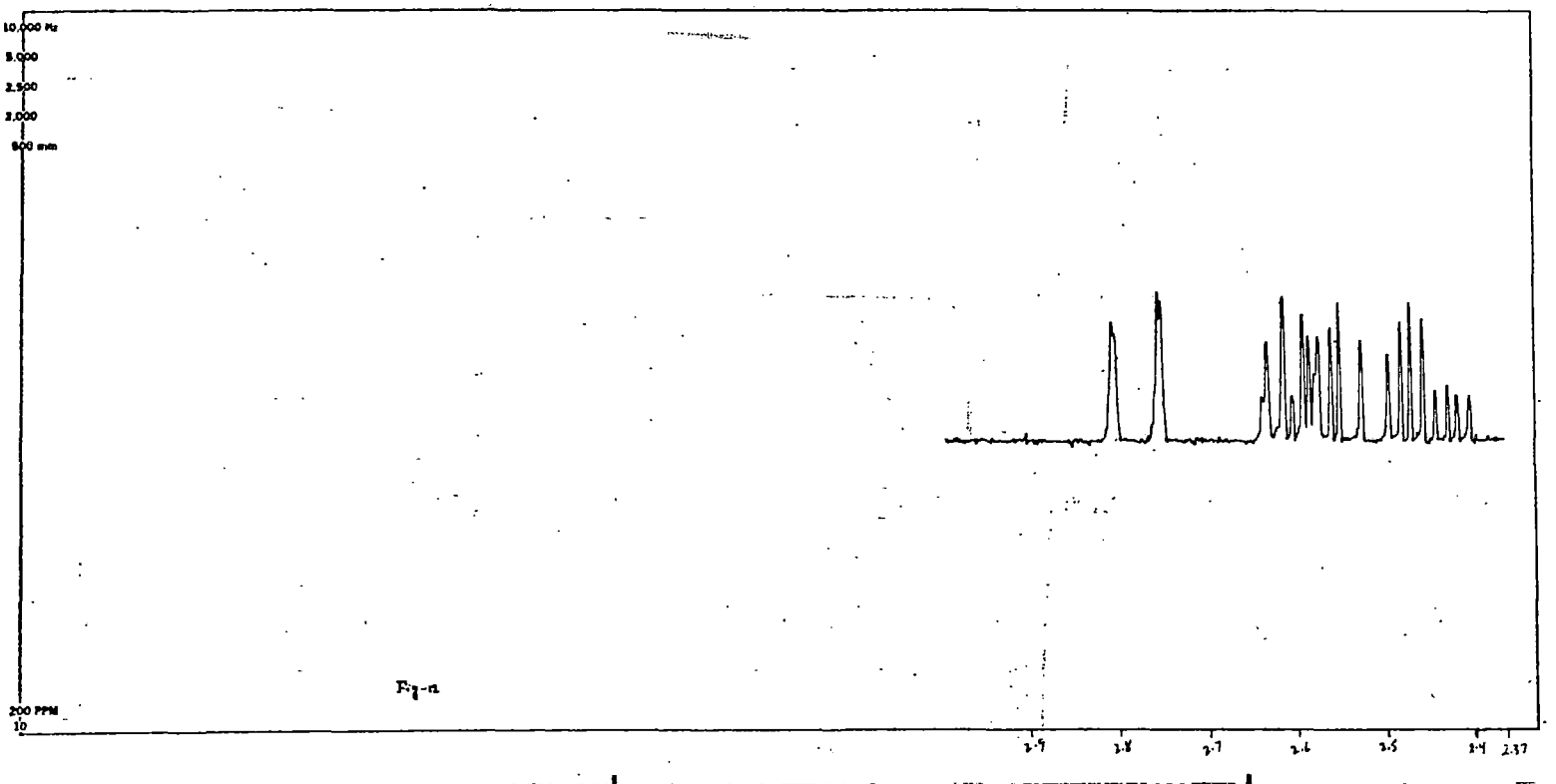
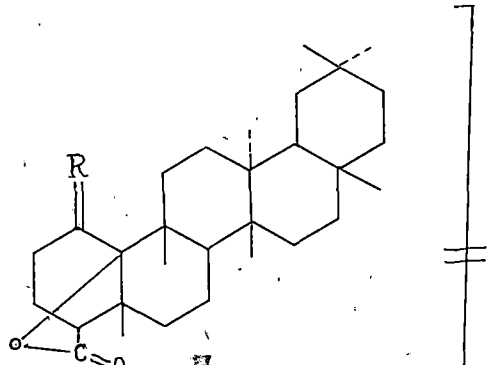


Fig.12: PMR spectrum of diketoleucolactone 5 (plot expansion).

Thus we may write the partial structure of leucolactone 1, leucolactone acetate 2 and diketoleucolactone 3 as:



Another R group may be at any of the positions C—11, C—12, C—15, C—16 and C—21 positions.

1, R = H / OH

2, R = H / OCOCH$_3$

3, R = O

Further discussion on the methyl signals of diketolactone 3:

The individual chemical shifts of the various methyl groups in the ^{1}H NMR spectra of friedelin have been assigned by Crowford *et al*[5]. It was suggested that the most downfield signal at 1.18 ppm to the C—28 methyl. Anjaneyulu *et al*[6] have assigned signals for different methyl groups of various D:A friedooleanane derivatives. A comparison of the methyl signals of the various derivatives of the compound 1 showed that the most downfield methyl signal appear at 1.28 ppm which must be the C—24 methyl group deshielded by the anisotropic effect of the lactone carbonyl group. The signal at

1.18 ppm as confirmed earlier[6], can be assigned to C—28 methyl group. Now these methyl protons split as a doublet (J = 0.5 Hz) as mentioned earlier which must be due to protons at either C—16 or C—22 position. Thus we may assume now that the second oxygen function is attached to either C—15 or C—21 position.

SECTION—D:

Mass spectral analysis of the compound leucolactone 1 and its derivatives:

Mass spectrum (Fig.13) of the compound 1 showed fragments at m/e 187, 189, 202, 203, 207, 220 (base), 221, 233, 234, 251, 384, 411, 421, 439, 454, 472 (M^+). The peak at m/e 472 being molecular ion peak, the other peak at m/e 454 must be due to loss of a molecule of water from the molecular ion. The peak at 439 is probably due to loss of a methyl group from the fragment at m/e 454, the one at m/e 421 may be due to loss of another water molecule from the fragment at m/e 439. Peak at m/e 411 may be considered due to the loss of —CO group from the fragment at m/e 439. The peak at m/e 202 may be due to loss of water from the fragment at m/e 220. The fragment at m/e 189 may be due to loss of water from fragment at m/e 207.

Mass spectrum (Fig.14) of the acetate of compound 1 viz. leucolactone diacetate 2 showed peaks at m/e 556, 496, 481, 453, 436, 421, 414, 393, 327, 301, 263, 262, 249, 233, 216, 212, 205, 189 (base), 187. The fragment at m/e 496 was due to the loss of CH_3COOH from M^+ ion peak. The peak at m/e 481 was due to loss of one methyl group from the fragment at m/e 496. The peak at m/e 453 was due to loss of CO from the fragment at m/e 481. The peak at m/e 436 was due to loss of CH_3COOH from the fragment at m/e 496. The peak at m/e 421 was due to loss of acetic acid from the fragment at m/e 481. The peak at m/e 202 was due to loss of CH_3COOH from the fragment at m/e 262 and the peak at m/e 189 might be due to loss of CH_3COOH from the fragment at m/e 249.

Mass spectrum of the ketolactone 3 showed molecular ion peak at m/e 468 and the other fragments at m/e 453, 425, 280, 250, 240, 218, 212, 210, 205, 196, 189, 182 etc.

The presence of fragments at m/e 220 and 202 in the hydroxylactone 1, 262 and 202 in the acetate lactone 2 and the fragments at m/e 218 in the diketolactone 3 shows that one of the oxygen must be present either in ring D or E, so as to form the fragment a.

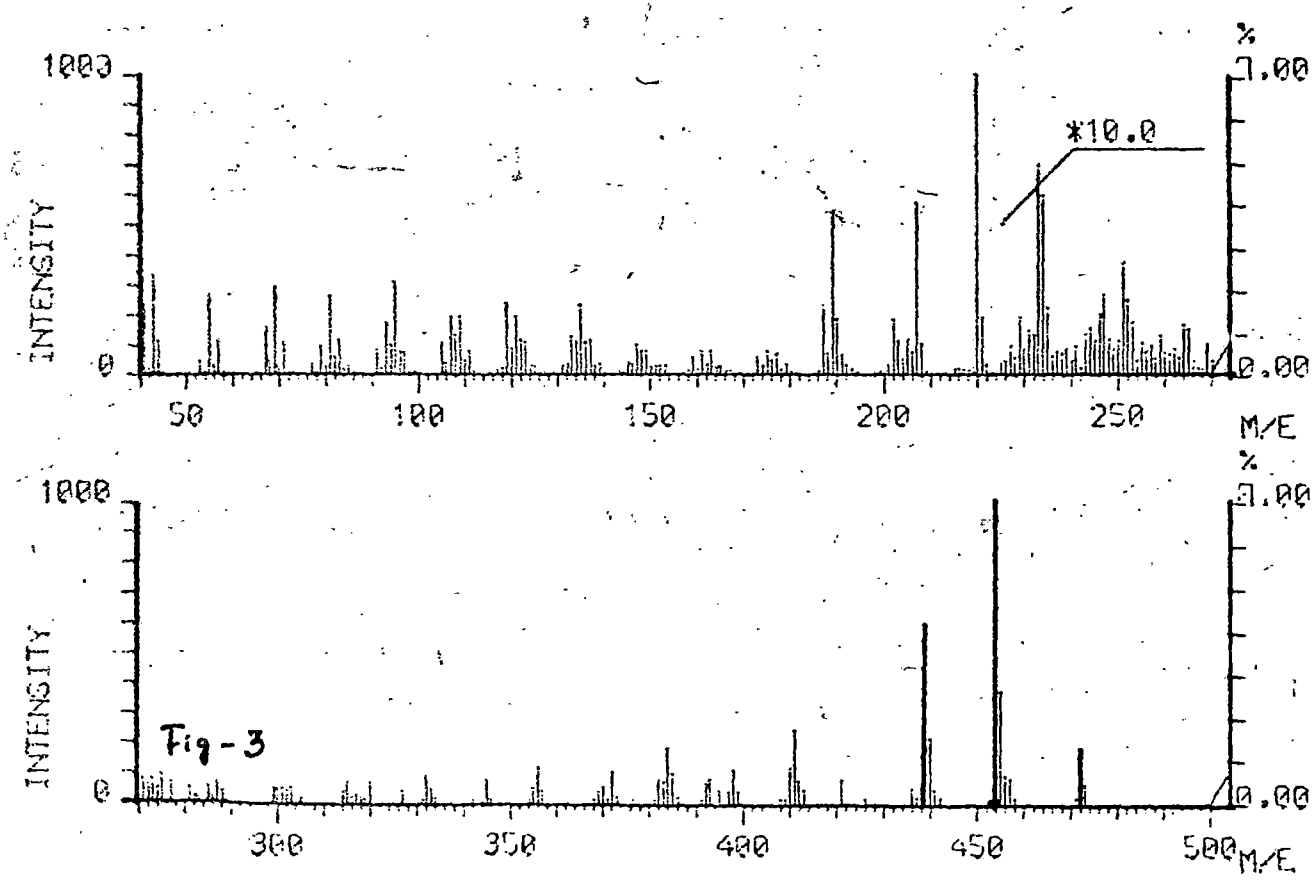


Fig.13: Mass spectrum of leucolactone 1.

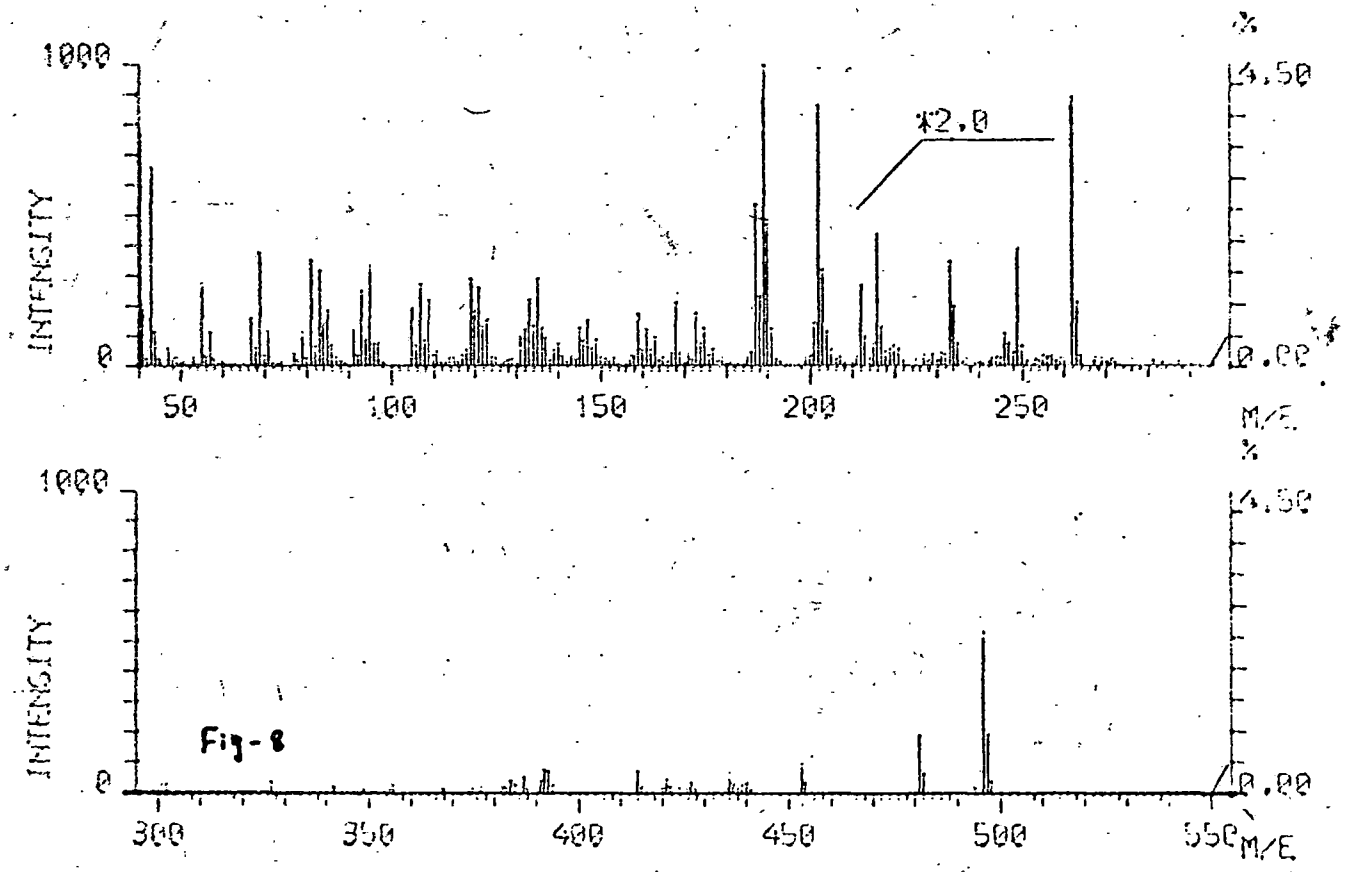
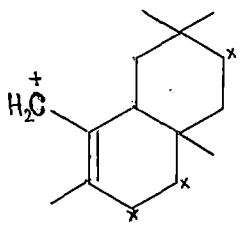


Fig.14: Mass spectrum of leuctolactone acetate 2.



a

\+ Any one of the positions containing group R.

When R = OH, a = m/e 220 $\xrightarrow{-H_2O}$ m/e 202

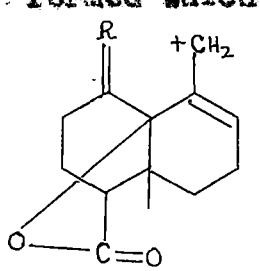
R=CH_3COO, a = m/e 262 $\xrightarrow{-CH_3COOH}$ m/e 202

R=O, a = m/e 218

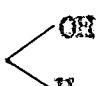
$\downarrow -CH_3$ $\qquad$ $\downarrow -CH_3$

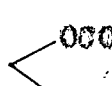
m/e 203 $\qquad$ m/e 187

Whereas the fragments at m/e 221, 203 in the hydroxy lactone 1 and fragments at m/e 263 and 203 in the spectra of the acetate lactone 2 and fragments at m/e 219 in the case of diketolactone 3 showes that the fragment b is formed which may be depicted as follows:



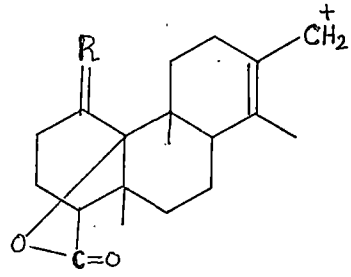
b

When, R=  b = m/e 221 $\xrightarrow{-H_2O}$ m/e 203

R=  b = m/e 263 $\xrightarrow{-CH_3COOH}$ m/e 203

R= O, b = m/e 219.

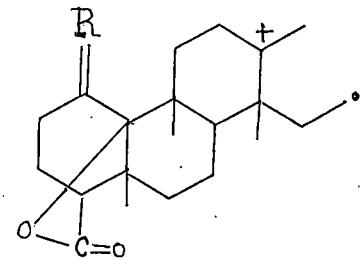
The presence of peaks at m/e 303 and 332 for the hydroxy lactone 1, fragments at m/e 301 and 330 for the diketolactone 3, indicated the formation of fragments c and d.



c

R=OH, H , c = m/e 303

R=O , c = m/e 301

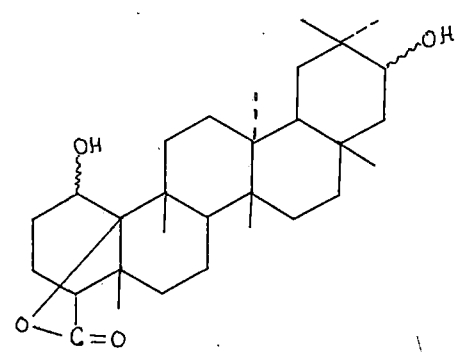


d

R=OH, H , d = m/e 332

R=O , d = m/e 330

The fragments a, b, c and d are typical fragments which have been found in other compounds belonging to the friedelane[7] skeleton. Thus from the above mass fragmentation analysis it may be inferred that the second oxygen function is probably not present in C—11, 12, 15 or 16 positions, thereby leaving only one probable position i.e. C—21 for the second hydroxyl group. Thus we may write the probable structural formula of the leucoplacotone as 1.



1

Though we have isolated the above compound once, attempts to reisolate the same, collected during the next following seasons failed to afford the above leucolactone thereby causing delay in further chemical investigation for the structural establishment of the compound. We are trying to reisolate the compound in a larger quantity currently, collection of which have already been started. It is hoped that we may be able to complete the establishment of the structure of this compound by further chemical and physical studies within next one year.

CHAPTER—IV

E X P E R I M E N T A L

Extraction:

Dried and powdered root of Leucas Aspera (5 kgs) was extracted directly with ethyl alcohol in a Soxhelt apparatus for twenty four hours. The solvent of the alcohol extract was concentrated to about 500 cc.

Hydrolysis of ethyl alcohol extract:

The ethyl alcohol extract was treated with 7% H_2SO_4 (20 cc) and the mixture was refluxed for 10 hours. Then it was cooled and extracted with ether. The ether layer was treated with 10% sodium hydroxide solution (3 x 100 ml) and the organic layer was washed with water to make it neutral and then dried over anhydrous sodium sulphate. The solvent was distilled off, which gave a gummy residue (5 gms).

Chromatography of the above gummy material:

The above gummy material (5 g) was dissolved in benzene (15 ml) and was placed on a column of alumina (400 g) deactivated with 16 ml of 10% aqueous acetic acid. The chromatogram was developed with petroleum ether and was eluted with the following solvents (Table—2):

Table—2

Chromatography of the above gummy material

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum (200 ml)	1—4	Nil
Petroleum ether:benzene (4:1) (200 ml)	5—8	Nil
Petroleum ether:benzene (3:2) (200 ml)	9—12	Nil
Petroleum ether:benzene (2:3) (300 ml)	13—18	White solid (300 mg) m.p.140—50°
Petroleum ether:benzene (1:4) (200 ml)	19—22	Nil
Benzene (200 ml)	23—26	Nil
Benzene:ether (4:1) (300 ml)	27—32	White solid (600 mg) m.p.270—90°

Further elution with more polar solvents did not afford any solid material

Isolation of stigmasterol:

Fraction 13—18 (table—2) were combined (300 mg) and was crystallised from a mixture of chloroform-methanol to afford crystals of $C_{29}H_{48}O$, m.p. 154—56°, $[\alpha]_D$ -50°. Its melting point was not depressed when mixed with an authentic sample of stigmasterol. IR spectra of the two were also superimposible.

Analysis report:

Found, C, 84.34% ; H, 11.71%

Required for $C_{29}H_{48}O$, C, 84.4% ; H, 11.72%

IR(nujol): ν_{max} at 3430 cm^{-1} (hydroxyl group)

Acetylation of stigmasterol : Preparation of stigmasteryl acetate:

Stigmasterol (0.1 g) was dissolved in 2 ml pyridine and acetic anhydride (2 ml) was added to the mixture and kept on water bath for 4 hours. It was cooled and poured into ice cold water. The white solid (0.09 g) separated out was filtered and washed with water. It was then crystallised from chloroform-methanol mixture which afforded crystals of $C_{31}H_{50}O_2$, m.p. 137--8°, $[\alpha]_D$ -34°. It showed no depression in m.p. when mixed with an authentic sample of stigmasteryl acetate. IR spectra of the two compounds were also superimposible.

Analysis report:

Found, C, 81.85% ; H, 11.07%

Calculated for $C_{31}H_{50}O_2$, C, 81.88% ; H, 11.08%

IR(nujol): ν_{max} at 1720 and 1240 cm^{-1} (acetate)

Mass : m/e at 454 (M^+), 453, 441, 439, 423, 395, 383, 255, 139 (base). Fig.1

Isolation of new triterpene dihydroxy lactone—leucolactone 1:

Fractions 27—32 (table—2) were combined (600 mg) and was crystallised from chloroform-methanol mixture to afford crystals of 1, m.p. 310° (500 mg).

Analysis report:

Found, C, 76.23% ; H, 9.58%

Required for $C_{30}H_{48}O_4$, C, 76.23% ; H, 10.23%

UV : No UV absorption between 200—350 nm.

IR(KBr): ν_{max} at 3607 and 3540 (two hydroxyl groups), 1750 (γ-lactone), 1380 and 1365 cm^{-1} (gem dimethyl group). Fig.2

PMR : 0.9, 0.98, 1.00, 1.21, 1.28 ppm (methyl groups), 2.6(1H, m), 3.18 (1H, $W_{\frac{1}{2}}$ = 8Hz), 3.85 (1H, $W_{\frac{1}{2}}$ = 18Hz) ppm.

Mass : m/e at 472 (M^+), 454, 439, 421, 411, 384, 251, 234, 253, 221, 220 (base), 203, 202, 207, 189, 187. Fig.13

Liebermann - Burtchard test positive, TNM test negative.

Acetylation of compound 1 ; Preparation of leucolactone diacetate 2 :

200 mg of the hydroxy lactone 1 was dissolved in 5 cc of pyridine and 5 cc of acetic anhydride was added.

The mixture was kept on water bath for 4 hours. Then it was cooled and poured into ice cold water when white solid separated out which was filtered. The residue (0.2 g) was dissolved in minimum amount of benzene and placed on a column of deactivated alumina (50 g). The chromatogram was developed with petroleum ether. The following solvents were used for chromatography (Table—3):

Table—3

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—20	White solid m.p. 270—80°

Further elution with more polar solvents did not afford any solid material

Fractions 13—20 (table—3) were combined and crystallised from a mixture of chloroform and methanol which afforded crystals of 2, m.p. 284—85° .

Analysis report:

Found, C, 73.35% ; H, 9.48%

Required for $C_{34}H_{52}O_6$, C, 73.35% ; H, 9.41%

IR(KBr): ν_{max} at 1728 and 1740, 1216 and 1235 cm^{-1} (two acetate), 1768 (lactone carbonyl), 1359 and 1379 cm^{-1} (gem dimethyl group) Fig.3

PMR : 0.85—1.28 (methyl groups), 2.05 (s) and 2.12 (s) (protons of acetomethoxy groups), 2.8 (d,d), 4.45(t), 4.9 (d) ppm Fig.5

^{13}C NMR: 176.09, 170.85, 169.79, 90.2, 80.53 and 71.64 ppm Fig.6

Mass : m/e at 556 (M^+), 496, 481, 453, 436, 421, 414, 393, 327, 301, 263, 262, 249, 233, 216, 212, 203, 189 (base), 187. Fig.14

Chromium trioxide - pyridine oxidation of the dihydroxy lactone 1 : Preparation of the diketoleucolactone 3 :

100 mg of the dihydroxy lactone, 1 dissolved in pyridine (2 ml), was added to CrO_3-Py complex prepared from pyridine (6 ml) and CrO_3 (0.6 g) at 10°C and the mixture was stirred for 12 hrs. Excess of CrO_3 was destroyed by adding methanol (4 ml), diluted with ethyl acetate and filtered. Ethyl acetate was removed and the concentrate was taken up in ether. The organic layer was washed with hydrochloric acid (5%), then with water till neutral and dried

over anhydrous sodium sulphate. Removal of solvent gave a gummy residue (0.9 g). The above residue was chromatographed over a column of deactivated alumina (30 g). The chromatogram was developed with petroleum ether and the product dissolved in benzene (5 ml) was poured on the column and eluted with the following solvents:

Table—4

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—20	White solid m.p. 310—20°

Further elution with more polar solvents did not afford any solid material

Fractions 13—20 (table—4) were combined and crystallized from chloroform-methanol mixture to give the crystals of 3, m.p. 323—4°.

Analysis report:

Found, C, 76.86% ; H, 9.45%

Required for $C_{30}H_{44}O_4$, C, 76.88% ; H, 9.46%

IR(nujol): ν_{max} at 1705—10 (broad peak), 1760 cm^{-1}

Fig.4

PMR : 0.82, 0.93, 1.02, 1.10, 1.18 and 1.30 ppm (tert-CH_3), 2.18 (d), 2.78 (d) ppm

Fig.9

Mass : m/e at 468 (M^+), 453, 425, 280, 250, 240, 219, 210, 205, 193, 189, 182 etc.

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Reprint no - 1.

Tetrahedron Vol. 39, No. 17, pp. 2819 to 2824, 1983
Printed in Great Britain.

0040-4020/83 $3.00+.00
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ACTION OF HYDROGEN PEROXIDE ON OLEAN-12,15-DIEN-3,11-DIOL: PREPARATION OF C-NOR-TRITERPENE LACTONES

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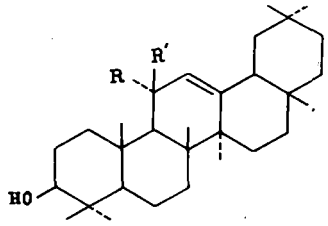
(*Received in UK 19 April 1982*)

Abstract—Treatment of olean-12,15-dien-3,11-diol with hydrogen peroxide containing p-toluenesulphonic acid furnished two isomeric γ-lactones identified as 3β-acetates of C-12-nor-olean-15-en-13α-carb→19α-olide and C-12-nor-olean-18(19)-en-13β-carb→15β-olide.

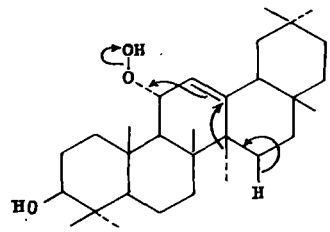
E. J. Corey *et al.*[1] synthesised epoxytaraxerol **2** from both the olean - 12 - en - 3β,11β - diol **1a** and olean - 12 - en - 3β,11α - diol **1b** and suggested that the formation of the same epoxide **2** from the isomeric (C-11) diols must proceed by C-11–O bond cleavage with the formation of the same C-11,12,13 allylic cation which then forms the hydroperoxide **3**. This **3** in turn undergoes O–O fission and carbon rearrangement to afford **2**.

that would undergo nucleophilic attack by OH^- ion producing a 16-hydroxytaraxerol derivative or to produce a cation at C-8 by migration of C-8–Me to C-14 position which was expected to eliminate a C-7 proton to give multiflorenol derivative **7**; but in actual practice, the findings were widely different from those expected and are discussed below:

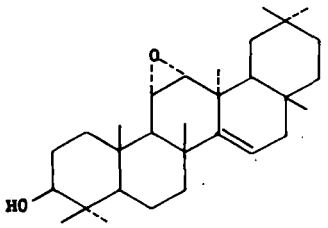
Treatment of taraxeryl acetate **8** with NBS afforded **9**,



1a, R=H, R'=OH
1b, R=OH, R'=H



3

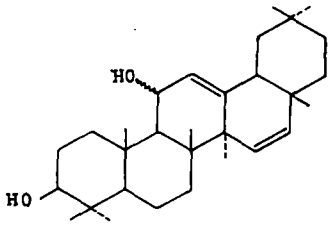


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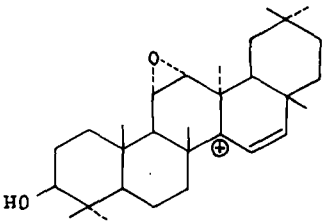
Chart I

This type of reaction under identical conditions was carried out on the diol **4** synthesised from taraxeryl acetate with a view to producing the allylic cation **5**. The intermediate **5** would further isomerize either to produce the allylic cation **6** by migration of C-15–16 double bond

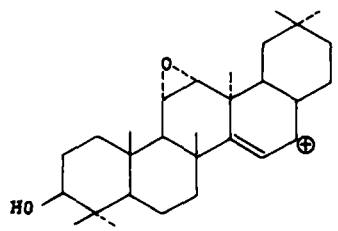
$C_{32}H_{51}O_2Br$ (M^+ 548, Br 81) m.p. 180–82°, $[\alpha]_D+47.4°$, ν_{max} 1720, 1250 (-$OCOCH_3$); ^1H NMR: δ 5.3 (m, 1H, $\diagdown C{=}C \diagup$ H), 4.53 (m, 1H, H–C–O–$COCH_3$), 4.3 (m, 1H, H–C–Br), 2.09 (s, 3H, –O–$COCH_3$) ppm. Treatment of **9**



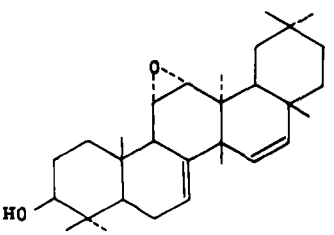
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5

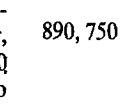


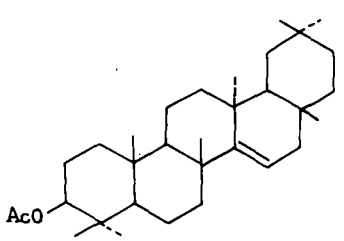
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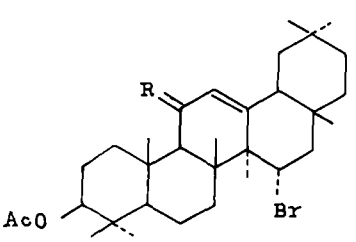
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Chart II

with sodium dichromate and acetic acid furnished 11 - oxo - 15 - bromo - β - amyrenyl acetate[2] **10** $C_{32}H_{49}O_3Br$, m.p. 240–41°, $[\alpha]_D + 88°$, λ_{max} 249 nm; ν_{max} 1725, 1250 ($-OCOCH_3$), 1680 ($-C{=}C-C{=}O$) cm^{-1}. Attempts to dehydrobrominate **10** with dimethyl aniline/collidine gave the starting compound **10**. A reverse route was followed to prepare the 11 - oxo - 12,15 - diene by dehydrobrominating **9** which furnished compound **11**, $C_{32}H_{50}O_2$, m.p. 199–200°, $[\alpha]_D + 42°$; no UV absorption between 220–300 nm; ν_{max} 1730, 1240 ($-OCOCH_3$), 1650, 890, 750 () cm^{-1}; NMR: 5.2–5.6 (*m*, 3H, vinyl protons), 4.5 (*m*, 1H, H–C–$OCOCH_3$), 2.08 (*s*, 3H, $-O-COCH_3$), 0.8–1.2 (8-t-CH_3) ppm. The diene **11** was oxidised to give the desired 11-oxo-12,15-diene **12**, $C_{32}H_{48}O_3$, m.p. 243–45°, $[\alpha]_D + 28.6°$, λ_{max} 244 nm; ν_{max} 1730, 1240 ($-O-COCH_3$), 1660 ($C{=}C-C{=}O$), 1650, 890, 750

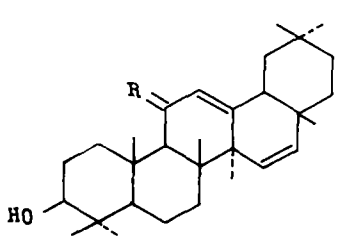


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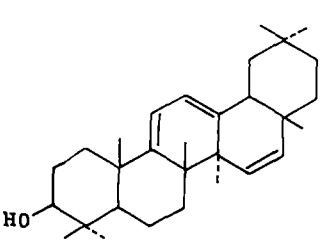
9 R = 

10 R = O



11 , R = 

12 , R = O



13

Chart III

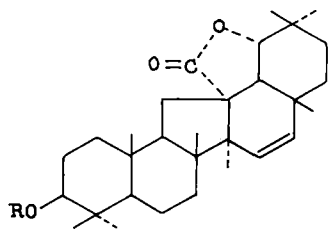
(), 820 () cm^{-1}. LAH reduction of the 11-oxo-diene **12** furnished a diene-diol **4**, $C_{30}H_{48}O_2$, m.p. 198–200°, ν_{max} 3460 (-OH), 820 () cm^{-1}; purification of the diol by chromatography yielded a homoannular diene **13**, $C_{30}H_{46}O$, m.p. 189–90°, λ_{max} 276 nm.

The diol **4** was directly treated with hydrogen peroxide containing p-toluenesulphonic acid following the procedure adopted by Corey *et al.*[1] The product furnished two compounds (**14** and **16**) of the same molecular formula $C_{30}H_{46}O_3$. The compound **14**, m.p. 240–41° showed IR bands at 3520 (-OH), 1775 (γ-lactone), 1650, 890, 870, 750 () cm^{-1}. Acetylation of **14** furnished the acetate **15** $C_{32}H_{48}O_4$, m.p. 215–16°; ν_{max} 1780 (γ-lactone), 1720, 1250 (-$OCOCH_3$), 1650, 890, 870, 750 () cm^{-1}. The formation of the lactone has thus been proved by the IR spectrum of the alcohol **14** and its acetate **15**. The structure **14** has been conclusively established from mass, ^1H and ^{13}C NMR spectra of the compound **15**.

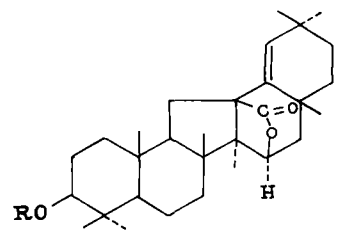
^1H NMR of **15** showed the presence of eight tertiary Me- groups between 0.5 and 1.5 ppm, the acetoxy Me-group at 2.05 ppm, the proton geminal to the lactonic O at 4.72 ppm as a doublet (J = 7.3 Hz), and the *cis*-disubstituted olefinic protons appeared at 5.27 ppm as an AB quartet (J = 10 Hz). Irradiation at 4.72 ppm gave a singlet at 1.7 ppm showing the proton at 1.73 ppm to be coupled to the one at 4.72 ppm with J = 7.5 Hz and weakly to other protons. Again irradiation at 1.73 ppm at a fairly low level gave a sharp line at 4.72 ppm which was a doublet otherwise. Hence the doublet at 4.72 ppm arises due to a single proton adjacent to the lactonic O that couples with the proton at 1.73 ppm. The multiplet at 4.5 ppm collapses to a broad singlet suggesting the presence of a proton at C-3 coupled with protons at C-2. The absence of any peaks between 2.2 and 3.5 ppm shows that the C-α to the lactonic -CO- possesses no proton. The above observations could be explained if the lactone -CO- group is attached to the C-13 position and the γ-lactone ring is formed with the C-19 carbon with a geminal β-H that has a single neighbouring β-axial proton at C-18 position, thus establishing the structure of **14**. This structure is corroborated by the ^{13}C NMR and mass (Experimental) spectra.

The compound **16** had m.p. 256–57° and showed IR absorptions at 3525 (OH), 1780 (γ-lactone), 820 () cm^{-1}. ^1H NMR showed a singlet at 5.3 ppm for the vinyl proton, a multiplet at 4.8 ($W_{1/2}$ = 20 Hz) ppm for the proton geminal to the lactonic oxygen which may have two neighbouring protons, a triplet at 3.23 ppm ($W_{1/2}$ = 20 Hz) for the proton geminal to the OH at C-3 present as an axial proton.[3] Acetylation of **16** gave the acetate **17**, $C_{32}H_{48}O_4$ m.p. 228–29°; ν_{max} 1780 (γ-lactone), 1715, 1250 (acetate), 820 (trisubstituted double bond) cm^{-1}. The mass spectrum of **17** was essentially identical with those of **15**.

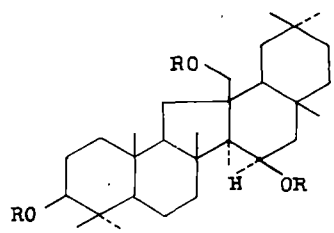
The ^1H NMR of **17** showed a multiplet at 4.8 ($W_{1/2}$ = 7 Hz) ppm (integrated for one proton) showing that the proton geminal to the lactonic O is equatorial which couples with axial and equatorial protons; the multiplet at 4.5 ppm was due to C-3 proton, the singlet at 5.3 ppm was due to C-19 olefinic proton. The absence of any peaks in the region 2.2–3.5 ppm showed that the lactone -CO- has no α-H as in **15**, and hence should be attached to the same carbon (C-13). The absence of AB quartet for the *cis* C-15–16 olefinic protons present in **4** indicated



14, R = H
15, R = CH_3CO



16, R = H
17, R = CH_3CO



18, R = H
19, R = CH_3CO

Chart IV

that this double bond must be involved in the formation of lactone **16** and the lactonic O should be attached to C-15 with an α-equatorial geminal proton that couples with C-16 protons. The structure **16** thus assigned to the lactone explains all the IR and NMR peaks.

LAH reduction of the lactone **16** afforded a triol **18** $C_{30}H_{50}O_3$ m.p. 280–82°, acetylation of which gave a triacetate **19**, $C_{36}H_{56}O_6$, m.p. 190–93°, ν_{max} 1735–40, 1250–40 (acetate groups), 820 cm^{-1}. ^1H NMR of the triacetate **19** showed peaks at 0.85–1.2 (eight t-Me groups), 2.05 (two acetoxyl Me- groups), 2.1 (one acetoxyl Me-), 3.65 (J = 11 Hz, AB-quartet for two methylene protons geminal to the acetate group), 4.45 (a multiplet for the proton at C-3 geminal to the acetate), 5.2 (J = 8 Hz, a doublet split into a multiplet for C-15-proton geminal to the acetate) and at 5.3 (a singlet for the C-19 vinyl proton which has no neighbouring proton) ppm.

Mechanism. Contrary to the formation of the proposed intermediates **5** or **6** the double bond at C-12–13 is pushed, during the formation of the epoxide **I-2**, from the hydroperoxide **I-1** towards ring E (to form a germanicol derivative) rather than to ring B or D due to the presence of double bond at C-15–16 position. Under the reaction condition the epoxide **I-2** is unstable due to the conformational strain caused by the double bonds at C-15–16 and C-18–19 positions and hence undergoes epoxide ring opening in presence of acid forming carbonium ion **I-4** probably by 1,3-hydride shift from C-13 to C-11 as shown in **I-3**. The intermediate carbonium ion **I-4** undergoes ring contraction producing the isomeric cations **I-5** and **I-5'** which in turn lose a proton to form isomeric aldehydes **I-6** and **I-6'** respectively. These aldehydes get oxidised in presence of hydrogen peroxide to the corresponding α and β carboxylic acids at C-13 positions.

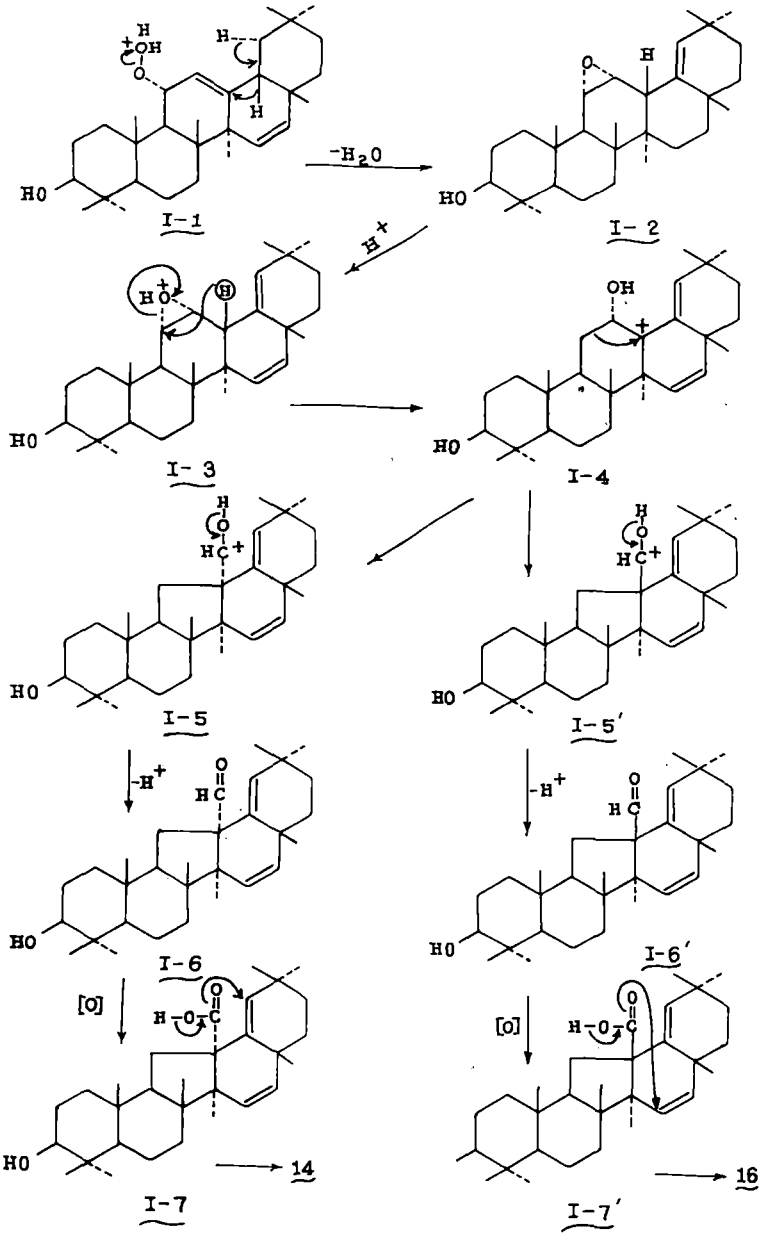


Chart V

The α-carboxyl group in **I-7** being at proximity to the double bond at C-18–19, undergoes lactonization furnishing the γ-lactone **14**, whereas the inetermediate **I-7'** having its carboxyl group in the β-position is at proximity to the C-15–16 double bond, lactonizes forming **16**. The Dreiding model of the lactones **14** and **16** showed that the lactones are strain free, thus stabilising the molecules. This is perhaps the first report on the formation of a lactone with the carbon skeleton rearrangement in ring C of a triterpenoid with hydrogen peroxide in presence of p-toluenesulphonic acid.

EXPERIMENTAL

M.ps are uncorrected. Petroleum used had b.p. 60–80°. All the rotations were determined in $CHCl_3$ at 1–2% solns. The ^1H NMR spectra were recorded with Varian A-60, T-60, FT-80A and XL-100 NMR spectrometers using $CDCl_3$ containing TMS as internal reference. All the UV spectra were determined in 95% ethanol and IR in nujol. Column chromatography were done on neutral alumina deactivated with 10% aqueous acetic acid (4 ml/100 g of alumina).

Oxidation of taraxeryl acetate **8** *with NBS in DMSO*

Preparation of 15-*bromo*-β-*amyrin acetate* **9**. To a soln of **8** (1 g) in $CHCl_3$ (50 ml) and DMSO (25 ml), was added NBS (1 g) in portions and kept in dark for 14 hr. The mixture was filtered and the filtrate extracted with $CHCl_3$, washed with water and dried ($NaSO_4$). Removal of the solvent and chromatography of the residue furnished **9** (0.9 g), m.p. 180–82°, $[\alpha]_D + 47.4°$. (Found: C, 70; H, 9.1. $C_{32}H_{51}O_2Br$ requires: C, 70.23; H, 9.3%.) Beilstein test for halogen—positive.

Oxidation of 15-*bromo*-β-*amyrin acetate* **9**

Preparation of 11-*oxo*-15-*bromo*-β-*amyrin acetate* **10**. To a refluxing soln of **9** (1 g) in benzene (40 ml) was added slowly a soln of $Na_2Cr_2O_7$ (1 g) in glacial AcOH (30 ml) with stirring while maintaining the temp at 70°. After the addition was complete the stirring was continued for 24 hr, maintaining the temp at 80–85°. At the end it was cooled and rectified spirit (5 ml) was added. The soln was concentrated to 1/3rd volume and poured into ice cold water. The ppt was filtered off, washed and crystallization from $CHCl_3$–MeOH gave **10** (0.75 g) m.p. 240–41°, $[\alpha]_D + 89°$. (Found: C, 68.2; H, 8.6. $C_{32}H_{49}O_3Br$ requires: C, 68.4; H, 8.7%.) Beilstein test—positive.

Attempted dehydrobromination of 11-*oxo*-15-*bromo*-β-*amyrin acetate* **10**

(a) *With dimethyl aniline.* Compound **10** (0.5 g) was refluxed with dimethylaniline (75 ml) for 6 hr. The mixture was cooled, acidified with HCl (6 N) and then extracted with ether. Removal of the solvent and chromatography of the residue furnished an eluate (with petroleum at eluent) which on crystallization with $CHCl_3$–MeOH afforded the starting **10**, m.p. (m.m.p.) 240–41°, Beilstein test—positive.

(b) *With s-collidine.* Compound **10** (0.2 g) was refluxed with s-collidine (15 ml) for 18 hr at 180° in an oil bath. Working up the mixture in the usual method gave back **10**, m.p. (m.m.p.) 240°, Beilstein test—positive.

Dehydrobromination of 15-*bromo*-β-*amyrin acetate* **9**

Preparation of olean - 12,15 - dien - 3 - β - yl acetate **11**. Acetate **9** (0.2 g) was refluxed with distilled dimethylaniline (30 ml) for 6 hr. The mixture was diluted with water, acidified with aq HCl and extracted with ether. The ether was distilled off and the residue chromatographed. Petroleum eluted a solid which on crystallization ($CHCl_3$–MeOH) gave **11**, m.p. 199–200°, $[\alpha]_D +$ 42°. (Found: C, 81.9; H, 10.8. $C_{32}H_{50}O_2$ requires: C, 82.35; H, 10.8%.) UV—no absorption in the region 220–300 nm. Beilstein test—negative.

Oxidation of olean-12,15-*dien*-3-β-*yl acetate* **11**

Preparation of 11 - *oxo* - *olean* - 12,15 - *dien* - 3 - β - *yl acetate* **12**. A soln of $Na_2Cr_2O_7$ (4 g) in glacial AcOH (100 ml) was added slowly during a period of 1 hr to a vigorously stirred soln of olean - 12,15 - dien - 3 - β - yl acetate (4 g) in refluxing benzene (50 ml). The mixture was refluxed for 24 hr and then cooled. Excess of dichromate was decomposed with EtOH (50 ml). The soln was concentrated to 1/3rd volume and the contents poured into ice cold water, extracted with ether, washed with water and dried (Na_2SO_4). Removal of the solvent and chromatography of the residue furnished a solid on elution with petroleum: ϕH (3:2). The solid on crystallization ($CHCl_3$–MeOH) gave 12, m.p. 243–45°, $[\alpha]_D + 28.8°$, λ_{max} 244 (ε, 11,376) nm, ^1H NMR: δ 0.82–1.04 (8 t-Me protons), 2.03 (s, 3H, $-OCOCH_3$), 2.23 (dd, 1H, C-1β-H), 2.42 (s, 1H, C-9α-H), 2.82 (t of d, 1H, C-1β-H),[4] 4.5 (t, 1H, H–C–3–O–CO–), 5.38 (s, 2H, 2 vinyl H), and 5.64 (s, 1H, 1 vinyl H) ppm. (Found: C, 79.86; H, 9.95. $C_{32}H_{48}O_3$ requires: C, 79.95; H, 10.06%.)

LAH reduction of 11 - *oxo* - *olean* - 12,15 - *dien* - 3 - β - *yl acetate* **12**

Preparation of olean - 12,15 - dien - 3,11 - diol **4**. A soln of **12** (2.5 g) in dry benzene (30 ml) and dry ether (70 ml) was refluxed with LAH (5 g) for 6 hr followed by stirring at room temp for another 12 hr. Excess LAH was destroyed by adding water dropwise at r.t. The etherial layer was separated and the aqueous layer was extracted with ether and the two ether soln were mixed, washed with water and dried (Na_2SO_4). The ether was removed and the residue yielded **4** (4.5 g), m.p. 198–200°, ν_{max} 3460, 1650, 890, 860, 820, 750 cm^{-1} (TLC single spot).

Compound **4** (0.2 g) was absorbed in a column of deactivated alumina (10 g) and elution with petroleum: ϕH (3:2) furnished **13** m.p. 189–90°, λ_{max} 276 nm, m/e 422 (M^+). (Found: C, 84.8; H, 11.30. $C_{30}H_{46}O$ requires: C, 85.25; H, 10.97% (TLC homogenous).)

Treatment of olean - 12,15 - dien - 3,11 - diol **4** *with* H_2O_2-*p*TsOH

Isolation of **14** *and* **16**. To a soln of **4** (1.9 g) in CH_2Cl_2 (100 ml) was added a soln (80 ml) prepared by mixing p-TsOH (3 g) and 30% H_2O_2 (5 ml) in t-BuOH (80 ml). The mixture was stirred slowly for 24 hr and then poured into water. It was then extracted with CH_2Cl_2, washed with water, dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue (1.5 g) was absorbed in an alumina column. Elution with benzene yielded a mixture of **14** and **16**, (Rf **14** = 49; **16** = 46 in EtOAc). The mixture was repeatedly crystallized from $CHCl_3$–MeOH when **14** (0.3 g) separated as the less soluble part. Further purification by crystallization from $CHCl_3$–MeOH afforded pure **14**, m.p. 240–41°, no UV absorption in the region 220–300 nm. (Found: C, 78.94; H, 9.89. $C_{30}H_{46}O_3$ requires: C, 79.25; H, 10.20%.)

Acetylation of lactone **14**. **14** (0.1 g) was heated with a mixture of Py (2 ml) and Ac_2O (2 ml) for 4 hr. The mixture was then poured into ice cold water and then filtered. The residue was washed with water and dried under suction. Crystallization of the solid furnished **15**, m.p. 215–16°; *m/e* 496 (M^+). 468 (M^+–CO), 452 (M^+–CO_2), 436 (M^+–AcOH), 421, 408, 392, 372, 313, 300, 269, 257, 244, 231, 217, 218, 206, 203, 191, 189, 187, 175, 171, 161, 147, 135; ^{13}C NMR (multiplicity): 175.5(*s*), 170.5(*s*), 132.75(*d*), 132.5(*d*), 82.0(*d*), 80.0(*d*), 75.8(*s*), 67.5(*d*), 55.75(*d*), 51.75(*d*), 45.25(*s*), 42.0(*s*), 38(3*t*, 1*s*), 36.8(*s*), 35.75(*t*), 34.8(*t*), 34.2(*s*), 32.8(*q*), 30.25(*s*), 29.0(*q*), 28.0(*q*), 25.0(*q*), 23.75(*q*), 23.5(*t*), 22.75(*q*), 21.25(*q*), 19.75(*t*), 18.6(*q*), 16.25(*q*) ppm. (Found: C, 77.25; H, 9.85. $C_{32}H_{48}O_4$ requires: C, 77.36; H, 9.74%.)

Isolation of lactone **16**. The more soluble fraction was crystallized twice and the purer product was isolated from the filtrate. Concentration of the filtrate gave **16** (0.2 g), m.p. 256–57°; *m/e* 454 (M^+), 436 (M^+–H_2O), 426 (M^+–CO), 410 (M^+–CO_2), 408, 392, 313, 269, 231, 205, 189, 187, 171. (Found: C, 79.45; H, 9.76. $C_{30}H_{46}O_3$ requires: C, 79.25; H, 10.20%.)

Acetylation of lactone **16**. **16** (0.01 g) was heated with Py (1 ml) and Ac_2O (1 ml) for 4 hr. The mixture on usual work up and crystallization from $CHCl_3$–MeOH gave the acetate **17**, m.p. 228–29°, *m/e* 496 (M^+), 468 (M^+–CO), 452 (M^+–CO_2), 436 (M^+–AcOH), 408, 392, 372, 313, 269, 257, 256, 217, 206, 203, 191, 189, 187, 171, 161, 147, 135. (Found: C, 77.38; H, 9.94. $C_{32}H_{48}O_4$ requires: C, 77.30; H, 9.74.)

LAH reduction of lactone **16**. A soln of 16 (0.2 g) dissolved in dry ether (50 ml) was refluxed with LAH 90.4 g) for 4 hr. Excess of LAH was decomposed with a saturated soln of Na_2SO_4 and the product extracted with ether (200 ml), washed with water and dried (Na_2SO_4). The solvent was distilled off and the residue when recrystallized from acetone furnished the triol **18** (0.15 g), m.p. 280–82° (TLC single spot). (Found: C, 78.42; H, 10.84. $C_{30}H_{50}O_3$ requires: C, 78.55; H, 10.99%.)

Acetylation of triol **18**—*preparation of triacetate* **19**. A mixture of 18 (0.12 g) in dry Py (2.0 ml) and Ac_2O (2.0 ml) was kept over a water bath for 12 hr. The mixture was poured into cold water when a solid which separated out was washed with water and dried under suction. It was then crystallized from $CHCl_3$–MeOH to afford the triacetate **19** (0.1 g), m.p. 190–93° (TLC-single spot); m/e 584 (M^+), 524 (M^+–AcOH), 432, 464 (M^+–2AcOH), 459, 389, 358, 343, 340, 269, 249, 215, 204, 189, 187; ^{13}C NMR (multiplicity): 170.5(*s*), 170.2(*s*), 170(*s*), 155.5(*s*), 122(*d*), 80.5(*d*), 78.5(*d*), 76.0(*t*), 55.5(*d*), 50.0(*d*), 46.8(*s*), 41.5(*s*), 39.5(*t*), 38.2(*s*), 38.0(*t*), 37.8(*s*), 37.5(*s*), 34.0(*t*), 33.9(*t*), 33.5(*t*), 32.0(*s*), 31.5(*t*), 31.0(*q*), 29.5(*q*), 28.0(*q*), 25.5(*q*), 23.8(*t*), 23.5(*q*), 22.5(*q*), 21.5(3*q*), 18.5(*t*), 16.0(*q*), 15.5(*q*) ppm. (Found: C, 73.50; H, 9.85. $C_{36}H_{56}O_6$ requires: C, 73.93; H, 9.65%.)

Acknowledgement—The authors' thanks are due to Dr. A. Duffield, University of New South Wales for the mass spectra; to Dr. S. K. Sengupta, East India Pharmaceutical Works Ltd. Calcutta for the optical rotations. Two of us (M. M. Mukherjee and D. K. Chakrabarti) are grateful to U.G.C. for the financial assistance.

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Reprint No. 2.

Indian Journal of Chemistry
Vol. 22B, January 1983, pp. 12-16

Action of N-Bromosuccinimide on Triterpene Acids & Esters in Dimethyl Sulphoxide

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Received 15 *April* 1982; *accepted* 11 *May* 1982

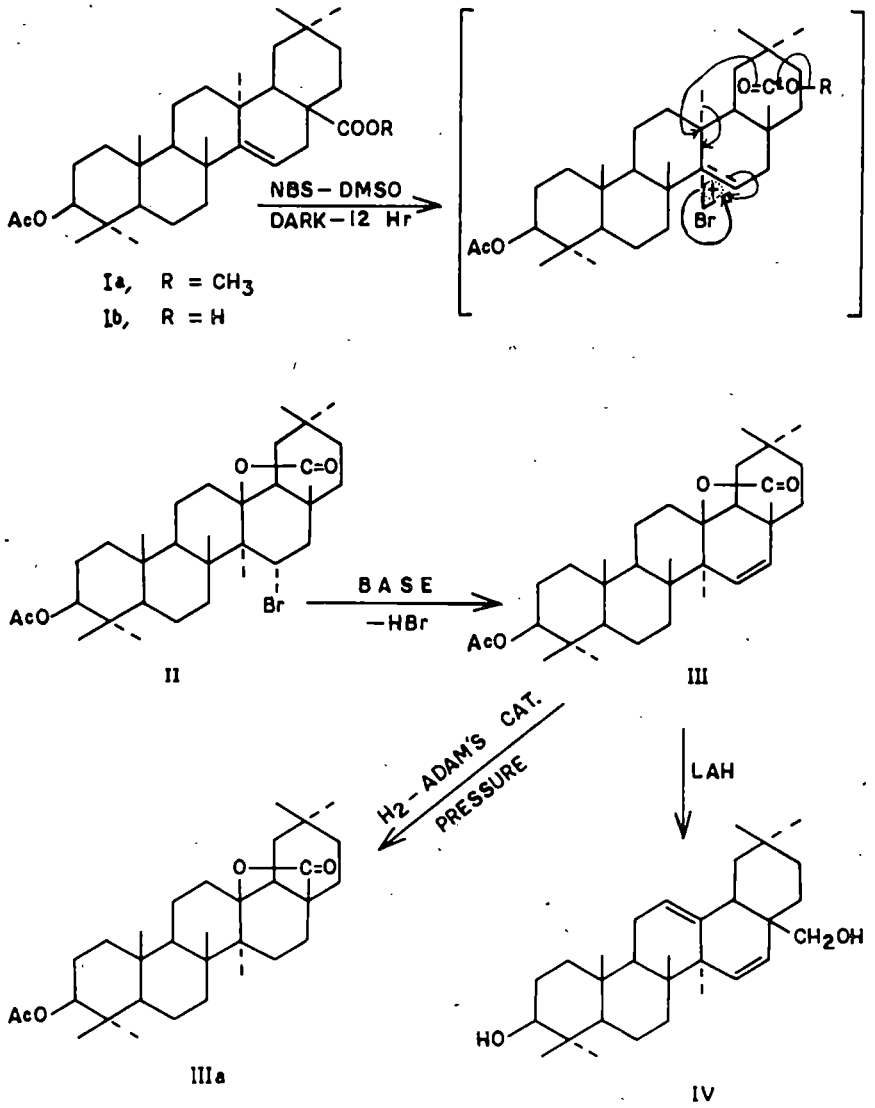
Action of N-bromosuccinimide on acetyloleanolic acid/methyl ester, acetylaleuritolic acid/methyl ester and acetylbetulenic acid/methyl ester in dimethyl sulphoxide has been studied and in each case a bromo γ-lactone is formed, and in the latter two compounds lactonization occurs with rearrangements. The compounds so formed have been identified as 3β-acetyl-12α-bromooleanan-28→13-olide (V), 3β-acetyl-15α-bromooleanan-28→13-olide (II) and 3β-acetyl-29,30-dibromo-18α-oleanan-28→19β-olide (IX) respectively.

Khastgir and coworkers[1] reported that taraxeryl acetate on treatment with N-bromosuccinimide (NBS) furnished 15-bromo-β-amyrin acetate. In order to examine the applicability of the reaction on other compounds of taraxerane skeleton, we have presently carried out the reaction on methyl acetylaleuritolate[2] (Ia), $C_{33}H_{52}O_4$, by treating it with NBS in dimethyl sulphoxide (DMSO) in dark for 12 hr. Usual work-up followed by chromatography and crystallization furnished a crystalline solid which analysed for $C_{32}H_{49}O_4Br$, m.p. 280-82°, $[\alpha]_D$ +19.51°. That it had γ-lactone moiety was supported by its IR spectrum in nujol, exhibiting bands at 1780, 1710, 1240 cm^{-1}. Its PMR spectrum indicated the absence of a vinyl proton and methoxycarbonyl protons originally present in the starting material, showing that the bromination was accompanied by the formation of a γ-lactone involving the double bond and the loss of a methyl group from the carbomethoxyl group. Repetition of the reaction on acetyl aleuritolic acid[2] (Ib), $C_{32}H_{50}O_4$, furnished the same bromolactone. The structure of the bromolactone has been established as II on the fact that the compound on dehydrobromination with dimethylaniline afforded 15,16-dehydro-lactone (III), $C_{32}H_{48}O_4$, m.p. 308-10°, $[\alpha]_D$ −8.42°; IR (nujol): 1770 (γ-lactone), 1730, 1250 (acetate), 900 cm^{-1} (*cis*-disubstituted olefin). III on LAH reduction furnished aegiceradiol[3] (IV), $C_{30}H_{48}O_2$, m.p. 235-36° identical (m.m.p., co-IR) with an authentic sample. III on catalytic hydrogenation over Adam's catalyst in acetic acid under pressure afforded 3β-acetyloleanan-28→13-olide[4] (IIIa), $C_{32}H_{50}O_4$, m.p. 293-94° (identical with the lactone prepared from 3β-acetyl oleanolic acid[4]). The formation of aegiceradiol (IV) showed that the double bond must be formed at 15-16 position on dehydrobromination of the bromine present at 15-position.

A study of the Dreiding model of the lactone (II) showed that the bromine at C-15 should be α-equatorial. This was confirmed by appearance of a proton centered at δ 4.3 (*d*, *d*) with coupling constants of 14 Hz (*J* a, a) and 3 Hz (*J* a, e) due to the axial proton geminal to the bromine having axial and equatorial protons on the vicinal carbon. The mechanism of formation of II probably involves the attack of the bromonium ion from NBS in DMSO at the double bond. Bromine being a bulky atom ultimately assumes the equatorial position so as to have the minimum strain and steric interaction. The next step involves concerted migration of the C-13 methyl to the C-14 position and elimination of the methoxyl methyl to form the 28→13-olide (II).

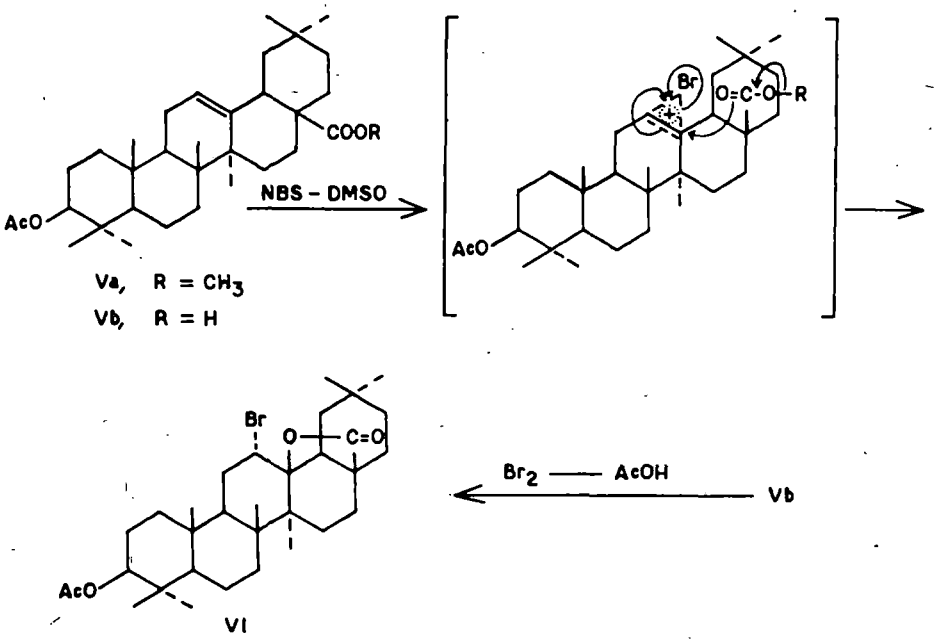
As no systematic work on the action of NBS in DMSO on a triterpene acid/ester has been reported, the formation of the lactone (II) prompted us to explore if the reaction could be applied to other triterpene acids and esters as well. Methyl acetyloleanolate (Va) was taken as another model compound for this reaction when we isolated the product (VI), $C_{32}H_{49}O_4Br$, m.p. 215-16°, IR (nujol): 1770 (γ-lactone), 1725, 1240 cm^{-1} (acetyl), which was found to be identical with 3β-acetyl-12α-bromo-oleanan-28→13-olide[5] (VI) prepared by treatment of 3β-acetyloleanolic acid with bromine in acetic acid. The same bromolactone (VI) was formed when 3β-acetyloleanolic acid (Vb) was treated with N-bromosuccinimide in dimethyl sulphoxide.

Methyl 3β-acetylbetulenate[6] (VIIa) on similar reaction with NBS in DMSO afforded two different bromo compounds, separated by chromatography.



The less polar one, $C_{33}H_{51}O_4Br$, m.p. 235-36°, $[\alpha]_D$ +42.55° was identified as methyl 30-bromo-3β-acetylbetulenate (VIII); IR (nujol): 1735 ($-COOCH_3$), 1725, 1240 ($-OCOCH_3$), 1260 ($-CH_2Br$), 1660, 876 cm^{-1} ($=CH_2$); PMR: δ 0.87-1.02 (15H, 5 tert. CH_3), 2.03 (*s*, 3H, $-OCOCH_3$), 4.1 (*m*, 2H, $-CH_2Br$), 3.75 (*s*, 3H, $-COOCH_3$), 4.9 (*m*, 2H, $=CH_2$), 4.5 (*m*, 1H, $CH-O-COCH_3$); VIII on treatment with zinc and acetic acid gave methyl 3β-acetylbetulenate (VIIa).

The more polar fraction (10%) was a dibromo lactone (IX), $C_{32}H_{48}O_4Br_2$, m.p. 303-4°, $[\alpha]_D$ +47; CD (*n*-hexane): 218 nm ($\varepsilon = -0.99$); IR (nujol): 1780 (γ-lactone), 1720, 1240 ($-OCOCH_3$), 1260 cm^{-1} ($-CH_2Br$); PMR: δ 0.87-0.92 (15H, 5 tert. CH_3), 2.03 (*s*, 3H, $-OCOCH_3$), 3.44 (*m*), 3.55 (*m*); 3.73 (*d*) and 3.78 (*d*) (4H, C-29-H_2Br and C-30-H_2Br, J_{AB} = 11 Hz), 4.34 (*s*, 1H, $HC-OCO$) and 4.5 (*m*, 1H, $HCOCOCH_3$). The methylene protons present at C-29 and C-30 as CH_2Br are non-equivalent and their position is fixed due to hindered rotation of these groups containing the bulky and highly electronegative bromine atoms which are at the farthest positions. The appearance of the C-19 proton adjacent to the lactonic oxygen as a singlet in the PMR spectrum of IX suggests that this proton has either no neighbouring proton or the conformation is such as to have no coupling with it. A Dreiding model of IX showed that the dihedral angle between the C-18 and C-19 protons is almost 90° if the C-18 proton is α-oriented. The appearance of a peak in the ^{13}C NMR of IX at 178.48 ppm revealed the presence of a lactonic carbonyl group in a five-membered ring whereas the one at 171 ppm indicated the presence of an acetoxyl carbonyl group. The doublets at 80.77 and 80.95 ppm due to *C*-H groups bonded to acetoxy and lactone oxygen atoms. The dibromolactone (IX) could not be dehydrobrominated with dimethylaniline but on debromination with Raney nickel-hydrogen gave X,



$C_{32}H_{50}O_4$, m.p. > 360°, $[\alpha]_D$ + 59°, identical with 3β-acetyloleanan-28→19β-olide prepared from 3β-acetylbetulenic acid. From these observations the dibromolactone (IX) has been assigned the structure of 3β-acetyl-29, 30-dibromo-18α-oleanan-28→19β-olide (IX).

3β-Acetylbetulenic acid (VIIb) also furnished the dibromolactone (IX) on treatment with NBS in DMSO and 3β-acetyl-30-bromobetulenic acid which was converted with the methyl ester (VIII) on esterification with diazomethane.

Methyl 3β-acetyl-30-bromobetulenate (VIII) is formed by allylic bromination of the methyl ester (VIIa) which on further attack by the bromonium ion on the double bond causes ring expansion and lactonization as in other acid isomerization[7] of the double bond to furnish the lactone (IX).

Methyl 3β-acetyldihydrobetulenate on similar treatment with NBS in DMSO gave back the starting material in 100% yield, suggesting that the attack by the bromonium ion on the double bond initiates the lactonization and no free radical is involved in this reaction.

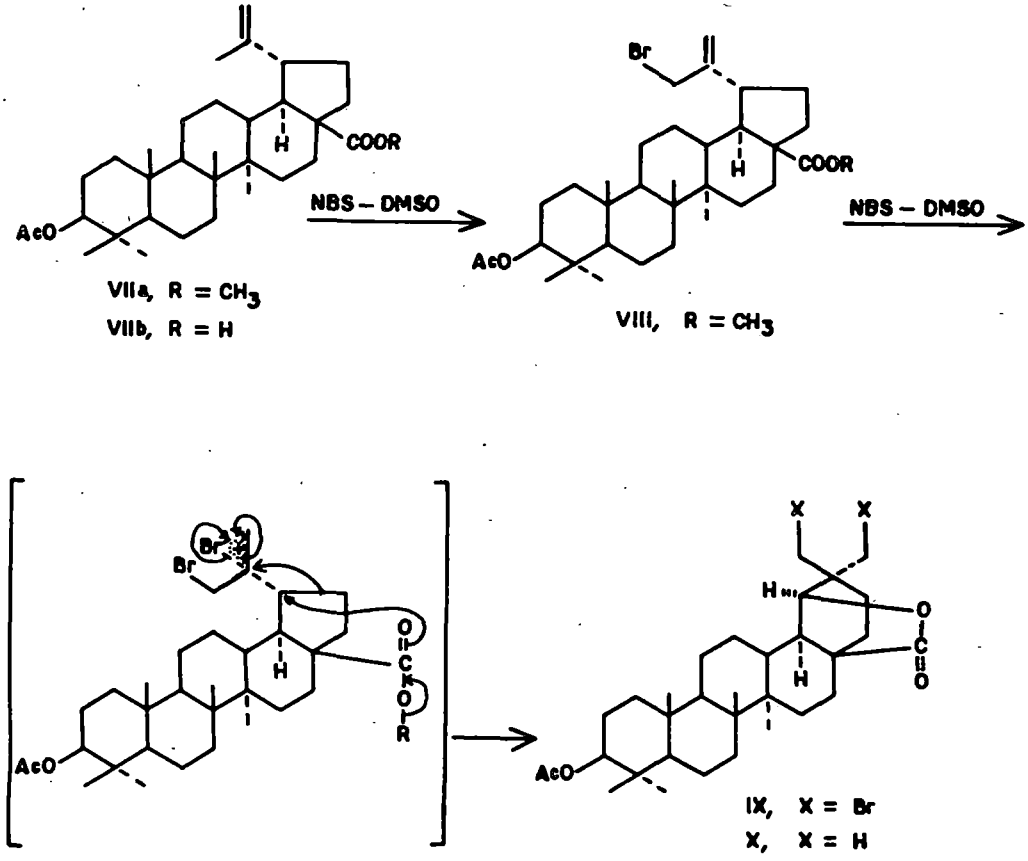
Experimental Procedure

Melting points are uncorrected. The petroleum used throughout had the b.p. 60-80°. Brockmann alumina (S. Merck) deactivated with 5% of 10% AcOH was used for column chromatography. TLC plates were coated with silica gel G (acc. to Stahl) having the thickness of about 0.2 mm and the spots located by exposing to iodine vapour. All the optical rotations were determined in chloroform solution. IR spectra were recorded as nujol mulls on a Beckman IR-20 spectrophotometer and the UV spectra in MeOH on a Beckman DU-2 spectrophotometer. NMR spectra were recorded on Varian A-60 or T-60 or EM-360 and FT-80A (^1H and ^{13}C) NMR instruments using $CDCl_3$ as solvent containing TMS as internal standard. Mass spectra were recorded by solid probe CI/CH_4 method.

Treatment of methyl 3β-acetylaleuritolate (Ia) with NBS: Formation of 3β-acetyloleanan-15α-bromo-28→13β-olide (II)—Ia (200 mg) dissolved in $CHCl_3$ (12 ml) was mixed with freshly distilled DMSO (6 ml). After cooling the reaction mixture, NBS (200 mg) was added, kept in dark for 12 hr, filtered, the filtrate extracted with $CHCl_3$, the organic layer washed with water and dried (Na_2SO_4). Chloroform was distilled off and the residue chromatographed over deactivated alumina. Elution with petroleum-benzene (3:2) furnished a solid which crystallized from $CHCl_3$ – MeOH to give II; PMR: δ 0.8 to 1.4 (7 tert. CH_3), 2.04 (*s*, 3H, O–$COCH_3$), 4.3 (*dd*, 1H, CHBr, J_{aa} = 14 Hz and J_{ae} = 3 Hz), 4.5 (*m*, 1H, H–C–O–$COCH_3$) (Found: C, 67.8; H, 8.8. $C_{32}H_{49}O_4Br$ requires C, 67.1; H, 8.6%).

Treatment of 3β-acetylaleuritolic acid (Ib) with NBS—Ib (150 mg) dissolved in $CHCl_3$ (10 ml) was mixed with DMSO (5 ml) and NBS (150 mg) and kept in dark for 24 hr. After usual work-up and chromatography, a solid was eluted by petroleum-benzene (3:2) which crystallized from $CHCl_3$ – MeOH to give II, identical (m.m.p., co-IR) with II obtained in the above experiment.

3β-Acetylolean-15,16-en-28→13β-olide (III)—II (150 mg) was refluxed for 4 hr with freshly distilled dimethylaniline (30 ml). The mixture was poured into water, acidified with 6*N* HCl and extracted with ether.



The ether layer was washed with water till neutral and dried (Na_2SO_4). The solvent was removed and the residue (130 mg) chromatographed. Elution with petroleum-benzene (3:2) furnished a solid which crystallised from $CHCl_3$–MeOH to furnish III; PMR: δ 0.8-1.3 (7 tert. CH_3), 2.04 (*s*, 3H, $-OCOCH_3$), 4.5 (*m*, 1H, $HC-OCOCH_3$), 5.5 (*AB* quartet $-\overset{|}{C}-CH=CH-\overset{|}{C}-$, J_{AB}=10 Hz) (Found: C, 77.1; H, 9.7. $C_{32}H_{48}O_4$ requires C, 77.4; H, 9.7%); Beilstein test-negative.

Hydrogenation of lactone (III): Formation of 3β-acetyloleanan-28→13β-olide (IIIa)—A mixture of III (200 mg) dissolved in ethyl acetate (50 ml), AcOH (50 ml) and Adam's catalyst (25 mg) was stirred under hydrogen atmosphere in a Pars hydrogenation apparatus (40 p.s.i). The solvent was removed under reduced pressure and the residue dissolved in benzene was passed through a column of alumina. The eluate on crystallization from $CHCl_3$–MeOH afforded IIIa, m.p. 293-94° (m.m.p. and co-IR identical with an authentic sample of 3β-acetyloleanan-28→13-olide[4]); IR: 1780 (γ-lactone), 1720, 1240 cm^{-1} (acetate) (Found: C, 77.0; H, 10.1. $C_{32}H_{50}O_4$ requires C, 77.1; H, 10.1%).

LAH reduction of III: Formation of aegiceradiol (IV)—III (50 mg) taken in dry ether (100 ml) was refluxed with LAH (100 mg) for 4 hr. The mixture was cooled, LAH decomposed with a saturated soln. of Na_2SO_4, extracted with ether, the ether extract washed with water, dried (Na_2SO_4) and the solvent removed. The residue (m.p. 225-28°) was chromatographed over alumina. Elution with benzene-ether (3:2) afforded a solid which crystallised from $CHCl_3$–MeOH to furnish aegiceradiol, m.p. 235-36° (m.m.p. and co-IR with authentic sample) (Found: C, 81.7; H, 10.9. Calc. for $C_{30}H_{48}O_2$: C, 81.6; H, 11.0%).

Treatment of methyl 3β-acetyloleanolate (Va) with NBS in DMSO: Formation of 3β-acetyloleanan-12α-bromo-28→13β-olide (VI)—Va (0.2 g) dissolved in $CHCl_3$ (10 ml) and DMSO (5 ml) was treated with NBS (0.2 g). The mixture was kept in the dark for 24 hr, washed with water, dried (Na_2SO_4) and the solvent removed. The oily residue (0.18 g) was chromatographed when a solid was eluted by petroleum-benzene (3:2). This on crystallization from $CHCl_3$–MeOH furnished VI (0.15 g), m.p. 215-16°; IR: 1770, 1180, 1160 (γ-lactone), 1725, 1240 cm^{-1} (acetate), identical (m.m.p., co-IR) with an authentic sample of 12α-bromo-3β-acetyloleanan-28→13-olide[5] (Found: C, 67.2; H, 8.6. Calc. for $C_{32}H_{49}O_4Br$: C, 67.1; H, 8.6%); Beilstein test-positive.

Treatment of 3β-acetyloleanolic acid (Vb) with NBS: Formation of bromo lactone (VI)—To a solution of Vb (0.2 g) in $CHCl_3$ (10 ml) containing DMSO (5 ml), NBS

(0.2 g) was added in one lot and the mixture kept in the dark for 24 hr. The mixture was diluted with water, extracted with $CHCl_3$, and the organic layer dried (Na_2SO_4). The solvent was distilled off and the residue again extracted with ether, stirred with aq. NaOH (5%) and separated into alkaline and neutral extracts. The alkali extract on acidification and extraction with ether did not furnish any solid residue indicating the absence of an acidic component in the reaction mixture. The neutral ethereal portion was concentrated to give a residue (0.15 g) which was homogeneous in TLC (single spot). It was crystallized from $CHCl_3$ – MeOH, m.p. 215-17° and found identical with VI obtained above.

Treatment of methyl 3β-acetylbetulenate (VIIa) with NBS: Formation of methyl 3β-acetyl-30-bromobetulenate (VIII) and 3β-acetyl-29,30-dibromooleanan-18α,28→19β-olide (IX)—To a solution of VIIa (0.4 g) in $CHCl_3$ (20 ml) and DMSO (10 ml), crystals of NBS (0.8 g) were added in lot of 100 mg each and the soln kept in dark for 24 hr. The mixture was diluted with water, washed the chloroform layer, dried and concentrated to give a residue (0.35 g) which was chromatographed. Elution with petroleum – benzene (4:1) furnished a solid which crystallized from $CHCl_3$ – MeOH to give VIII, m.p. 235-36°, $[\alpha]_D$ +42.55° (Found: C, 65.2; H, 8.6; Br, 13.5. $C_{33}H_{51}O_4Br$ requires C, 65.3; H, 8.6; Br, 13.5%). Beilstein test-positive.

Further elution of the column with petroleum-benzene (2:3) furnished a solid which crystallized from $CHCl_3$ – MeOH to give the dibromo lactone (IX), m.p. 303-4°, $[\alpha]_D$ +47.22°; CD: 218 nm, ε = 0.99; CMR: δ 178.48 (*s*, lactone $-O-C=O$), 171 (*s*, $-O-CO-CH_3$), 80.77 (*d*, $HC-O-C=O$), 80.95 (*d*, $HC-O-C=O$) and 28 other peaks between 55.60 to 13.67 ppm; MS†: 659 (M_1^+H, 10%), 657 (M_2^+H, 19%), 655 (M_3^+H, 10%), 599 ($M_1^+H-CH_3COOH$), 597 ($M_2^+H-CH_3COOH$), 595 ($M_3^+H-CH_3COOH$), 503 (599-CH_3Br† and 597-CH_3Br‡, 5%), 501 (597-CH_3Br† and 595-CH_3Br‡, 5%), 407 (503-CH_3Br† or 501-CH_3Br‡, 31%), 517 (599-HBr† and 597-HBr‡), 515 (597-HBr† and 595-HBr‡), 489 (517-CO), 487 (515-CO), 437 ($MH-Br_2$), 435 (517 – HBr† and 515 – HBr‡), 407 (503-CH_3Br† and 501-CH_3Br‡ or 487-HBr‡ and 489 – HBr†), 249, 191 (base), 189 (Found: C, 58.5; H, 7.3. $C_{32}H_{48}O_4Br$ requires C, 58.6; H, 7.4%).

Debromination of lactone (IX): Formation of 3β-acetyloleanan-18α,28→19β-olide (X)—X (0.2 g) dissolved in EtOH (50 ml) was refluxed with freshly prepared Raney Nickel (5 g) for 6 hr. The mixture was filtered and the filtrate on concentration furnished X (0.14 g), m.p. > 360°, $[\alpha]_D$ +59° identical (m.m.p. and co-IR) with an authentic sample of 3β-acetyloleanan-28 →19β-olide.

†M_1^+H refers to the protonated molecular mass with the two bromine atoms of isotopic mass 81; M_2^+H to mass with isotopic masses 79 and 81; and M_3^+H to mass with isotopic mass 79. Br† and Br‡ refer to bromine atoms of isotopic masses 81 and 79 respectively.

Acknowledgement

The authors thank Dr A Duffield, University of New South Wales, for mass spectra; Dr P M Scopes, Department of Chemistry, Westfield College, London for the CD and Dr S K Sengupta, East India Pharmaceutical Works Ltd, Calcutta for optical rotations. Two of the authors (MMM and DC) are grateful to the UGC, New Delhi for financial assistance.

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