CHEMICAL INVESTIGATION ON INDIAN MEDICINAL PLANTS AND PLANT PRODUCTS

Thesis submitted for the Degree of
Doctor of Philosophy (Science)

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Shri Samarendra Nath Bose, M. Sc.

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The present thesis submitted for the Degree of Philosophy (Science) of the University of North Bengal, Dist. Darjeeling consists of the following:

Part I:

Studies on oxidation of 3β-acetoxy methyl betulinate by mercuric acetate and establishment of the stereochemistry of the isopropenyl substituent at C-19 of the oxidation product.

Part II:

The assignment of the correct structure of the lactone, obtained by mercuric acetate oxidation of 3β-acetoxy betulinic acid and studies on the novel base-catalysed E-homorearrangement of the keto lactone derived thereof.

Part III:

Studies on the lead tetra-acetate oxidation of 3β-acetoxy betulanic acid. The conversion of the olefine mixture obtained by Pb(IV) acetate oxidation to the corresponding 13(18)-unsaturated hydrocarbon and identity of this hydrocarbon with that prepared from the Hg(ic) acetate oxidation product of 3β-acetoxy methyl betulinate (Part I).

Part IV:

Chemical investigation on the neutral part of the benzene extract of the stem-bark of Macaranga denticulata, Muell. Arg. and

isolation of a new triterpene alcohol, 3-epi-taraxerol.

Part V:

Chemical investigation on the stem bark of Bischofia javanica Blume.

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Department of Chemistry
University of North Bengal
Dist. Darjeeling

Samarendra Nath Bose

August, 1969.

I N D E X

SUMMARY

The work embodied in the present thesis has been divided into five parts:

A. The first part deals with the preparation of the hydrocarbon, A, 28-nor lup-13(18)-ene from mercuric acetate oxidation product of 3β-acetoxy methyl betulinate.

B. The second part describes the elucidation of correct structure of the lactone B obtained by mercuric acetate oxidation of 3β-acetoxy betulinic acid and also studies on the novel E-homorearrangement product D obtained from the nor keto lactone C, and elucidation of its structure D.

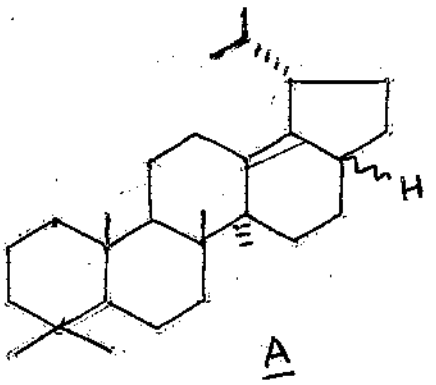
C. The third part describes the preparation of the same hydrocarbon A, 28-nor-lup-13(18)-ene by lead tetraacetate oxidation of 3β-acetoxy betulanic acid.

D. The fourth part deals with the chemical investigation on the neutral part of Macaranga denticulata, Muell Arg.

E. The last part (Part V) consists of investigations carried out on the benzene extract of the trunk bark of Bischofia javanica Blume.

A. Part I, Chapter II deals with the mercuric acetate oxidation of 3β-acetoxy methyl betulinate and conversion of the oxidation product by a series of reactions to the desired hydrocarbon A, 28-

nor-lup-13(18)-ene. Mercuric acetate oxidation of 3β-acetoxy methyl

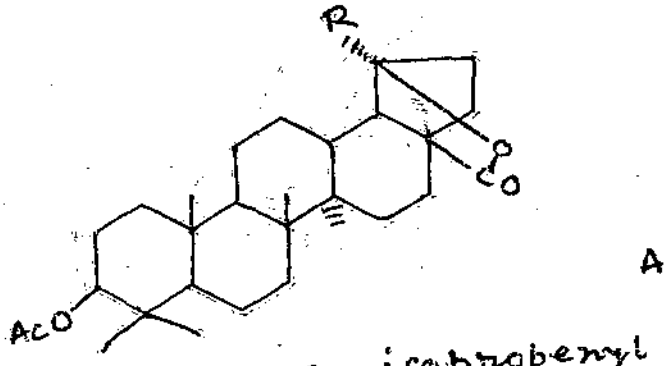


A

betulinate gave a non conjugated diene m.p. 218-19°, $(\alpha)_D$+58° which on hydrogenation afforded 3β-acetoxy lup-13(18)en-28-oate, m.p. 214-16°, $(\alpha)_D$ + 20°. The latter on hydrolysis furnished the corresponding hydroxy acid, m.p. 287-8°, $(\alpha)_D$ + 10° which on pyrolysis gave 28-nor-lup-17(18)-en-3β-ol characterised as the 3-acetate, m.p. 210-12°, $(\alpha)_D$-9°. The nor-alcohol on CrO_3-Py oxidation followed by Huang-Minlon reduction afforded the hydrocarbon 28-nor-lup-17(18)-ene which on isomerisation with 2N H_2SO_4 furnished the desired hydrocarbon A, 28-nor-lup-13(18)-ene m.p. 193-4°, $(\alpha)_D$ + 70°.

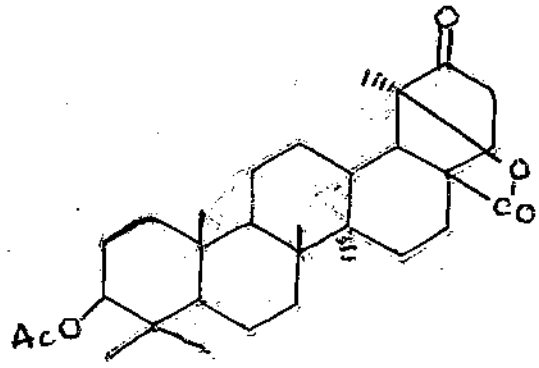
B. Part II, Chapter I deals with the assignment of the correct structure of the lactone B obtained by mercuric acetate oxidation of 3β-acetoxy betulinic acid. Physical (spectroscopic) studies particularly circular dichroism Cotton effect considerations coupled with various chemical degradations involving lead tetra-acetate cleavage of the tetra-ol derived from the lithium aluminium hydride

reduction of the nor-keto lactone C, established unequivocally the correct structure of the lactone as depicted in B. Treatment of the nor-keto lactone C by K-tertiary butoxide gave the E-homo rearrangement product for which the structure D has been proposed. This structure D is in accord with the physical evidences obtained for the compound. This rearrangement is only explicable if the original lactone has got the structure B. The mechanism for this reaction has been discussed.



B, R = isopropenyl

C, R = -CO.CH3

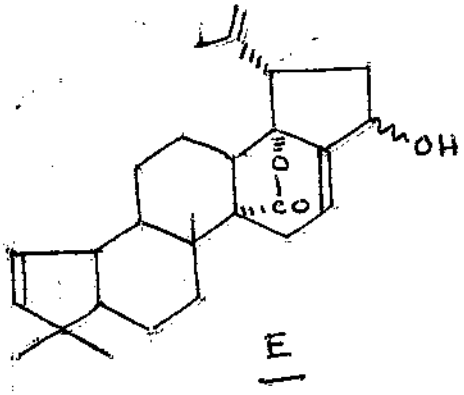


D

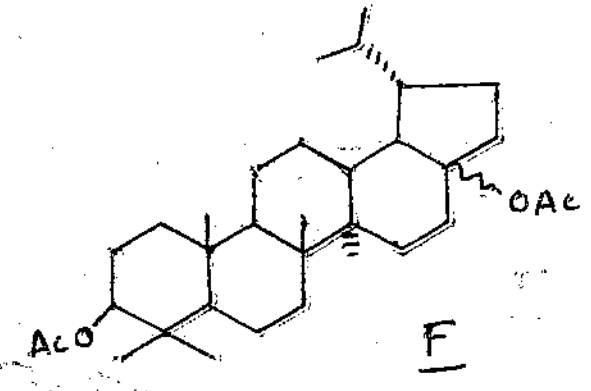
C. Part III, Chapter III deals with the lead tetra acetate oxidation of 3β-acetoxy betulanic acid whereby a mixture of hydrocarbons and a diacetate were isolated. The hydrocarbon mixture was converted by a series of reactions to the same hydrocarbon A, 28-nor-lup-13(18)-ene.

Lead tetraacetate oxidation of 3β-acetoxy betulanic acid furnished a hydrocarbon mixture m.p. 170-6°, $(\alpha)_D$ + 28.27° and a diacetate m.p. 207-9°, $(\alpha)_D$ + 23.01°. The hydrocarbon mixture on hydrolysis followed by CrO_3-Py oxidation gave a ketone m.p. 145-50°, $(\alpha)_D$ + 48.19° which on Huang Minlon reduction, gave a hydrocarbon mixture m.p. 156-8°, $(\alpha)_D$ - 7.00°. The latter was isomerised in 2N H_2SO_4 to the same hydrocarbon A, 28-nor-lup-13(18)-ene. Their identity was confirmed by m.m.p. and by comparison of IR, NMR and mass spectra. This observation unequivacally established the stereochemistry of C-19 isopropenyl substitutent in $Hg(OAc)_2$ oxidation products as α i.e. trans with respect to the C-17β-substituent as is present in the original compounds. In view of the above results emmolactone can now be fully represented by structure E.

The diacetate, m.p. 207-9°, $(\alpha)_D$ + 23.01° was shown by spectral and chemical evidences to possess the structure F.



E



F

D. Part IV, Chapter III comprises of the work on the constituents of the neutral part of Macaranga denticulata. Isolation and identification of taraxerone, a new triterpene alcohol - 3-epi-taraxerol, and β-sitosterol have been discussed.

Section C, deals with the isolation and identification of taraxerone.

Section D, deals with the elucidation of the structure of 3-epi-taraxerol. The configuration of the hydroxyl group at C-3 position was indicated by NMR. CrO_3-Py oxidation gave taraxerone. Its structure and stereochemistry was confirmed by partial synthesis from taraxerone by Paton's method as well as by Na-isoamyl alcohol reduction.

Section E, deals with the isolation and identification of β-sitosterol.

E. Part V. Chapter II deals with the investigations on the benzene extract of Bischofia javanica, Blume. Betulinic acid has been isolated and identified from acid fraction and epifriedelanol acetate, friedelin and β-sitosterol have been isolated and identified from the neutral fraction.

List of Published and Communicated Papers. :

1. StereoChemistry of the C-19 isopropenyl Substituent in the mercuric acetate oxidation product of the triterpenes of Lupane series. H. N. Khastgir and S. N. Bose, Tetrahedron letters No. 1, 39, 1968.

2. Mercuric acetate oxidation of 3 β -acetoxy methyl betulinate. S. N. Bose and H. N. Khastgir. J. Ind. Chem. Soc., XLVI, P.660 September issue, 1969.

3. Terpenoid and Related Compounds Part VI : Chemical investigation of Biscofia Javanica Blume, S. N. Bose and H. N. Khastgir; J. Ind. Chem. Soc., XLVI, 757, 1969.

4. Chemical investigation of Macaranga denticulata : H. N. Khastgir and S. N. Bose. Part III Abstracts , Proc. 56th. Ind. Sc. Congress. P. 127. 1969

5. "Chemical investigation on some plants of Euphorbiaceae family" - a short report : S. N. Bose, B. P. Prodhan, D. R. Misra and H. N. Khastgir, J. University of North Bengal. P. 20, November, 1968.

6. Revised structure of the mercuric acetate oxidation product of acetyl betulinic acid and a novel E-homorearrangement of the Ketolactone derived thereof: communicated.

PART I

Mercuric Acetate Oxidation of 3β-acetoxy methyl betulinate

Chapter I

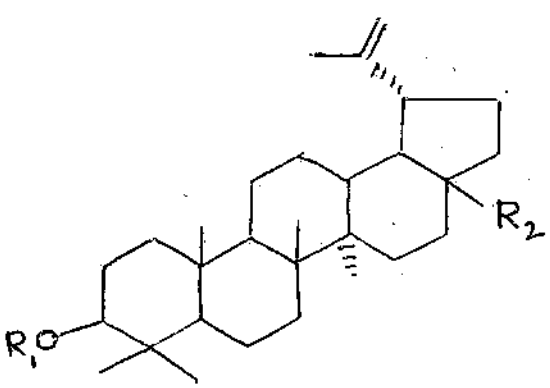
A short review on the mercuric acetate oxidation of triterpenes of lupane series

A. Dehydrogenation with mercuric acetate in the lupane series[2,4]

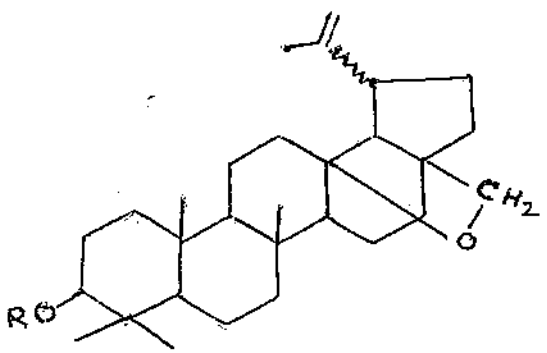
Biedebach[1] first published a paper on the Hg (II) acetate oxidation on the triterpenes of lupane series. Betulin and lupeol gave dehydro compounds of unknown structure. Since their esters also underwent dehydrogenation but not their dihydro derivatives, it was concluded that this dehydrogenation was associated with the presence of olefinic bond.

Allison and co-workers[2] carried out mercuric acetate oxidation on betulin 1a and isolated a cyclic ether assigned as 2a, IR ν max 1630, 836 cm^{-1} (vinylidene group). NMR of the corresponding acetate 2b exhibited peaks at 63 and 89 cps (-C-C$\underline{H}_2$-O). Hydrogenation of 2b followed by oxidation gave a lactone 3b, IR ν max 1770 cm^{-1} (γ-lactone). 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

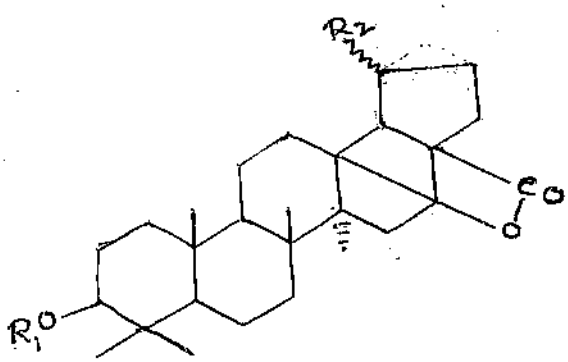
nature of the third hydroxyl group and the product obtained in this reaction was assigned structure 4e, λmax 206 mμ (ϵ 7900), IR ν max 1650 and ~~3050 and~~ 3050 cm^{-1}. The decision regarding the lactone termination was made on the basis of experiments with betulinic acid and its methyl ester. Acetyl betulinic acid 1b with Hg(ic) acetate gave a γ-lactone, assigned as 3a, IR ν max 1792 cm^{-1}, which on hydrogenation gave the same dihydrolactone 3b as that obtained from betulin. Consequently the lactone termination and the ether 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-C19 lactone structure by Djerassi et al.[3] in 1955. Non-identity of thurberogenin with this lactone led Allison and coworkers to assign structure 3a (13-18 lactone) for the lactone.

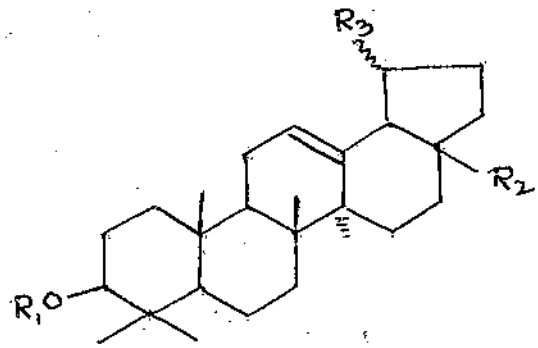


1a , $R_1 = H$, $R_2 = CH_2OH$
1b , $R_1 = Ac$, $R_2 = COOH$
1c , $R_1 = Ac$, $R_2 = COOCH_3$
1d , $R_1 = Ac$, $R_2 = CH_2OAc$



2a , R = H
2b , R = Ac





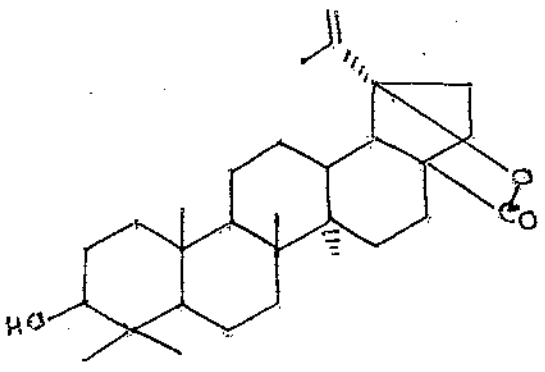
3a , R_1 = Ac , R_2 = isopropenyl
3b , R_1 = Ac , R_2 = isopropyl
3c , R_1 = H , R_2 = isopropyl

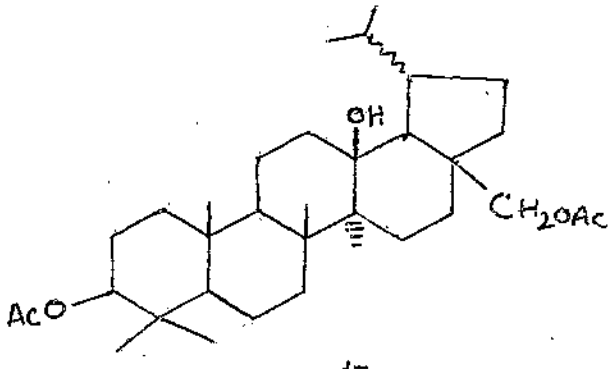
4a , R_1 = Ac , R_2 = COOMe , R_3 = isopropenyl
4b , R_1 = Ac , R_2 = COOMe , R_3 = isopropyl
4c , R_1 = H , R_2 = COOH , R_3 = isopropyl
4d , R_1 = Ac , R_2 = CH_2OAc , R_3 = isopropenyl
4e , R_1 = Ac , R_2 = CH_2OAc , R_3 = isopropyl
4f , R_1 = Ac , R_2 = CH_3 , R_3 = isopropyl

3β-Acetoxy methyl betulinate 1c on similar oxidation with Hg(ic) acetate gave an unconjugated diene, λmax 206 mμ (ε7100), IR νmax 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 mμ (ε5300), νmax 876 cm^{-1}, on hydrolysis with sodium ethoxide gave the acid 4c. The latter on treatment with hydrogen chloride in chloroform gave the lactone 3c.

Betulin diacetate 1d on Hg(ic) acetate oxidation gave a non-conjugated diene assigned as structure 4d, which was also prepared from the ester 4a and the lactone 3a. Reduction of the ester 4a with LAH gave a diol which on acetylation furnished the same

dienyl acetate 4d. Similar reduction of the lactone 3a followed by acetylation gave the acetoxy alcohol depicted as 5, which on $POCl_3$-pyridine dehydration furnished the dienyl acetate 4d. Since 4d was formed by dehydration of the tertiary hydroxyl group at C-13 and hydrogenation of the product gave 4e, which showed no I.R. absorption for vinylidene group but only exhibited U.V. absorption at λmax 206 mμ, ϵ 7900, it was concluded that the double-bond was trisubstituted and should be placed at 12-13 position.





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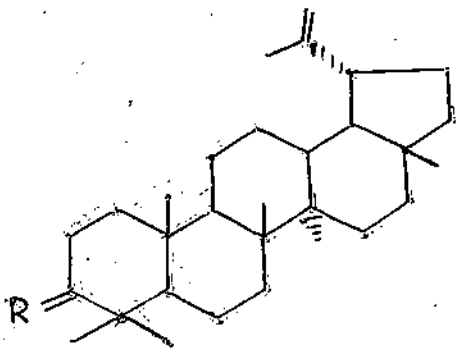
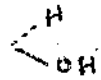
(Thunbergogenin, Djerassi et.al. 1955)

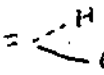
Allison et al.[4] also carried out Hg(ic) acetate oxidation on lupeol 6a, lupenyl acetate 6b, lupenyl benzoate 6c, lupenone 6d and α-lupene 6e and the corresponding dehydroproducts were assigned structures 7a, 7b, 7c, 7d and 7e respectively. Inter-conversion among these products indicated that in each case the unsaturated linkage was present at the same position. That the dehydrocompounds contained two non-conjugated double bonds was evident from U.V. absorption and from perbenzoic acid titration. The perbenzoic acid reaction products neither responded to tetra-

nitromethane test nor did they show any absorption in the U.V. region. It was also evident from the isolation of formaldehyde by Pb (iv) acetate oxidation of the pentaol derived by osmylation of 7a. The newly introduced double bond was placed at 12-13 position by analogy with their work on betulindiacetate 1d and 3β-acetoxy methyl betulinate 1c.

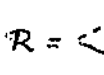


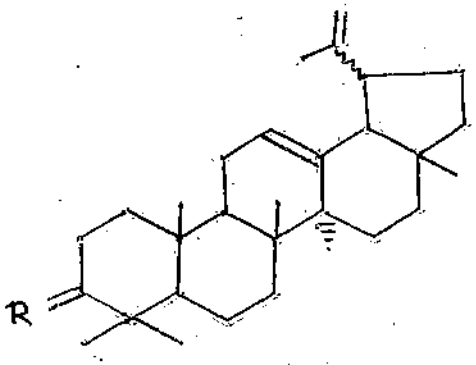
6a, R = 

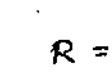
6b, R = 

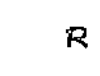
6c, R = 

6d, R = O

6e, R = 



7a, R = 

7b, R = 

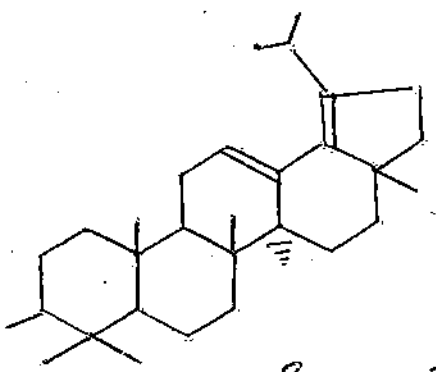
7c, R = 

7d, R = O

7e, R = 

The product 7b on treatment with HCl in acetic acid gave a compound which was assigned the cisoid heteroannular structure 8, on the basis of its U.V. absorption at λmax 234 mμ and a second compound which was assigned the transoid structure 9, λmax 244, 252, 260 mμ.

Hydrogenation of both gave the fully substituted.mono-unsaturated acetate 10. The molecular rotation differences resulting on hydrogenation of the cis-oid diene 8 (-1324°) and the transoid diene 9 (+254) were in agreement with their formulations. Hydrogenation of 7b, furnished 3β-acetoxy lup-12-ene 4f, λ max 206 mμ, which did not show any I.R. absorption in vinylidene region indicating that only the vinylidene group had been reduced.



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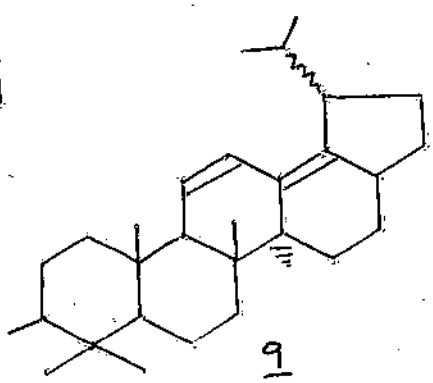

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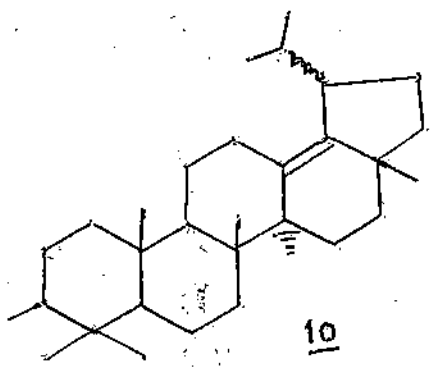
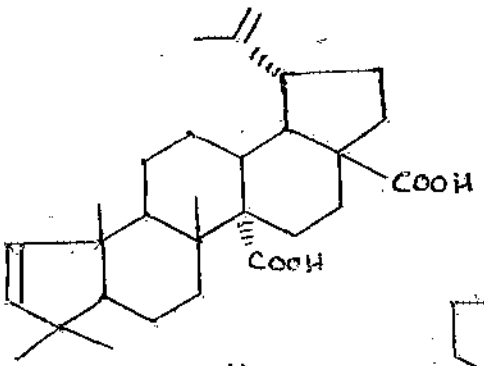


10

B. Mercuric acetate oxidation of Ceanothenic acid derivatives:

P. deMayo et al.[5] isolated from Ceanothus americans a C-29 A-nor-lupene derivative, ceanothenic acid 11, $C_{29}H_{42}O_4$, having two carboxyl groups. One of the carboxyls was located at C-17, and chemical evidences led them to two alternatives for the position of the other carboxyl group either C-8 or C-14. In order to decide between these two alternative positions they carried out Hg (ic) acetate oxidation on methyl dehydro ceanothenate 12 (R or R' = COOMe, R' or R=Me).



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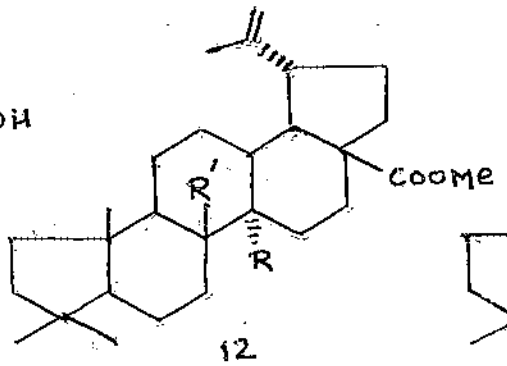

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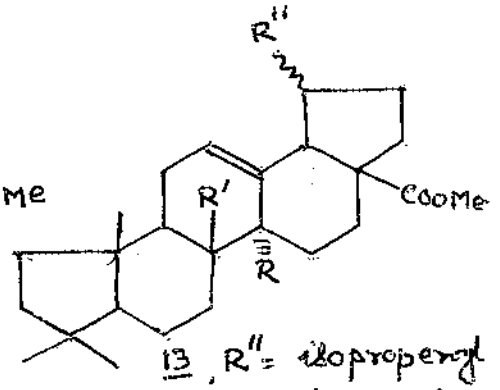
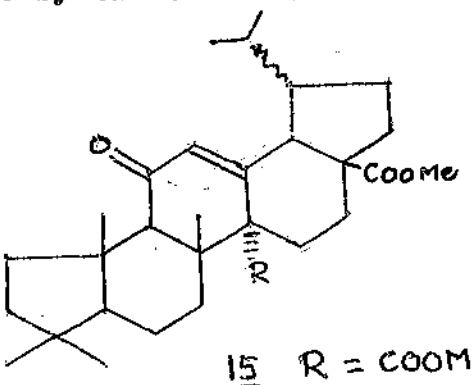


13, R'' = isopropenyl
14, R'' = isopropyl

The dehydrocompound resulting from this oxidation was assigned structure 13 (R or R' = COOMe, R' or R=Me) by analogy with Allison and coworkers' work on methyl betulinate. Hydrogenation of 13 afforded the dihydro compound 14 (R or R' = COOMe, R' or R = Me), which was converted to the corresponding acid by lithium iodide halolysis. Although this acid evolved carbondioxide at its melting point as expected for a βγ-unsaturated acid, the decarboxylated product could not be obtained crystalline. However, CrO_3-oxidation of 14 in acetic acid, afforded the conjugated ketone 15, which being a vinylogous β-ketoester was readily hydrolysed by 5% KOH solution to a non-crystalline dicarboxylic acid mono ester[6] 16. The latter underwent smooth decarboxylation at 145° to furnish the crystalline monoester assigned as structure 17. The above series of reactions could best be explained if the second carboxyl were placed at C-14. U.V. spectra of 17, showed that it still retained the αβ-unsaturated carbonyl moiety, indicating that the original double bond did not change its position.

On the basis of these chemical evidences ceanothenic acid, was formulated as 11. Although no convincing evidence was available it was thought by the authors that configuration of the isopro-



15, R = COOMe

16, R = COOH

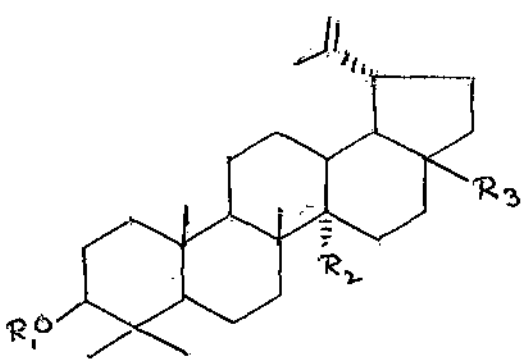
17, R = H

penyl substituent in the Hg (ic) acetate oxidation product might be β, that is, opposite to that in lupeol. The NMR spectrum showed a single broad band at τ 5.24, instead of original two multiplets present in the parent compound. In addition, they thought that inversion would seem necessary to allow approach of the reagent at C-12.

C. Mercuric acetate oxidation of Melaleucic acid derivative - methyl melaleucate

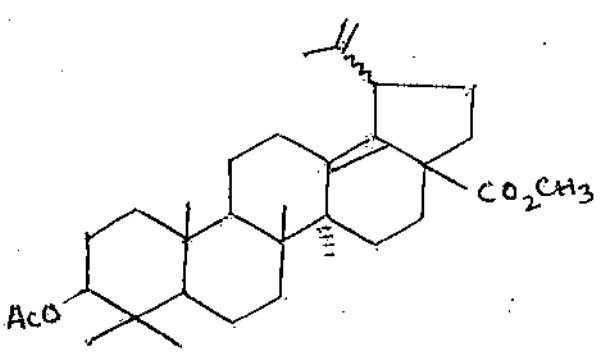
Chopra et al.[7] applied mercuric acetate oxidation in order to establish the position of the hindered carboxyl group in melaleucic acid 18a. But before that they repeated the oxidation on 3β-acetoxy methyl betulinate 1c in order to establish unequivocally the position of the newly introduced double bond. They

observed that the NMR spectrum of the oxidation product, which was formulated as 4a by Allison et al.[2], and its dehydroderivative 4b, did not show any absorption for vinyl proton whereas methyl oleanolate 19 and its acetate 20 both exhibited a multiplet[8] centred at δ 5.25. This observation coupled with the U.V. absorption[9] at 210 mμ (ε=5700), 215 mμ (ε=4500) and 220 mμ (ε=3700) indicated that the double bond was tetrasubstituted. Thus, provided no skeletal rearrangement is involved, the most likely position of the tetrasubstituted double bond would be at 13-18 position, which requires that 4a and 4b should be represented as 21a and 21b. 21b on osmylation followed by Pb (IV) acetate cleavage gave the diketoester 22a, which on hydrolysis and decarboxylation afforded the nor-ketone 22b, IR ν max 1750 (five membered ring ketone), 1708 cm^{-1} (six membered ring ketone). Both 22a and

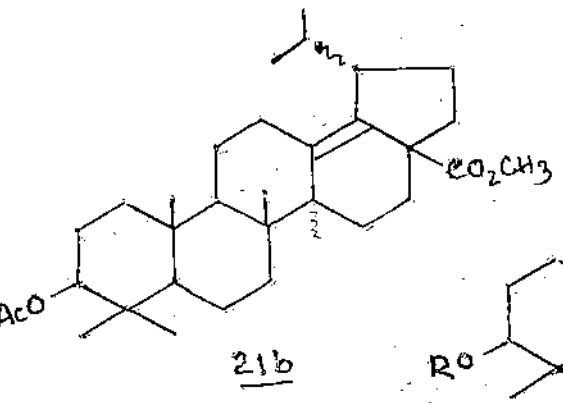


18a , $R_1 = H$, $R_2 = R_3 = COOH$

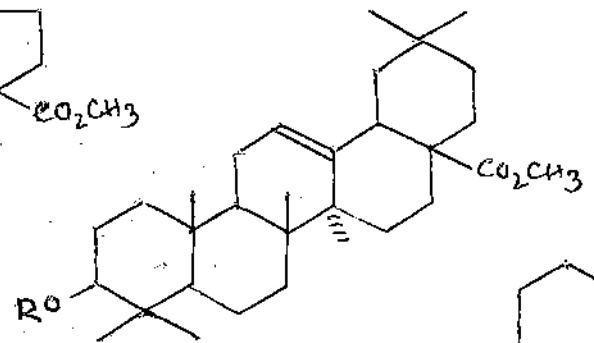
18b , $R_1 = H$ $R_2 = R_3 = COOCH_3$



21a

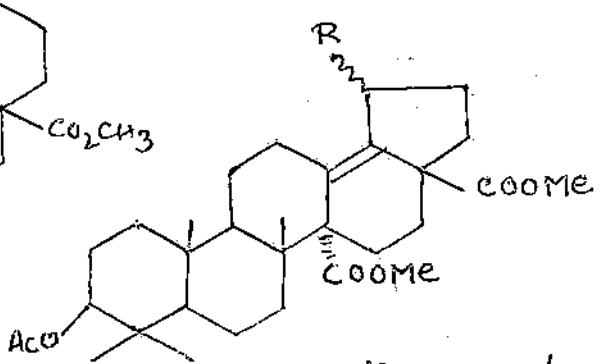


21b



19 , R = H

20 , R = Ac

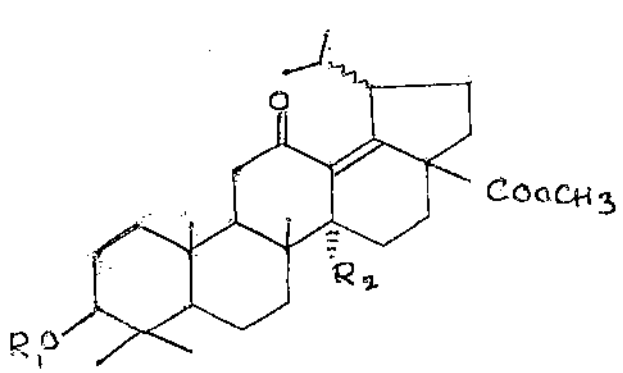


23a , R = isopropenyl

23b , R = isopropyl

22b showed absence of any absorption attributable to vinyl proton in their NMR spectra.

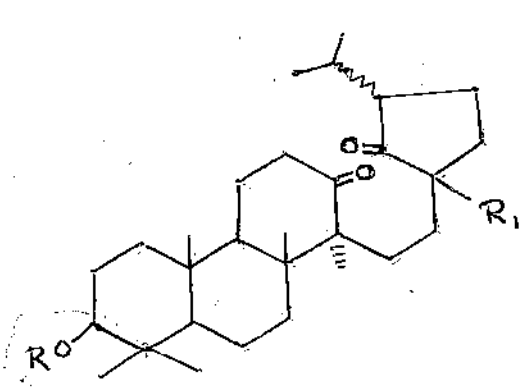
Oxidation of melaleucic acid derivative 18b with Hg (ic) acetate gave a non-conjugated diene formulated as 23a, λmax 201 mμ (ϵ 7400), NMR signal at δ 4.75 (2H, multiplet, C=C$\underline{H}_2$), which on reduction furnished 23b, having no vinylic proton absorption in NMR, but showing yellow colour with tetranitromethane.



24a , R_1 = Ac , R_2 = $COOCH_3$

24b , R_1 = H , R_2 = COOH

24c , R_1 = R_2 = H



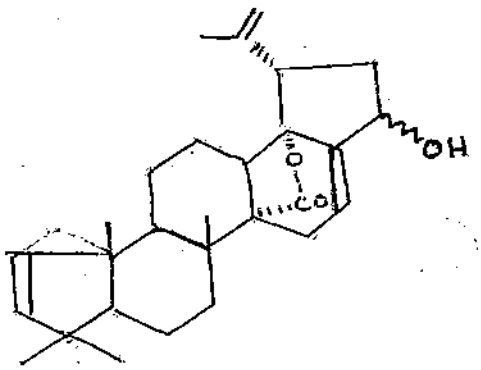
22a , R = Ac , R_1 = $COOCH_3$

22b , R = R_1 = H

23b was successfully converted by CrO_3-HOAc oxidation to the conjugated ketone 24a, IR ν max 1690 cm^{-1} (conjugated ketone), U.V. λ max 242 mμ (ϵ, 13500), NMR spectrum showed absence of any peak due to vinylic proton at C-12. These evidences definitely confirmed the structure of the conjugated ketone as 24a. The ketoester 24a on partial hydrolysis with 5% MeOH-KOH[6] gave the dicarboxylic acid monoester 24b, the methoxy carboxyl group at C-14 only being hydrolysed due to the acceleration[10] by the introduction of a conjugated carbonyl at C-12. The monoester 24b was decarboxylated to nor-keto ester 24c, thus establishing the βr-relationship of the double bond to the carbonyl group. 24c, still contained a conjugated ketone group as revealed by IR absorption at 1690 cm^{-1} and U.V. absorption at 240 mμ (ϵ, 13200). On the basis of the chemical evidences discussed above, Chopra et al.[7] established the structure of melaleucic acid as 18a.

D. Mercuric acetate oxidation of a Ceanothenic acid derivative and its correlation with a product obtained from Emmolactone

During their work on the elucidation of the structure of emmolactone 25, a bis-nor triterpene of A-nor-lupane skeleton, Eade and his co-workers[11] isolated a product 26a, by reductive hydrogenolysis of emmolactone with palladium-on-charcoal catalyst. The acid 26a, which was characterised as its methyl ester 26b, on treatment with hydrogen chloride in chloroform cyclised to a saturated r-lactone $C_{28}H_{44}O_2$, 27, IR ν max 1758 cm^{-1} and showing negative tetranitromethane test.



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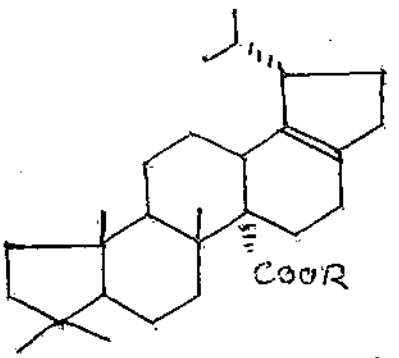
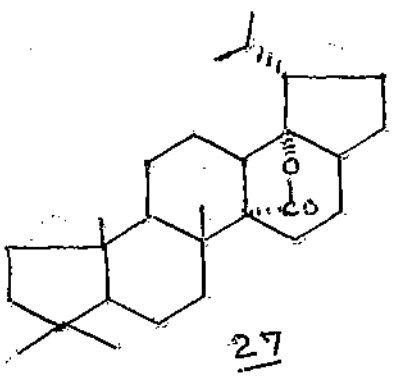


26a, R = H

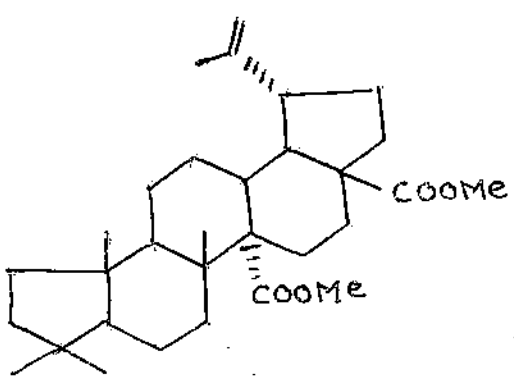
26b, R = CH_3



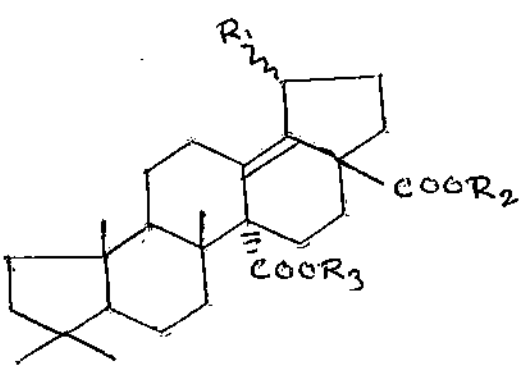
27

Mercuric acetate oxidation of dihydro dimethyl ceanothenate 28, furnished a product, which has been assigned the structure 29a. Hydrogenation of 29a furnished 29b which on halolytic cleavage with lithium iodide afforded a partially hydrolysed product, the acid 29c. The latter being a $\beta\gamma$-unsaturated acid readily underwent smooth decarboxylation[12] to give 26b. The halogenolysis of the methoxy carbonyl group at C-14 could now be effected to give 26a. This monocarboxylic acid 26a on being heated at 320-340° under nitrogen gave a mixture of products, one of which was the lactone 27 already described above. The convergent synthesis established the stereo-

chemistry of emmolactone with the exception of the orientation of the hydroxyl at C-22 and the isopropenyl side chain at C-19. P. de Mayo and Starratt[5] have suggested that the change in the NMR signal of the isopropenyl olefinic protons from two multiplets in dimethyl dihydro ceanothenate 28 to a single broad band (δ 4.76) in the dehydro derivative 29a was probably due to inversion of the isopropenyl group during mercuric acetate oxidation. But Eade et al.[11] have observed a precisely similar spectral change in the conversion of emmolactone 25 to a number of products when such inversion could not have occurred. The near equivalence of the isopropenyl group olefinic protons in all those compounds may well be due to long range shielding effects which in turn could be expected to be sensitive to changes in the geometry about the D/E ring function. They suggested, on biogenetic grounds, that the isopropenyl group in emmolactone 25 was probably in the normal α-orientation.



28



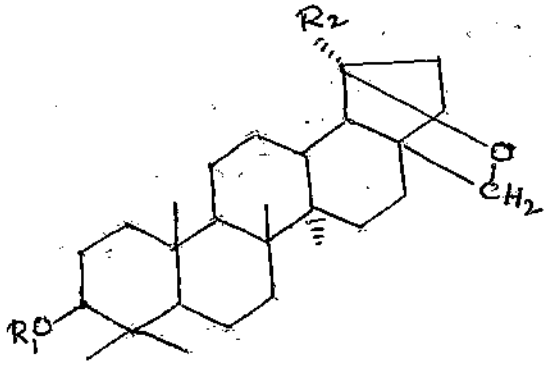
29a, R_1 = Isopropenyl, R_2=R_3=Me

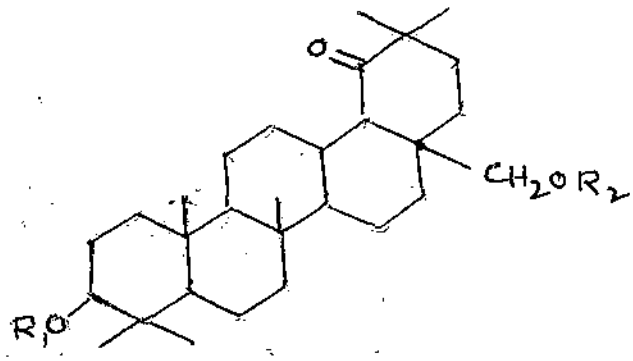
29b, R_1 = Isopropyl, R_2=R_3=Me

29c, R = Isopropyl, R_2 = H, R_3 = Me

E. A revised structure of the mercuric acetate oxidation product of betulin

Vystrčil et al.[13] very recently have shown that the primary product of oxidation of betulin 1a with Hg (ic) acetate has got the structure 30a. The conclusion is based on the following evidences. The oxidation product 30a of betulin 1a readily undergoes acid catalysed isomerisation. The corresponding acetate 30b, m.p. 242-44°C, $(\alpha)_D$ + 64.5°, ν max 1725, 1260, 1648, 905 cm^{-1}, prepared by the original method of Allison et al.[2], on treatment with 85% formic acid at elevated temperature yielded a mixture of 3β-acetoxy-28-formyloxy derivative 31b and 3β, 28-diformyloxy derivative 31c, which were converted on alkaline hydrolysis to a uniform product identified as the known 3β, 28β-dihydroxy-19-oxo-18 α-H-oleanane 31a. From the established structure of the keto





30a, R_1 = H, R_2 = isopropenyl

30b, R_1 = Ac, R_2 = isopropenyl

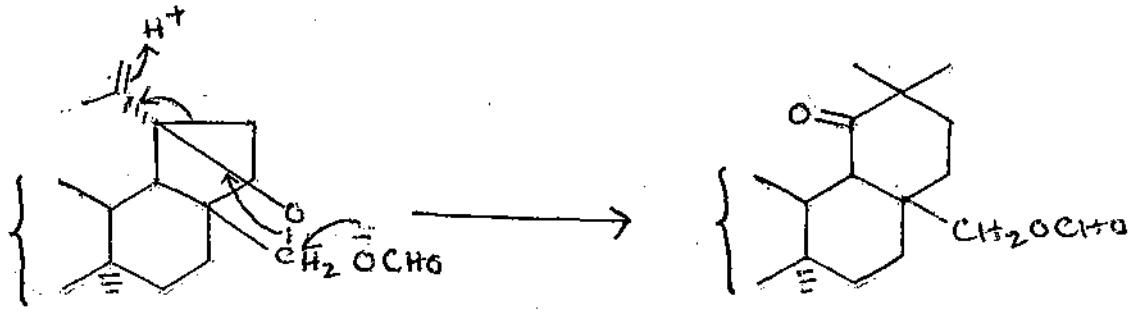
30c, R_1 = Ac, R_2 = $-COCH_3$

31a, $R_1 = R_2$ = H

31b, R_1 = Ac, R_2 = CHO

31c, $R_1 = R_2$ = CHO

diol 31a and isolation of the mixed ester 31b, the course of acid-catalysed isomerisation can be explained only by assuming the structure 30a for the Hg (ic) acetate oxidation product instead of the 13β-28 epoxy structure of Allison et al.[2] The isomerisation is initiated in a similar manner[14] as in the case of betulin but the transiently formed electron deficiency at C-19 is compensated by oxygen in the same position bound in an oxo-group which is further made possible by the simultaneous attack of the formate nucleophile at C-28. From the primary product, mixed ester 31b, the diformate 31c is formed by transesterification of the 3β-acetoxy group.



The properties of the derivatives of the oxidation product are also in agreement with the suggested structure. For example, the positive Cotton effect[15] of 3β,28-diacetoxy-30-nor-lupan-20-one 32, Φ_{308} + 1284°, Φ_{272} - 3925°, a = 52 (dioxan) is changed to a negative value in 3β-acetoxy-19β-28-epoxy-30-nor-lupan-20-one 30c, Φ_{307} - 1883°, Φ_{270} + 203°, a =-21 (dioxan), which is a familiar effect of α-substitution of methyl ketone with restricted rotation[16]. Moreover, the NMR spectrum of the diacetoxy nor-

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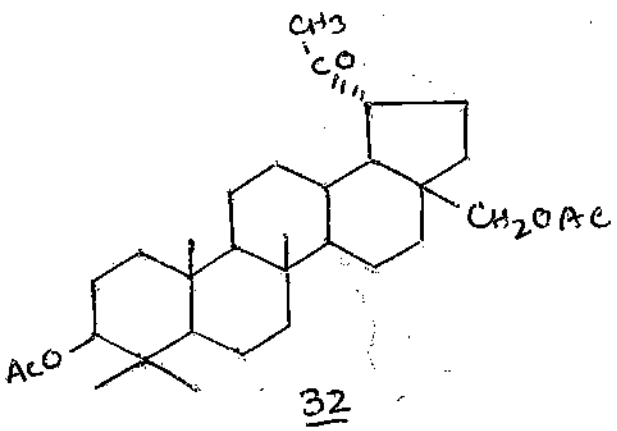
ketone 32 showed a clear signal of 19β-H at τ 7.38 (multiplet) which was completely missing in the spectrum of 30c. The latter on condensation with benzaldehyde followed by reacetylation furnished the benzal derivative 33a, $C_{38}H_{52}O_4$, m.p. 276-7°, $(\alpha)_D$ + 34°, which on sodium borohydride reduction or Meerwein Pondorf reduc-



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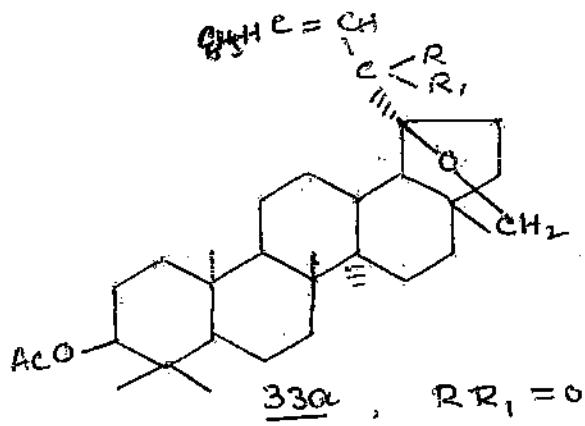


33a , $RR_1 = O$

33b , $R = H, R_1 = OH$

tion gave a mixture of isomeric alcohols 33b, $C_{38}H_{54}O_4$, λ max 252 mμ (log ε 4.3), ν max 3600, 3520, 1083 (OH). 33b could not be dehydrated to the phenylbutadiene system C_6H_5 CH=CH-CH=C as would be expected by analogy with literature reports[17], had the epoxide bridge terminated at a different position than C-19.

CHAPTER II

Mercuric acetate oxidation of 3β-acetoxy methyl betulinate

Introduction

Allison and coworkers[2] carried out mercuric acetate oxidation on 3β-acetoxy methyl betulinate 1c, and assigned structure 4a for the resulting product, for which they placed the newly introduced double bond at C-12, but did not assign the stereochemistry of the C-19 isopropenyl substituent. Later Chopra[7] et al. during their work on the elucidation of the structure of melaleucic acid 18a, reexamined the product of oxidation of 3β-acetoxy methyl betulinate. From chemical degradation as well as physical evidences they have unequivocally established its structure as 21a, where the newly introduced double bond has been placed between C-13 and C-18. But they also did not assign the stereochemistry of the C-19 isopropenyl group.

We became interested in the problem of the stereochemistry of the isopropenyl group at C-19 and designed a series of experiments, described below, which would finally settle beyond doubt the stereochemical orientation of the C-19 substituent.

Betulinic acid on esterification with diazomethane followed by acetylation with acetic anhydride - pyridine method furnished 3β-acetoxy methyl betulinate 1c, m.p. 199-200°, $(\alpha)_D$ + 14° (lit.[18] m.p. 202-3° $(\alpha)_D$ + 17.1), I.R. peaks at 1730 (broad, $COOCH_3$ and $-O.CO.CH_3$), 1243 cm^{-1} ($-O.CO.CH_3$), and 1640 and 905 cm^{-1} ($C=CH_2$, vinylidene group). The latter on oxidation with

mercuric acetate in chloroform-acetic acid solution exactly following the procedure adopted by Allison et al.[2] furnished methyl 3β-acetoxy lup-20 (30); 13(18)-diene-28-oate 21a, m.p. 218-19°, $(\alpha)_D$ + 58° (lit.[2] m.p. 217-19°, $(\alpha)_D$ + 60°); I.R. peaks at 1735 (-$COOCH_3$ and -$O.CO.CH_3$), 1240 (-$O.CO.CH_3$) and at 890, 855, 1650 cm^{-1} (vinylidene). NMR spectrum revealed a peak at δ 3.65 (singlet, 3H, $COOC\underline{H}_3$), a multiplet centred at δ 4.78 (2H, $C=C\underline{H}_2$), a broad multiplet centred at δ 4.42 (1H, $\underline{H}$-C-O-$CO.CH_3$ at C-3) but showed the absence of any peak due to vinyl group (tri-substituted double bond), U.V. spectrum did not show any absorption between 220 to 300 mμ. Hydrogenation of 21a in presence of PtO_2 catalyst in ethyl acetate-acetic acid mixture at room temperature furnished methyl 3β-acetoxy lup-13 (18)-en-28-oate 21b, m.p. 215-16°, $(\alpha)_D$ + 18°. (Lit[2] m.p. 215-17°, $(\alpha)_D$ + 19°), I.R.(Fig 1) peaks at 1735 (-$COOCH_3$ and -$OCOCH_3$) and 1240 cm^{-1} ($O.COCH_3$). Its NMR spectrum exhibited peaks at δ 3.66 (3H, $COOC\underline{H}_3$), δ 4.47 (1H, $\underline{H}$-C-$O.CO.CH_3$) and at δ 2.01 (singlet, 3H, -$O.CO.C\underline{H}_3$) but did not show any absorption attributable to any vinyl proton. It developed a strong colour with tetranitromethane indicating the presence of unsaturation. Mass spectra of the compound showed the molecular ion peak at 512 and several other peaks at m/e 453, 410, 249 and 189 consistent with the 13-18 double bond structure ~~19a~~, ~~27~~, 21b (Fig. 2, for discussion see page 31).

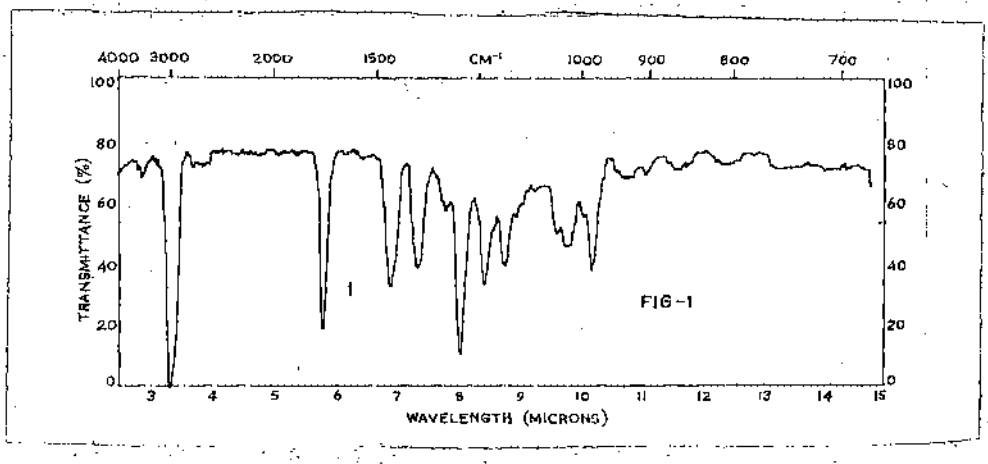

4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
TRANSMITTANCE (%)
100
80
60
40
20
0
1
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

FIG-1

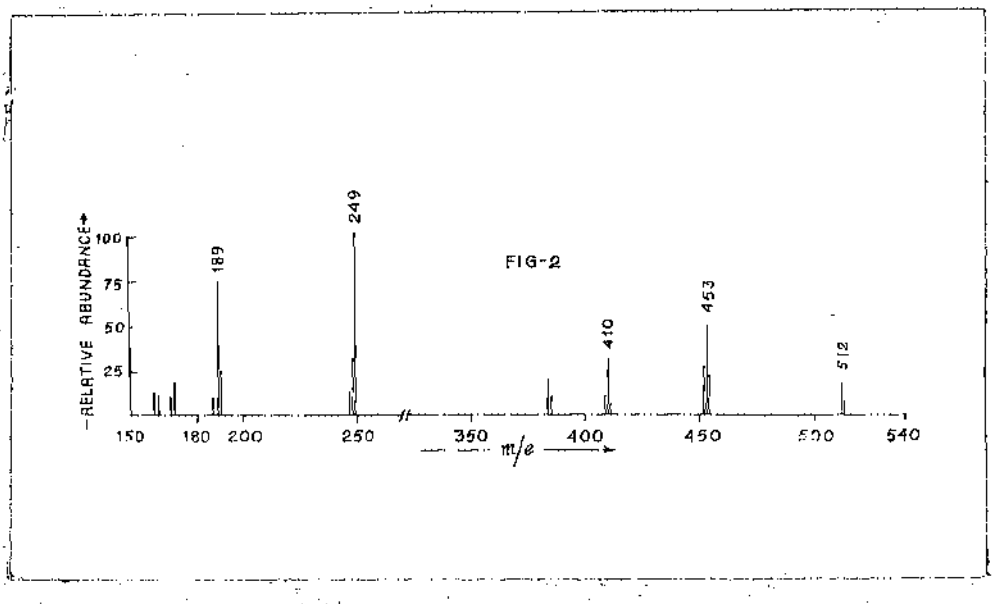
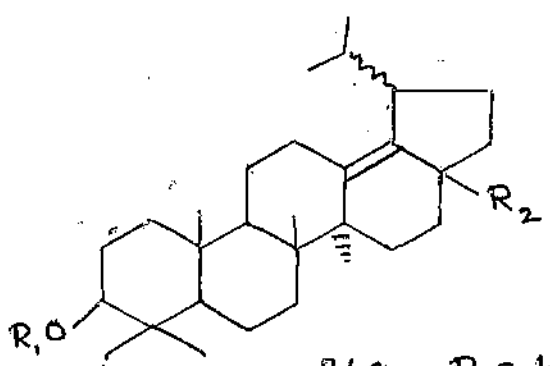

RELATIVE ABUNDANCE
100
75
50
25
189
249
410
453
512
150 180 200 250 350 400 450 500 540
m/e

FIG-2

Hydrolysis of 21b with 5% methanolic potassium hydroxide solution gave methyl 3β-hydroxy lup-13(18)-en-28-oate 34a, m.p. 198-9°, I.R. peaks at 3410 (OH), 1740 (-$COOCH_3$). The corresponding acid 34b was prepared in good yield by the method of Eschenmoser et al.[20] with lithium iodide in 2,4,6,- collidine solution. Chang et al.[21] have recently shown that potassium tertiary butoxide in dimethyl sulfoxide solution serves as a unique method for hydrolysis of hindered ester functions. Good yields have been reported by carrying out the reaction at comparatively low temperature (around 100°) for a short period (usually four hours). This method was also successfully applied on the ester 34a and the acid 34b, thus obtained in good yield, had m.p. 287-9°, $(\alpha)_D$ + 11°. It may be mentioned here that Allison et al.[2]



34a , R_1 = H , R_2 = $COOCH_3$

34b , R_1 = H , R_2 = COOH

carried out the hydrolysis of the ester 34a by heating with sodium and alcohol in a sealed tube, but the resulting acid 34b

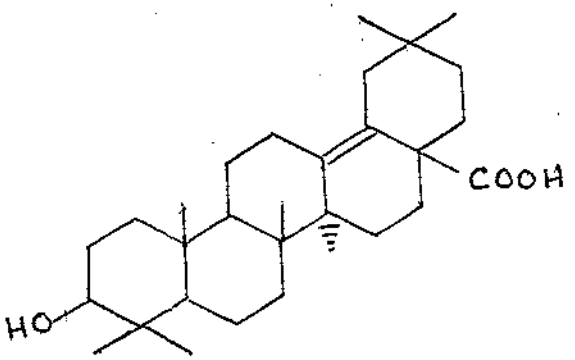
was isolated in a very poor yield. The I.R. spectrum of the hydroxy acid 34b showed absorption bands at 3390 (OH), 1700 cm^{-1} (-COOH). The latter was esterified with diazomethane followed by acetylation whereby the parent ester acetate 21b was regenerated (m.p. and m.m.p.) in good yield. This clearly demonstrated that during halolytic or dimethyl sulfoxide-tertiary butoxide cleavage of the ester no skeletal rearrangement had taken place.

Pyrolytic decarboxylation of the acid 34b

It has been observed[12,22] that β-γ unsaturated acids are generally decarboxylated with ease on pyrolysis, carbondioxide being eliminated accompanied by a shift of the double bond to the αβ-position. Although many αβ-unsaturated acids are readily decarboxylated but in the case of tertiary β-γ -unsaturated acids a shift of the double bond cannot precede decarboxylation.

A very important observation was made by Barton[23] when he found that δ -oleanolic acid 35 and morolic acid 36 underwent pyrolytic decarboxylation smoothly within a few seconds just above their melting point to give the nor-compound 37 in excellent yield, while oleanolic acid 38, a γδ -unsaturated acid was decarboxylated very slowly at a very high temperature (100° above the decomposition (melting) temperature of morolic acid), took a longer time (above half an hour) and the yield was very poor. The ease of decarboxylation of morolic acid 36, similar to δ -oleanolic acid 35, and in contrast to oleanolic acid 38, led Barton to place

the double bond in the 8Y- position with respect to the carboxyl group at C-17 in morolic acid.



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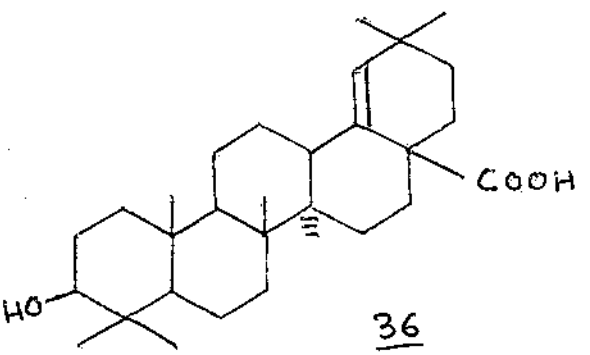

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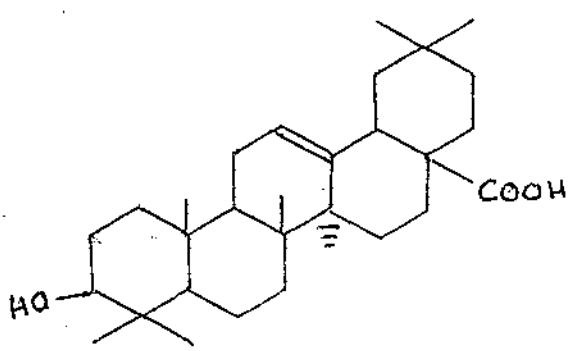

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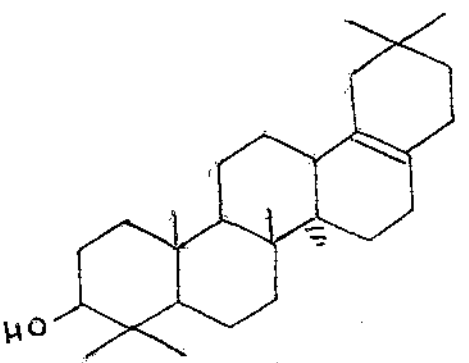
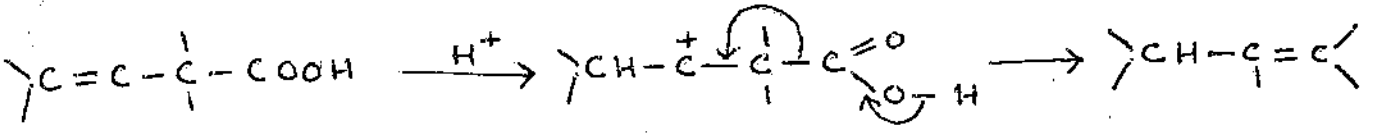


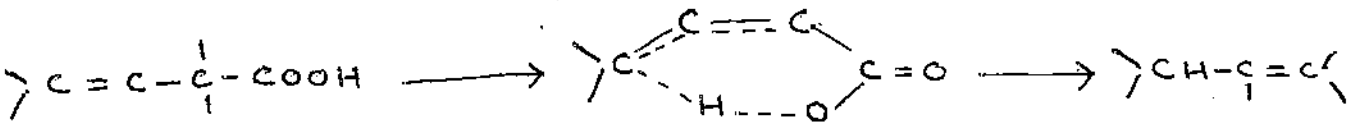
37

As regards the mechanism of the reaction there are two possibilities which can explain the shift of the double bond from

βγ- to αβ position during decarboxylation of the βγ- unsaturated acids. In the first of these: [A] a β-carbonium ion is formed and in the second [B] the transition state is of the intramolecular type without separation of charge[24]. Mechanism [A] should proceed in presence of acids while mechanism [B] should be the preferred one in the gaseous phase.

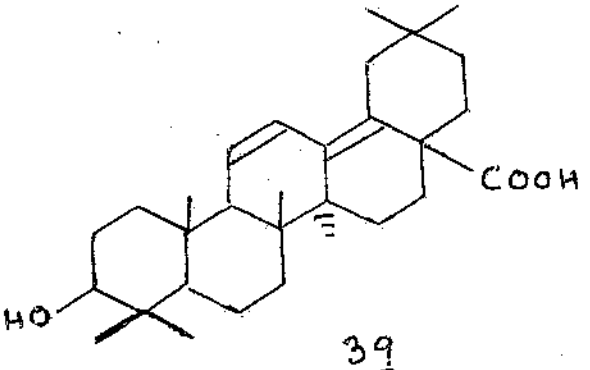


[A]

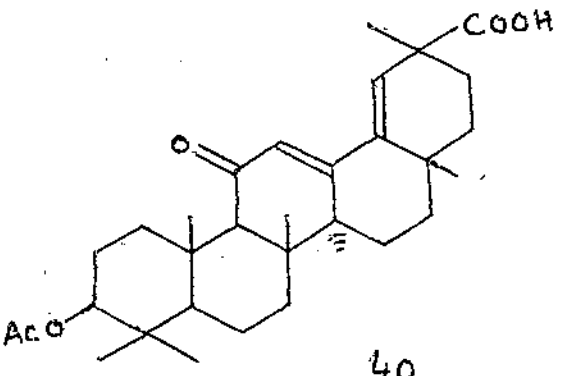


[B]

By studying the pyrolysis of βγ: δε dienoic acids, dehydro-oleanolic acid 39 and dehydroglycyrrhetic acid acetate 40, Barton established that mechanism [B] could explain the results

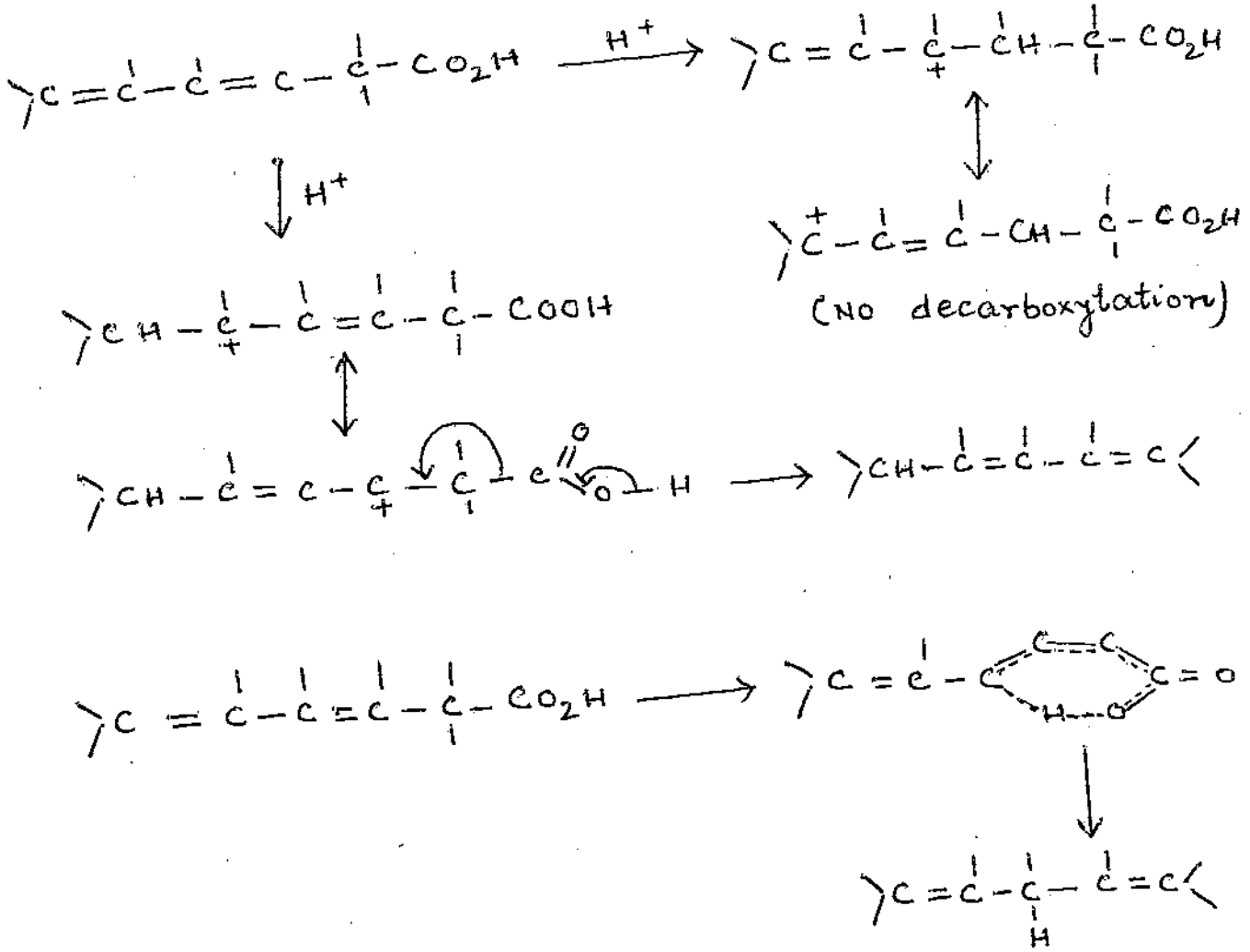


39



40

better. The product diene was not a conjugated one; on the contrary, the double bond shifted out of conjugation, which lends support in favour of 6-membered transition state mechanism as depicted in [B].



By similar pyrolytic decarboxylation of the unsaturated hydroxy acid 34b just above its melting point an excellent yield of the nor-alcohol 41a has been obtained. The facile decarboxylation at the melting point of the compound provides additional support for

the position of the double bond at βγ- position (C13-C18) with respect to the carboxyl group at C-17 in 34b.

The nor-alcohol 41a, thus obtained by pyrolytic decarboxylation was an oil and resisted all attempts at crystallisation from different solvents. It was subsequently characterised as its acetate 41b obtained by the usual acetic anhydride-pyridine method. The acetate 41b, m.p. 210-12°, $(\alpha)_D$ - 9.00°, corresponded to molecular formula $C_{31}H_{50}O_2$ (M^+ 454). Its mass spectra (Fig. 3) showed molecular ion peak at 454 and several other peaks at m/e 411, 249, 189, 204, 175, 163. I.R. spectra exhibited peaks at 1740, 1242 (-O.COCH$_3$). NMR spectrum showed signals at δ 1.98 (3H, singlet, -O.CO.CH$_3$), δ 4.4 (1H, H-C-O.COCH$_3$) and several peaks between δ 0.85 to δ 1.02 accounting for seven methyl groups. The alcohol 41a was converted to the ketone 41c by oxidation with CrO_3-pyridine complex at 15°C. The ketone had m.p. 121-23°, $(\alpha)_D$ + 31.37°, I.R. peaks at 1705 cm^{-1} (6 membered ring ketone), U-V spectrum exhibited a peak at 280 mμ (ϵ=75). The latter on Huang-Minlon reduction afforded the hydrocarbon 41d, m.p. 141-2°, $(\alpha)_D$ - 37.21°. Both its analytical data and molecular weight determination by mass spectra established its molecular formula as $C_{29}H_{48}$ (M^+ 396). NMR spectrum (Fig. 4) was devoid of any peak attributable to vinylic proton but showed signals for seven methyl group at δ 0.78 to δ 1.04. Mass spectra of the hydrocarbon (Fig. 5) exhibited peaks at 396 (M^+), 353, 204, 191 and 163, 161 (m/e)

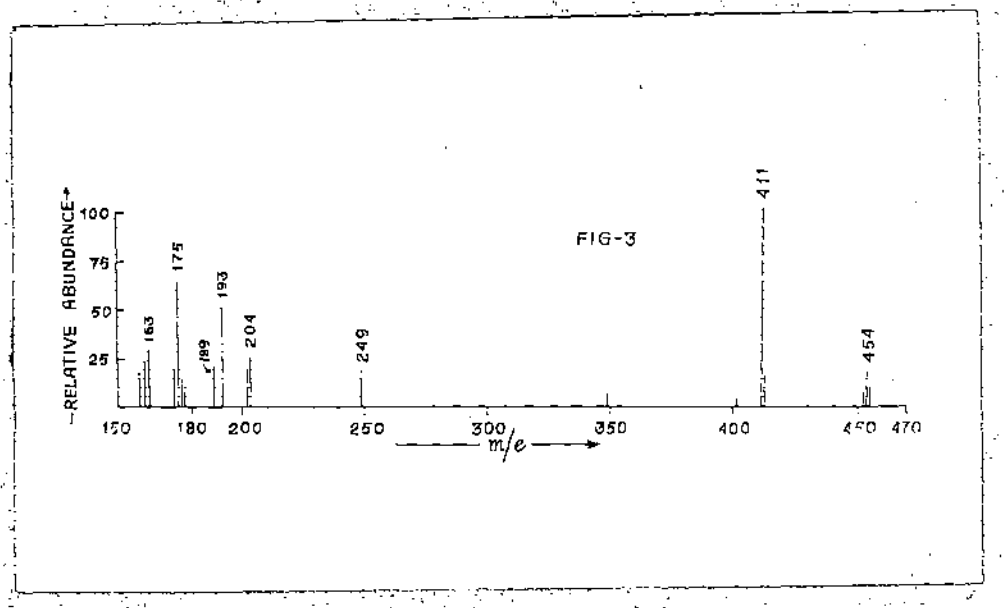


FIG-3

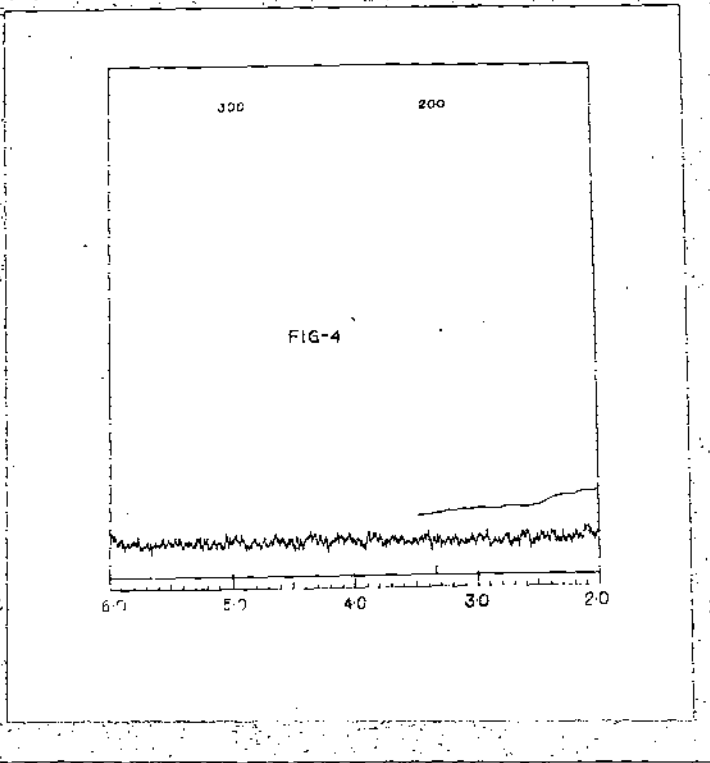


FIG-4

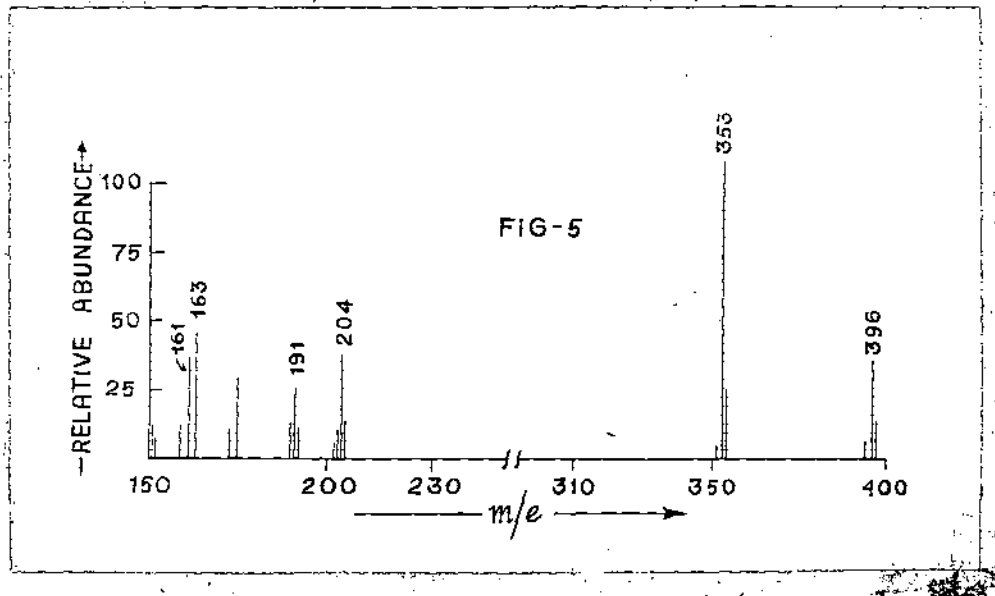
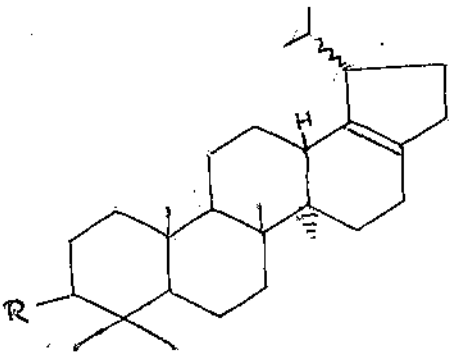
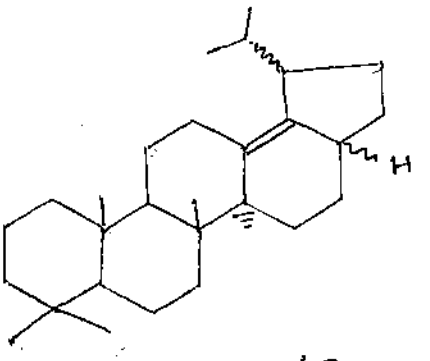


FIG-5

consistent with 17-18 double bond structure (for detailed discussion of mass spectra see page 32).



41a, R = OH
41b, R = OAc
41c, R = O
41d, R = H

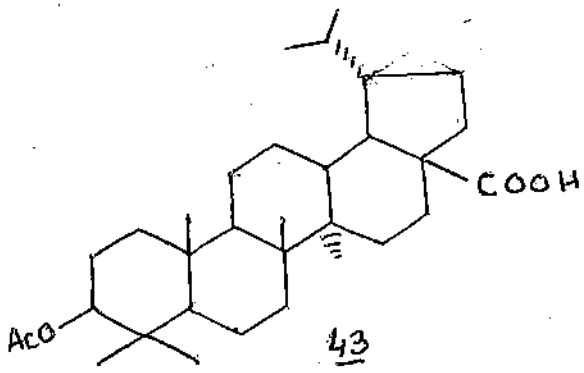


42

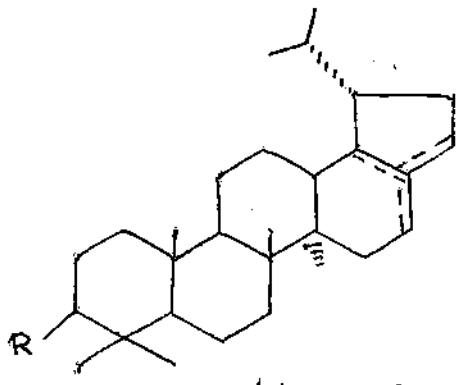
Isomerisation of the hydrocarbon 41d to the new hydrocarbon 42

The hydrocarbon 41d described above was isomerised by 2N sulfuric acid in acetic acid under the identical conditions described by Jeger et al.[25] and furnished a new hydrocarbon 42, $C_{29}H_{48}$ (M^+ 396), m.p. 193-94°, $(\alpha)_D$ + 70.00. NMR spectrum (Fig.6) of the latter did not show any peak due to vinylic proton but showed signals for seven methyl groups at δ 0.62 to δ 1.0, I.R. spectrum (Fig.8) exhibited peaks at 845, 1615-25 cm^{-1} (broad). In its mass spectrum (Fig. 7) besides the molecular ion peak at 396 it exhibited several other peaks at m/e 353, 204, 191 and 161, consistent with the 13-18 double bond structure 42 (for detailed dis-

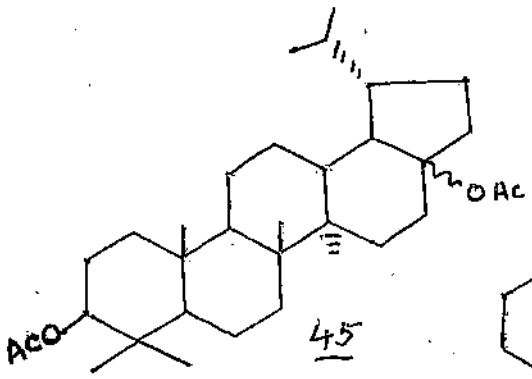
cussion see page 29). In order to establish the stereochemistry of the isopropyl group in 42, the following sequence of reactions were also carried out.



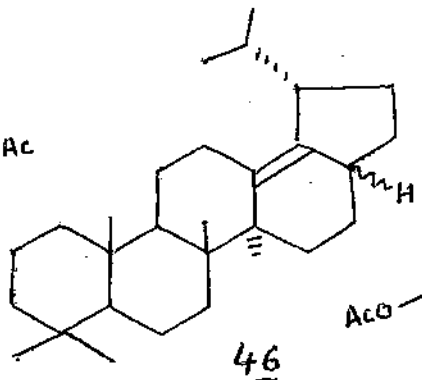
43



44a, R = OAc
44b, R = OH
44c, R = O
44d, R = H

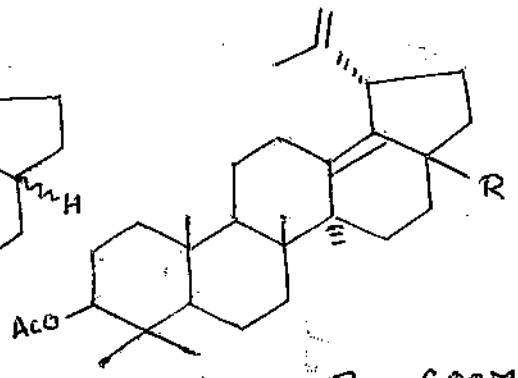


45



46

(identical with 42)



47a, R = COOMe
47b, R = CH_3
47c, R = CH_2OAc

-3β-acetoxy betulanic acid 43, m.p. 304-5° was subjected to oxidative decarboxylation by Pb (IV) acetate according to the method of Cambie et al.[26]. Two solid products were isolated after

chromatography on alumina. The compound isolated from the petroleum eluate m.p. 170-76°, has been assigned structure 44a (a mixture of olefins) on the basis of chemical and physical evidences. The second product isolated with petroleum:benzene mixture (4:1) corresponded to the molecular formula $C_{33}H_{54}O_4$ (M^+ 514) and has been assigned structure 45 on the basis of I.R., NMR and mass spectral evidences coupled with chemical evidences. (The detailed discussion on the Pb (IV) acetate oxidation of 43 has been made in Part III, ChapterIII). Hydrolysis of 44a with 5% methanolic sodium hydroxide solution for three hours gave the alcohol 44b $C_{29}H_{48}O$, m.p. 110-4°, $(\alpha)_D$ + 36.6°. The latter on CrO_3-pyridine oxidation afforded the ketone 44c, $C_{29}H_{46}O$, m.p. 145-50°, $(\alpha)_D$ + 48.19°. The ketone 44c on reduction by Huang-Minlon procedure gave the hydrocarbon mixture 44d, $C_{29}H_{48}$(M^+ 396), m.p. 156-8°, $(\alpha)_D$ -7°. The hydrocarbon mixture 44d, was isomerised by 2N sulfuric acid in acetic acid exactly following the procedure laid down by Jeger et al.[25] to yield the desired hydrocarbon 46, $C_{29}H_{48}$(M^+ 396), m.p. 192-3°, $(\alpha)_D$ + 67.2° (TLC homogeneous). The hydrocarbon (46) was found to be identical with the hydrocarbon 42 (Fig. 8) in all respects (m.p., m.m.p., I.R., NMR and mass spectra comparison). The identity of hydrocarbons also proves that the isopropyl substituent at C-19 as well as the hydrogen at C-17 ~~both~~ has identical stereochemistry. Whereas the exact stereochemistry of the C-17 H cannot be ascertained the stereochemistry at C-19 isopropyl group in 46 is obviously as depicted

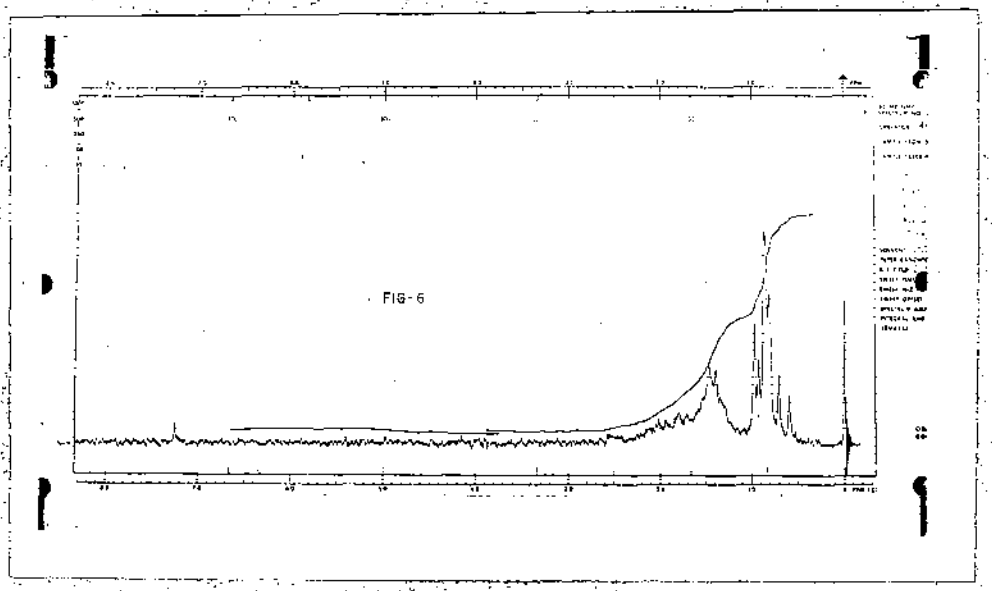


FIG-6

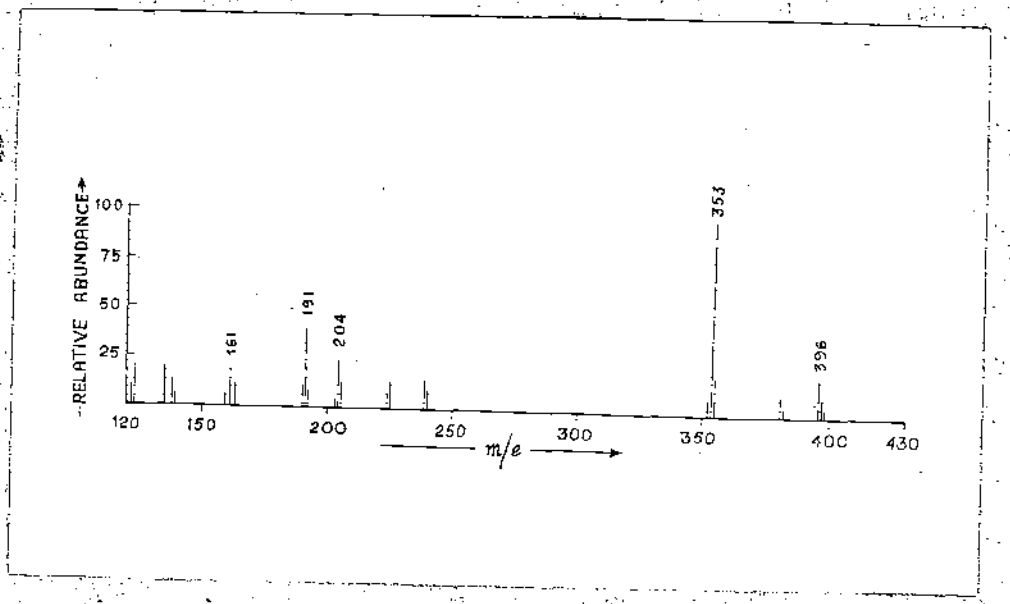
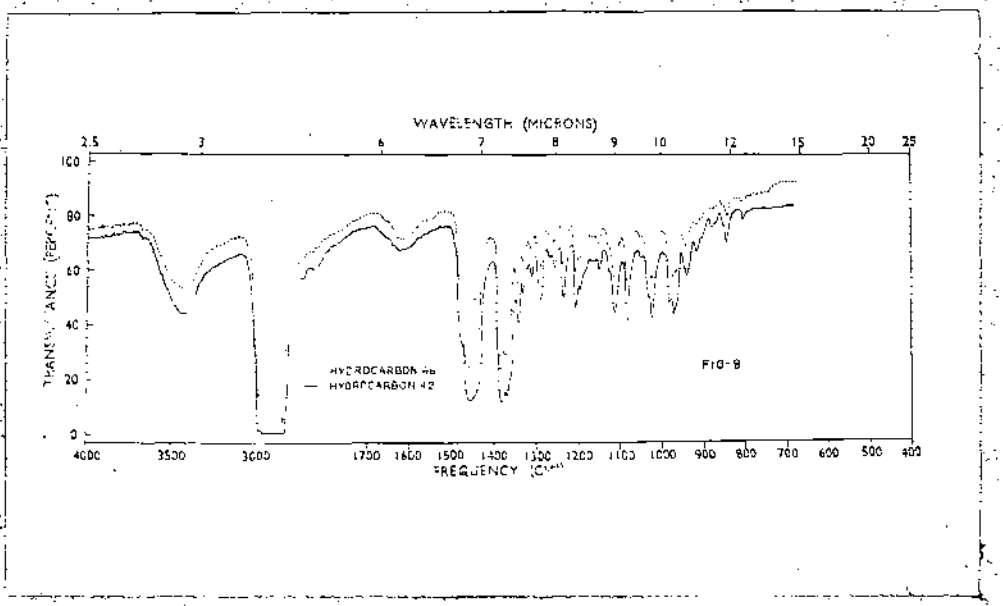
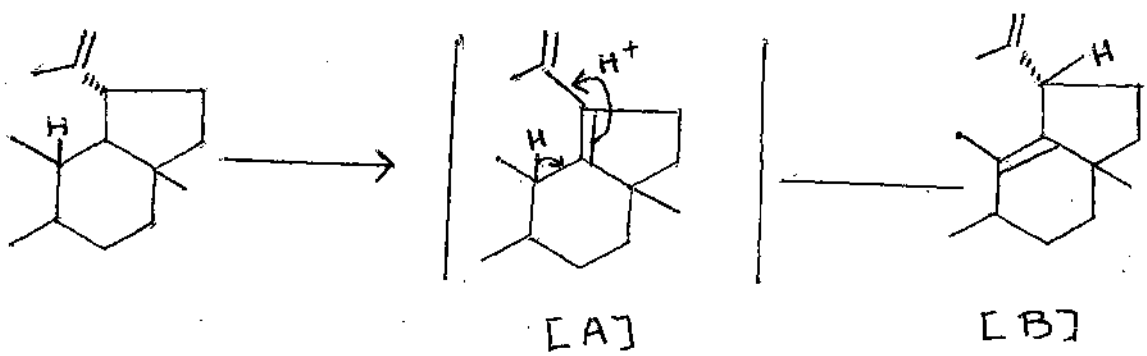

RELATIVE ABUNDANCE
100
75
50
25
120
150
200
250
300
350
400
430
m/e
161
191
204
353
396

Fig. 7


WAVELENGTH (MICRONS)
2.5
3
6
7
8
9
10
12
15
20
25
100
80
60
40
20
0
4000
3500
3000
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
FREQUENCY (CM⁻¹)
HYDROCARBON 41
HYDROCARBON 42
FIG-8

in the formula as it has been derived from 3β-acetoxy betulanic acid by reactions which precluded the possibility of epimerisation at C-19. It follows, therefore, that the sterochemistry of the isopropyl substituent in the oxidation product 21a must be trans with respect to the β-carbomethoxy substituent at C-17 and can now be fully expressed by structure 47a. In view of the above results the Hg $(OAc)_2$ oxidation product of lupeol acetate[4], m.p. 229-31°, and betulin deacetate[4], m.p. 177-78°, may be assigned structures 47b and 47c respectively.

As regards the mechanism of mercuric acetate oxidation it can be stated that the initial product of dehydrogenation is probably A, which then isomerizes to the thermodynamically more stable B, the addition of proton at C-19 taking place from β-face and thus retaining the α-orientation of the isopropenyl substituent.



[A] [B]

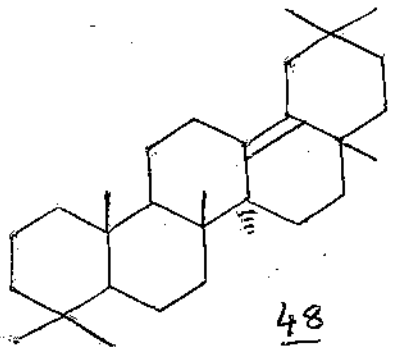
Application of mass spectrometry

Recently mass spectroscopy has been extensively applied in the elucidation of structure as well as in stereochemical problems in organic chemistry. Djerassi and coworkers[19a] measured the mass

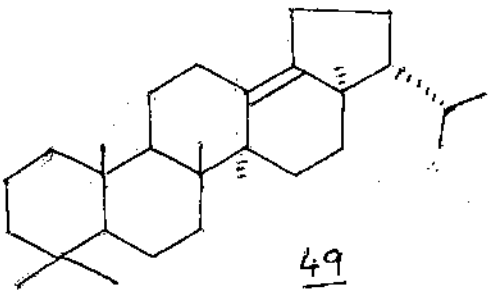
spectra of various triterpenoids and suggested mechanisms for the fragmentation patterns recorded. They also recorded the mass spectra of $\Delta^{13(18)}$-oleanene 48 and suggested the mechanism for the genesis of the various fragments formed from the molecular ion. Pandey et al.[27,28] applied mass spectroscopy in their structure elucidation of hopen-II 49 (hop-13(18)-ene) and found that the fragmentation pattern can be best explained by applying the same mechanism suggested by Djerassi et al.[19a].

Since the compound 49 is structurally very similar to 28-nor-lup-13(18)-ene 42 having 13-18 double bond and cyclopentane E ring with an isopropyl side-chain, it is expected that both the compounds would exhibit the same mass fragmentation pattern. Comparison of the mass spectra of the two compounds in fact revealed completely identical patterns, which provided additional support for the placement of the double bond at 13-18 position in 42. The molecular ion peak in the spectra of 42 (Fig. 7) appeared at M^+ 396. The successive peaks at m/e 381 (M-15) and m/e 353 (M-43) corresponded to the loss of methyl (probably allylically activated one at C-17) and isopropyl groups respectively. The cleavage of ring C and the formation of retro-Diels-Alder fragments have been found to be similar to that observed in the case of 48 and 49. The base peak 50a appeared at m/e 191 and the other important peaks appeared at m/e 204 and m/e 191. Following the mechanism of Djerassi and co-workers[19a,b] in the present case various fragments from the molecular ion of 42 would be 51a

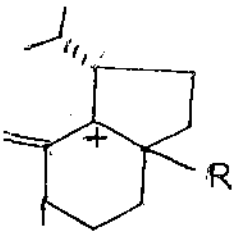
(m/e 204), 52a (m/e 191) and the fragment due to the ion 50a (m/e 191) originating from the left hand portion of the molecule (A/B ring).



48

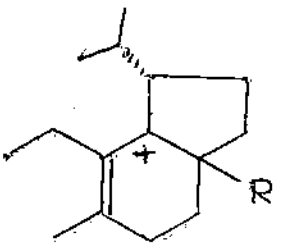


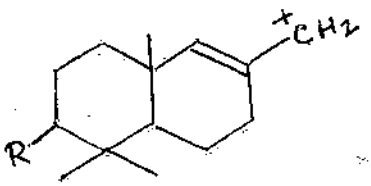
49



52a, R = H (m/e 191)

52b, R = COOMe (m/e 249)





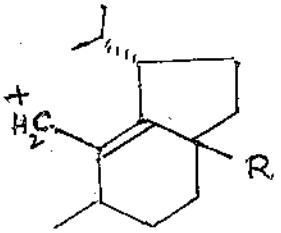
50a, R = H (m/e 191)

50b, R = OAc (m/e 249)

51a, R = H (m/e 204)

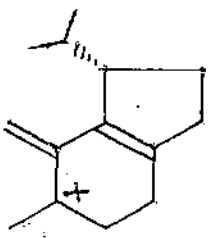
51b, R = COOMe (m/e 262)

The appearance of a peak at m/e 189 has been attributed to the species 54 formed from the ion 53a appearing at m/e 190, by the loss of the substituent at C-17. This is analogus to that



53a, R = H (m/e 190)

53b, R = COOMe (m/e 248)

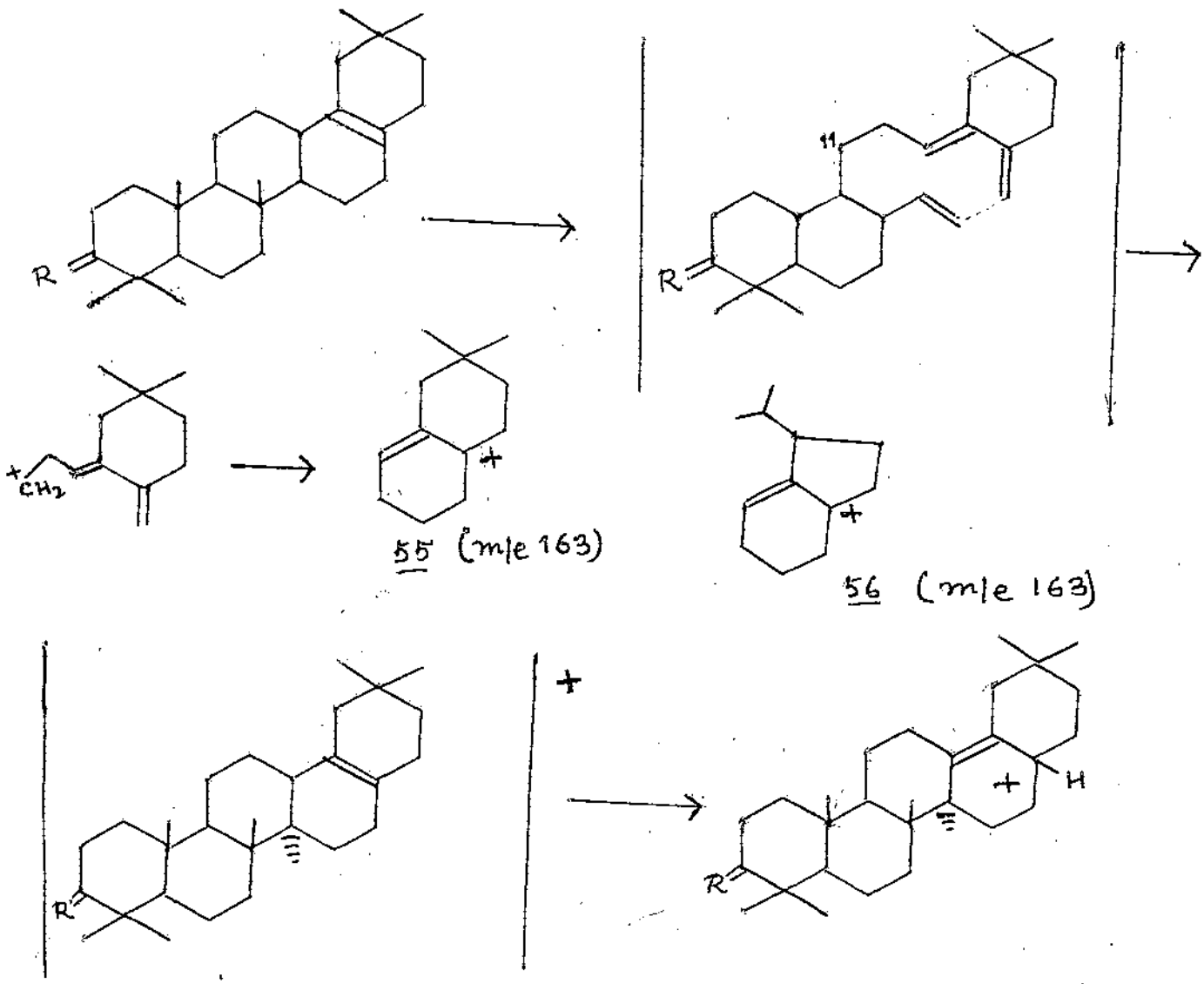


54 (m/e), 189

observed in the case of δ -amyrene[19a]. Another significant feature in the mass spectra of 42 is the formation of a fragment exhibited at m/e 161 by the extrusion of the isopropyl side chain (43 mass units) from 51a. This observation is similar to that shown by the mass spectra of hopene-II[27]. Mass spectral fragmentation pattern of 3β-acetoxy lup-13(18)-en-28-oate 21b, presented a similar spectral picture as that observed in the case of 28-nor-lup-13(18)-en 42 described above and is thus consistent with its 13-18 double bond structure. Chopra et al.[7] placed the double bond formed by mercuric acetate oxidation of 3β-acetoxy methyl betulinate at 13-18 position from spectroscopic and chemical evidences but did not adduce any mass spectral evidence. The molecular ion peak of 21b (Fig.2) appeared at M^+ 512. The successive peaks at m/e 453 and m/e 410 are due to the species ($M^+ - COOCH_3$) and ($M^+ - COOCH_3 - CH\langle^{CH_3}_{CH_3}$) respectively. The ion peak at m/e 453 formed by loss of carbomethoxyl group is very prominent, the expulsion being facilitated due to its situation at an allylically activated position. The splitting of rinc C and the retro-Diels-Alder fragmentation takes place as in δ -amyrene 48 and hopene-II 49 and gives rise to the fragments 52b (m/e 249), 51b (m/e 262) and the peak due to 50b (m/e 249), originating from the left hand portion of the molecule. The fragment 54 (m/e 189) is also formed here by the loss of the C-17 carbomethoxyl substituent from another ion 53b appearing at m/e 248.

Mass spectra of 28-nor-lup-17(18)-ene derivatives, 41b and 41d

Djerassi et al.[19a] also recorded the mass spectra of 28-nor-$^{17(18)}$-oleanene derivatives and suggested very reasonable mechanisms for the formation of the various fragments. In all these derivatives the mass spectra are characterised by the fragment ion 55 which appeared at m/e 163 arising out of the retro-Diels-Alder reaction involving the opening of ring D followed by rupture of 9-11 bond and rearrangement to ion 55. The mechanism is represented below.



55 (m/e 163)

56 (m/e 163)

The other fragments which are similar to that of $\Delta^{13(18)}$-triterpenes have been explained on the basis of bond migration from 17-18 to 13-18 position as shown below. Several cases of this type of bond migration in triterpenoids have been reported in the literature[19a,b]. Similar fragmentations for $\Delta^{17(18)}$-nor-lupene derivatives 41b and 41d have been observed. The molecular ion peaks of 41b (Fig. 3) and 41d (Fig. 5) appeared at M^+ 454 and M^+ 396 respectively. The respective fragments at m/e 411 and m/e 353 are formed by the loss of isopropyl group. The prominent fragment 56, which appeared at m/e 163 has been observed for both the compounds and can be explained very logically by Djerassi's[19a,b] mechanism involving opening of ring D, cleavage of 9-11 bond and rearrangement. Besides these, the other peaks observed for the two compounds are very similar to those recorded for lup-13(18)-ene. This is explicable in terms of bond migration to 13-18 position already referred to above in the case of 28-nor-17(18)-oleanene derivatives. The peaks due to ions 51a, 52a, 53a and 54 are observable for both the acetate 41b and the hydrocarbon 41d arising from the right hand portion of the molecule. The ions 50b for the acetate 41b and 50a for the hydrocarbon 41d arise from the left-hand portion of the molecule. Thus the 17-18 double bond structures 41b and 41d for the acetate and the hydrocarbon respectively rest on firm grounds on the basis of the mass spectral evidences.

Emmolactone a bis-nor triterpene of lupane series, has been assigned the structure 25 where Eade et al.[11], preferred the ∝-orientation of the isopropenyl substituent, on biogenetic grounds. The Australian workers[11] co-related a product of emmolactone with a product obtained by Hg $(OAc)_2$ oxidation of dimethyl dihydroceanothenate (vide supra). Since the Hg $(OAc)_2$ oxidation products retain the original ∝-orientation of the C-19 isopropenyl substituents the assignment of ∝-orientation to C-19 isopropenyl substituent in emmolactone is now fully confirmed on the basis of our present observations.

Chapter III

Experimental

Melting points are uncorrected. Petroleum used had b.p. 60-80°. All optical rotations were measured in chloroform unless otherwise stated. NMR spectra were taken in varian-60 spectrophotometer IR spectra recorded were taken in Perkin-Elmer spectrophotometer 337. UV spectra were taken in Zeiss VSU-1 spectrophotometer.

Mercuric acetate oxidation of 3β-acetoxy methyl betulinate 1c - Preparation of methyl 3β acetoxy lup-13(18); 20(10) diene-28-oate 21a

3β-acetoxy methyl betulinate (24 gr) dissolved in chloroform (1000 ml) was added to mercuric acetate (350 gr) in acetic acid (5000 ml) and the mixture was heated under reflux for 5 hrs. and then kept overnight. The white mercurous acetate was filtered off, diluted with water and extracted with chloroform. The chloroform extract was washed with water, 5% sodium hydroxide and then with water again until neutral. The orange solid obtained after evaporating chloroform was dissolved in pyridine (200 ml) and hydrogen sulphide was passed in the solution for 3 hrs. The black precipitate was removed by filtration from Kieselguhr and the solvent was removed under reduced pressure. The brownish black solid (20 gr.) was dissolved in benzene (60 ml) and was placed over a column of alumina (1 kg) deactivated with 40 ml of 10%

aqueous acetic acid. The chromatogram was developed with petroleum and was eluted with the following solvents (Table 1).

Table 1

Chromatography of the above brownish black solid (20 g).

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (1000 ml)	1-20	Yellow sulphur
Petroleum (500 ml)	21-30	Nil
Petroleum:benzene (9:1) (1000 ml)	31-50	Solid with orange colour (12 gr.) m.p. 210-5°C

Further elution with more polar solvents did not yield any crystalline material

Fractions 31-50 (Table I) were combined (12 gr), dissolved in benzene (25 ml) and was rechromatographed over a column of alumina (500 g, deactivated with 20 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum (Table II).

Table II

Chromatography of the above solid (12 gr.)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (500 ml)	1-10	Nil
Petroleum:benzene (9:1) (1000 ml)	11-30	White crystalline solid (9 gr.). m.p. 214-16°C

Further elution with more polar solvent did not afford any crystalline material.

The combined solids from fractions 11-30 (Table II) were combined and crystallised from a mixture of chloroform-methanol, when fine prismatic crystals of m.p. 218-19°, $(\alpha)_D$ + 58° (Lit. m.p. 217-219° $(\alpha)_D$ + 60°) were obtained. The melting point could not be raised by further crystallisation.

Found: C, 77.6; H, 9.8%

$C_{33}H_{50}O_4$ requires: C, 77.6; H, 9.9%

U.V. (in 95% ethanol) : 205 mμ (ϵ = 7200)

I.R. ($CHCl_3$) νmax 1735 (broad, -O-$COCH_3$, -$COOCH_3$), 1240 (-O-$COCH_3$), 890, 855, 1650 cm^{-1} (unsaturation).

NMR (60 Mc) 3.65 δ (singlet 3H, -$COOC\underline{H}_3$) 4.42 δ (1H, multiplete 1H-CO-$OCCH_3$), 2.02 δ (singlet 3H, -O-$COCH_3$), 4.78 δ (2H, multiplete

-C-=C$\underline{H}_2$). No absorption attributable to the vinylic proton at C_{12}.

Hydrogenation of 21a : Preparation of methyl 3β-acetoxy lup-13(18)-en-28-oate 21b.

Methyl 3β-acetoxy lup-13 (18); 20 (29)-dien-28-oate (8 g.) in ethyl acetate - acetic acid (2:5, 200ml) solution was reduced catalytically over PtO_2-catalyst (200 mg) for 3 hours. After separating the catalyst ethyl acetate was removed, and diluted with water. The white solid (7.5 g) thus obtained was collected by filtration. It was crystallised from chloroform-methanol mixture when crystals in the form of plates were obtained , m.p. 215-16°, $(\alpha)_D$ + 18° (Lit. m.p. 215-17°, $(\alpha)_D$ + 19°).

Found: C, 77.1; H, 10.3%

Calculated for: $C_{33}H_{52}O_4$: C, 77.3; H, 10.2%

NMR (60 Mc) 3.66 δ (singlet 3H, -COOC$\underline{H}_3$) 4.47 δ (1H, multiplet $\underline{H}$-CO-OCH$_3$), 2.01 δ (3H, singlet -O-COC$\underline{H}_3$). No absorption attributable to the vinylic proton at C 12.

I.R. (KBr) : 1735 (acetate and carbomethoxyl) 1240 (acetate) cm^{-1}

Mass : 512, 453, 410, 249 and 189 m/e

Hydrolysis of Methyl 3β-acetoxy lup-13(18)-en-28-oate (21b) and preparation of methyl 3β hydroxy lup-13(18)-en-28-oate (34a)

To a solution of 6 gr of 21b in 50 ml of benzene 200 ml of 7% methanolic potassium hydroxide solution was added and the mixture refluxed on water both for 3 hours. After the reaction it

was concentrated and then diluted with water when white solid separated out. It was filtered and crystallised from a mixture of chloroform and methanol to afford crystals m.p. 198-199°.

Found: C, 78.79; H, 10.61%

$C_{31}H_{50}O_3$ requires: C, 79.10; 10.71%

IR ($CHCl_3$) : 3410 (-OH), 1740 ($-COOCH_3$) cm^{-1}.

Halolytic cleavage of tertiary ester, methyl 3β hydroxy lup-13(18)-en-28-oate (34a) and preparation of 3β hydroxy lup-13(18)-en-28-oic acid (34b)

A solution of 3β-hydroxy lup-13(18)-en-28-oate (2 g.) in 2.4.6 collidine (30 ml) containing lithium iodide (4 g.) was heated under reflux for 4 hours. The cooled solution was poured into crushed ice, acidified with hydrochloric acid and extracted with ether. To the ether solution was added a 10% sodium hydroxide solution (60 ml) when an insoluble sodium salt of the acid was obtained. The salt was collected by filtration, suspended in water and then acidified with 10% hydrochloric acid. The latter was extracted with ether, washed with water until neutral and dried (Na_2SO_4). Removal of ether gave a white solid which was crystallised with a mixture of chloroform and methanol where by needle shaped crystals, m.p. 287-8°, $(\alpha)_D$ + 10° (lit. m.p. 287-9°, $(\alpha)_D$ + 11°) were obtained.

Found: C, 78.62; H, 10.41%

Calculated for $C_{30}H_{48}O_3$: C, 78.93; H, 10.52%

I.R. (Nujol) : 3390 (-OH), 1700 (-COOH) cm^{-1}.

Dimethyl sulfoxide-potassium tertiary butoxide hydrolysis of methyl 3β-acetoxy lup-13(18)-en-28-oate (21b): preparation of 3β-hydroxy lup-13(18)-en-28-oic acid (34b):

Potassium (10 gm) was dissolved in 100 ml of tertiary-butyl alcohol by heating in an oil bath and excess alcohol was removed under suction. To the potassium tertiary butoxide thus prepared was added dimethyl sulfoxide (250 ml) followed by the addition of 2.5 gr 3β acetoxy lup-13(18)-en-28-oate. The reaction mixture was heated at 100° for 4 hours. It was cooled and then poured into crushed ice and acidified with hydrochloric acid (10%). The latter was extracted with chloroform washed well with water until neutral and dried (Na_2SO_4). The chloroform solution was decolourised by heating with active charcoal and then filtered. After removal of chloroform the white solid obtained was crystallised from chloroform and methanol when fine niddle shaped crystals m.p. 287-8° were obtained.

The acid (34b) prepared by the above two different methods was found to be identical (m.m.p.).

Esterification of 3β hydroxy lup-13(18)-en-28-oic acid (34b): preparation of 3β hydroxy lup-13(18)-en-28-oate (34a):

To a solution of 3β-hydroxy lup-13(18)-en 28-oic acid (300 mg) dissolved in ether (30 hrs) was added an ethereal solution of diazomethane (20 ml), prepared from nitrosomethyl urea (200 mg). It was kept overnight at room temperature. Excess diazomethane was destroyed by adding 1 ml of acetic acid, washed well with 5% sodium hydroxide solution and then with water until neutral and dried (Na_2SO_4). Ether was removed when a gummy solid was obtained (250 mg).

It was dissolved in 5 ml of benzene and chromatographed on a column of alumina (20 g.) deactivated by 1 ml of 10% aqueous acetic acid (Table III).

Table III

Chromatography of above gummy solid (250 mg)

Eluent	Fractions 25 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-4	Nil
Petroleum ether:benzene (4:1) (100 ml)	5-8	Nil
Petroleum ether:benzene (3:2) (100 ml)	9-12	White solid m.p. 190-5°C

Further elution with more polar solvents did not yield any crystalline material.

Fractions (9-12) table III were combined (200 mg) and crystallised from chloroform methanol to afford plates, m.p. 198-199°. It was found to be identical with (34b) by mixed m.p.

Acetylation of 3β hydroxy lup-13(18)-en-28-oate 34a : Preparation of 3β acetoxy lup-13(18)-en-28-oate 21b

The hydroxy ester (0.1 g.) was acetylated in the usual manner with 2 ml of pyridine and 2 ml of acetic anhydride. The crude acetate thus obtained was crystallised from chloroform and methanol mixture to furnish crystals, m.p. 215-16°. This was found to be identical (m.m.p.) with the original ester acetate 21b.

Pyrolytic decarboxylation of the hydroxy acid 34b: Preparation of 28-nor-lup-17(18)-en-3 β-ol 41a

3β hydroxy lup-13(18)-en 28 oic acid (5 g.) was taken at the bottom of a pyrex test tube and was heated in a Wood's metal bath at 290-5°C for 5 minutes until evolution of carbon dioxide subsided. The brownish residue was taken up in ether, washed with sodium hydroxide solution (5%) and than with water until neutral. It was dried by anhydrous sodium sulphate. Removal of ether furnished a gummy residue (3.3 g) which was dissolved in benzene (25 ml) and subjected to chromatography in a column of alumina (200 g.) deactivated by 8 ml. of 10% aqueous acetic acid. The chromatogram was developed with petroleum (table IV).

Table IV

Chromatography of the above gum (3.3 g.)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (200 ml)	1-4	Slight oil could not be induced to solidification
Petroleum (200 ml)	5-8	Nil
Petroleum:benzene (4:1) (200 ml)	9-12	Nil
Petroleum:benzene (3:2) (300 ml)	13-18	Solid (2.7g), m.p. 112-16°

Elution with more polar solvents did not afford any crystalline solid

The solid (2.7g) from fractions 13-18 (table IV) could not be induced to crystallise well from different solvents. Shining crystalline shape, however, appeared after complete removal of the solvents.

Acetylation of the nor alcohol 41a: Preparation of 28 nor-lup-17(18)-enyl-3β acetate 41b

28 nor lup-17(18)-en-3β-ol (0.2 g) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml). The solid obtained was dissolved in benzene (5 ml) and placed on a column of alumina (15 g) deactivated by 0.6 ml of 10% aqueous acetic acid solution.

The chromatogram developed with petroleum (Table V).

Table V

Chromatography of the above solid (190 mg)

Eluent	Fractions 25 ml each	Residue left on evaporation
Petroleum	1-5	White crystalline solid m.p. 207-9°

Further elution with more polar solvents did not afford any material

The fractions 1-5 (table V) were combined (150 mg) and crystallised from a mixture of chloroform and methanol to afford fine needle shaped crystals m.p. 210-12°, $(\alpha)_D$ - 9.00°.

Found: C, 81.68; H, 10.81%

Calculated for $C_{31}H_{50}O_2$: C, 81.94; H, 11.01%

TLC developed a single spot

Mass spectra : m/e 454, 411, 249, 204, 189, 175, 163

NMR (60 Mc) : δ1.98 (3H, singlet, -0.COC$\underline{H}_3$), δ 4.4 (1H, multiplet, $\underline{H}$-C-O-CO.CH_3). δ 0.85 to δ 1.02 (7 CH_3)

I.R. (Nujol) : 1740, 1242 cm^{-1} (-OCOCH_3).

Chromic acid-pyridine oxidation of the nor alcohol 41a : Preparation of 28-nor-lup-17-(18)-en-zone, 41c

3β hydroxy 28-nor lup-17(18)-ene 41a (2 g.) was oxidised with CrO_3-Py complex prepared from pyridine (20 ml) and CrO_3 (2 g) at 15°C. Excess CrO_3 was destroyed by adding 5 ml methanol, diluted with ethyl acetate and filtered. Ethyl acetate was removed, the concentrate was taken up in ether. The ether solution was washed with 5% hydrochloric acid solution, then with water until neutral and dried (Na_2SO_4). Removal of ether gave a gummy residue (1.4 g.). It was chromatographed over a column of alumina (active, 80 g). The chromatogram was developed with petroleum and the product (1.4 g.) dissolved in benzene (10 ml) was placed on the column. It was eluted with the following solvents (Table VI).

Table VI

Eluent	Fractions 50 ml each	Residue
Petroleum (150 ml)	1-3	Nil
Petroleum:benzene (4:1) (150 ml)	4-6	Solid m.p. 120-2°

Further elution with more polar solvent did not yield any material

Fractions 4-6 (table VI) (1.0g) on recrystallisation from chloroform and methanol furnished needle shaped crystals, m.p. 121-3°, $(\alpha)_D$ + 31.37°.

Found: C, 84.51; H, 10.99%

Calculated for $C_{29}H_{46}O$: C, 84.87; H, 11.22%

UV (95% ethanol) : λ max 280 mμ (ϵ , 75)

I.R. (KBr) : max 1705 cm^{-1} (6-ring ketone)

Modified Wolff-Kishner reduction of the nor ketone, 41C : Preparation of the hydrocarbon 28-nor-lup-17(18)-ene, 41d.

3-oxo-28-nor-lup(17)-18-ene (0.8 g.) in diethylene glycol (400 ml) was refluxed with hydrazine hydrate (90%, 15 ml) for 30 minutes. After addition of KOH (1 g.) the mixture was further refluxed for one hour. The condenser was removed and the mixture heated to 125°. After refluxing for another 2½ hours the reaction mixture was cooled and diluted with water when a solid separated out. The solid (0.71 g.) was chromatographed over a column of active ~~chromatographed over a column of active~~ alumina (60 g.). The chromatogram was developed in petroleum. The solid (0.71 g) dissolved in petroleum (6 ml) was placed on the column and it was eluted with the following solvents (Table VII).

Table VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (250 ml)	1-5	Solid (0.66 g) m.p. 135-7°

Further elution with more polar solvents did not furnish any material.

Fractions 1-5 (Table VII) were combined and crystallised from chloroform and methanol to afford the hydrocarbon 28-nor-lup-17 (18)-ene 41d, m.p. 141-2°, $(\alpha)_D$ - 37.21°.

Found: C, 87.64; H, 12.05%

Calculated for $C_{29}H_{48}$: C, 87.87; H, 12.12%

Mass spectra : m/e 396, 353, 204, 191, 163, 161

UV (95% ethanol) : No absorption in the region 220-300 mμ

NMR spectra (60 Mc) : δ 0.78 to δ 1.04 (7 CH_3) no peak for vinylic proton.

Acid isomerization of 28-nor-lup-17(18)-ene, 41d: Preparation of of 28-nor-lup-13(18)-ene, 42

To 28-nor-lup-(17)-18-ene (500 mg), dissolved in acetic acid (35 ml) was added 2N sulfuric acid solution (3.5 ml) and the mixture was refluxed for 2½ hours. On cooling the reaction mixture was

diluted with water, and the precipitated solid was collected by filtration. The yellow coloured solid (400 mg) was chromatographed on a column of active alumina (30 g). The column was developed in in petroleum and the solid dissolved in petroleum (5 ml) was poured on the column (Table VIII).

Table VIII

Chromatography of above yellow solid (400 mg)

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum 150 ml	1-3	Crystalline solid m.p. 180-3°C
Further elution with more polar solvents did not afford any solid		

The fractions 1-3 (table VIII) were combined and crystallised from chloroform-methanol to furnish fine prisms of 42, m.p. 193-4° $(\alpha)_D$ + 70.°00, TLC homogeneous.

Found : C, 87.55; H, 11.95%

Calculated for $C_{29}H_{48}$: C, 87.87, H, 12.12%

I.R. (KBr palets) : 845, 1625-15 cm^{-1} (broad)

NMR (60 Me) : δ0.62 to δ1.0 (21H, 7 methyl groups). No peak corresponding to vinylic proton

Mass spectra : m/e 396, 353, 204, 191, 161.

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PART II

Mercuric acetate oxidation of acetyl betulinic acid

Introduction

Allison and coworkers[1] carried out mercuric acetate oxidation of acetyl betulic acid 1 and obtained a γ-lactone, m.p. 315-17°, $(\alpha)_D$ + 60°, to which they assigned structure 2, but did not assign the stereochemistry of the isopropenyl substituent at C-19. Our chief objectives in investigating the lactone 2 has been to gain insight (i) into its chemistry and stereochemical behaviour and (ii) the stereochemical disposition of the isopropenyl group at C-19.

Section A

Towards this end, we prepared the lactone 2, by mercuric acetate oxidation of acetyl betulic acid 1 in chloroform and acetic acid solution, essentially following the method of Allison et al.[1] The lactone 2, m.p. 302°, $(\alpha)_D$ + 58°, showed I.R. peaks (Fig. 9) at 1780 (γ-lactone), 1730 and 1245 (acetate) and 1640 and 890 cm^{-1} (vinylidene group). Its NMR spectrum (Fig. 10), besides peaks for five methyl groups at saturated carbons at δ 0.96, δ 0.89 and δ 0.81, exhibited peaks at δ 1.98 (3H, -O.CO.CH_3), δ 1.65 (3H, C=C(CH$_3$)), δ 4.95, δ 5.35 (2H, vinylidene = CH_2) and at δ 4.4 (1H, H-C-O.CO.CH_3).

To provide reasonable evidences regarding the sterochemical assignment of the isopropenyl substituent at C-19, we thought it desirable to examine the Dreiding model of the lactone and found that if the C-18H were α-oriented the lactone could be constructed

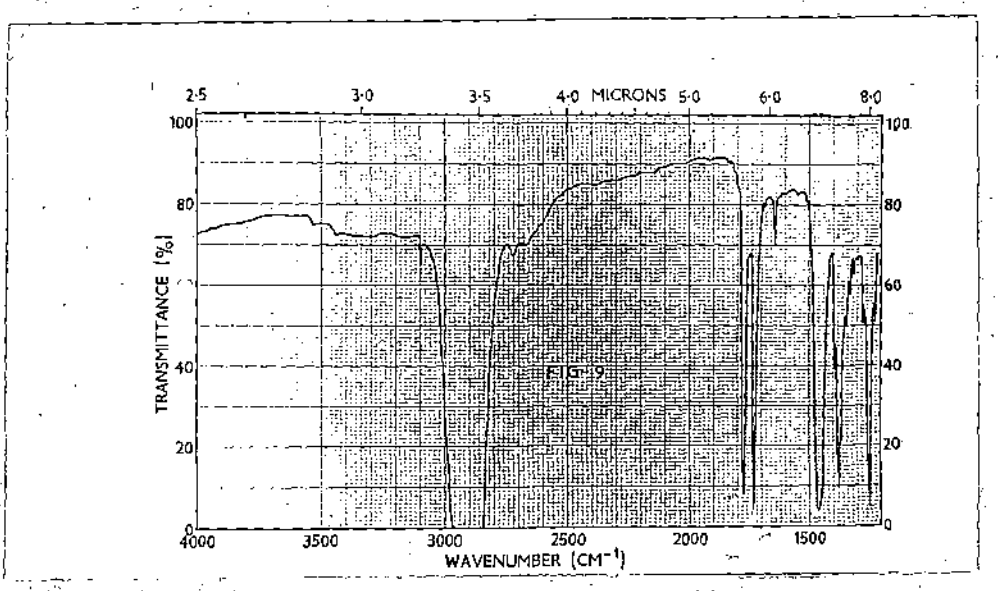


FIG-9

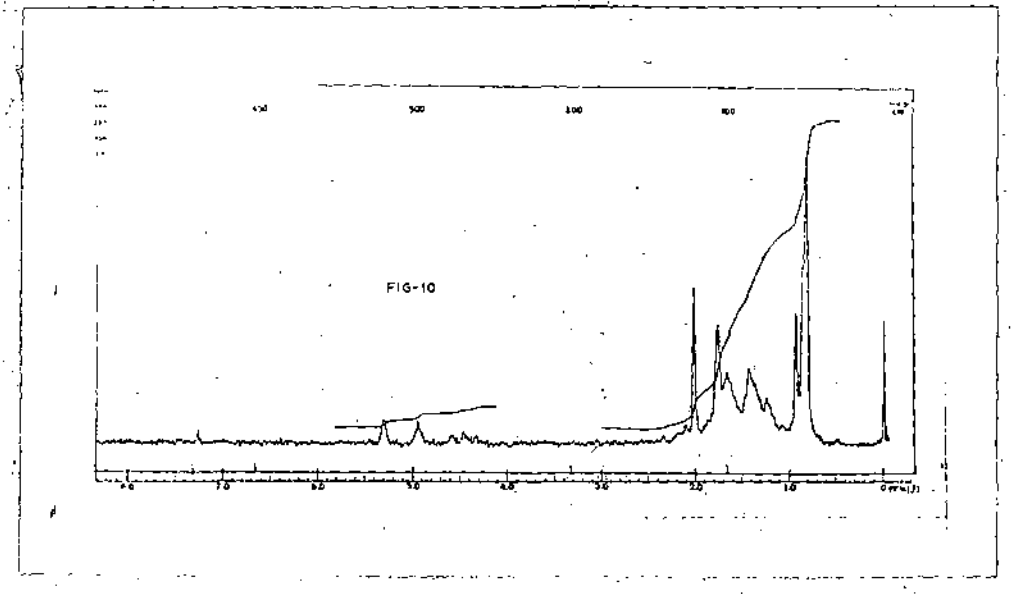


FIG-10

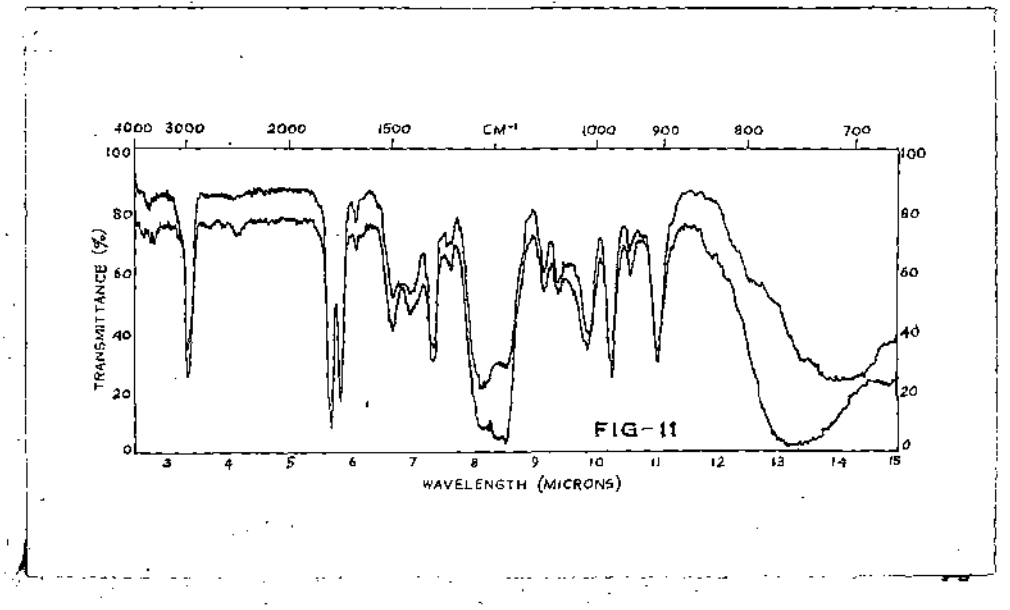
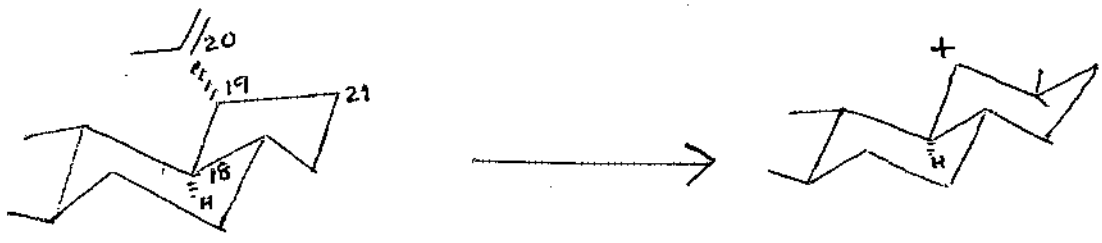
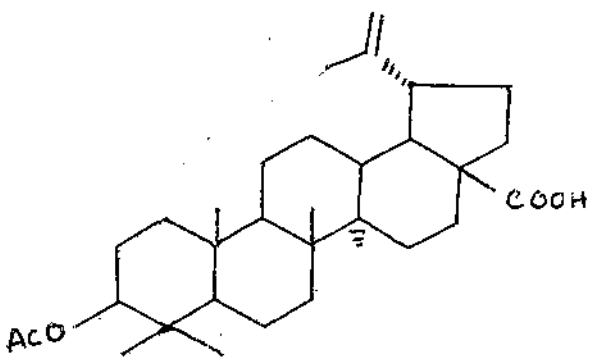


FIG-11

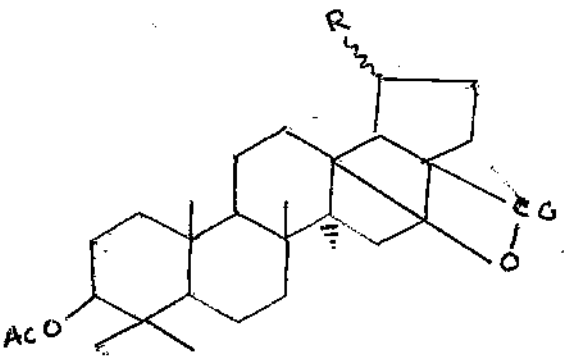
easily. And if the C-18H retained its original ∝-orientation and the C-19 isopropenyl group were <u>trans</u> (∝-oriented) to the C-17 group, as in lupeol - betulinic acid series, it should be amenable to acid induced isomerisation[2] as observed in the lupeol series, with expansion to six-membered ring E with chair conformation as shown below. The expansion results from the co-planarity of the



carbon atoms at C-19, C-20 and C-21. But if the orientation were <u>cis</u> no skeletal rearrangement would occur as the carbon atoms at C-19, C-20 and C-21 would no longer be co-planar. Accordingly, the lactone <u>2</u> was exposed to the action of (i) HCl-$CHCl_3$[3,4] (ii) 98% formic acid[5,6] (iii) HCl-acetic acid and in each case the starting material was recovered in good yield (mixed m.p. and I.R. comparison) (Figs. 11 and 12) and no isomerisation product could be detected. These experiments suggested that the skeleton of the lactone and the stereochemistry of the substituents in the compound is preserved unchanged.



1



2 , R = isopropenyl
3 , R = $-CO.CH_3$
4 , R = isopropyl

The lactone 2 was next ozonised at 0°C in chloroform solution and the ozonide on decomposition under neutral conditions furnished the nor-ketolactone 3, m.p. 301-3°, ~~m.p. 301-3°~~, $(\alpha)_D$ - 9° (lit.[1] m.p. 317°, $(\alpha)_D$ - 2°). Elemental analysis and molecular weight determination by mass spectrometry established its molecular formula as $C_{31}H_{46}O_5$. The mass spectra (Fig. 13) of the keto-lactone 3, in addition to the molecular ion peak at 498, showed peaks at m/e 438 (M^+ - ACOH) and m/e 395 (M^+ - CH_3COOH - $COCH_3$), and a peak at m/e 249 resulting from the C-ring cleavage as observed in other saturated 3-acetoxy triterpenes[7]. It showed an U.V. absorption at 275 mμ (log ε 1.4) attributable to the carbonyl group. I.R. spectra (FIG.14) of the compound showed peaks at 1721 (composite for acetate and carbonyl), 1788 (γ-lactone) and 1255 cm^{-1} (acetate). The O.R.D. curve (Fig. 15) of the compound showed a negative Cotton effect with

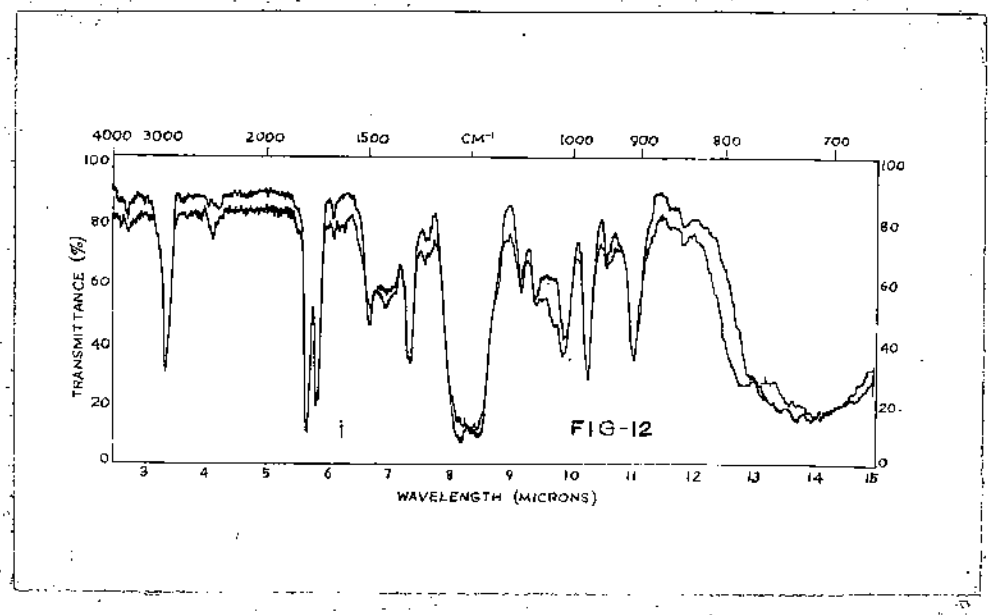


FIG-12

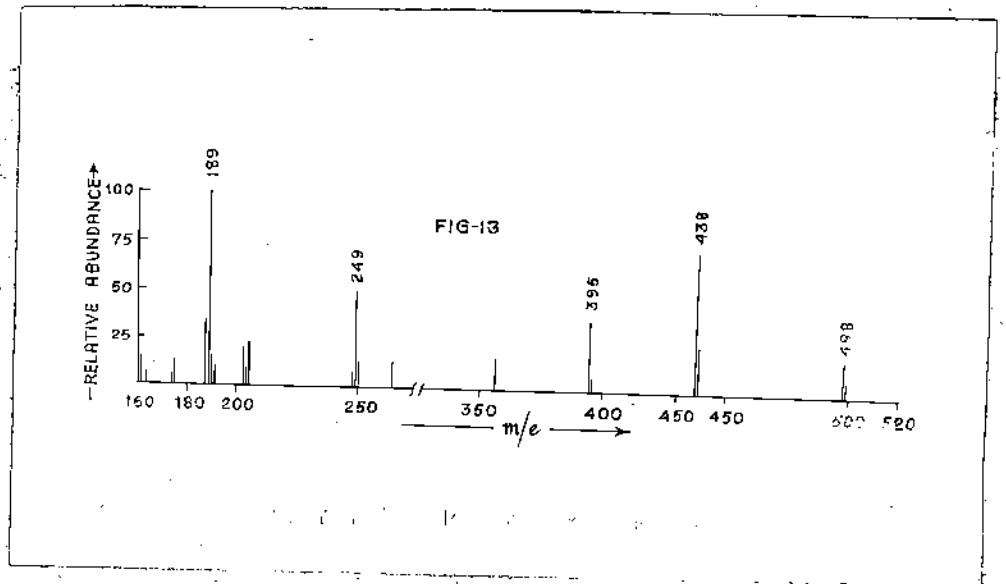


FIG-13

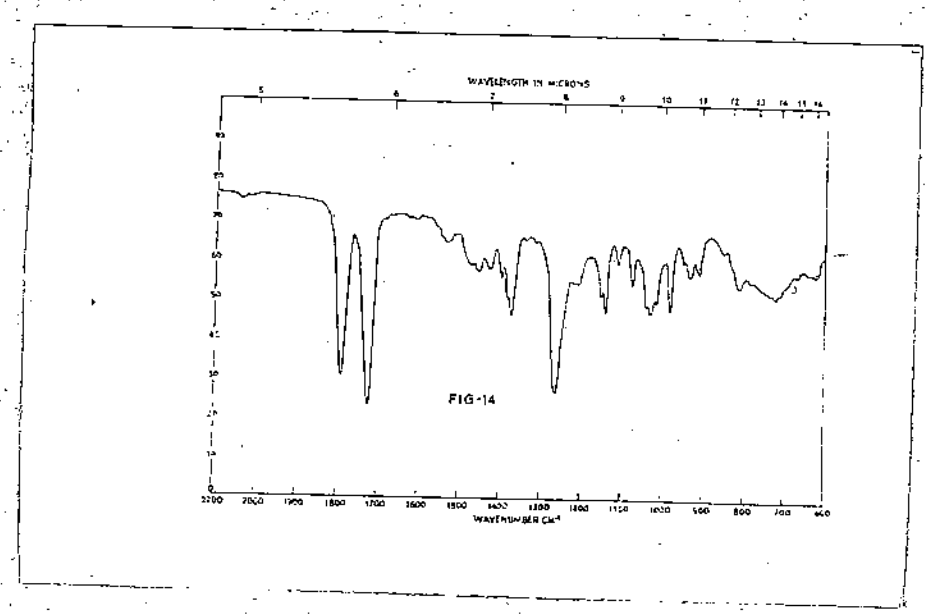


FIG-14

the following characteristics $(\phi)_{306}$ - 381° (trough), $(\phi)_{273}$ + 114° (peak) and $(\phi)_{234}$ - 1765° (trough). In NMR spectrum (Fig. 16) it exhibited peaks at δ 2.35 (3H, -CO.C<u>H</u>$_3$), δ 2.04 (3H, -O.CO.C<u>H</u>$_3$), δ 4.4 (1H, <u>H</u>-C-O-COCH$_3$) and peaks at δ 0.95, δ 0.90 and δ 0.85 attributed to five methyl groups situated at saturated carbon atoms.

Baeyer-Villiger oxidation of nor-ketone <u>3</u>

With a view to converting the acetyl side chain in <u>3</u> to the corresponding acetate (-O.CO.CH$_3$) Baeyer-Villiger oxidation was attempted. Both perbenzoic acid and trifluoro peracetic acid has been applied but no oxidation of the acetyl (COCH$_3$) side chain could be effected, indicating thereby a strong steric hindrance about the acetyl side chain.

At this stage we thought it appropriate to ask what these chemical and physical evidences mean. The facts that lactone <u>2</u> could not be isomerised under strong acid conditions and that Baeyer-Billiger oxidation was not successful on <u>3</u>, seemed most significant. Consideration of the above results led us to think that most probably the isopropenyl side chain in <u>2</u> was probably β-oriented and hence the -CO.CH$_3$ group in the nor-ketone <u>3</u> was also in the unstable β-orientation. Since equilibration by base and acid[8] is an expected process for a conformationally unstable ketone, we thought that if the side-chain at C-19 (CO.CH$_3$) in <u>3</u> were β-oriented it would undergo epimersation to the stereochemically more stable α-isomer as is observed in the case of steroids and

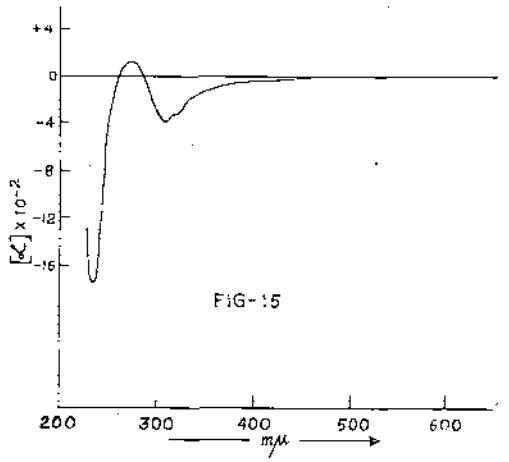


FIG-15

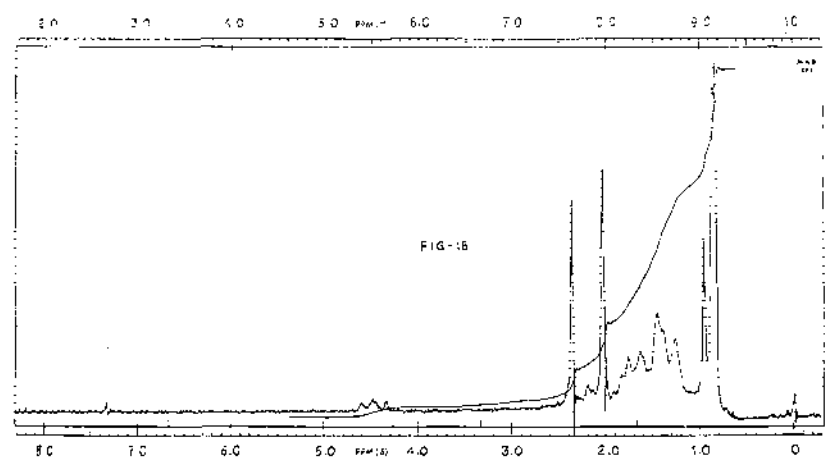


FIG-16

triterpenoids[8]. This is because, examination of Dreiding model shows that ∝-equatorial orientation of the $CO-CH_3$ group is more stable than the β-(axial) orientation, since in the former case it would experience less steric interaction with C-17 β-axial substituent.

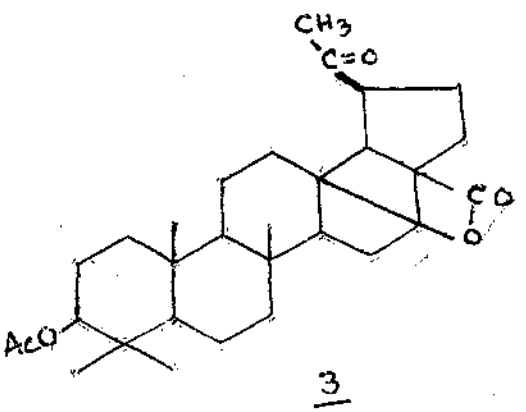
Action of 5% methanolic potassium hydroxide and 2N H_2SO_4 on the nor-keto lactone 3

The nor-keto-lactone 3 was accordingly treated with 5% methanolic potassium hydroxide solution by refluxing the reaction mixture for three hours. The crude product of the reaction on acetylation and chromatography gave back the starting material 3 in good yield (m.m.p. and I.R.). It was also treated with 2N H_2SO_4 in ethanol solution by refluxing the solution for four hours. On working up the reaction mixture, the starting material was recovered unchanged (m.m.p. and I.R. comparison).

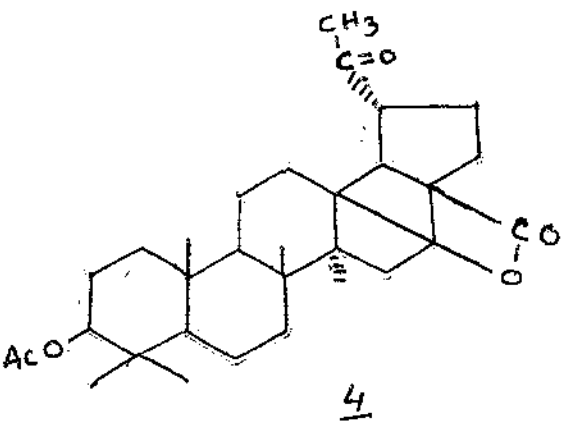
Action of potassium tertiary butoxide - in t-butanol on 3

A more drastic basic condition was then applied. The benzene solution of the compound was added to potassium tertiary butoxide in tertiary butanol and the reaction mixture was refluxed for four hours. The crude product isolated was acetylated which on chromatography on alumina furnished a new compound m.p. 300-2°, $(\alpha)_D$ -24°. The mixture melting point of this compound with the original keto lactone showed considerable depression. TLC analysis of the two

compounds also showed that they were different, the compound 3 had R_f = 0.55 and the new compound had R_f = 0.63 in the solvent system benzene:chloroform (1:1). Both elemental analysis and mass spectroscopic molecular weight determination established its molecular formula as $C_{31}H_{46}O_5$ (M^+ 498). Its I.R. spectrum (Fig. 17A)showed absorption bands at 1765 (γ-lactone), 1725 (acetate and ketone, composite) and 1240 cm^{-1} (acetate). It also showed U.V. absorption at 290 mμ (ε 90) indicating the presence of a saturated carbonyl group. Since equilibration by base to the stereochemically more stable isomer is an expected process, the product obtained by K-tertbutoxide treatment on 3 was believed to be the epimer of 3 with acetyl side chain at C-19 α-oriented and was assigned[9] structure 4.



3



4

Subsequently, the O.R.D. of the compound was measured and the O:R.D. curve (Fig. 18) revealed a strong negative Cotton effect, much more stronger than the original compound 3, and had the following

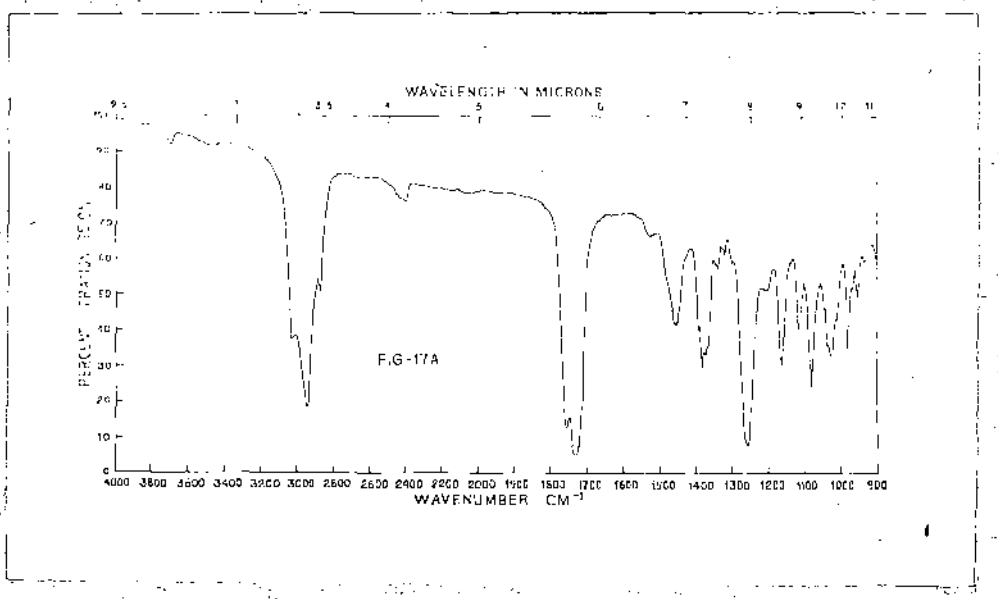


FIG-17A

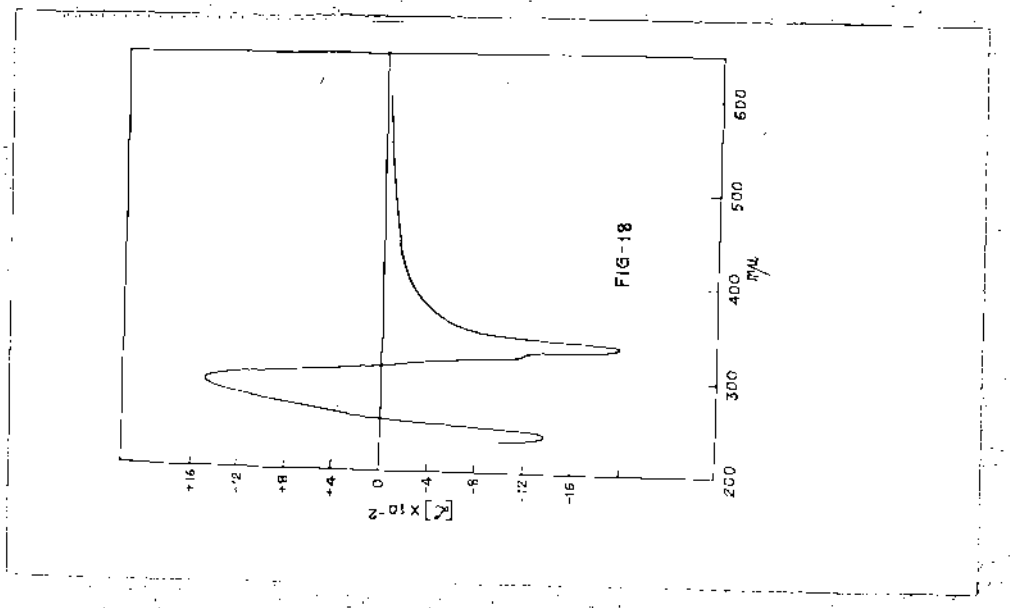
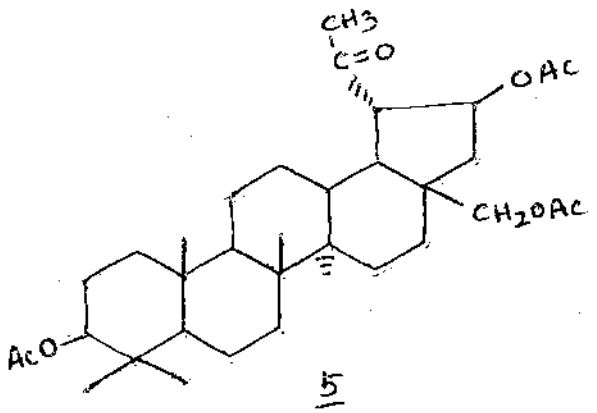


FIG-18

characteristics: $(\phi)_{335}$ - 1794° (trough), $(\phi)_{295}$ +1480° (peak) and $(\phi)_{240}$ - 1380° (trough). This result was unexpected for a structure depicted in 4. Survey of literature showed that compounds 5[10] and 6[11] obtained from thurberogenin and methyl 3β-acetoxy betulinate where the $-CO.CH_3$ group is α-oriented have been reported to exhibit positive Cotton effect. ORD curve of compound 5 showed the following characteristics[10]: $(\phi)_{589}$: 0° $(\phi)_{305}$ + 1848° (peak), $(\phi)_{305}$ + 1848° (peak), $(\phi)_{257}$ - 2184° (trough) and O.R.D. curve of 6 showed the following characteristics[10,11]: $(\phi)_{305}$ + 1368° (peak), $(\phi)_{260}$ - 1938° (trough).



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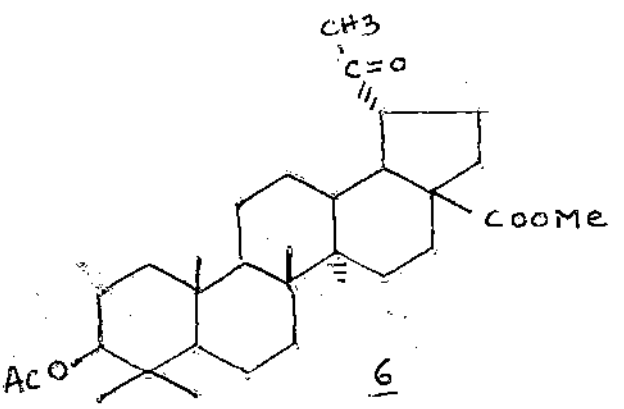
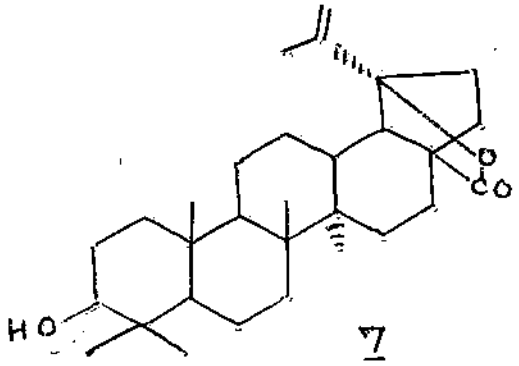


6

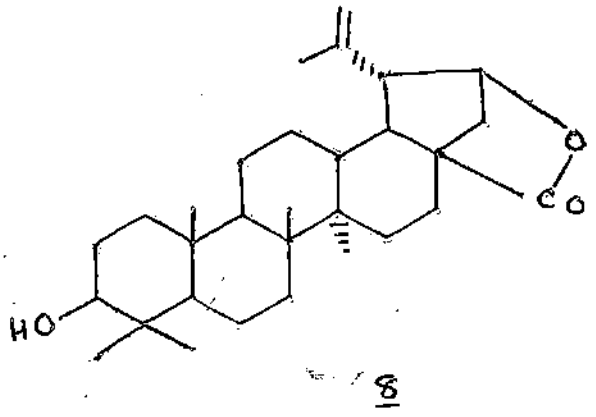
At this point we thought that there was no reason why our compound should exhibit such a strong negative cotton effect if it really represented structure 4. This consideration led us to suspect the validity of the structure 2 of the lactone put forward by Allison and coworkers[1] and raised the question that the lactone termination might not be at C-13.

Allison and coworkers[1] assigned the structure of the lactone as 2 from the following considerations. In the formation of the γ-lactone from acetyl betulic acid by mercuric acetate oxidation the lactone termination may be at C-13, C-19, C-21 or C-15. Since the triol obtained by LAH reduction gave a diacetate and not a triacetate it was concluded that the third alcoholic group was tertiary in nature and the lactone termination at C-21 or C-15 was ruled out. This was further confirmed by dehydration experiment on the above diacetate with $POCl_3$-pyridine. Thus the lactone termination could either be at C-13 or C-19. Allison et al.[1] discarded the C-17-19 lactone structure on the ground that the lactone was not identical with thurberogenin, which was previously assigned structure 7 by Djerassi and coworkers[11,12]. But recently, the structure of thurberogenin was revised[10] and was assigned the 17-21 lactone structure 8. Therefore, in the light of the recent revised



7

Thurberogenin (old structure)



8

(Revised structure)

structure of thurberogenin as well as our observation regarding the unexpected negative O.R.D. data strengthened our belief that the structure of the lactone in mercuric acetate oxidation of acetyl-betulic acid needs revision.

Application of circular dichroism studies

Recently a great deal of work has been done on the application[13] of circular dichroism measurements in structural elucidation and stereochemical aspects of unknown natural products, specially in the steroid and terpene series. We thought that circular dichroism measurements on the lactone might offer valuable clues not only to the structure of the lactone but also the stereochemical environment of the compound. Klyne and coworkers[14-16] have extensively applied this physical tool for the determination of nature and structure of lactones derived from various steroid and terpene derivatives. A lactone sector rule has been devised which enable the Cotton effect of many lactones to be predicted from consideration of assymetric surroundings of the chromophore. The sector rule affords a satisfactory explanation of the empirical data for the great majority of the compounds studied.

In the triterpene field Klyne et al.[16] studied the circular dichroism curves of various lactones of the β-amyrin series of both 18α and 18β stereochemistry. Chart A shows the results obtained by them for some of the lactones studied.

consideration also predicted positive Cotton effects for both the compounds. If the lactone termination were at C-13, in our compound, as depicted in 2, then by analogy with oleanane series a positive C.D. curve would be expected. But on the contrary a negative lactone Cotton effect was actually observed, for the nor-keto lactone 3 (Fig. 19A) with a strong negative maximum at 218 mμ (Δε,-7.02) and a negative Cotton effect at 290 mμ (Δε,-0.9) for the ketone carbonyl chromophore. Since the ketonic carbonyl chromophore might have some effect on the C.D. of lactone chromophore, the lactone devoid of any other chromophore was also prepared and C.D. measured. The lactone 2, was hydrogenated in the presence of PtO_2 catalyst at room temperature to furnish the dihydrolactone 9, m.p. 296-8°, $(\alpha)_D$ + 45° (lit.[1] m.p. 299-300°, $(\alpha)_D$ + 49°), I.R. 1780 (γ-lactone), 1735 and 1240 cm^{-1} (acetate) and absence of bands for vinylidene group. The circular dichroism curve of this dihydrolactone 9, exhibited (Fig. 20) a negative lactone Cotton effect with a maximum at 216 mμ (Δε = -6.05). This observation was a compelling evidence against the 13-18 lactone structure 2, and coupled with the chemical evidences so far recorded opened up the possibility that the lactone termination could be at C-19. Klyne et al.[17], have indeed, measured the C.D. of a 28-19β lactone of lupane series and found a negative lactone cotton effect which is in accord with our observation for 3 as well as 9. On the basis of these physical evidences, it may be argued that the lactone termination should be at C-19 and the structure of the lactone should be represented by 10 (dihydro lactone by 10a) and the nor-keto lactone by 11. The positive Cotton effect (Δε, + 1.58 at

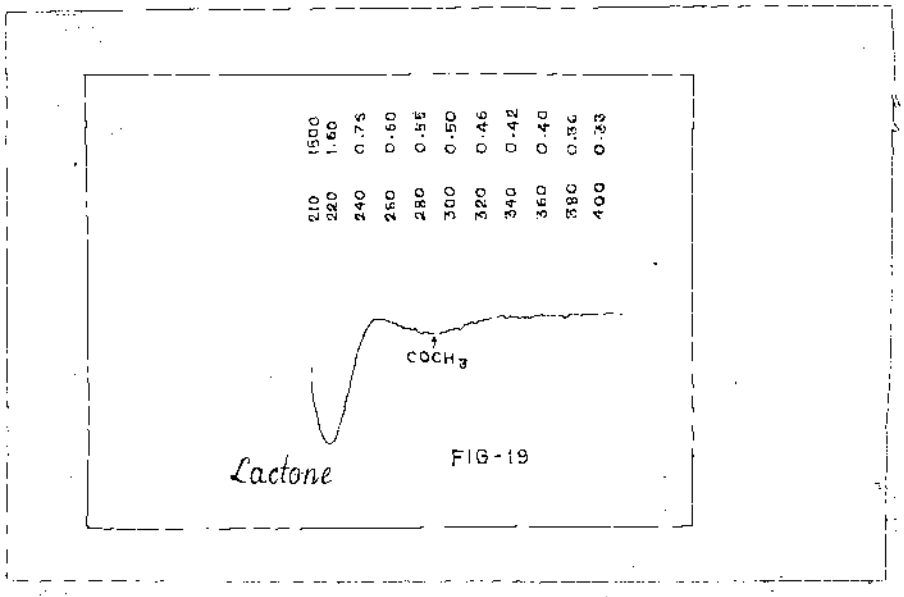


FIG-19

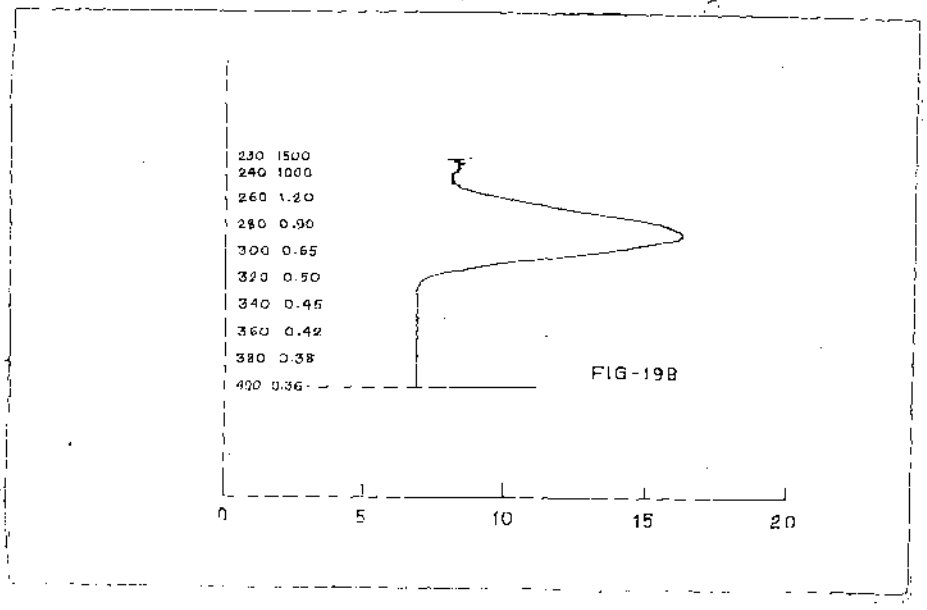
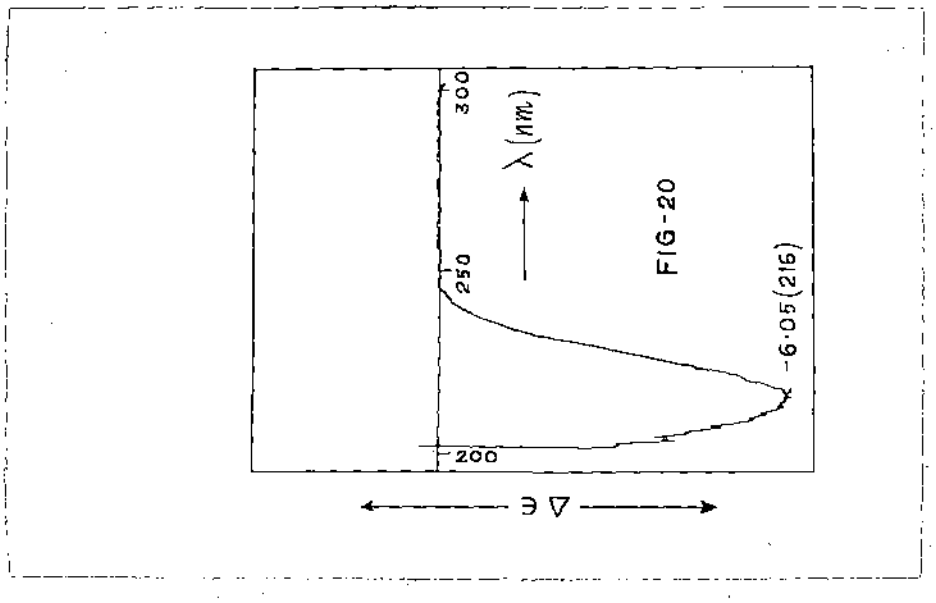
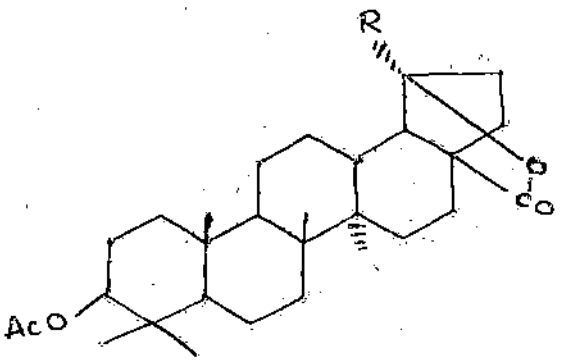


FIG-19B





10 , R = isopropenyl

11 , R = $-CO.CH_3$

10a , R = isopropyl

288 mμ, Fig. 19B) due to carbonyl group of methyl 3β-acetoxy 20-oxo-30-nor lupan-28-oate which has been recorded by us changed to a negative value in nor ketolactone 11 (Δε, -0.99 at 290 mμ; Fig. 19). This change to a negative value for the compound 11 is a familiar effect of α-substitution of methyl ketone with restricted rotation[18,19].

The resistance of the lactone 11 towards acid-catalysed isomerization can now be explained as due to considerable steric hindrance arising out of the C-17-C-19 lactone bridge. The same consideration also explains the inertness of the compound to Baeyer-Villiger oxidation even under most drastic conditions of trifluoroperacetic acid.

Section B

Structure of the product m.p. 300-2°, $(\alpha)_D$ - 24° obtained by K-tertbutoxide treatment of lactone 11

In view of the revised structure 11 of the nor-ketolactone, re-investigation of the structure of the compound m.p. 300-2°, $(\alpha)_D$- 24°, obtained by K-tertiary butoxide treatment of lactone 11, and reacetylation, was undertaken. NMR spectrum of the compound was taken and revealed a new picture with very interesting features. The spectra (Fig. 21) showed the absence of the peak at δ 2.35 due to $-CO-CH_3$ group present in the original compound 11. It also exhibited peaks at δ 2.04 ($-O.CO.C\underline{H}_3$, 3H), δ 4.4 ($\underline{H}$-C-O.CO.CH_3) and tall peaks δ 0.95 and δ 1.0. attributable to five methyl groups on saturated carbons. In addition to these, an additional peak appeared at δ 1.40 (singlet) which integrated for three protons. This observation, proved that it was not at all a simple case of epimerisation, but a skeletal rearrangement had taken place during the reaction. The molecular weight determination by mass spectra and elemental analysis showed that it had the same molecular formula $C_{31}H_{46}O_5$ as the original compound proving thereby that no elimination in the molecule had taken place. The new peak at δ 1.4 was assigned to a methyl group on a carbon bound by a oxygen bridge, in this case, probably by a lactonic oxygen bridge, which contributes to deshielding[20] and the consequent shift of the new methyl peak.

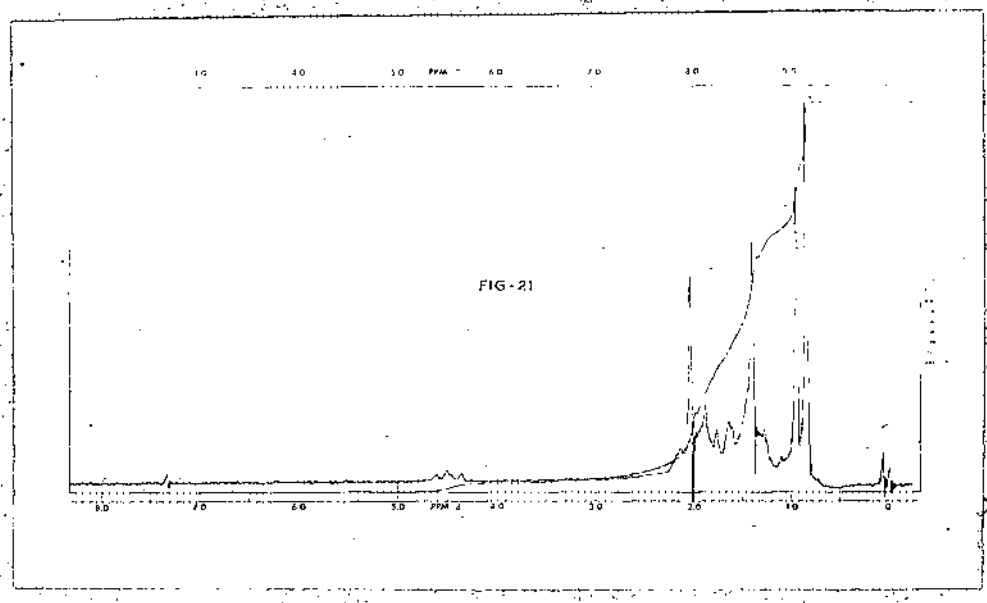


FIG-21

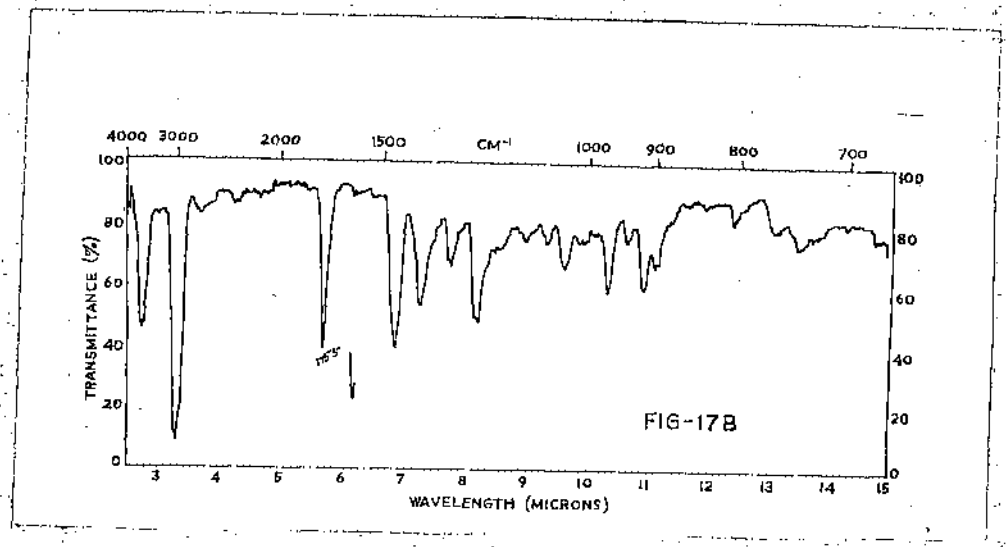
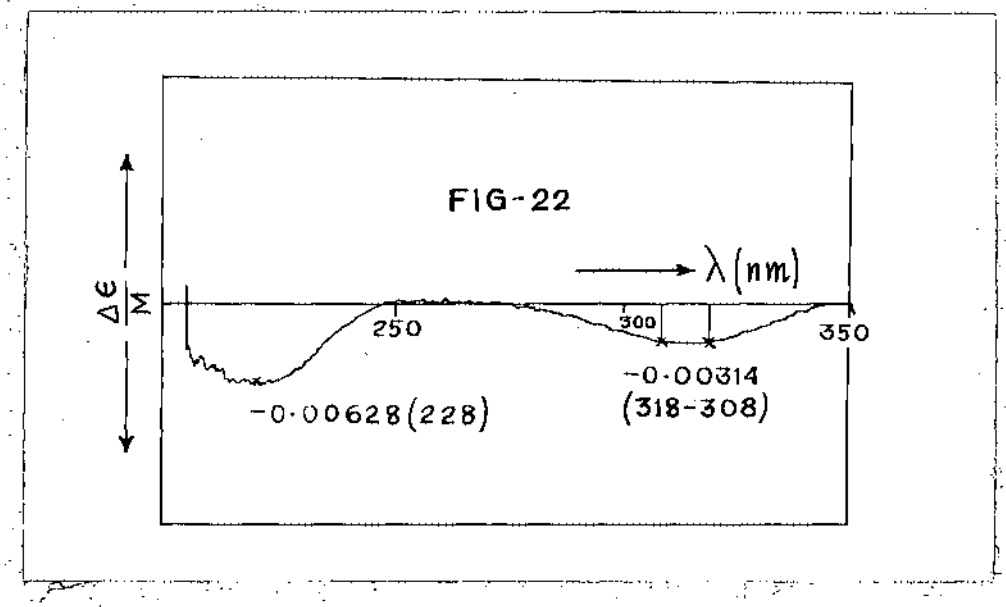
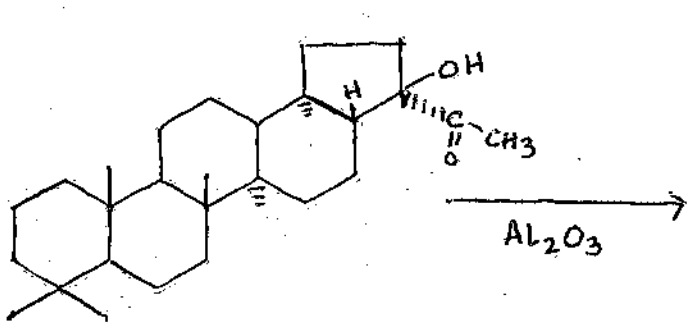


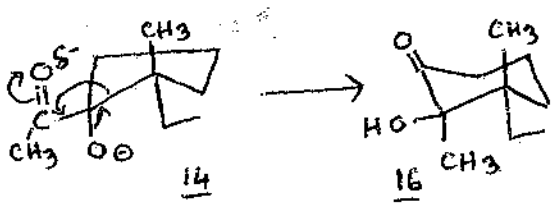
FIG-17B



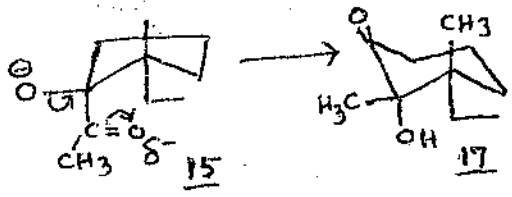
It is interesting to observe that the structure 11, represented for the nor-keto-lactone, possesses all the structural requirements for ring enlargement to an E-homo derivative under basic conditions. The basic condition employed in the present case, K-tert-butoxide-tert-butyl alcohol, is sufficient to break the lactone ring and then undergo E-homo rearrangement as have been observed in the case of 17-hydroxy-20-keto steroids[21] and E-homo rearrangement[22] in 21-hydroxy ~~iso~~adiantone 12 which in contact with basic alumina furnished 13. The stereochemical requirements of this rearrangement have been considered in detail by Turner[23]. For this type of base-catalysed reaction the suggestion was made that removal of a proton from the hydroxyl group and conference of a full negative charge on oxygen will, as a consequence of an electrostatic repulsion, lead to a particular orientation (s-trans[24] i.e. trans rotational isomer about a single bond) of the carbonyl dipole in 14 and 15. Rearrangement in this case will then proceed from the 17α-hydroxy in 14 to the 17β-OH configuration in 16 and 17β- OH in 15 to 17 α-OH D-homoderivative 17 would be anticipated. This prediction was found to agree with the experimental results.



12 13



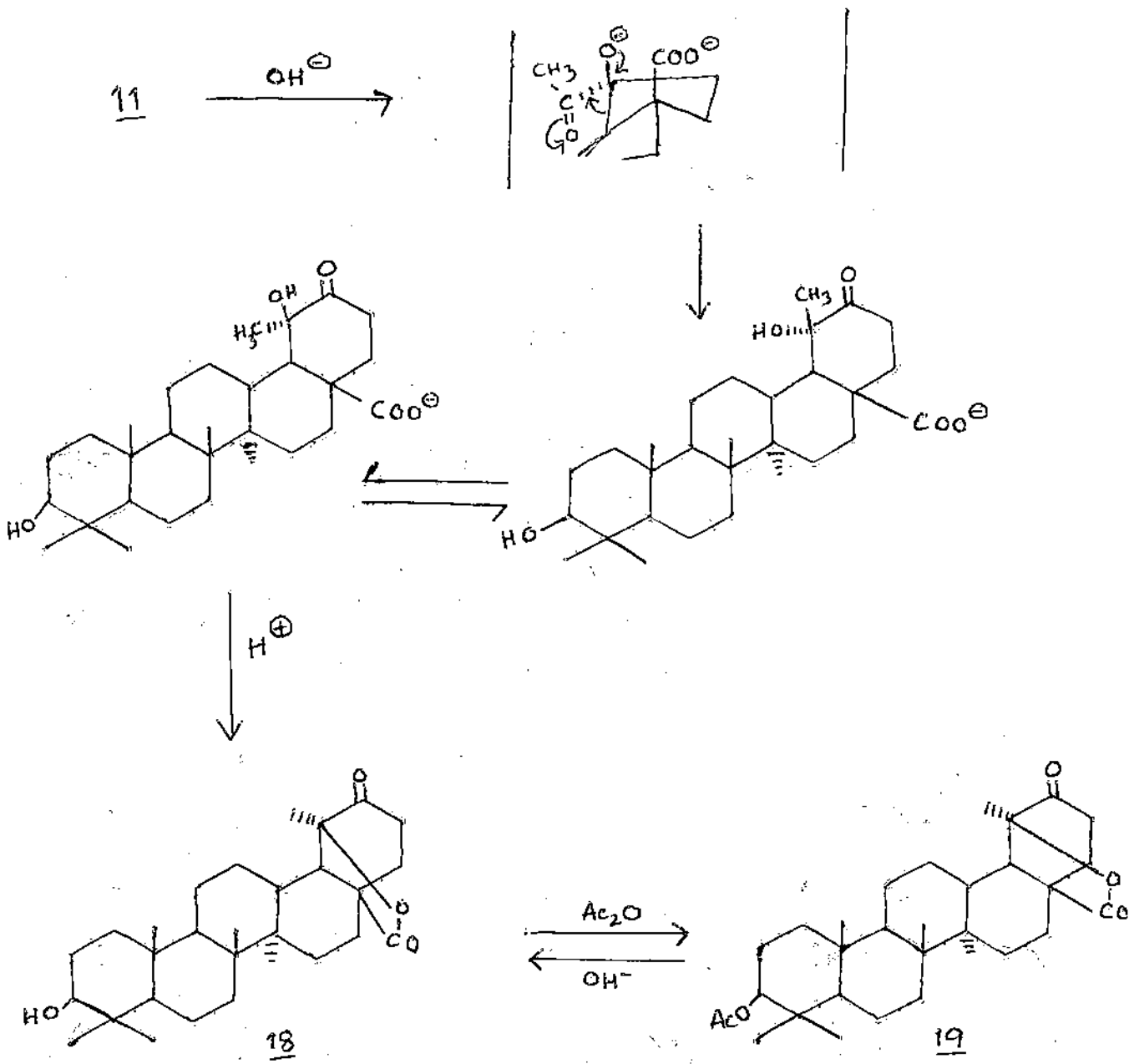
14 16



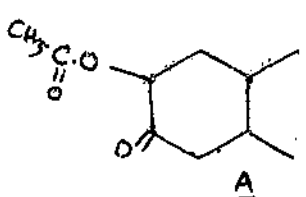
15 17

On the basis of the above argument the base catalysed ring expansion of the nor-keto lactone 11, should be represented by 18, which would be formed according to the mechanism shown below in chart B.

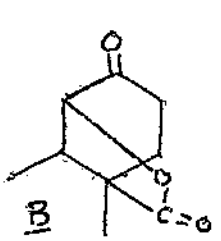
Chart B



If the above mechanism, demanding stereo specificity of the reaction, predicted by Turner[23] is applicable here, then the resulting hydroxy group at C-19 would be equatorial in the chair E-ring conformation. But as the product in the reaction obtained after acidification, was a γ-lactone (I.R. ν max 1755 cm^{-1}) (vide supra), the mechanism of lactonisation requires to be discussed. There are four possibilities: (i) The lactonisation is possible during acetylation, when the C-19 carbonium ion may be generated leading to lactone 18, (ii) during working up of the reaction mixture which involved acidification with mineral acid (see experimental) the carbonium ion at C-19 may be formed, (iii) the intermediate 19α-ketol which is first formed may undergo equilibriation to a mixture of 19α and 19β ketols under the strong basic conditions (K-t butoxide) utilised, and then undergo lactonisation to give 18. This is consistent with the observations[25] in the case of D-homo rearrangements in the steroid series where an equilibrium mixture of 17α and 17β ketols were obtained in several cases under strong alkaline conditions, (iv) ring E-may assume boat conformation which probably facilitate lactonisation between C-19 OH and C-17 COOH groups. In order to understand the lactonisation process the intermediate product 18 was isolated prior to acetylation. It had m.p. 258-60°, molecular formula $C_{30}H_{44}O_4$ and showed U.V. absorption at 280 mμ (ε84). IR spectrum of the compound (Fig. 17B) also showed a broad peak at 1755 cm^{-1} attributable to the composite contribution of a γ-lactone and a six membered ring ketone situated at the α-position to an ester group (in this case γ-lactone). Such shifts



A



B

in the I.R. absorption of ketone adjacent to ester group (acetoxy) of structural type A have been recorded in the literature[26]. Since the compound 18 has got the structural type B having the lactone termination α to the keto group of a six membered ring ketone (analogous to A structurally), the absorption at 1755 cm^{-1} is explicable for the keto-group as well. The same hydroxy lactone 18 was also isolated by mild alkaline hydrolysis of the corresponding acetate. This clearly demonstrates that lactonisation did not take place during acetylation and hence it may be concluded that lactonisation probably took place during acidification of the reaction product with mineral acid.

The structure of the rearranged 3β-acetoxy-lactone as shown in 19, from mechanistic grounds can well explain all the spectroscopic observations. The peak at δ 1.40 in the NMR spectrum (Fig.21) can be explained for the CH_3- group in the system $CH_3-\overset{1}{C}-O-C-$ in structure 19. The U.V. absorption at 290 (ϵ 95) and I.R. peak at 1755 cm^{-1} are also in accord with the above structure. Circular dichroism measurements also strongly support the structure assigned to the rearranged lactone 19. C.D. curve showed two maxima (Fig. 22), The negative lactone effect at 220 mμ ($\Delta\epsilon$ -3.12) has the sign and order of magnitude expected for a 28-19β lactone of 18α-oleanone system. The second maxima due to the ketone Cotton effect at 318-308 mμ ($\Delta\epsilon$, -1.56), however, cannot be correlated with the stereochemistry of the molecule because the octant rule is not applicable to ketones with an oxygen function (lactone) on the α-

carbon atom (P.M. Scopes, Westfield College, London, Private communication). Thus the spectroscopic evidences coupled with the mechanistic considerations of E-homo rearrangement lead to the tentative structure 18 for the rearrangement product. It also becomes clear now that this homo-rearrangement is only explicable in terms of the lactone termination at C-19 in the original nor-keto-lactone 11 and provides additional support to the structure 10 for the γ-lactone obtained by Hg(ic) acetate oxidation of adetyl betulic acid. The failure of the E-homo rearrangement with 5% methanolic potassium hydroxide may be attributed to the inability of the mild conditions used, to effect cleavage of the γ-lactone.

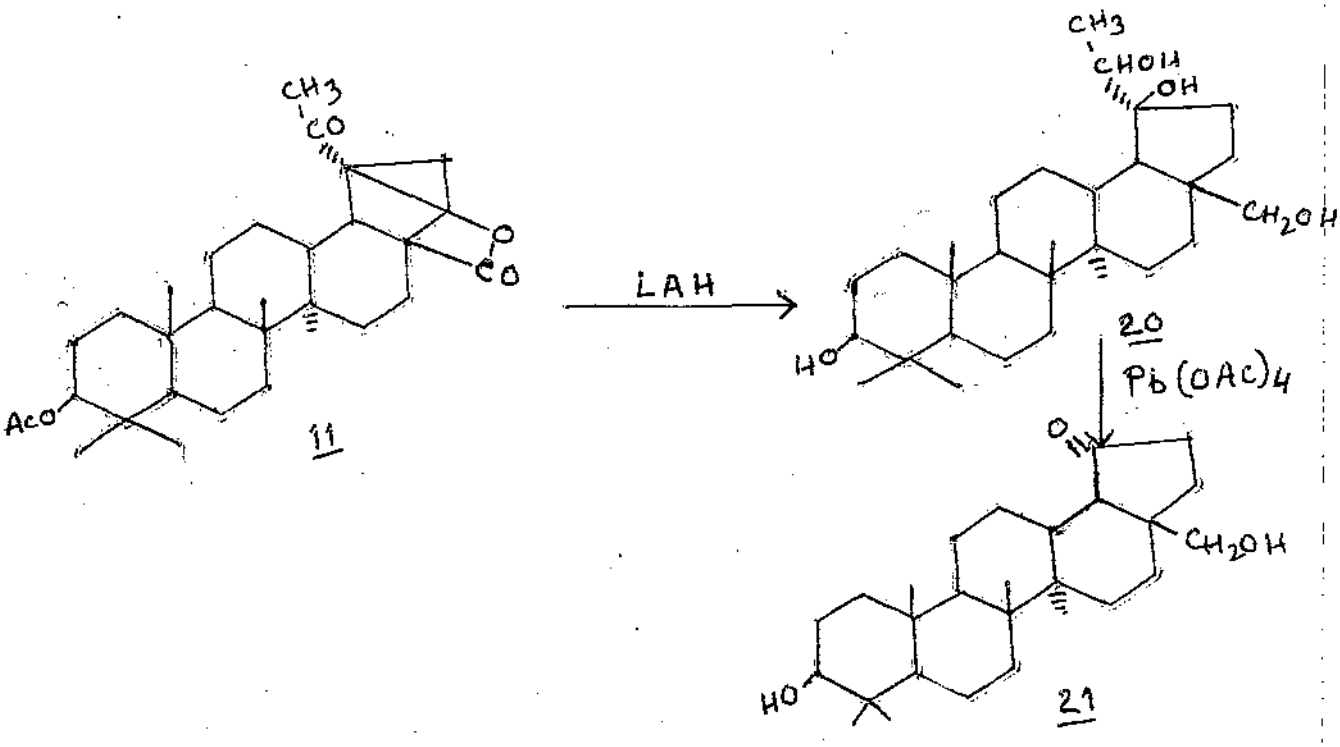
Section C

Chemical evidences for structure 10 assigned to the lactone obtained by Hg (ic) acetate oxidation of acetyl betulic acid

Additional evidence regarding the lactone termination at C-19 for the Hg(ic) acetate oxidation product of acetyl betulic acid, has been obtained from the chemical degradation studies described below.

The nor-keto-lactone 11 was reduced by lithium aluminium hydride to furnish a tetra-ol 20, $C_{29}H_{50}O_4$, m.p. 281-3°, $(\alpha)_D$ + 20°, I.R. ν max 3420 (OH), the absorption in the carbonyl region being totally absent, no U.V. absorption in the region 220-300 mμ. The tetraol was treated with sodium periodate in methanol solution at room temperature, but only the starting material could be recovered in good yield. Treatment of 20 with Pb (iv) acetate at room

temperature in acetic acid solution also resulted in the isolation of the starting material. The tetraol was then exposed to the action of Pb (iv) acetate in glacial acetic acid at 120° for six hours. The gummy reaction product thus obtained was hydrolysed with methanolic potassium hydroxide solution and then chromatographed over alumina. Elution with benzene:chloroform (1:1) resulted in the isolation of a product m.p. 240-2°, $(\alpha)_D$ +32° which analysed for $C_{27}H_{44}O_3$. Its I.R. spectrum showed a peak in the carbonyl region at 1740 cm^{-1} attributed to a ketonic carbonyl group present in a five membered ring and a peak at 3310 cm^{-1} assigned to hydroxyl stretching. In U.V. spectrum it showed abosrption at 285 mμ (ε 75) indicative of the presence of a carbonyl group. On the basis of the above evidences the Pb (iv) oxidationproduct has been assigned structure 21.

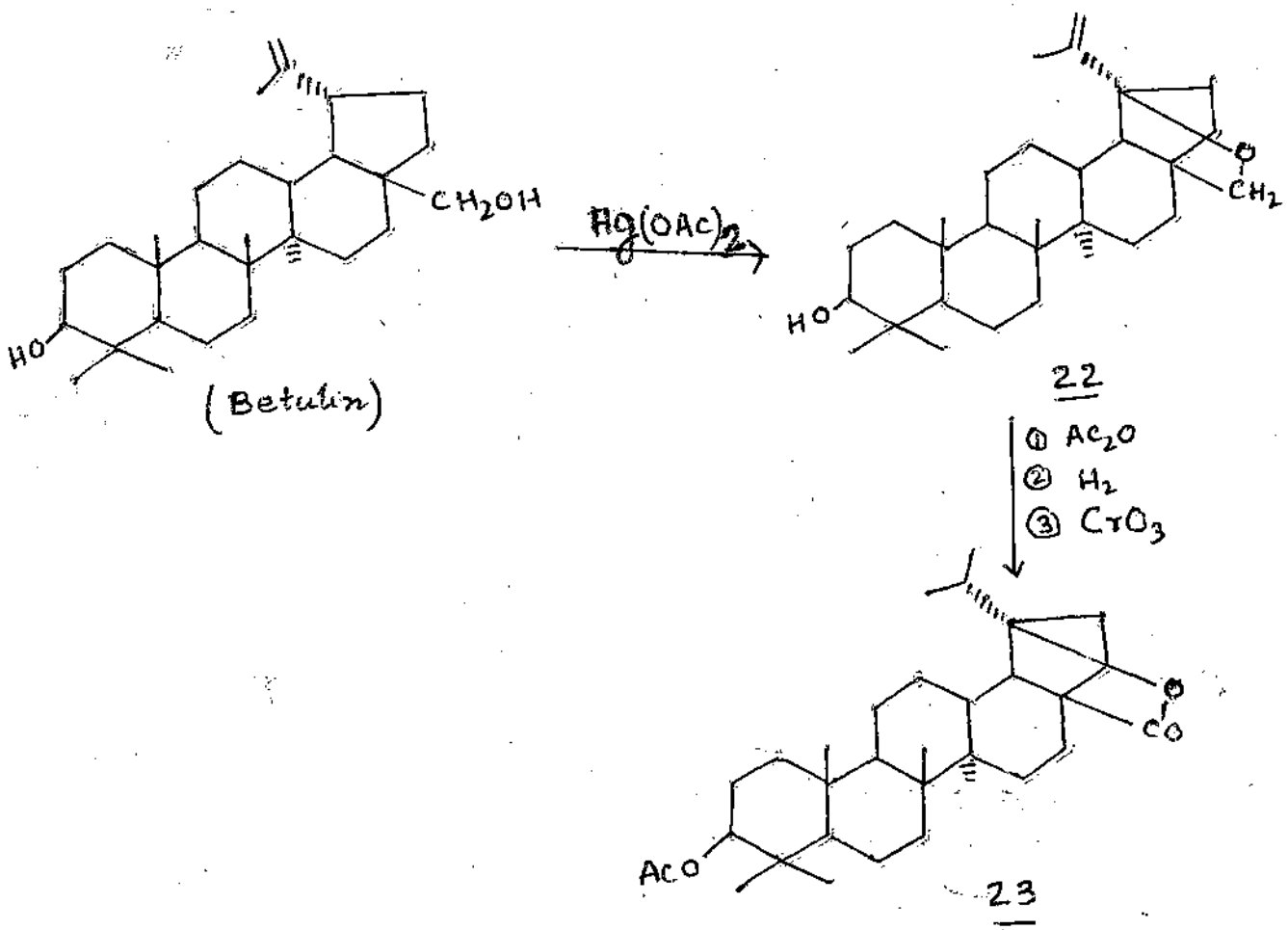


The isolation and characterisation of the tris-nor-ketone 21 establishes definitely the presence of a 1,2-glycol system in the compound. Hence the nor-keto-lactone and the parent lactone must be represented as shown in 11 and 10 respectively.

The failure of glycol cleavage by lead tetraacetate or sodium periodate under mild conditions (room temperature) must be ascribed to steric hindrance around C_{19}-C_{20} bond.

When we had completed these studies a communication[27] leading to the same conclusion regarding the structure of the lactone obtained by Hg(ic) acetate oxidation of acetyl betulic acid has been published. However, the Australian group[27] did not provide any physical evidence from circular dichroism measurements nor did they encounter the novel rearrangement, the base catalysed E-Homo rearrangement, discussed above.

Very recently Vystrčil[19] et al. have shown that the Hg(ic) acetate oxidation product of betulin was 22, the oxygen bridge being present between C-17-C-19. They have established their structure mainly from acid-induced E-ring expansion, which has been discussed in detail (vide supra). Since this ether on acetylation, hydrogenation, followed by CrO_3 oxidation afforded the same dihydro 3β-acetoxy lactone 23 as that obtained by Hg (ic) acetate oxidation of acetyl betulic acid followed by hydrogenation, the revised structure 22 of the ether lends further good support to our structure 10 having lactone termination at C-19.


CH2OH
HO
(Betulin)
Hg(OAc)2
CH2
HO
22
① Ac2O
② H2
③ CrO3
CO
AcO
23

Experimental

Mercuric acetate oxidation of acetyl betulinic acid 1 : Preparation of the lactone 2

To acetyl betulinic acid (10 g) dissolved in chloroform (250 ml) was added a solution of mercuric acetate (175 g) in hot acetic acid (1.2 lit.), reaction mixture was maintained at 100° in an oil bath for 4 hours. On cooling the precipitated mercurus acetate was filtered off and the whole filtrate was diluted with water and extracted with more chloroform. The orange coloured chloroform solution was washed well with water and dried (anhydrous Na_2SO_4). Removal of chloroform gave an orange solid which was dissolved in pyridine and hydrogen sulfide was passed for three hours. The black reaction mixture was filtered from Kieselguhr and pyridine was removed under reduced pressure when a brownish black gummy product (9 gr) was obtained. It was dissolved in benzene (40 ml) and chromatographed over alumina (350 gr) column deactivated by 14 ml of 10% aqueous acetic acid. Chromatogram was developed in petroleum ether and eluted by the following solvents (Table I).

Table I

Eluent	Fractions 50 ml each	Residue left on evaporation
Petroleum ether (200 ml)	1-4	Nil
Petroleum ether (200 ml)	5-8	Yellow sulphur
Petroleum ether (300 ml)	9-14	Nil
Petroleum ether:benzene (4:1) (350 ml)	15-21	Solid, m.p. 295-8°

Further elution with more polar solvent did not afford any crystalline material

The fractions (15-21) (table I) were collected and crystallised from a mixture of chloroform and methanol to afford fine needle shaped crystals m.p. 301-2°, $(\alpha)_D$ + 58°. Recrystallisation did not improve the melting point.

Found : C, 77.58; H, 9.54%

Calculated for $C_{32}H_{48}O_4$: C, 77.42; H, 9.67%

I.R. (KBr) : 1780 (γ-lactone), 1730, 1245 (acetate), 1640 and 830 (vinylidine) cm^{-1}.

NMR (60 Mc) : δ 0.96, δ 0.89 and δ 0.81 (5 CH_3), δ 1.98 (3H, -O.CO$\underline{CH_3}$) δ1.65 (3H, -C=C), δ 4.95, δ 5.25 (2H, vinylidene, δ 4.4 ($\underline{H}$-C-O.$\underset{CH_3}{C}$OCH$_3$).

Attempted isomerisation of 2 with hydrogen chloride in chloroform

To the solution of the lactone 2 (0.2 g) in chloroform (15 ml), hydrogen chloride was passed through for 4 hours. Chloroform and HCl were removed by evaporation and the product was dissolved in ether. The ether solution was washed well with water and dried (anhydrous Na_2SO_4). Removal of ether gave a solid (0.18g). The product was recrystallised from a mixture of chloroform and methanol to afford fine needle shaped crystals, m.p. 299-301°. Mixed m.p. with 2 was found undepressed. (I.R. comparison identical with 2).

Attempted isomerisation of 2 with 98% formic acid in acetic acid

To the lactone 2 (0.2g) a mixture of acetic acid (10 ml) and formic acid (98%) (10 ml) was added and the mixture was heated for 16 hours at 100°. The reaction mixture was cooled and poured into water and the precipitated solid was collected by filtration. The brown solid (.16 g), thus obtained, was chromatographed on an active column of alumina (12 g). The chromatogram was developed in petroleum ether. The solid was dissolved in 5 ml benzene and poured into the column and eluted by the following solvents (Table II).

Table II

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-2	Nil
Petroleum ether:benzene (4:1) (100 ml)	3-4	Nil
Petroleum ether:benzene (3:2) (100 ml)	5-6	Nil
Petroleum ether:benzene (2:3) (150 ml)	7-9	Solid m.p. 296-8°

Elution with more polar solvents did not afford any crystalline material.

Fraction (7-9) (Table II) were combined and crystallised from $CHCl_3$-MeOH, when needle shaped crystals m.p. 301-2°, was obtained. It was found to be identical with 2 by m.m.p. and I.R. comparison.

Attempted isomerisation of 2 with hydrochloric acid in acetic acid

The lactone 2 (.25 g) was dissolved in AcOH (30 ml) and conc. HCl (3 ml) was added to it. The reaction mixture was heated at 100° for one hour. It was cooled and diluted with water, the brown solid (0.17 g) was collected by filtration. It was dissolved in 6 ml of benzene and poured on an active column prepared from alumina (1.5 g.). The chromatogram was developed in petroleum ether and eluted with the following solvents (Table III).

Table III

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (150 ml)	1-3	Nil
Petroleum ether:benzene (4:1) (100 ml)	4-5	Nil
Petroleum ether:benzene (3:2) (100 ml)	6-7	Nil
Petroleum ether:benzene (2:3) (150 ml)	8-10	Solid, m.p. 296-9°
Further elution with more polar solvent did not afford any solid		

The fractions (8-10), Table III) were combined and crystallised from $CHCl_3$-MeOH, when needle shaped crystals m.p. 301-3° was obtained. It was also found to be identical with 2 by m.m.p. and I.R. comparison.

Ozonolysis of the lactone 2 : Preparation of nor-keto lactone 3

The lactone 2 (4 g) was dissolved in $CHCl_3$ (40 ml). A stream of ozonised oxygen was passed through it for 3 hours while the temperature was kept at 0°C. It was then treated with 2 ml of AcOH and 2 gr. Zn dust to decompose the ozonide. After decomposition the mixture was ~~then~~ extracted with ether, washed well with water and dried (anhydrous Na_2SO_4). Evaporation of the solvent gave a white solid (3.4 g). It was dissolved in benzene (25 ml) and poured on a

column prepared from silica gel (220 g). The chromatogram was developed in petroleum ether and eluted with the following solvents. (Table IV).

Table IV

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether (150 ml)	1-3	Nil
Petroleum ether:benzene (4:1) (200 ml)	4-7	Nil
Petroleum ether:benzene (3:2) (150 ml)	8-10	Nil
Petroleum ether:benzene (2:3) (300 ml)	11-16	White solid, m.p. 294-6°

Elution with more polar solvent did not afford any more solid

The fractions 11-16 (3.2 g, Table IV) were combined and crystallised from a mixture of chloroform and methanol when prismatic crystals, m.p. 301-3°, $(\alpha)_D$ - 9° (lit.[1] m.p. 317°, $(\alpha)_D$ - 2°) were obtained. Recrystallization did not improve the melting point.

Found : C, 74.19; H, 9.08%

Calculated for $C_{31}H_{46}O_5$: C, 74.68; H, 9.23%

I.R. ($CHCl_3$) : 1721 (compositive for acetate and carbonyl), 1780 (γ-lactone), 1255 cm^{-1} (acetate)

U.V. (Ethanol): λ max 275 mμ (log ϵ 1.4)

NMR (60 Mc) : δ 0.95, δ .90 and 0.85 (5 CH_3), δ 2.35 (3H, -CO.$\underline{CH_3}$), δ 2.04 groups on saturated carbon) (3H, -O.$COCH_3$), δ 4.4 (1H, $\underline{H}$-C-O-$COCH_3$)

Mass : 498, 438, 395, 249 m/e

O.R.D. : $(\phi)_{306}$ - 381° (trough), $(\phi)_{273}$ + 114° (peak) and $(\phi)_{234}$ - 1765° (trough)

C.D. : Negative Cotton effect with a negative maximum at 218 mμ ($\Delta\epsilon$, -7.02), 290 mμ ($\Delta\epsilon$-0.99)

R_f (Benzene:$CHCl_3$:1:1) : 0.55

Attempted epi-merisation of the nor-ketone 3 with 2N sulfuric acid in ethanol solution:

To the norketone 3 (200 mg) dissolved in 15 ml ethanol was added 2N H_2SO_4 (0.2 ml) and the reaction mixture was refluxed for 4 hours. The reaction mixture was cooled and diluted with water. The precipitated solid was collected by filtration. The crude product (180 mg) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml). The acetylated product (170 mg) thus obtained on working up in the usual manner, was subjected to chromatography on an alumina column, prepared from alumina (12 g.) and deactivated by 0.4 ml of 10% aqueous AcOH. The chromatogram was developed in petroleum ether. The solid (170 mg) dissolved in benzene (6 ml) was put on the column and eluted by the following solvents (Table V).

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Table V

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-2	Nil
Petroleum ether:benzene (4:1) (100 ml)	3-4	Nil
Petroleum ether:benzene (3:2) (100 ml)	5-6	Nil
Petroleum ether:benzene (2:3) (150 ml)	7-9	Solid, m.p. 299-301°

The fractions 7-9 (table V) (150 mg) were collected and crystallised from $CHCl_3$- MeOH, m.p. 301-3°. The m.m.p. of this and 3 remained undepressed.

Attempted epimerisation of 3 with methanolic KOH

The norketone 3 (200 mg) dissolved in benzene (10 ml) was added to a methanolic KOH solution (5%, 10 ml) and the mixture was refluxed on water bath for 3 hours. It was then concentrated and diluted with water. The precipitated solid (155 mg) was collected by filtration. The latter was reacetylated with acetic anhydride (2 ml) and pyridine (2 ml). On working up the reaction mixture in usual way, a solid (160 mg) was obtained. It was dissolved in benzene (8 ml) and put on a column prepared from alumina (15 g.) deactivated by 0.6 ml of 10% aqueous acetic acid. The chromatogram

was prepared in petroleum ether and eluted with the following solvents (Table VI).

Table VI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (100 ml)	1-2	Nil
Petroleum ether:benzene (4:1) (100 ml)	3-4	Nil
Petroleum ether:benzene (3:2) (100 ml)	5-6	Nil
Petroleum ether:benzene (2:3) (100 ml)	7-8	Solid, m.p. 298-300°

Further elution with more polar solvent did not give any solid.

Fractions 7-8 (Table VI, 130 mg) were combined and crystallised from a mixture of chloroform and methanol m.p. 301-2°.
Mixed m.p. with 3 remained undepressed.

Attempted Baeyer-Villiger oxidation of 3

The nor-ketone 3 (200 mg) dissolved in chloroform (10 ml) was added to a solution of perbenzoic acid in chloroform (10 ml) and toluene-p-sulphonic acid (30 mg). The reaction mixture was refluxed for 6 hours and kept at room temp. over night. The excess perbenzoic acid was destroyed by adding potassium iodide solution acidified with acetic acid, followed by the addition of a dilute sodium thiosulfate

solution. The chloroform layer was washed with aqueous NaOH solution and then with water and dried (Na_2SO_4). Removal of the solvent gave a solid (180 mg) which was recrystallised from chloroform-methanol, m.p. 302-3°C. Mixed m.p. with starting material 3 remained undepressed.

Modified Baeyer-Villiger oxidation of 3

The norketone 3 (150 mg) was dissolved in chloroform followed by a solution of trifluroperoxyacetic acid. The peracid reagent was prepared from trifluroacetic anhydride (1.2 ml), 90% hydrogen peroxide (0.16 ml) and methylene chloride (2 ml). Following a 3 hr. period of reflux, the reaction mixture was successively cooled, filtered, washed with water, dried (Na_2SO_4) and concentrated from a mixture of chloroform and methanol; m.p. 301-3°. Mixed m.p. with the starting norketone 3 remained undepressed.

Base catalysed reaction of 3 with K.t. butoxide in t-butyl alcohol: Preparation of 18

The nor-keto lactone 3 (200 mg) dissolved in dry benzene (10 ml) (10 ml) was added to a solution of 0.060 gr of potassium in 8 ml of dry tertiary butyl alcohol and the whole reaction mixture was refluxed for 4 hours. The solvent was removed and water was added followed by acidification with hydrochloric acid (10%, 5 ml). The crude product (140 mg) thus obtained was crystallised twice from acetone when constant melting product 18 was obtained, m.p. 258-60°.

Found : C, 76.02; H, 9.46%

$C_{29}H_{44}O_4$ required : C, 76.31; H, 9.64%

I.R. (Nujol) : 3600 (OH), 1755 (γ lactone and carbonyl) cm^{-1}.

U.V. (Ethanol) : λ max 280 mμ (ϵ 84)

Acetylation of 18 : Preparation of 19

The compound 18 (200 mg) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) in the usual manner. The solid obtained by usual work up was crystallised from a mixture of chloroform and methanol when fine needle shaped crystals m.p. 300-2°, $(\alpha)_D$ - 24° was obtained.

Found : C, 74.17; H, 9.28%

$C_{31}H_{46}O_5$ required : C, 74.68; H, 9.23%

I.R. : 1765 (γlactone), 1725 (acetate and ketone) and 1240 cm^{-1} (acetate)

U.V. (ethanol) : λmax 290 mμ (ϵ90)

N.M.R. (60 Mc) : δ 1.40 (3H, singlet $C\underline{H}_3$-C-O-C) δ 2.04 (3H, singlet $C\underline{H}_3$-C(=O)-O-C), δ 4.4 (1H, multiplet AcO-C(H)-), δ.95 and δ1.0 (5 CH_3 group on saturated carbon).

O.R.D. : $(\phi)_{335}$ - 1794° (trough), $(\phi)_{295}$ + 1480° (peak), and $(\phi)_{240}$ -1380° (trough)

C.D. : 318-308 mμ ($\Delta\epsilon$, -1.56), 250-2 mμ, ($\Delta\epsilon$, -0.16), 220 mμ ($\Delta\epsilon$, -3.12)

Rf (Benzene:$CHCl_3$ (1:1) : 0.63

Mass : 498, 438, 249 m/e

Hydrolysis of 19 : Preparation of 18

The compound 19 (100 mg) was hydrolysed with methanolic alkali (10%, 10 ml) under reflux for 3 hours. After concentrating the reaction mixture, it was diluted with water. A solid (80 mg) was obtained which on crystallisation from acetone afforded crystals of m.p. 257-9°c. This hydrolysed product was found to be identical with 18 (m.m.p. and I.R. comparison).

Hydrogenation of 2 : Preparation of 9

A solution of the lactone 2 (200 mg) dissolved in a mixture of ethyl acetate and acetic acid (10 ml each) was shaken in an atmosphere of hydrogen in presence of PtO_2 catalyst at room temperature for 3 hours. After hydrogenation was complete the catalyst was removed by filtration and the filtrate was concentrated to a small volume and diluted with water. The solid precipitate, thus obtained, was crystallised twice from chloroform and methanol mixture to afford fine needle shaped crystals of 9, m.p. 296-8°, $(\alpha)_D$ + 45° (lit.[1] m.p. 299-300° $(\alpha)_D$ + 49°).

Found : C, 76.86; H, 10.21%

Calculated for $C_{32}H_{50}O_4$: C, 77.06; H, 10.04%

I.R. (KBr) : 1780 (γ lactone), 1735 and 1240 cm^{-1} (acetate) band for vinylidene group was absent

C.D. : Negative lactone Cotton effect with a maximum at 216 mμ ($\Delta\epsilon$, -6.05).

L.A.H. reduction of 11 : Preparation of 20

The nor-keto lactone **11** (0.3 g.) was dissolved in dry tetrahydrofuran (80 ml) and to it LAH (1.2 g.) was added. The reaction mixture was then refluxed for 4 hours. Excess LAH was destroyed by ethyl acetate and a saturated aqueous solution of sodium sulphate was added dropwise until inorganic salts coagulated at which point anhydrous sodium sulfate was added. The supernatant solution was decanted and the residue was washed with fresh ether. The combined ether solutions were washed with water, dried evaporated and the crystalline residue was recrystallized from 95% ethanol to give the tetra-ol **20** m.p. 281-3° $(\alpha)_D$ + 20°.

Found: C, 75.58; H, 10.98%

Calculated for : $C_{29}H_{50}O_4$ C, 75.32; H, 10.82%

I.R. (Nujol) : 3420 cm^{-1} (hydroxyl)

U.V. (Ethanol) : Optically transparent in the region 220-300 mμ

Attempted glycol cleavage of 20 with sodium periodate

The tetraol **20** (150 mg) was dissolved in methanol (25 ml) and to it $NaIO_4$ solution (5 ml prepared from 1 gr. of $NaIO_4$ dissolved in 30 ml water), was added and the reaction mixture was kept at room temperature for 48 hours. After concentration on water bath it was diluted with water. The precipitated solid was collected by filtration and crystallised from 95% ethanol m.p. 281-2°.

It was found to be identical with the starting material (by m.m.p. and I.R. comparison).

Attempted glycol cleagave of tetra-ol-20 with lead tetraacetate at room temperature

A solution of the tetra-ol **20** (200 mg) in glacial acetic acid (10 ml) was added to a solution of Pb $(OAc)_4$ (400 mg) in acetic acid (10 ml). The reaction mixture was kept at room temperature for 24 hours. The excess Pb $(OAc)_4$ was destroyed by ethylene glycol (2 ml) and then diluted with water, extracted with ethyl acetate, washed with Na_2CO_3 solution and water until neutral. The solvent was removed and the solid thus obtained was recrystallised from 95% ethanol to afford crystals m.p. 289-2°.

It was found to be identical with the starting material (m.m.p. and I.R. comparison).

Glycol cleavage of tetra-ol 20 by lead tetra acetate oxidation at 120° : Preparation of tris nor-ketone 21

A solution of the tetra-ol **20** (400 mg) in glacial acetic acid (20 ml) was added to a solution of Pb $(OAc)_4$ (1 gr.) in acetic acid (25 ml) and the reaction mixture was kept at 120°C for 3 hours. Excess Pb $(OAc)_4$ was destroyed by adding ethylene glycol (2 ml). It was diluted with water, extracted with chloroform, washed with Na_2CO_3 solution and then with water until neutral. ~~Remove of the~~ Gummy solid, (280 mg) thus obtained, by evaporating the solvent was

hydrolysed with methanolic alkali (10%) for 3 hours. After concentrating somewhat the reaction mixture was diluted with water. The precipitated solid (220 mg) was collected by filtration. It was dissolved in benzene (10 ml) and poured on the column, prepared from alumina (20 gr.), deactivated by 0.6 ml of 10% aqueous acetic acid. The chromatogram was developed with petroleum ether and eluted with the following solvent (Table VII).

Table VII

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether (150 ml)	1-3	Nil
Petroleum ether:benzene (3:1) (150 ml)	4-6	Nil
Petroleum ether:benzene (2:2) (100 ml)	7-8	Nil
Petroleum ether:benzene (1:3) (100 ml)	9-10	Nil
Benzene (100 ml)	11-12	Nil
Benzene:chloroform (3:1) (100 ml)	13-14	Nil
Benzene:chloroform (2:2) (150 ml)	15-17	Solid

Elution with more polar solvent did not afford any solid.

The fractions 15-17 (table VII) were combined and crystallised from methanol to furnish crystals, m.p. 240-2°, $(\alpha)_D$ + 32°.

Found : C, 77.57; H, 10.51%

Calculated for $C_{27}H_{44}O_3$: C, 77.88; H, 10.57%

U.V. (Ethanol) : λ max 285 mμ (ϵ 75)

I.R. ($CHCl_3$) : 1740 (cyclopentanone) and 3310 cm^{-1} (hydroxy group).

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PART III

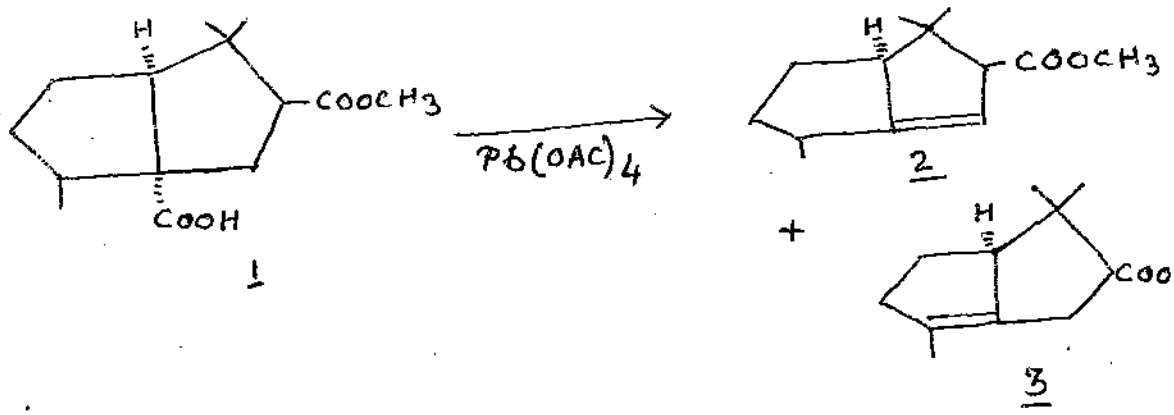
Lead tetraacetate oxidation of 3β-acetoxy betulanic acid

Chapter I

A short Review on Pb (IV) acetate oxidation on some tertiary acids in terpene series

A. Lead tetraacetate oxidation of nor-cedrene dicarboxylic acid monomethyl ester and nor patchoulidicarboxylic acid monomethyl ester

During their work on elucidation of the structure of patchouli alcohol and absolute configuration of cedrene, Buchi et al.[1] carried out Pb (IV) acetate oxidation on nor-cedrene dicarboxylic acid monomethyl ester 1 in benzene solution with approximately two equivalents of $Pb(OAc)_4$ and obtained two isomeric unsaturated esters, separable by gas chromatography. One of these 2 had I.R. (CCl_4) bands at 1732, 1663 and 846 cm^{-1} and NMR peaks at 8.95 (3H), 8.85 (3H) ($C\underline{H}_3$-C-$C\underline{H}_3$), a doublet at 8.78 (J=8 cps, $C\underline{H}_3$-C-H), 6.16 (3H, $COOC\underline{H}_3$) and 4.54 τ (broad, 1H, C=C$\langle^{H}$). The second isomer 3, was not an $\alpha\beta$-unsaturated ester and showed NMR signals at 9.17(3H), 9.07(3H) (CH_3-C-$C\underline{H}_3$), 8.37 (3H, $C\underline{H}_3$-C=C) and 6.40 τ (3H, $COOC\underline{H}_3$). When they applied the same reaction on nor-patchoulidicarboxylic acid mono-methyl ester 4, four products were obtained which were separated by gas chromatography.



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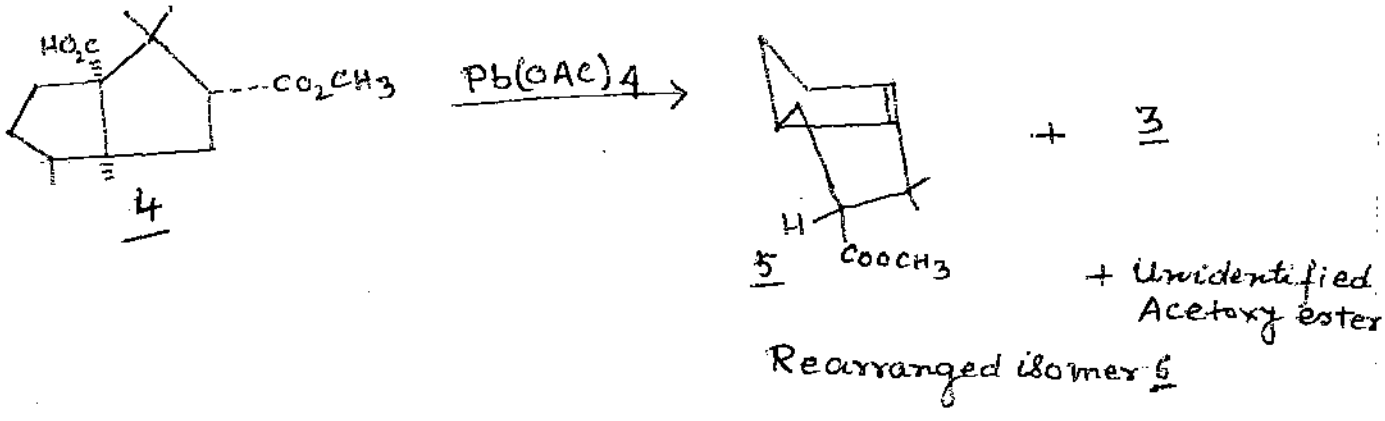
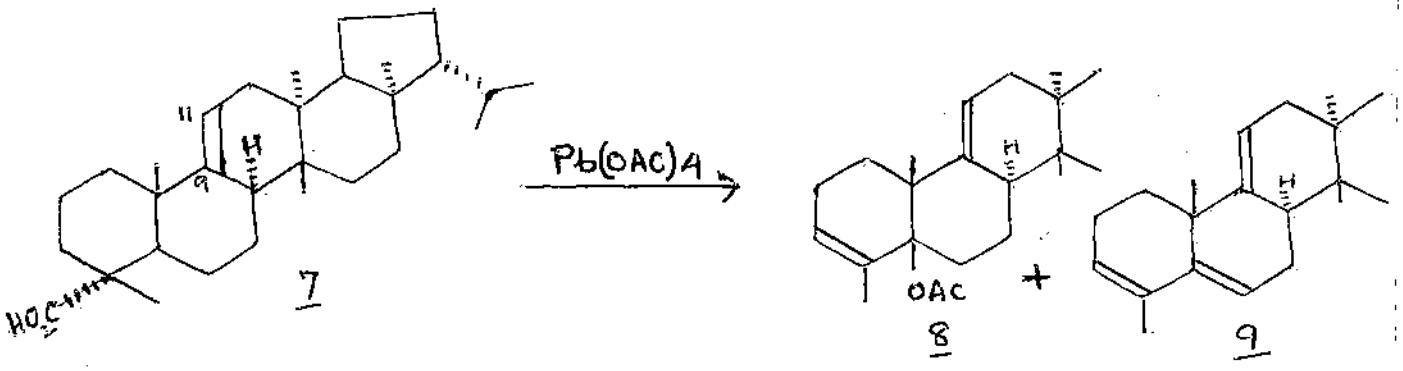


The structure of the product 5 was elucidated by I.R. and NMR spectra. One of the products was identified as 3. The third isomer 6 seemed to be the result of a rearrangement because its NMR spectrum did not show gem-dimethyl system. The fourth product was an unidentified acetoxy ester. Its I.R. spectrum showed bands at 1740, 1443, 1275, 1683, 2880 and 2960 cm^{-1} and NMR signals at 9.16, 4.97, 6.35, 8.78, 8.93 and 9.04 τ.

B. Lead tetraacetate oxidation of davallic acid

While working on the constitution of the triterpene acid, davallic acid 7, Nakanishi and coworkers[2], subjected the acid to Pb (IV) acetate decarboxylation. The products were (i) acetoxy nordavalladiene 8, m.p. 195°, IR ν max 1740 cm^{-1}, NMR peaks at 1.54 (C=C-Me), 1.97 (-O.CO.CH_3), 5.33 ppm (diffuse) and (ii) triene 9 m.p. 165°, λ max 233, 241, 250 mμ (2000, 2200, 1400), NMR 1.54

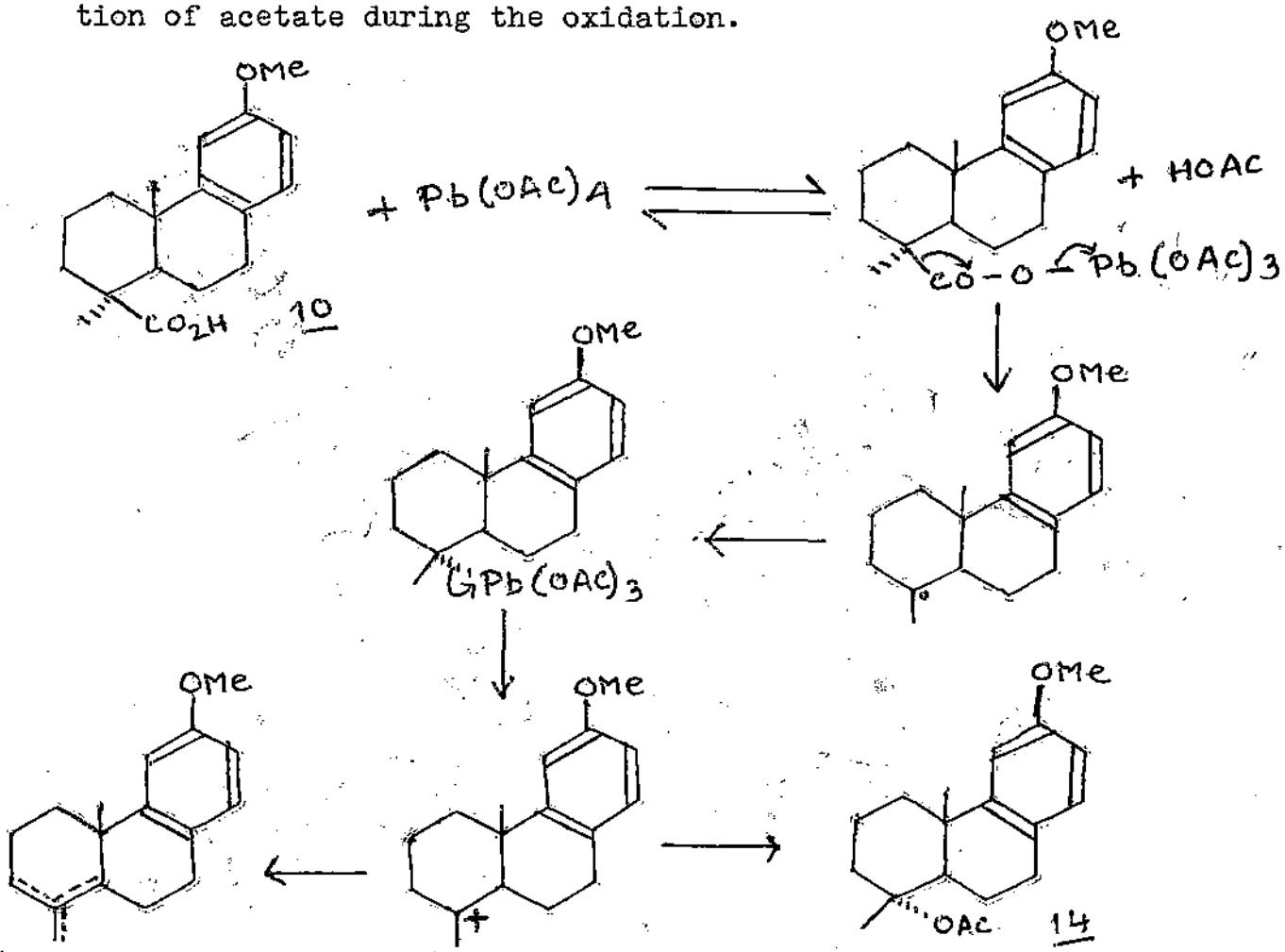
(=C-Me), 5.36 (diffuse) ppm. The triene 9 was also produced by treatment of 8 with dry HCl in chloroform solution. The low λ max intensity of the triene is caused by contamination (V.P.C.) but suffice to indicate the presence of a conjugated diene and not a triene system and suggests that the original double bond is located at 9-11 position rather than at Δ^7-position in davallic acid.

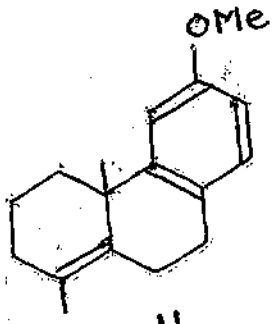


C. Lead tetraacetate oxidation of O-methyl podocarpic acid

Bennet and Cambie[3] carried out Pb(IV) acetate oxidation of O-methyl podocarpic acid 10 and the products were subjected to chromatography. The least polar material (76%) was a mixture of three isomeric alkenes $C_{17}H_{22}O$, in which GLC and NMR spectrum indicated the presence of 63% of the $\Delta^{4(5)}$ isomer 11, 28% of the $\Delta^{4(15)}$ isomer 12 and 9% of the Δ^3-isomer 13. The ratio and identification of the products was confirmed by subsequent isolation of pure samples of the alkenes 11 and 12 from other reactions. Next a liquid compound, $C_{19}H_{26}O_3$, was isolated from the chromatogram, which has been assigned structure 14. Its IR spectrum showed bands

at 1735 and 1255 cm^{-1} and NMR spectrum exhibited features expected for structure 14. Hydrolysis of 14 gave an alcohol having IR peak at 3560 cm^{-1} and 1125 cm^{-1} attributed to tertiary OH stretching. A strongly deshielded C-10 methyl signal at δ1.47 suggested α-configuration of the C_4-acetoxyl group. In the NMR spectrum of the corresponding alcohol the signal for this methyl group appeared in the normal position at δ1.15. A paramagnetic shift of 19 cps on acetylation of the alcohol is in agreement with the equatorial and hence α-configuration of the alcohol. This assignment is also in agreement with the mechanistic considerations regarding the formation of acetate during the oxidation.





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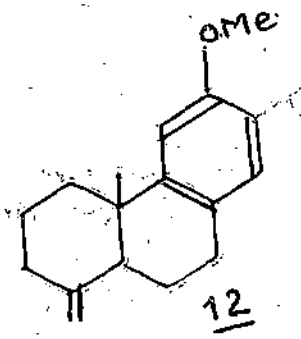

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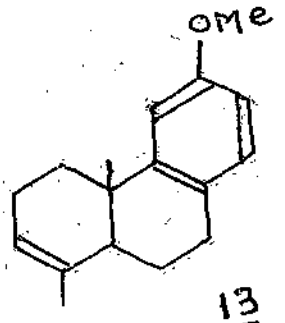

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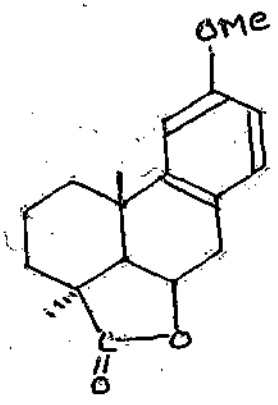

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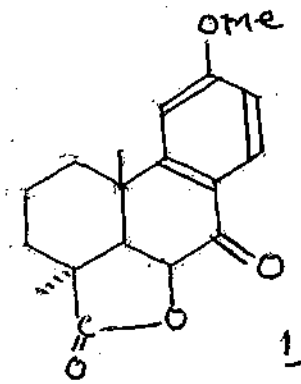
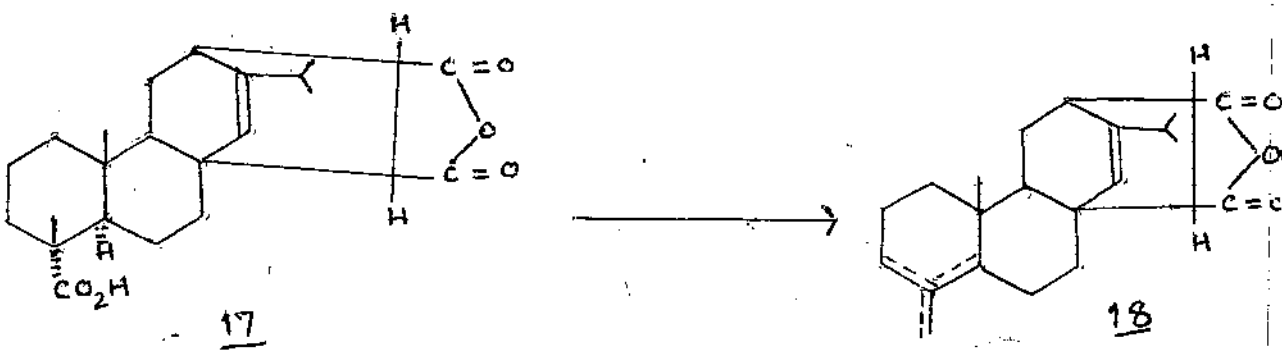


16

The other two products which were isolated by these authors are represented by structures 15 and 16. The mechanism for the formation of alkene mixtures and the acetate 14, as envisaged by Cambie et al.[3] has been shown in chart above.

D. The oxidative decarboxylation of maleopimaric acid and fumaropimaric acid by Pb(IV) acetate

Maleopimaric acid 17 on treatment with lead tetraacetate[4] in pyridine at 50° gave the diene mixture 18, NMR spectra and VPC analysis of which indicated the presence of 50% of the $\Delta^{4(18)}$ isomer 19, 35% of the $\Delta^{4(5)}$ isomer 20 and 15% of the Δ^{3}-isomer 21. The diene readily absorbed a molar equivalent of hydrogen to afford



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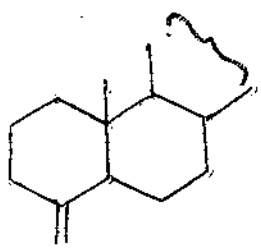

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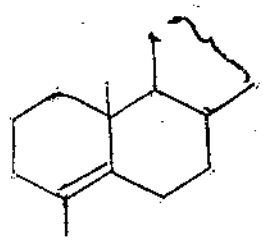

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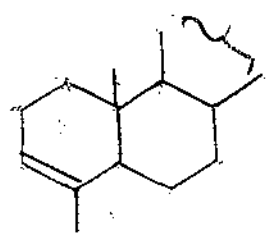
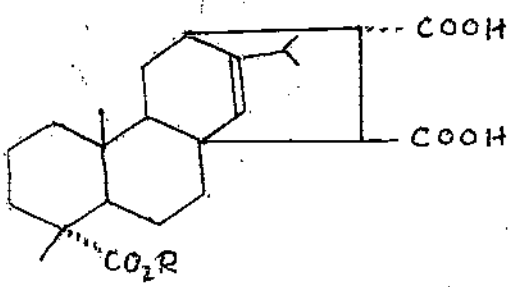


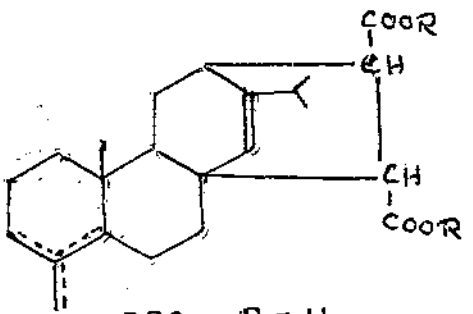
21

the Δ^{13}-compound.

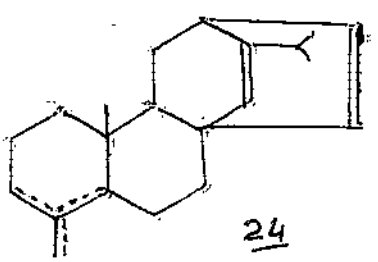
Under similar oxidation conditions fumaropimaric acid 22a, gave 23a as the major product along with some of the triene 24



22a, R = H
22b, R = CH_3



23a, R = H
23b, R = CH_3



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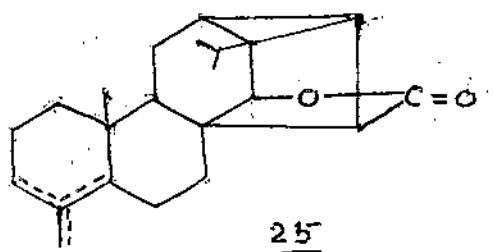
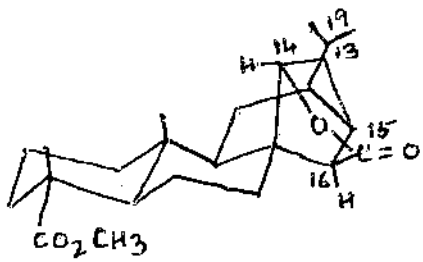


25

When compound 23a was resubmitted to oxidative decarboxylation, the triene 24 was obtained in 25% yield and a lactone 25 was obtained in 65% yield. The reaction of methyl fumaropimarate with lead tetraacetate gave unexpected products. The major crystalline compound has been assigned structure 26, ν max 1730 (ester carbonyl), 1775 cm^{-1} (γ-lactone). NMR spectrum of the compound exhibited a signal at τ 5.5 for C-14 proton but showed absence of olefinic protons, the isopropyl methyl groups appeared in the region τ 8.90-9.10 showing the absence of 13(19) double bond.



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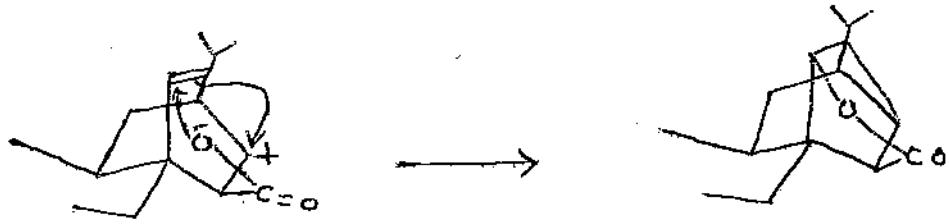
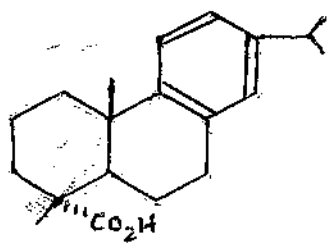
The formation of the olefins (A-ring) is consistent with the generation of a carbonium ion at C-4 and the rearrangement required in the formation of the compounds 25 and 26 is particularly suggestive of a carbonium ion mechanism and could arise as shown below.



E. Lead tetraacetate oxidation of dehydroabietic acid

With a view to preparing the ketone 28, Huffman and coworkers[5] carried out Pb(IV) acetate oxidation on dehydroabietic acid 27 in benzene solution in presence of pyridine under nitrogen and claimed to have isolated 29, Δ^1-exo-dehydroabietene in 80% yield.



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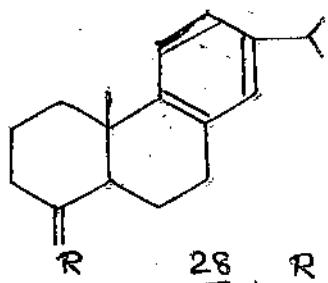
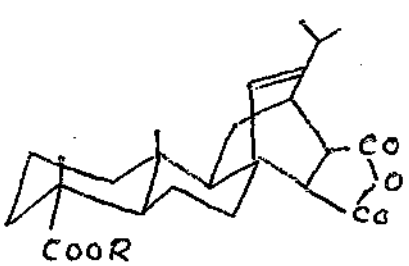


28, R = O

29, R = CH_2

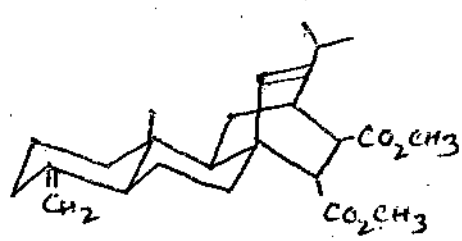
Lead tetraacetate oxidation of maleopimaric acid and methyl fumaropimarate:

While working on the stereochemistry of maleo pimaric acid and the longe range shielding effect of the olefinic bond Ayer et al.[6] carried out Pb (IV) acetate oxidation of maleo pimaric acid 30 in pyridine. Compounds 31 and 32 resulted from this oxidation followed by (in the case of 31) methanolysis and esterification with diazomethane. The configuration of C-1 in 32 rests on the observation that the C-17 protons are not shifted relative to methyl maleopimarate 33. If the acetoxy group were β, a downfield shift of the C-17 protons would be expected.



30, R = H

33, R = CH_3



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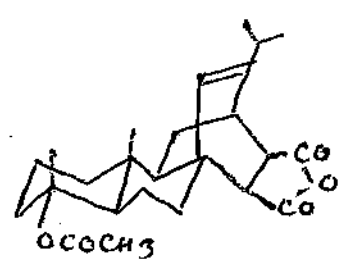
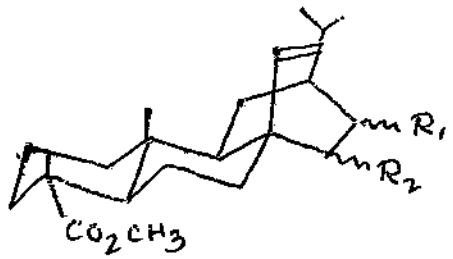
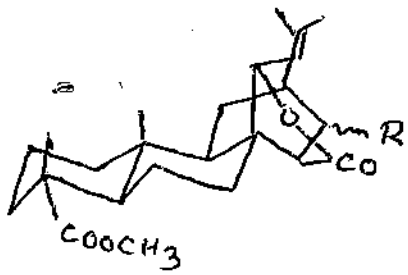


32

The compound 34 (prepared by the addition of fumaric acid to methyl abietate) with Pb (IV) acetate in pyridine yielded the γ-lactone 35. A second product of this oxidation was the $\Delta^{21,22}$ compound corresponding to 36 which on hydrogenation over Pt yielded 36.



34 , R_1 = exo-COOH
R_2 = endo-COOH



35 , R = exo-COOH

36 , $R_1 = R_2 = H$

PART III

CHAPTER II

Mechanism of Pb(IV) acetate Decarboxylation of Acids

Mechanism of Pb^{IV} acetate Decarboxylation

Lead tetraacetate has been used for the oxidative decarboxylation of aliphatic carboxylic acids[1-6] and bis-decarboxylation of 1,2-dicarboxylic acids[7,8]. Lead (IV) carboxylate is converted to lead (II), carbondioxide and products of oxidation of the alkyl moiety. The relative ease of decarboxylation of various carboxylic acids, represented by R-COOH, and isolation of products characteristic of a carbonium intermediate derived from R, led Mosher and Kehr[9] to formulate a direct 2-equivalent process. Direct formation of Pb (II) salt and an acyloxonium intermediate 36a, which underwent subsequent decarboxylation, was postulated.

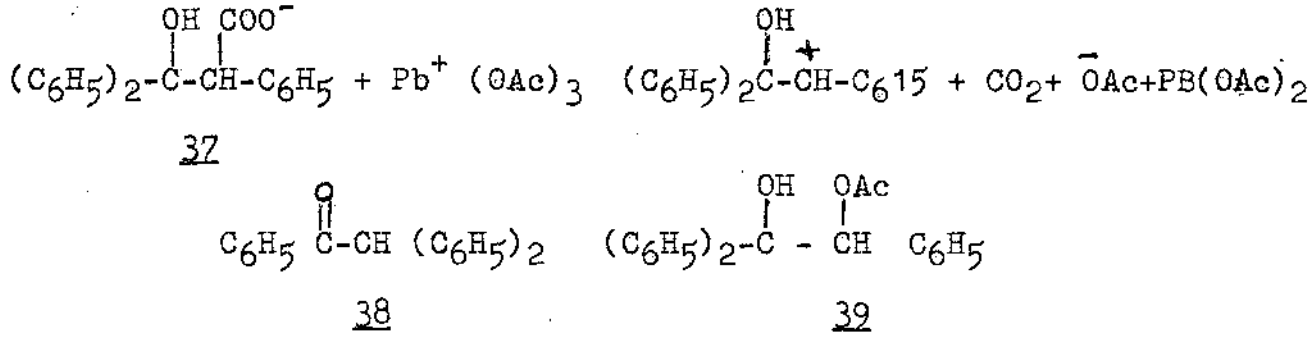
$$R.CO_2Pb^{IV} \longrightarrow RCO_2^{+} + Pb^{II} \quad .....$$

36a

They adduced no evidence for free radicals and attributed the formation of alkanes to hydride transfer processes.

Kharash and coworkers[10] and Benson et al.[11] studied the decomposition of lead tetraacetate itself in acetic acid and found that the reaction was accelerated by sodium acetate. Both investigators concluded that the decomposition proceeded via 1-equiv. changes. However, it is important to note that the reaction catalysed by sodium acetate gave no conspicuous amounts of carbondioxide or methane. Also, in the above reaction, no methyl acetate, the product expected for an ionic pathway, could be detected. This

datum raised the question of whether in general the formation of carboxylic ester from the radical pair, $R(CO_2)_3Pb.\ RCOO.$ can occur competitively. The study of oxidative decarboxylation of aliphatic acids by lead (IV) acetate leading to alkenes, alkylacetates and related products indicated that the reaction involves carbonium ion intermediates (or a transition state of cationic character). Similarly, the facile oxidation of some acids, particularly formic acid[9,12], is probably a direct 2-equiv. change from Pb(IV) to Pb(II) which does not involve intermediate radicals. Corey and Casanova[13] studied the Pb(IV) oxidative decarboxylation of α,β,β-triphenyl-β-hydroxy propionic acid 37 and isolated the rearranged benzhydryl phenyl ketone 38 as the major product (71% in benzene and 76% in acetonitrile) along with the acetate 39 in much smaller amounts (14% and 18% respectively).



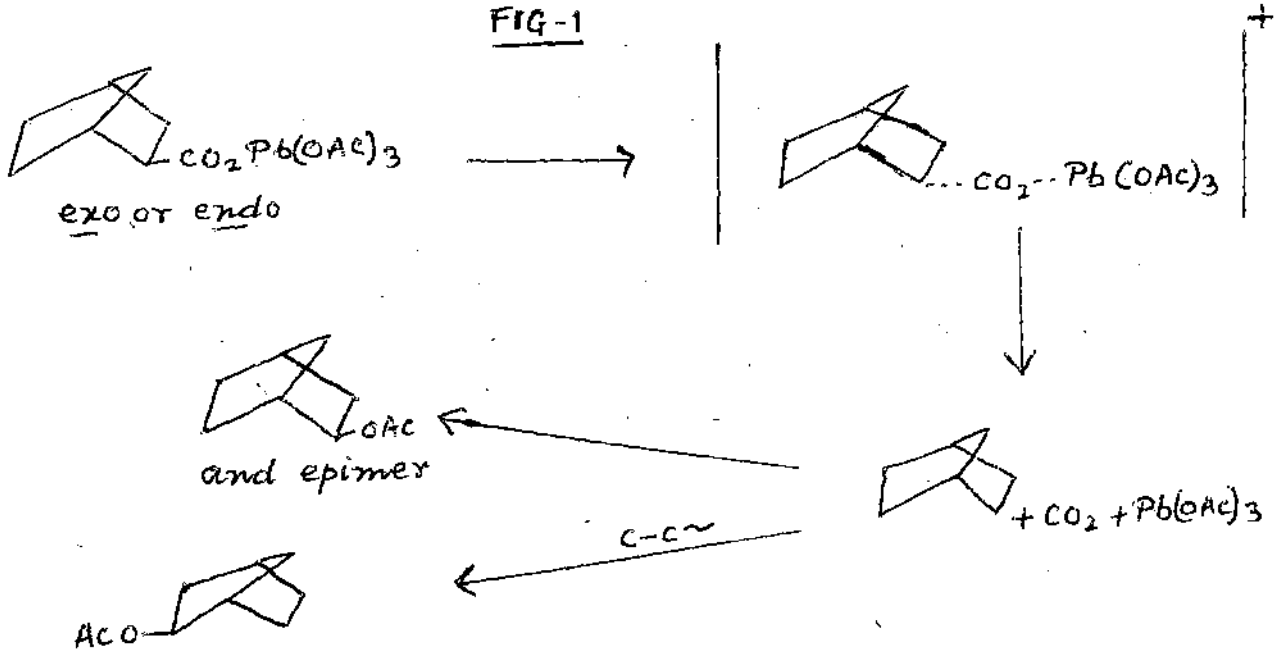
These data indicate a cationic intermediate for the ketone 38. That this cation is also a precursor of the acetate 39 follows from the relatively small influence of solvent polarity on product distribution. Corey[13] also studied Pb (IV) acetate decarboxylation in the norbonyl series and Buchi and coworkers in the cyclobutyl series.

Both endo- and exo- norbornane-2-carboxylic acids underwent smooth conversion to predominantly exo-norbornyl acetate (24-67% yield) upon treatment with lead-tetra acetate-pyridine in benzene. The acetate from the exo-acid was essentially pure exo-isomer uncontaminated by appreciable amounts of endo-acetate, whereas the product from the endo-acid consisted of Ca. 97% exo- and 3% endo-acetates. When either the optically active exo- or endo-acid was subjected to oxidative decarboxylation in benzene solution, the exo-acetate which resulted was optically active with a rotation corresponding in each to 43% net retention of optical purity. In acetonitrile as solvent, optically active exo- and endo- norbornane-2-carboxylic acids again afforded endo-acetate each with the same net retention of optical purity, 33%, somewhat less than observed for benzene as solvent. The invariance of the optical result with both isomers and in the two solvents of widely different polarity provided a good case for offering a plausible mechanistic hypothesis.

Since the major product of reaction, was exo-norbornylacetate, it was suggested that, it must come from a carbonium ion precursor.[14] It is also known that the 2-norbornyl radical is not subject to carbon rearrangement in the temperature range[15] of the reactions studied (80°C). As regards the occurrence of the optically active product two possibilities have been suggested:

(1) both rearranged and non-rearranged acetate are derived from the classical norbornyl cation-OAc^- (or $Pb(OAc)_3^-$) ion-pair in a close competition between rearrangement and ionpair collapse as shown in Fig. 1.

FIG-1



and (2) rearrangement occurs via a cation and non-rearrangement via the nor-bornyl radical. The latter explanation was disfavored as the solvent effects referred to above (benzene and acetonitrile) were considered not compatible with the assumption of competing processes, one of which does and the other does not involve charge separation. In their view, all the norbornyl acetate is formed from the cation and in the case of the optically active acetate the precursor must be the classical cation. Further, the evidence for a common behavior of exo- and endo - acids was also put as an argument for the intervention of classical ion as a precursor for rearranged acetate as well. However, they also suggested that it was also not improbable that the mechanism might be represented as homolytic decarboxylation followed by very fast electron transfer from the norbonyl radical to $Pb(OAc)_3$.

Pb(IV) acetate oxidative decarboxylation of cyclobutane carboxylic acids by Buchi and coworkers gave an acetate mixture of cyclobutyl, cyclopropyl carbinyl and allyl carbinyl acetates which showed a close similarity with the product mixture previously obtained by sulfonate solvolysis and deamination[12]. This again points to a cationic intermediate in the process. The conversion of α-β-diphenyl glutaric acid[16] (either diastereoiosmer) to β-diphenyl-γ-butyrolactone is also interpreted in terms of the cationic pathway. Kochi[17] studied the oxidative decarboxylations of pentanoic acids viz. n-valeric, isovaleric and 2-methyl butyric acids with lead tetraacetate in benzene solutions at 81° and proposed a free radical chain mechanism which includes butyl radicals as transient and Pb(III) as metastable intermediates. On the basis of the rate studies, catalysis by copper salts, inhibition of the reaction by oxygen and product analysis, they concluded that the Pb(III) is implicated in the following manner.

Pre-equilibration

$$Pb^{IV}(OAc)_4 + nR\,CO_2H \rightleftharpoons Pb^{IV}(OAc)_{4-n}(O_2CR) + nHOAc$$

$$\underline{40}$$

$$\underline{40} + mPy \rightleftharpoons Pb^{IV}(OAc)_{4-n}(O_2CR)_n\,Pym$$

$$\underline{41}$$

(Rate is enhanced by pyridine or valeryl peroxide)

$$n = 1,2,3,4$$

Initiation

$$(40,\ 41) \longrightarrow Pb^{III} + R. + CO_2$$
$$42$$

Propagation

$$RCOO\ Pb^{III} \longrightarrow Pb^{II} + R. + CO_2$$
$$43$$
$$R + Pb^{III} \longrightarrow R^+ \text{ (alkene, ester)} + Pb^{III} \text{ etc.}$$
$$44$$

Termination

$$R. + Pb^{III} \longrightarrow R^+ \text{ (alkene, ester)} + Pb^{II}$$
$$45$$
$$R.\ \text{(Radical reactions)} \xrightarrow{HS} RH + S.\ \text{(disprop, recomb)}$$
$$46$$

(HS = solvent)

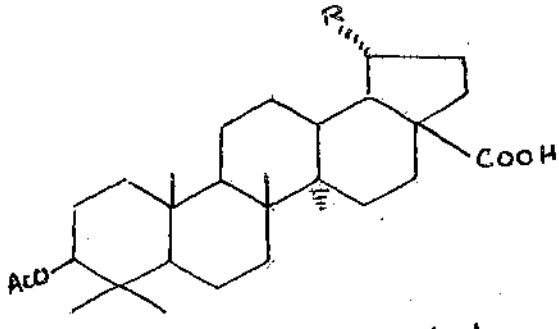
The general concept of oxidation of a free radical by a metal salt has ample chemical support[17]. Butenes and butyl esters derived from the pentanoic acids are products of oxidation and butanes are products of reduction. The author believes that butane arises from butyl radicals by either direct chain transfer from hydrogen donors (step 46) or reduction to carbanionic intermediates followed by protonation or both.

Thus proposals that have been made for the mechanisms of the oxidative decarboxylation of acids include both radical and ionic schemes. Although it seems highly probable that carbonium ions may be involved in the final stage of the reaction[8], the possibility

that these species could have been formed by oxidation of radical precursors cannot be ignored. Thus the fundamental question of whether the initial oxidative attack is a homolytic or heterolytic process has still remain unanswered.

The products derived from the related oxidative cleavage and cyclisation[18,19] of alcohols by Pb (IV) acetate are symptomatic of free radicals as intermediates. Cyclic ethers formed in a number of cases have been interpreted[20] as arising via free alkoxy radicals: these are known to undergo intramolecular[21,22] 1,5-hydrogen abstraction. Fragmentation and cyclisation are characteristic of alkoxy radicals. However, similar behavior patterns has also been postulated via heterolytic oxonium routes[23].

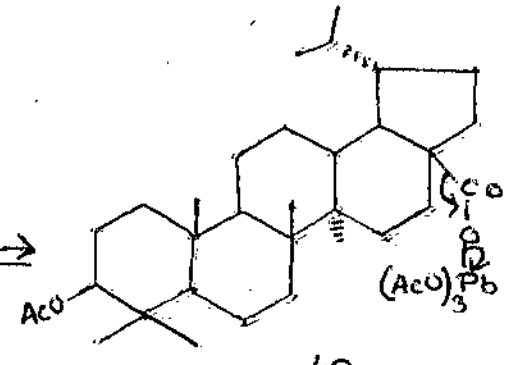
Though the present work adds no further evidence, similar initiation and termination steps can be rationalised for the formation of alkene mixtures 52a and the acetate 53a during the oxidation of 3β-acetoxy betulanic acid 47b, according to the following scheme.



47a, R = isopropenyl

47b, R = isopropyl

47b ⟶ + $Pb(OAc)_4$



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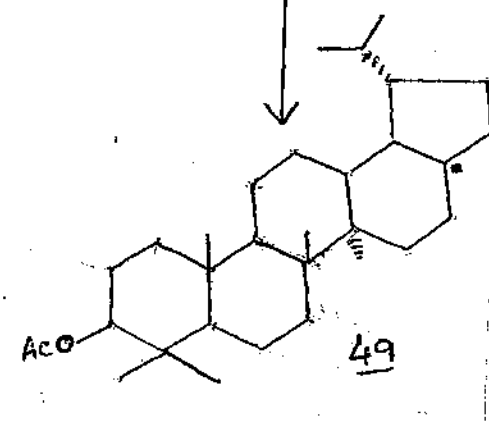

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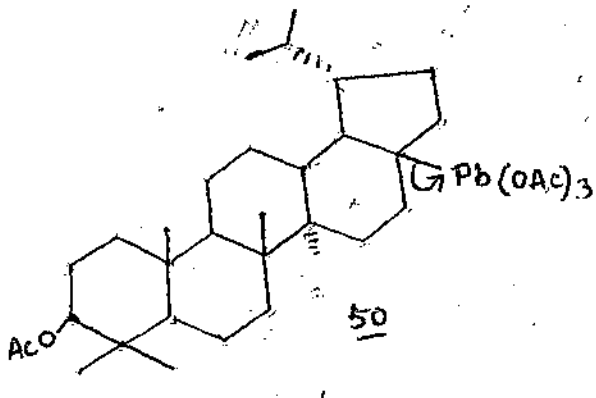

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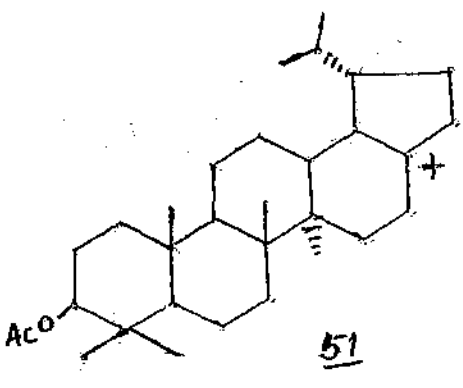
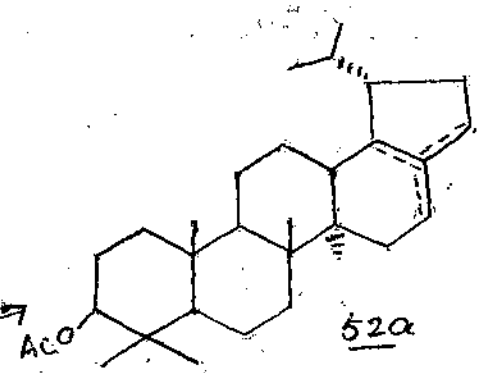
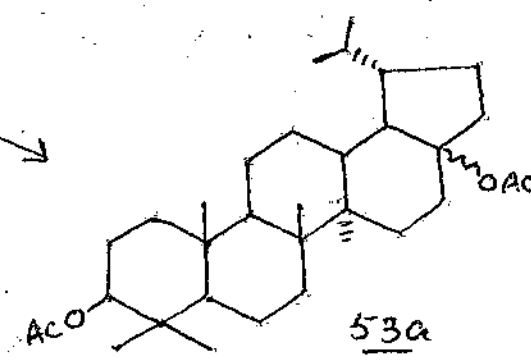


51



52a

^{-}OAc



53a

Homolytic fission of the ester 48 would give the alkyl radical 49, carbondioxide and the bulky lead triacetate radical, attack by which on 49 from the less hindered β-face would furnish the unstable organo-lead compound 50. Fragmentation of the latter or of the radical 49 by oxidation would then yield the tertiary carbonium ion 51, lead diacetate and acetate ion. Elimination of proton from either C-16, C-18 or C-22 would then give rise to the alkene mixture 52a while attack by acetate anion would then afford the tertiary acetate 53a. Since attack generally takes place from less hindered face, in the present case it is expected that the stereochemistry of C-17 acetate group would probably be β; attack of acetate anion being favoured from the less hindered β face whereby the generating acetate will experience less steric interaction with bulky C-19 α-isopropyl group. However, studies to establish the stereochemistry of C-17 acetate is under way.

CHAPTER III

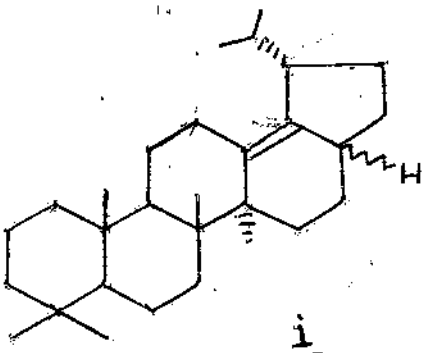
Oxidation of acetyl betulanic acid by lead tetra acetate

Introduction

Literature is rapidly growing on the oxidative decarboxylation of saturated acids with Pb(IV) acetate which give rise to olefin or olefin mixtures accompanied by, very often, an acetate (ester). The unsaturation and acetoxylation generally takes place at the position where carboxyl group is attached.

But very few studies have been concerned with the oxidative decarboxylation of tertiary acids in general[1-6] and even less are oxidation of tertiary acids of triterpene series.[2]

With a view to get further insight into the oxidation produets as well as to prepare the hydrocarbon (i) in which the isopropenyl substituent at C-19 will retain the same stereochemistry as in the parent lupeol-betulinic acid series, the oxidative decarboxylation of 3β-acetoxy betulanic acid was undertaken. A survey of literature showed that this type of oxidation with Pb(IV) acetate has not been attempted on saturated triterpene acids where the tertiary carboxyl group is situated at the ring juncture (in this case at C-17).



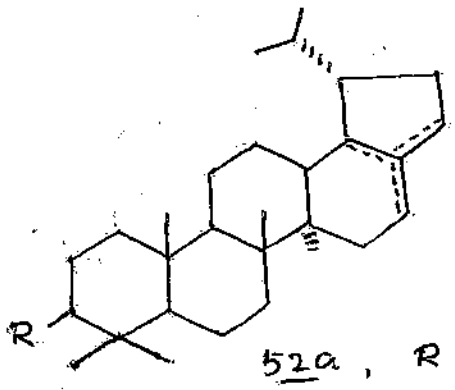
i

$Pb(OAc)_4$ oxidation of 38-acetoxy betulanic acid

Betulinic acid was acetylated with acetic anhydride and pyridine in the usual manner to afford acetyl betulinic acid[47a] m.p. 288-90°, $(\alpha)_D$ + 18.5° (lit.[24] m.p. 290-291°, $(\alpha)_D$ + 20°). The latter on hydrogenation in the presence of PtO_2 catalyst in acetic acid-ethyl acetate solvent mixture at room temperature afforded acetyl betulanic acid 47b, m.p. 304-5°, (lit.[25] m.p. 311-12°). It developed no coloration with tetranitromethane and did not consume perbenzoic acid even after 48 hours. The I.R. spectrum of 47b showed absorption peaks at 1700 (carboxyl) 1730, 1242 cm^{-1} (acetate) and was optically transparent in the U.V. region 220 to 300 mμ.

A mixture of acetyl betulanic acid, 47b, dry benzene, pyridine and lead tetra-acetate was heated under nitrogen atmosphere for three hours, according to the method of Cambie et al.[3] A vigorous evolution of carbon dioxide occurred and two solid products were isolated after chromatography on alumina. The less polar material (60% yield) recovered from the column with petroleum analysed for $C_{31}H_{50}O_2$, (M^+ 454), m.p. 170-6°, $(\alpha)_D$ + 28.27°. Its I.R. spectrum (Fig. 23) showed absorption peaks at 1728, 1246 (acetate) and at 900, 870 cm^{-1} due to tri-substituted double bond. The NMR spectrum showed several peaks for seven methyl groups at δ 0.78 to δ 0.98, a peak at δ 1.98 (3H, singlet) assigned to acetate methyl, and peak at δ 5.14 (multiplet) assigned to vinylic proton (trisubstituted double bond) and a peak at δ 4.4 (due to the proton

of the carbon bearing the acetate group). It gave an yellow colour with tetranitromethane. Perbenzoic acid titration indicated the presence of active unsaturation. TLC analysis showed two spots very close to each other (R_f values0.82 and0.81) in the solvent system benzene:methanol (4:1). Separation of the hydrocarbons by GLC using 2% SE-30 column at 250°C under N_2 was not very effective as will be evident from the Fig. 23G. Although GLC indicated that the sample was fairly pure, TLC showed that it was a mixture of two isomers. Attempts to separated the two by preparative TLC has as yet proved futile. On the basis of the consideration of the above data and the present knowledge about the mechanism[3-5] of lead tetraacetate oxidation, it has been assigned the structure depicted in 52a, a mixture of double bond isomers. This structure also explains the chemical reactions described below. Further work for the separation of the isomers is in progress.



52a, R = OAc

52b, R = OH

52c, R = O

52d, R = H

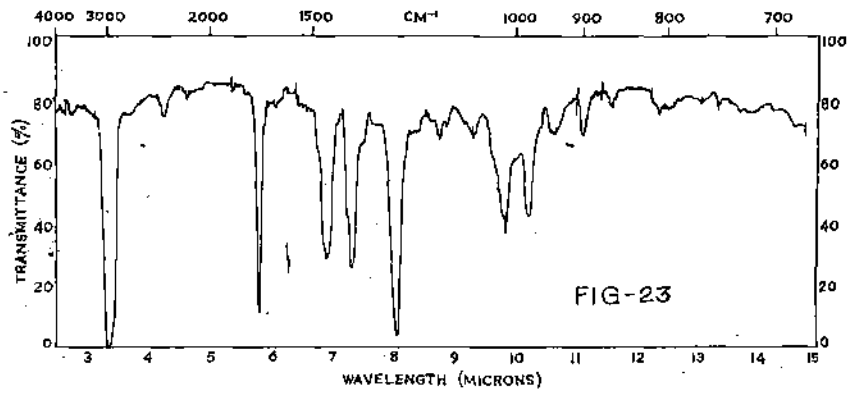


FIG-23

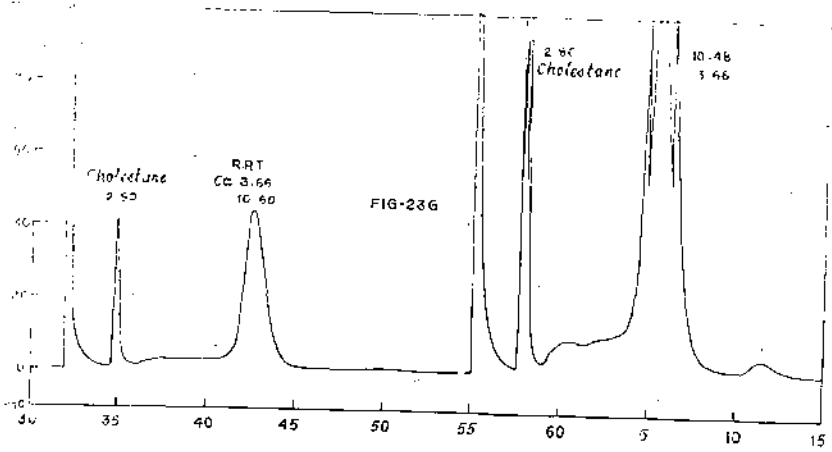


FIG-23G

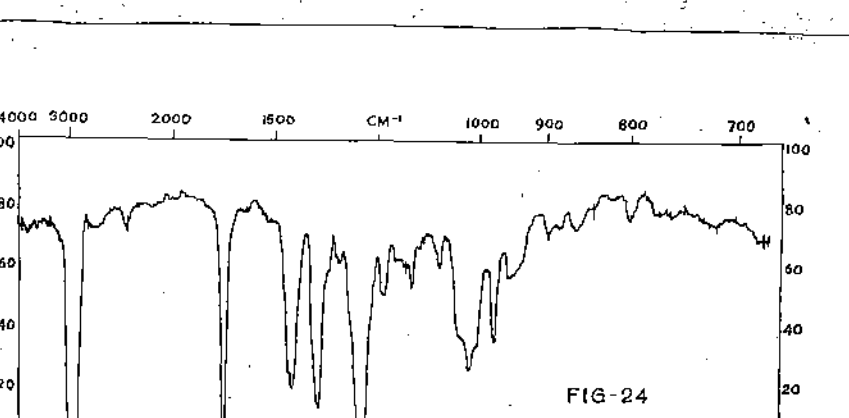
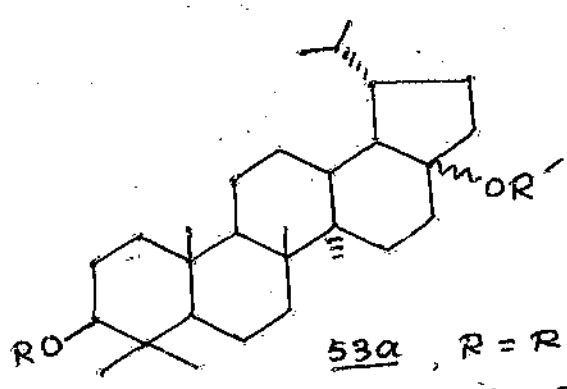


FIG-24

The more polar material (20% yield) eluted from the column with petroleum : benzene mixture (4:1) corresponded to the molecular formula $C_{33}H_{54}O_4$ (M^+ 514), m.p. 207-9°, $(\alpha)_D$ + 23.01°. In its I.R. spectrum (Fig. 24) it showed peaks at 1726, 1244 cm^{-1} (acetate) It did not develop any colour with tetranitromethane and did not consume ~~any~~ perbenzoic acid even after 48 hours showing the absence of any unsaturation. NMR spectrum, in addition to the peaks for CH_3-groups, also showed two peaks respectively at δ 2.1 (3H, singlet, -O.CO.C$\underline{H}_3$) and δ 1.96 (3H, singlet, -O.CO.C$\underline{H}_3$) and a peak at δ 4.4 (1H, multiplet, proton at the carbon bearing the acetate group). Since the acetate at C-3 would remain unchanged during Pb(IV) acetate oxidation, the peak at δ 4.4 (1H, multiplet) may be assigned to the axial proton at C-3 bearing the acetate group. Thus it may be inferred that the newly introduced acetate group is tertiary in nature having no proton adjacent to it. On the basis of the above argument and the physical evidences structure 53a has been tentatively assigned for it, which places the acetate group at C-17. This assignment is also in conformity with the mechanism of lead tetraacetate oxidation[3,4,5]. Mass spectral (Fig. 25) fragmentation of the diacetate 53a, showed peaks at m/e 514, 454, 411, 249, 189, which are in complete agreement with the structure proposed[26].



53a , R = R' = Ac

53b , R = R' = H

53c , R = Ac, R' = H

Although nothing specifically can be said regarding the stereochemistry of the C-17 acetate group, it appears that the acetate ion would probably attack from the less hindered β- face of the C-17 carbonium ion in the molecule to give the C-17 β-acetate. Work is in progress to settle this point.

The diacetate 53a on treatment with lithium aluminium hydride furnished the diol 53b, $C_{29}H_{50}O_2$, m.p. 230-2°, I.R. peaks (Fig. 26) at 3500 cm^{-1} (hydroxyl), the peak due to -O.CO.CH_3 present in the parent compound, was absent indicating that both the acetate groups have suffered reduction. Acetylation of the diol 53b with acetic anhydride and pyridine, gave the hydroxy monoacetate 53c, m.p. 270-1°, $C_{31}H_{52}O_3$. I.R. spectrum (Fig. 27) of the latter showed absorption peaks at 3600 (-OH), 1730 and 1245 cm^{-1} (-O-CO.CH_3) indicating that one of the hydroxy group had remained unaffec-

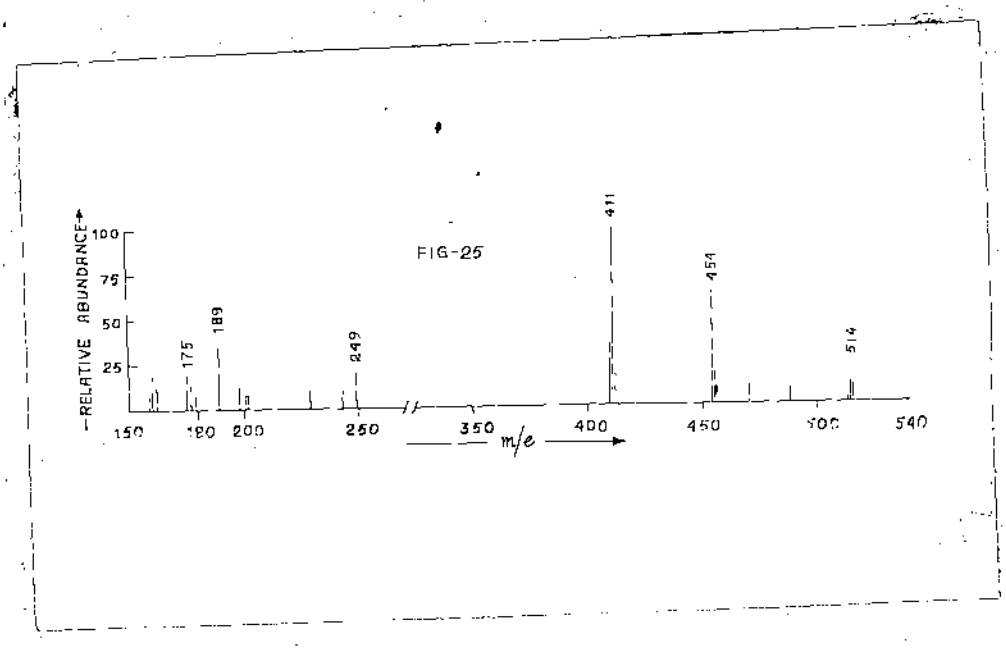


FIG-25

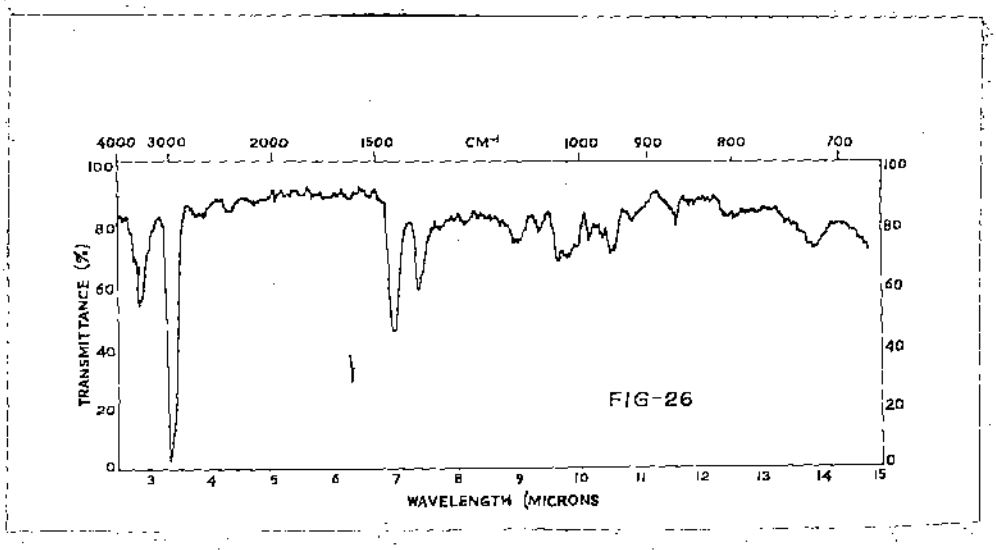


FIG-26

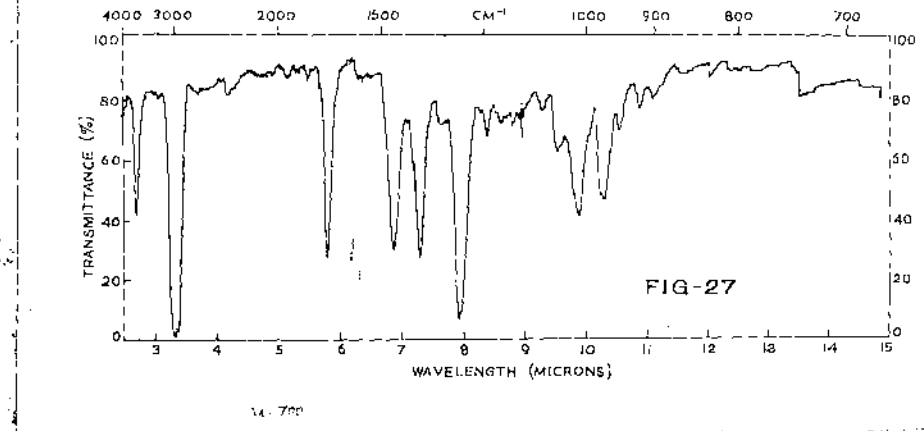


FIG-27

ted under the acetylation conditions. Since the hydroxyl group at C-3 would be easily acetylated under the conditions described, the hydroxyl group which survived acetylation must be either tertiary or a very hindered secondary one in nature. In order to clafify this point CrO_3-Py oxidation on 53C was attempted, but only the starting material could be recovered in good yield. In view of this failure of oxidation it can be inferred that non-acetylable hydroxyl group is tertiary in nature and is situated at C-17 as expressed in 53C.

Conversion of the olefen mixture 52a to the hydrocarbon mixture 52d

Hydrolysis of 52a with 5% methanolic sodium hydroxide solution for three hours gave the alcohol 52b, m.p. 110-4°, $(\alpha)_D$ +36.6°. Its analytical data corresponded to the molecular formula $C_{29}H_{48}O$. In I.R. spectrum it showed absorption at 3400 cm^{-1} assigned for the hydroxyl group and peaks at 890,870,815 cm^{-1} attributable to tri-substituted double bond. It developed a deep yellow colour with tetranitromethane. The alcohol 52b on CrO_3-Py oxidation yielded the ketone 52C, $C_{29}H_{46}O$, m.p. 145-50°, $(\alpha)_D$ + 48.19°. U.V. spectrum showed a peak at 285 mμ (ϵ 75) and I.R. spectrum showed peaks at 1705 cm^{-1} (six membered ring ketone) and at 887, 875 and 810 cm^{-1} indicating the presence of tri-substituted double bond. The ketone 52C on Huang-Menlon reduction afforded the hydrocarbon mixture 52d $C_{29}H_{48}$, m.p. 156-8°, $(\alpha)_D$ - 7.0°. All attempts to separate the constituents in a long active column resulted in the

isolation of the same hydrocarbon mixture. TLC analysis showed two spots very close to each other with the solvent system benzene: methanol (85:15). Attempts at separation of the mixture by preparative TLC have not yet been successful but further experiments are in progress to this end. I.R. spectrum (Fig. 28) of this mixture showed absorption peaks at 1680, 812, 900 and 800 cm^{-1} due to trisubstituted double bond. NMR spectrum (Fig. 29) showed peaks for seven methyl groups at δ0.75 to δ1.05 and a peak at δ5.2 (multiplet) which integrates for one proton due to tri substituted double bond ($C=C\overset{H}{<}$). The latter evidence shows the absence of tetrasubstituted double bond in the olefin mixture 52d and can now be represented by the structure 54.

Isomerisation of 54 to 55

The hydrocarbon mixture 54 was isomerised by 2N sulfuric acid in acetic acid exactly following the conditions described by Jeger[27] et al. to yield the desired hydrocarbon 55, $C_{29}H_{48}$ (M^+ 396) m.p. 192-3°, $(\alpha)_D$ + 67.2°. Its NMR spectrum (Fig. 30) showed peaks in the region δ0.62 to δ1.0 attributed to 7 methyl groups and the absence of any peak attributable to vinylic proton. I.R. spectrum (Fig. 8) showed absorption at 845, 1615-1625 cm^{-1} (broad). Homogeneity of the compound was checked by TLC analysis.

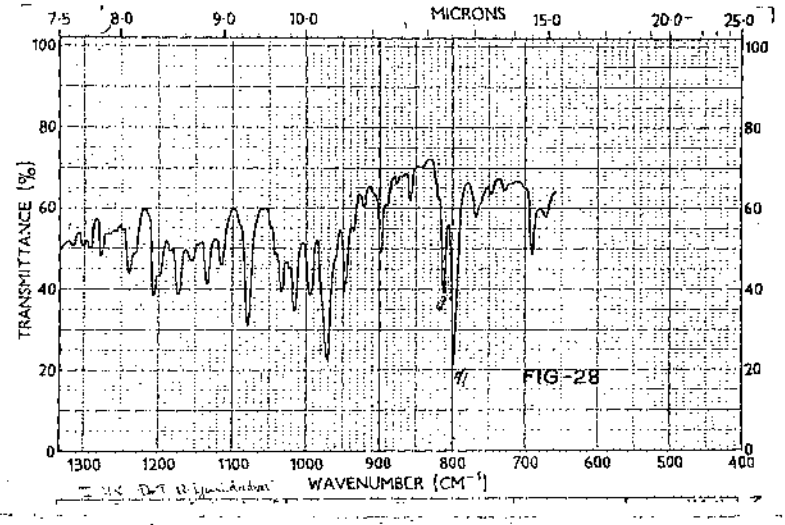


FIG-28

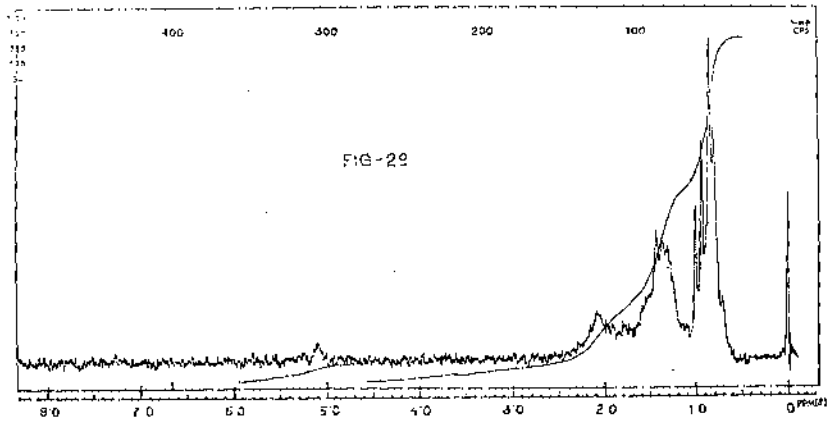


FIG-29

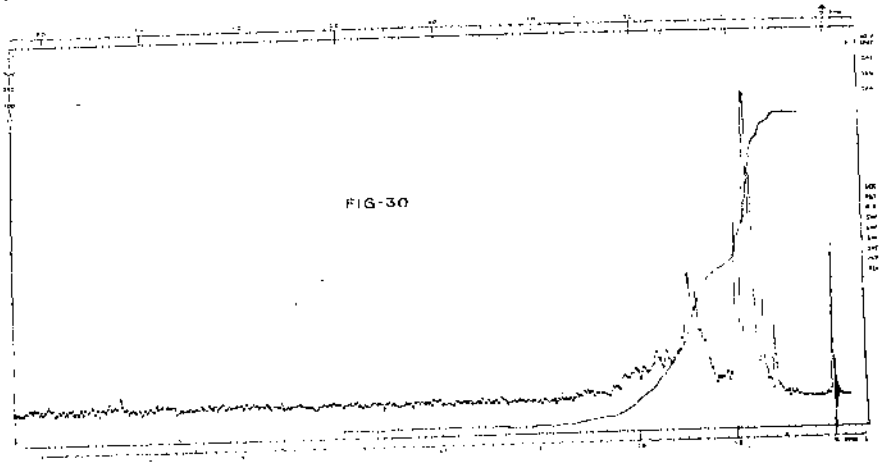
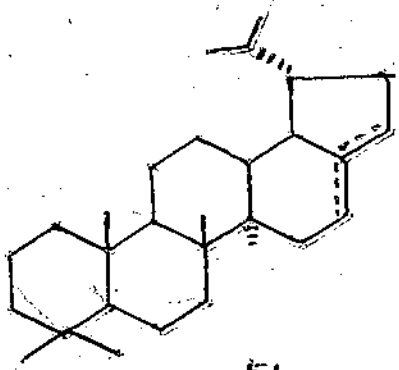
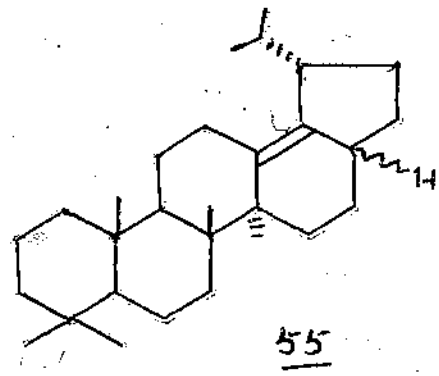


FIG-30



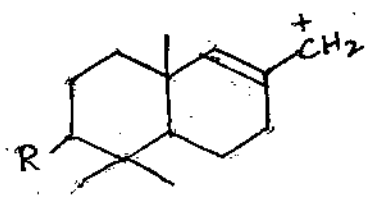
54



55

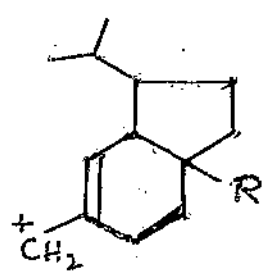
Application of mass spectrometry

Djerassi et al.[26] recorded the mass spectra of saturated lupane derivatives and observed that loss of methyl and isopropyl groups are very pronounced in certain members but becomes minimal in highly substituted derivatives. The most abundant fragment, however, corresponded to species 56 observed to some extent in the spectra of all pentacyclic triterpenes. Besides this, a peak corresponding to the species 57 is also observed in certain derivatives.



56a, R = OAc

56b, R = OH



57a, R = OAc

57b, R = OH

We have measured the spectra of the diacetate 53a and the diol 53b. The molecular ion peak in 53a (Fig. 25) appeared at m/e 514. The successive peaks at m/e 454 and m/e 411 corresponded to the species formed by the loss of acetic acid and acetic acid plus isopropyl groups respectively. This is consistent with the observations that angular substituents are readily lost. The species 56a appeared at m/e 249. Another peak at m/e 189 is formed by the loss of acetic acid from the fragment ion 56a. The peak at m/e 235 corresponding to the species 57a has not been observed but a peak at m/e 175 formed by the loss of acetic acid from fragment ion 57a has been observed.

The diol 53b exhibits the molecular ion peak (Fig. 31) at m/e 430 of very low intensity. The peaks at m/e 412 and m/e 369 are formed from the moleculerion by the loss of water and water plus isopropyl groups respectively and are quite pronounced. The peak corresponding to the species 56b appears at m/e 207. The peak at m/e 189 is formed from 56b by the loss of water. In this case the peak at m/e 193 corresponding to 57b is observed but with very low intensity; however, the peak at m/e 175 formed by the loss of water from 57b is much pronounced.

The mass spectrum (Fig. 32) of the nor-lup-13(18)-ene 55 has already been discussed in Chapter II page 29.

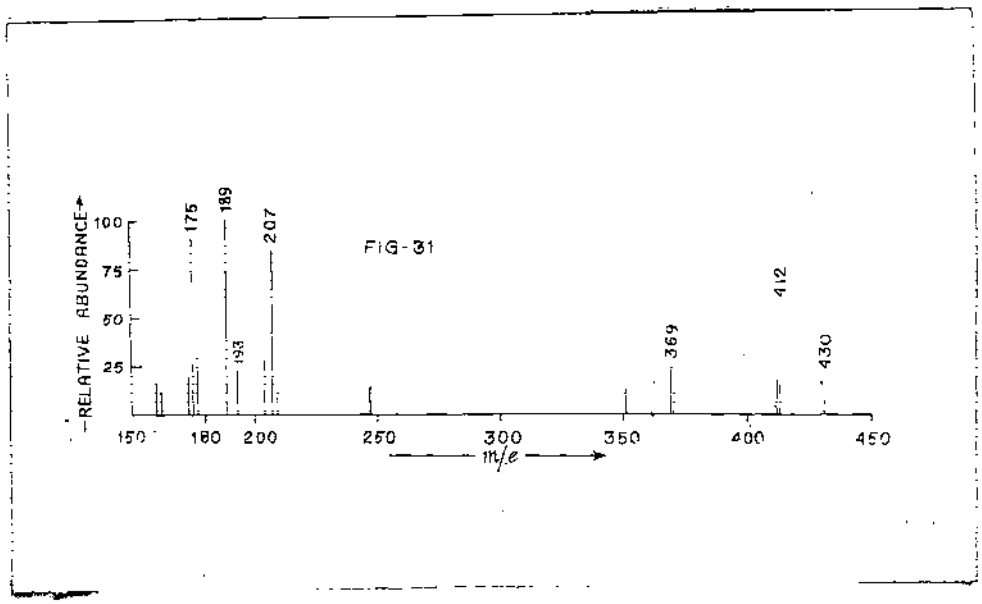

RELATIVE ABUNDANCE
100
75
50
25
175
189
193
207
369
412
430
150
180
200
250
300
350
400
450
m/e

FIG-31

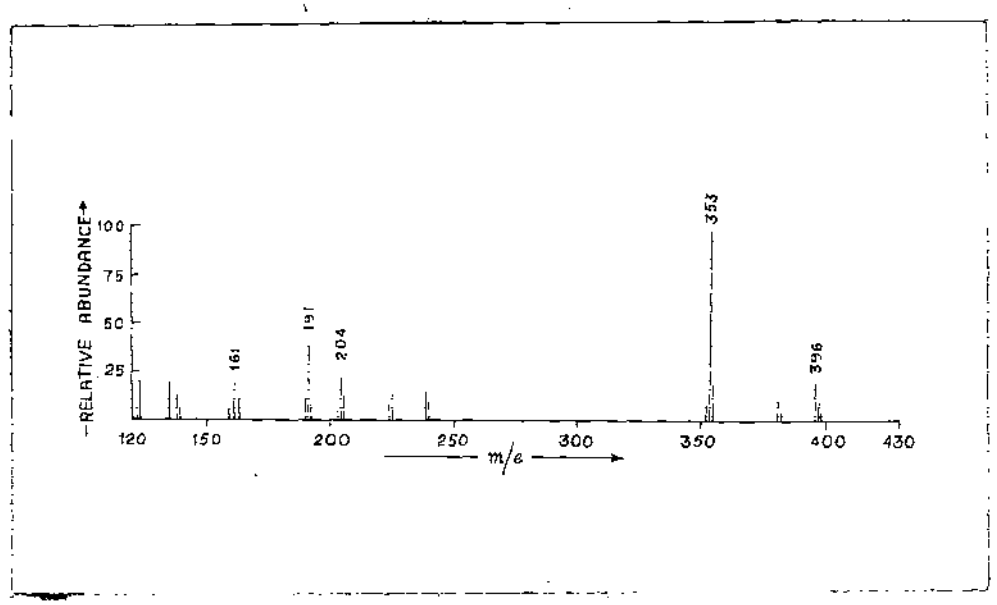

RELATIVE ABUNDANCE
100
75
50
25
161
191
204
353
396
120
150
200
250
300
350
400
430
m/e

Fig. 32

Chapter IV

Experimental

Preparation of acetyl betulinic acid 47a

A mixture of betulinic acid (12 g), pyridine (100 ml) and acetic anhydride (100 ml) was heated on the water bath for 3 hours. The reaction mixture was cooled and poured in ice cold water. The solid thus obtained, was collected by filtration and crystallised from a mixture of chloroform-methanol to give needle shaped crystals of acetyl betulinic acid 47a, m.p. 288-90°, $(\alpha)_D$ + 18.5° (lit. m.p. 290-2° $(\alpha)_D$ + 20.1°).

Found :	C, 76.81; H, 9.72%
Calculated for $C_{32}H_{50}O_4$:	C, 77.06; H, 10.04%

Hydrogenation of 47a : Preparation of acetyl betulanic acid 47b

Acetyl betulinic acid (8 g), dissolved in a mixture of ethyl acetate and acetic acid (100 ml each) was shaken in an atmosphere of hydrogen in presence of PtO_2 catalyst (0.2 g) for 3 hours until absorption of hydrogen ceased. Ethyl acetate was removed by distillation and the solution was diluted with water whereby a white solid (7.5 g) separated out which was collected by filtration. Crystallisation from a mixture of chloroform and methanol furnished crystals, m.p. 304-5° (lit. m.p. 311-12.5°).

Found : C, 76.65; H, 10.55%

Calculated for $C_{32}H_{52}O_4$: C, 76.80; H, 10.40%

U.V. (95% ethanol) : transparent in the region 220 to 300 mμ

I.R. (KBr) : 1242, 1730 and 1700 cm^{-1}.

Lead tetra-acetate oxidation of acetyl betulanic acid 47b : Preparation of hydrocarbon mixture 52a and diacetate 53a:

A mixture of acetyl betulanic acid (6.6 g), dry benzene (60 ml), dry pyridine (3 ml) and lead tetraacetate (9 g.)was heated under reflux under nitrogen atmosphere for 3 hours. The cooled mixture was filtered and the filtrate was concentrated under vacuum to yield a pale yellow gum (6 g.). The ether was dissolved in benzene (20 g) and poured on a column of alumina (500 g) deactivated with 20 ml of a 10% aqueous acetic acid. The chromatogram was developed with petroleum.

Table 1

Chromatography of above gum (6 g)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (1000 ml)	1-20	White solid m.p. 165-73°
Petroleum (300 ml)	21-26	Nil
Petroleum:benzene (4:1) (500 ml)	27-36	White solid m.p. 202-5°

Elution with more polar solvent did not afford any crystalline material

Solid of fractions 1-20 (table 1) (4 g) were collected and was rechromatographed on active alumina (400 g). The chromatogram was developed with petroleum. The solid fraction (4 g) dissolved in benzene (12 ml) was poured on the column and eluted with the following solvents.

Rechromatography of white solid fractions 1-20 (4 g) (Table I):

Table II

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (400 ml)	1-8	Nil
Petroleum:benzene (9:1) (800 ml)	9-24	White solid m.p. 168-74° (same m.p. for each fractions)

Further elution with more polar solvents did not yield any solid

The solid from fractions 9-24 (Table II) (3.8 g) was crystallised from a mixture of chloroform and methanol when crystals of 52a m.p. 170-6°, $(\alpha)_D$ + 28.27° were obtained.

Found : C, 82.14; H, 10.62%

Calc. for $C_{31}H_{50}O_2$: C, 81.94; H, 11.01%

I.R. (KBr) : 1728, 1246 (acetate) 900 and 870 cm^{-1} (trisubstituted double bond).

NMR (60 Mc) : δ.78 to δ.98 (7 methyl), δ1.98 (-O.CO$\underline{CH}_3$), δ 4.4 ($\underline{H}$-C-OCOCH_3), δ5.14 (vinylic proton).

Tetranitromethane : Yellow colour

T.L.C. (benzene:methanol::4:1) : two very close spots R_f 0.82 and 0.81

Estimation of double bond : Perbenzoic acid titration of hydrocarbon mixture 52a

Hydrocarbon mixture 52a (0.055 g) was dissolved in chloroform (5 ml) in a volumetric flask (25 ml). A solution of perbenzoic acid in chloroform (5 ml) was pipetted out and added to the solution and the volume made upto 25 ml by addition of chloroform. A blank solution was similarly prepared by taking 5 ml of perbenzoic acid solution as above in a 25 ml volumetric flask and the volume made up to 25 ml with chloroform. Perbenzoic acid was prepared by the method of Mayer and Manley[28]. 5 ml aliquot portions were taken from each of the above solutions and titrated against standard sodium thiosulphate solution as shown in the table III below.

Strength of sodium thiosulphate solution = 0.0296N

Results

Table III

Time of interval	Blank	Thio required ml.	Reaction mixture	Thio required	Diff. in ml.	No. of double bond
5 minutes	Blank (5 ml) + 2% KI (10 ml) + ACOH (2 ml) + starch soln.	4.8	Aliquot (5 ml) + 2% KI (10 ml) +ACOH (2 ml) starch soln.	4.6	0.2	.26
30 minutes	"	4.8	"	4.3	0.5	.68
1 hour	"	4.8	"	4.1	0.7	.98
2 hours	"	4.8	"	4.1	0.7	.98
4 hours	"	4.8	"	4.1	0.7	.98
8 hours	"	4.8	"	4.1	0.7	.98
16 hours	"	4.8	"	4.1	0.7	.98

Calculation showed that one mole equivalent of perbenzoic acid was consumed within 1 hour, showing thereby that the hydrocarbon mixture 52a contains one double band.

Isolation of the diacetate 53a

Solid fractions 27-36 (1.4 g. Table I) were crystallised from a mixture of chloroform and methanol when fine crystals of 53a m.p. 207-9°, $(\alpha)_D$ + 23.01° were obtained.

Found :	C, 77.21; H, 10.27%
Calculated for $C_{33}H_{54}O_4$:	C, 77.04; H, 10.50%
I.R. (KBr)	: 1726, 1244 cm^{-1} (acetate)
NMR (60 Mc)	: δ 0.78-0.97 (7 CH_3), δ 2.1 (3H, singlet, acetate), δ 4.5 (1H, multiplet, H-C-OCOCH$_3$), δ 1·96 (3H, singlet, acetate)
Mass spectrum	: m/e 514, 454, 411, 249, 189, 175.

Perbenzoic acid titration of the diacetate 53a : Estimation of double bond :

The diacetate 53a (0.072 g) was dissolved in chloroform (5 ml) in a 25 ml volumetric flask. A solution of perbenzoic acid in chloroform (5 ml) pipetted out and added to the solution and the volume made upto 25 ml by addition of chloroform. A blank solution was similarly prepared by taking 5 ml of perbenzoic acid solution as above in a 25 ml volumetric flask and the volume made up to 25 ml with chloroform. 5 ml aliquot portions were taken and titrated

from each of the above solutions against standard sodium thiosulphate solution as shown in the Table IV below.

Strength of sodium thiosulphate solution = 0.0265(N).

Results

Table IV

Time of interval	Blank	Thio required ml.	Reaction mixture	Thio required	Diff. in ml.	No. of double bond
5 minutes	Blank (5 ml) +2% KI (10 ml)+ACOH (2 ml) + starch	4.3	Aliquot (5 ml) +2% KI (10 ml) +ACOH (2 ml) starch soln.	4.3	0.0	0.0
30 minutes	"	4.3	"	4.3	0.0	0.0
1 hour	"	4.3	"	4.3	0.0	0.0
4 hours	"	4.3	"	4.3	0.0	0.0
8 hours	"	4.3	"	4.3	0.0	0.0
16 hours	"	4.3	"	4.3	0.0	0.0
30 hours	"	4.3	"	4.3	0.0	0.0
48 hours	"	4.3	"	4.3	0.0	0.0

It was found that there was no uptake of perbenzoic acid even in 48 hours indicating absence of unsaturation in 53a.

Hydrolysis of 52a and preparation of alcohol 52b

To a solution of 1.5 g. of the acetate 52a in benzene (20 m) 5% methanolic potassium hydroxide solution (100 ml) was added , and the mixture was refluxed on water bath for 3 hours. After removal of benzene and methanol, the residue was diluted with water when white solids separated out. The solid was filtered and crystallised from a mixture of chloroform and methanol to afford needles of crystals m.p. 110-4°, $(\alpha)_D$ + 36.6°.

Found :	C, 84.22; H, 11.34%
Calculated for $C_{29}H_{48}O$	C, 84.46; H, 11.65%
I.R. ($CHCl_3$) :	3400 (hydroxyl), 890, 870, 815 cm^{-1} tri-substituted double bond.

Chromic acid-pyridine oxidation of 52b : Preparation of the ketone 52C

The alcohol 52b (1.1 g.) dissolved in pyridine (10 ml) was added to a CrO_3-Py complex prepared from pyridine (12 ml) and CrO_3 (1.2 g.) at 10°C and the mixture was allowed to stand for 15 hours. 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 until neutral and dried

(Na_2SO_4). Removal of ether gave a gummy residue (1 g.). The above residue was chromatographed over a column of active alumina (60 g.) The chromatogram was prepared with petroleum and the product dissolved in benzene (5 ml) was poured on the column and eluted with the following solvents (Table V).

Table V

Eluent	Fractions 50 ml each	Residue
Petroleum (100 ml)	1-2	Nil
Petroleum:benzene (4:1) (150 ml)	3-5	Solid, m.p. 140-6°

Further elution with more polar solvents did not yield any solid.

Fractions 3-5 (0.75 g. Table V) on crystallization from chloroform and methanol furnished crystals of 52C, m.p. 145-50°, $(\alpha)_D$ + 48.19°. Further crystallisation did not raise the melting point.

Found : C, 85.02; H, 11.05%

$C_{29}H_{46}O$ requires : C, 84.87; H, 11.22%

U.V. (95% ethanol) : 285 mμ (ε 75)

I.R. (KBr) : 1705 (6-ring ketone), 887, 875, 810 cm^{-1} (trisubstituted double bond)

Huang Minlon reduction of ketone 52C : Preparation of the hydrocarbon 52d

A mixture of ketone 52C (0.6 g), diethylene glycol (200 ml) and hydrazine hydrate (90%, 10 ml) was refluxed for 30 minutes. Solid KOH (0.8 g) was added and the mixture was further refluxed for one hour. The condenser was removed and the mixture was heated to 195°. After refluxing for another 2½ hours the reaction mixture was cooled and diluted with water when a solid separated out. The solid (0.5 g.) was chromatographed over a column of active alumina (40 g.). The chromatogram was developed with petroleum. The solid (0.5 g.) dissolved in petroleum (5 ml) was placed over the column and was eluted with the following solvents (Table VI).

Table VI

Eluent	Fractions 25 ml each	Residue on evaporation
Petroleum (100 ml)	1-4	Solid m.p. 152-5°
Further elution with more polar solvents did not afford any solid		

Fractions 1-4 (Table VI) were collected (0.35 g.) which on crystallization from chloroform and methanol afforded crystals of 52d (revised 54) m.p. 156-8°, $(\alpha)_D$ - 7.00° (lit.[27] m.p. 153-4°, $(\alpha)_D$ - 12.0°).

Found : C, 87.44; H, 11.98%

$C_{29}H_{48}$ requires : C, 87.87; H, 12.12%

I.R. (KBr) : 1680, 812, 800 cm^{-1}, (trisubstituted double bond)

NMR (60 Mc) : δ 0.75 to δ 1.05 (7 CH_3) and δ 5.2 (vinylic proton)

Acid isomerisation of hydrocarbon mixture 54 to $\Delta^{13(18)}$ hydrocarbon 55:

The hydrocarbon mixture 54 (0.3 g.) was dissolved in acetic acid (21 ml) and 2N sulfuric acid (2.1 ml) was added to it. The reaction mixture was refluxed for 2½ hours. It was then cooled in ice bath and diluted with water. The solid which separated out was collected by filtration. The yellow solid (0.2 g.) was chromatographed on an active alumina (20 g.) column. The column was developed in petroleum and the substance dissolved in petroleum was placed on the column (Table VII).

Table VII

Chromatograph of above yellow solid (0.2 g.)

Eluent	Fractions 25 ml each	Residue on evaporation
Petroleum (100 ml)	1-4	Crystalline solid m.p. 183-5°C

Further elution with more polar solvents did not afford any solid

The fractions 1-4 (Table VII) were combined and crystallised from chloroform and methanol mixture to afford fine prisms of 55, m.p. 192-3°, $(\alpha)_D$ + 67.2 (lit.[27] m.p. 188-9°, $(\alpha)_D$ + 59°).

Found :		C, 87.53; H, 12.01%
Calculated for $C_{29}H_{48}$:		C, 87.87; H, 12.12%
I.R. (KBr)	:	845, 1615-25 cm^{-1} (broad)
NMR (60 Mc)	:	δ 0.62 to δ 1.00 (7 CH_3), no peak corresponding to vinylic proton
Mass spectrum	:	m/e 396, 353, 204, 191, 161.

LAH reduction of 53a : Preparation of diol 53b

The diacetate 53a (0.2 g) was dissolved in ether (50 ml) and to it added LAH (0.5 g.) in ether (30 ml). The reaction mixture was refluxed for 3 hours. Excess reagent was destroyed with saturated sodium sulphate in cold. It was then extracted with ether, washed with water and dried (Na_2SO_4). Evaporation of the solvent left a white residue (0.15 g.) which was recrystallised from methanol to furnish crystals m.p. 230-2°.

Found :		C, 80.65; H, 11.71%
Calculated for $C_{29}H_{50}O_2$:		C, 80.93; H, 11.63%
I.R. (Nujol)	:	3440 cm^{-1} (hydroxy)
Mass spectra	:	m/e 430, 412, 369, 207, 193, 189, 175

Acetylation of 53b : Preparation of hydroxy acetate 53c

The diol 53b (0.1 g) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) at room temperature in the usual manner. The reaction mixture on usual work up afforded a solid (0.08 g) which was crystallised from a mixture of chloroform and methanol to furnish crystals m.p. 270-1°.

Found :		C, 78.68; H, 11.12%
Calculated for $C_{31}H_{52}O_3$	:	C, 78.81; H, 11.01%
I.R. (Nujol)	:	3500 (hydroxy), 1730 and 1245 cm^{-1} (acetate).

Attempted chromic acid oxidation of 53C

The hydroxy acetate 53c (0.2 g) was dissolved in pyridine (5 ml) and was added to CrO_3-Py complex prepared from pyridine (2 ml) and CrO_3 (0.2 g.) and was kept at room temperature for 20 hrs. The crude product (140 mg) obtained by working up in the usual way was chromatographed over an alumina column (.10 g.) deactivated by 0.4 ml of 10% aqueous acetic acid. The chromatogram was prepared with petroleum ether and the product dissolved in benzene (6 ml) was poured on the column. It was eluted with the following solvents (table X).

Table X

Eluent	Fractions 50 ml each	Residue
Petroleum	1-2	Nil
Petroleum:benzene (3:1)	3-4	Nil
Petroleum:benzene (1:1)	5-6	Solid, m.p. 266-8°C

Further elution with more polar solvent did not yield any material

Fractions 5-6 (table X) (120 mg) on recrystallisation from chloroform and methanol furnished crystals m.p. 269-71°. It was found to be identical with the starting material by m.m.p. determination and I.R. comparison.

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PART IV

Chemical Investigations on the stem bark of Macraranga Denticulata, Muell, Arg.

Chapter I

Morphological feature of the plants of Euphorbiaceae family

Euphorbiaceae[1(a)] is a family of two hundred genera and more than three thousand species, which are chiefly tropical and very rare in cold countries.

Morphological features: Members of this family are usually shrubs, herbs and trees often with milky juice.

Leaves alternate or opposite, rarely divided or compound, usually small, often minute, always unisexual. Perianth simple and calveine, rarely petioled, often wanting in one or both sexes, rarely double, with the inner of 4-5 minute patals. Stamens various, anthers two celled, often didymous. Ovary superior, of 3, rarely more or two carpels, free or united, entire or divided, stigmatic surface usually on the inner face of the style or stylerams, ovules 1-2 in each carpel, pen dulons, from the inner angle of the cell, funnicle often thickened. Fruit either a capsule or of two valved 1-2 seeded cocci separating from a persistent axis, or a drupe with 1-3 cells or of one or more combined nuts. Seeds latterally attached at or above the middle of the cell with or without an aril or thickening of the hilum. Embryo straight, in a fleshy albumen, with flat cotyledons and a superior radicle, very rarely exalbuminous with fleshy cotyledons.

Macaranga, thouars: Trees or shrubs, leaves alternate, often large and peltate, entire or lobed, more or less glandular, beneath, 3-5 plinerved. Flowers in axillary racemes or branched panicles, usually dioecious, opetalous, males many, clustered, fem. one or few under each bract; bracts often large, entire or toothed. Males Fl. minute. Calyx globose or obovoid; sepals 3-4, valvate. Stamens one or more, central, filaments flexuous; anthers 3-4' loceuate, cells more or less two valved. Pistillide 0. Fem Fl. Calyx 2-4 lobed or toothed. Ovary 1-6 celled; styles entire, long or short; cells 1-ovuled. Capsules small, of 1-5 2 valved naked or armed cocci, often glandular or with a waxy coat. Seeds globose, testa crustaceous or osseous albumen fleshy; cotyledons broad, flat - species about 80, in the tropics of the old world.

Macaranga denticulata, Muell. Arg. Leaves deltoid ovate acuminate or obtuse base rounded or trumcate peltate or cordate 9-13 nerved glabrous or puberulous beneath, male panicles slender, bracts minute, stamens 6-30, styles very short, capsule small didymous more or less clothed with waxy glands.

M. denticulata is a small evergreen tree[1(b)] growing to a height of 40 to 60 feet, having a clear stem of from 20 to 40 feet, and girth at breast height of from 3 to 11 feet. It is available chiefly at Terai and Duars of North Bengal. Wood white, prettily grained, but not durable and exuding a red gum when a branch is cut. Leaves shortly peltate, palmately 11-12 nerved, with glands on the margin chiefly near the apex, 4-12 in diam. thinly coriaceous, base truneate with 2 glands above, glands beneath dense nerves 6 or more

pairs above the basal, strong beneath; petiols 2-4 in, stipules small, ovate - lanceolate, tomentose, fugacious. Capsules ¼ in. diam didymous, black, with yellow glands. Seeds globose, black.

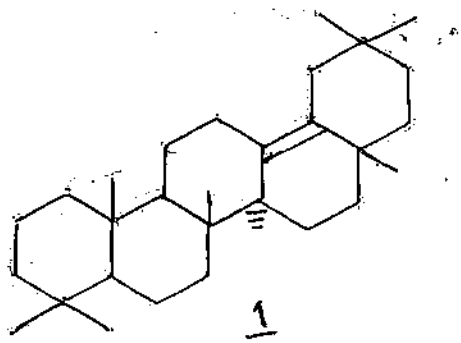
Chapter II

A short review on Δ^{14}-Taraxerene triterpenoids

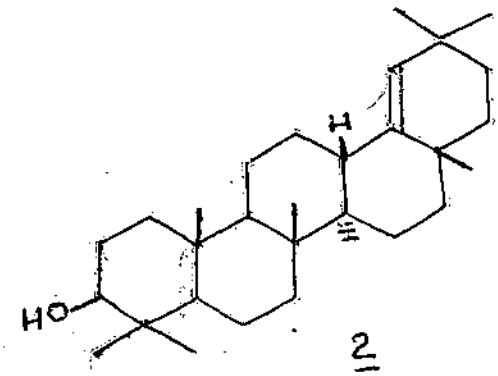
1. Taraxerol and taraxerone

An alcohol named taraxerol first isolated from Taraxacum officinale[2] and Litsea dealbata[3] was shown to be identical with alnulin[4-7] obtained from the bark of grey alder, by Jeger[8] et al. in 1950. An alcohol, skimmiol, isolated from Skimmia japonica by Takeda[9-11] in 1945, was also shown to be identical with taraxerol by Brooks[12]. The triterpenoid nature of taraxerol was proved by Burrows[2,12] and also by Takeda and his coworkers[13,14]. On selenium dehydrogenation 1:2:3:4-tetramethylbenzene, 2:7-dimethyl-, 1:2:7-trimethyl- and 1:2:5:6-tetramethyl-napthalene along with 1:8 dimethyl-picene, were isolated. These facts suggested a general relationship to amyrins with normal triterpenoid rings A and B. The presence of unsaturation in taraxerol was shown by conversion of its acetate to an epoxide $C_{32}H_{52}O_3$, m.p. 257-60°, $(\alpha)_D$ + 47.3° with perbenzoic acid. The unsaturation was probably present as the group C=CH (I.R. band at 814 cm^{-1}). Conversion of taraxerol to dihydrotaraxerol, $C_{30}H_{52}O$, m.p. 261-2°, $(\alpha)_D$ + 47.3° was claimed by Takeda. Taraxerol by CrO_3-Py oxidation gave taraxerone $C_{30}H_{48}O$, m.p. 238-40°, $(\alpha)_D$ + 8°. The latter on further oxidation furnished dicarboxylic acid which on ring closure gave a cyclopentanone derivative. These sequence of reactions showed the secondary nature of hydroxyl group present in a six membered ring.

That taraxerol belongs to β-amyrin group was proved by Takeda[10] and subsequently by Jeger[8]. Clemmensen reduction of taraxerone gave a hydrocarbon m.p. 190-91°, $(\alpha)_D$ - 34°, identical with δ'-amyrene 1 which was also obtained by pyrolysis of taraxeryl benzoate followed by hydrogenation. This and other work led Takeda to formulate taraxerol as 2 (olean-18-en-3β-ol). This was invalidated[15] when this structure was assigned to germanicol 2.

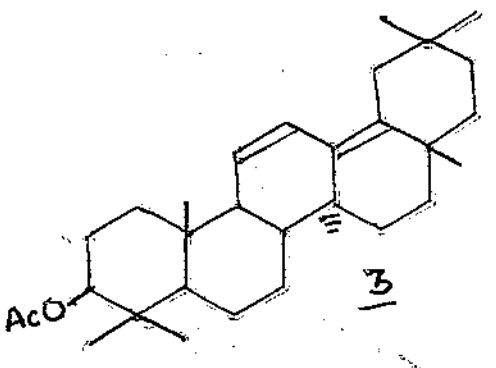


1



2

Oxidation of taraxeryl acetate with SeO_2 gave two isolable products, olean-11:13(18)-dienyl acetate 3, m.p. 226°, $(\alpha)_D$ - 58°, λmax 242 mμ, log ε 4.35, 250 mμ, log ε 4.4; 259 mμ, log ε 4.2 and the diene-dione 4, m.p. 237-40°, $(\alpha)_D$ - 90°, λmax 276 mμ log ε 4.1. These results established decisively the presence of the hydroxyl group at C-3. Now with the clearly made proviso that no skeletal change had taken place during SeO_2 oxidation the structure 5 was advanced for taraxerol.



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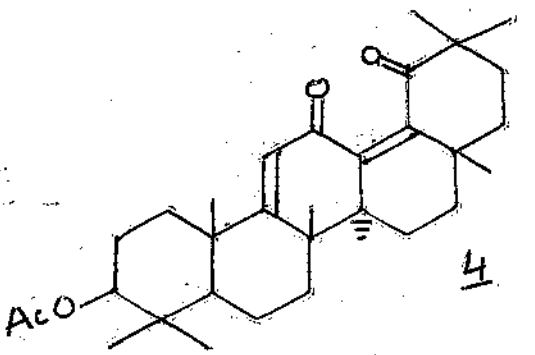

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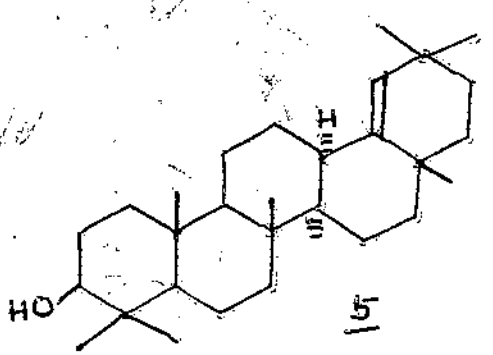
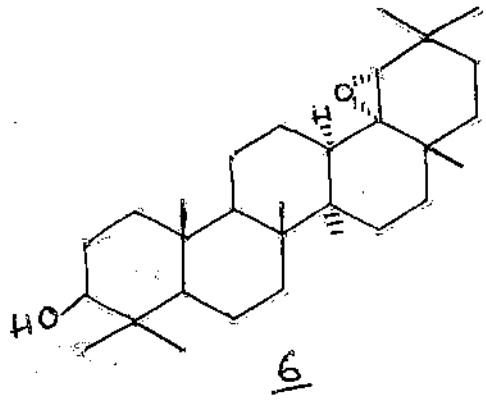


5

If 5 was indeed the correct structure then the oxide derived would be 6 and the unsaturated alcohol (as was claimed by Takeda) would be 7. But 7 being an allylic alcohol would be unlikely to survive in the conditions of its genesis. This structure also could not explain the formation of oleana-2:12 diene on dry distillation of taraxeryl benzoate, as reported by Takeda, as it would require the migration of the double bond at 18:19 past the thermodynamically more stable 13(18) position to the less stable 12:13 position leaving also, the less stable configuration at C_{18}.



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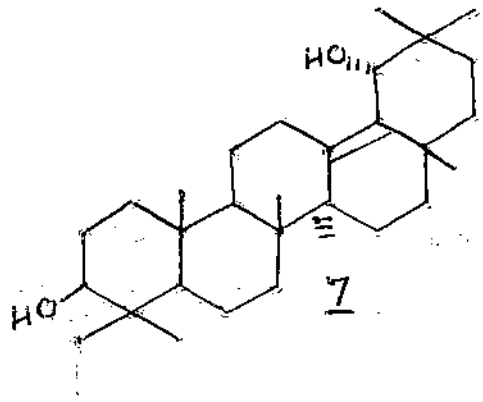
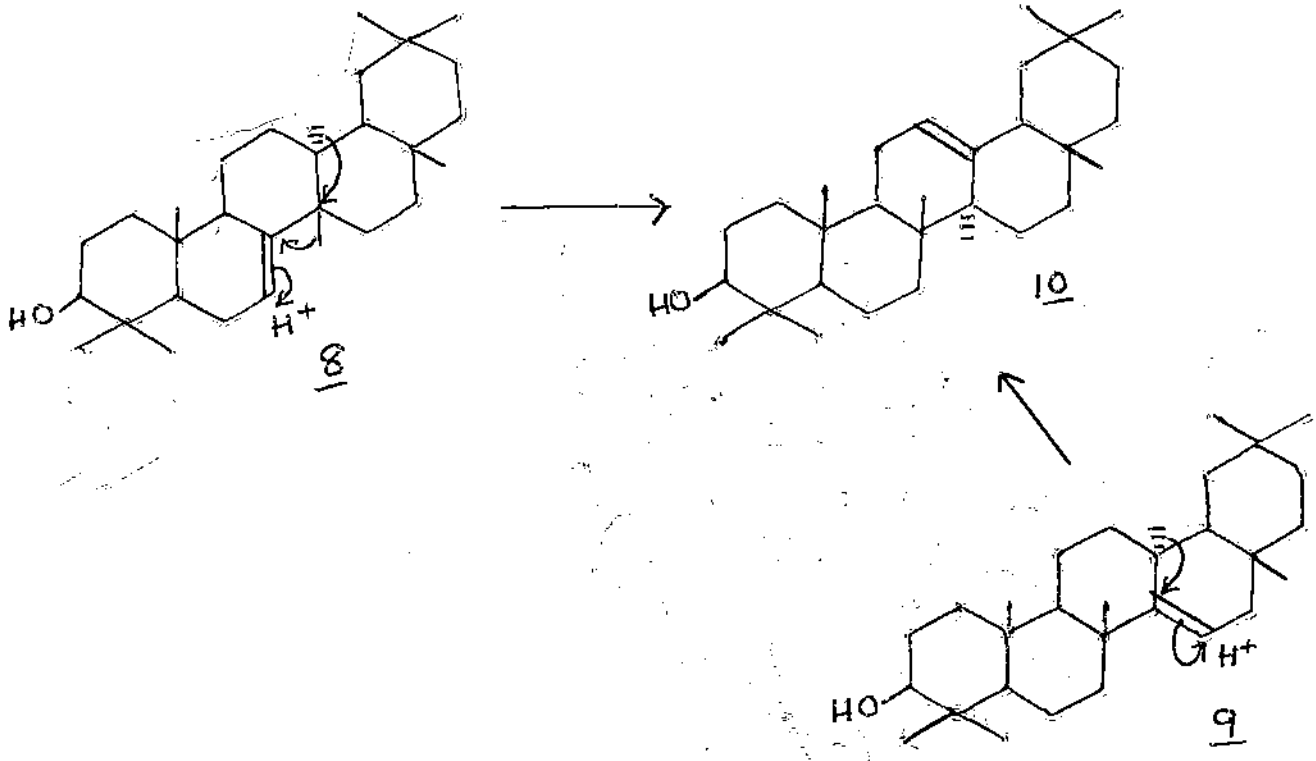
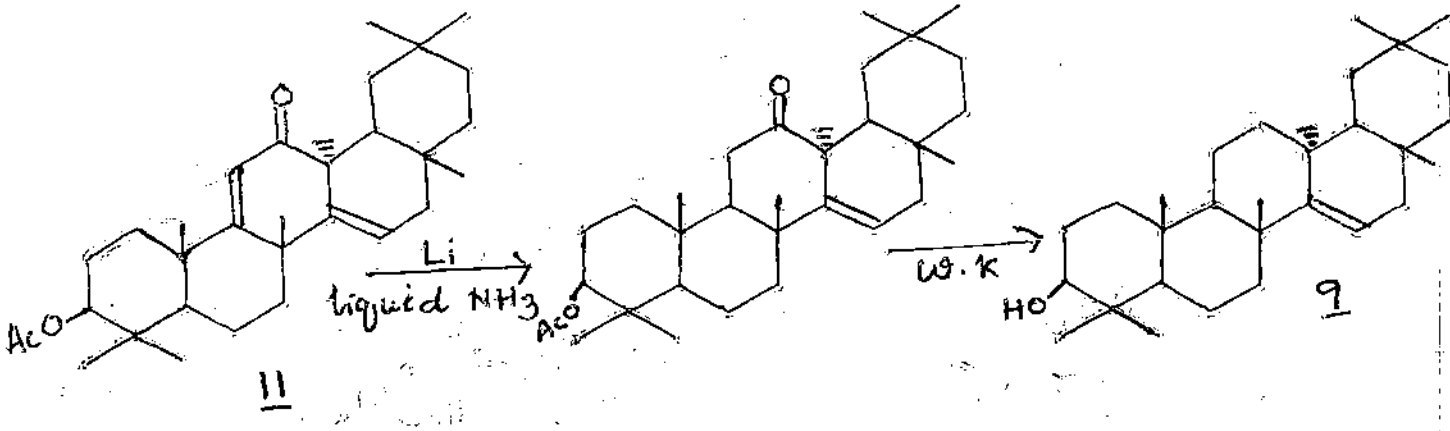


7

When a suspension of taraxeryl acetate in acetic acid at 90° was treated with hydrochloric acid in a very short time an excellent yield of β-amyrin acetate was obtained. This indicated that in the formation of oleanane derivatives from taraxerol a rearrangement had taken place. Since rearrangement has led to the β-amyrin structure taraxerol cannot already possess it. Two structures, at least, could be presented 8 and 9. The conversion of 8 to β amyrin is strongly reminiscent of euphol → isoeuphol rearrangement[16] which involved protonation of the double bond with concerted methyl migration from C_{14} to C_{13} as represented below. A similar rearrangement for 9 is also possible. The formulation 9 was preferred because of certain analogies with previous work on β-amyrin series[17].



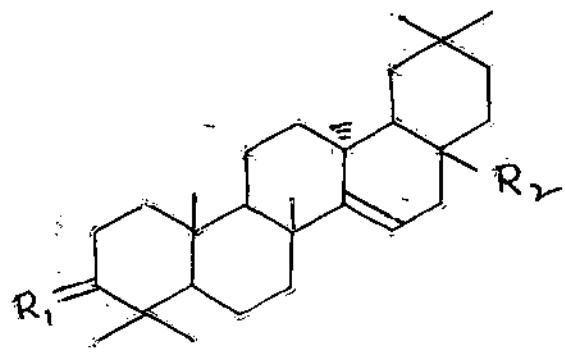
The partial synthesis from 12 keto-iso-oleana $\Delta^{9(11):14(15)}$ dienylacetate[19] 11 by Spring[18] and co-workers as shown below established beyond doubt that taraxerol possesses the structure 9.



2. Myricadiol 12

From the bark of Myrica gale Ryabimin and Matyukhina[20] isolated a triterpene diol, myricadiol, m.p. 273-4°, which has been assigned structure 12 on the basis of the following arguments. It gave a diacetate, $C_{34}H_{54}O_4$ m.p. 256-58°, $(\alpha)_D$ + 1°. Oxidation of myricadiol with chromium trioxide-pyridine gave myricanal 13a an oxo-aldehyde, $C_{30}H_{48}O_2$, m.p. 256-7° (disemicarbazone m.p. 298° bis 2:4 dinitrophenyl hydrazone m.p. 247°). Treatment of the latter with diethylene glycol, N_2H_4 and KOH gave taraxerene m.p. 201-2°, which was also prepared from taraxerone by similar Wolff-Kishner reduction. The latter on being treated with HCl-chloroform gave olean-12-ene m.p. 161-5°-162.5°. Acid isomerisation of myricadiol diacetate with acetic acid-HCl mixture gave erythrodiol diacetate, $C_{34}H_{54}O_4$, m.p. 184.5-85.5°, $(\alpha)_D$ + 60° which on hydrolysis gave erythrodiol[21] m.p. 231.5°-32.5°, $(\alpha)_D$ + 83°. Myricadiol evidently contained a primary and a secondary hydroxyl group as indicated by the spectrum of the oxidation product described above and evidently was taraxen-14-ene-3β, 28 diol.

Myricadiol was also isolated by Dhar and Agarwal[22] from Myrica esculenta. Recently Bose and Paul[23] isolated myricadiol and have recorded mass spectra of the compound.



12 , $R_1 = H(OH\beta)$, $R_2 = CH_2OH$

13 , $R_1 = H(OH\beta)$, $R_2 = CHO$

13a , $R_1 = O$, $R_2 = CHO$

14 , $R_1 = H(OCH_3\beta)$, $R_2 = CH_3$

15 , $R_1 = H(OH\alpha)$, $R_2 = CH_3$

3. Myriconal 13

The Russian workers[24,25] also isolated another new triterpene $C_{30}H_{48}O_2$ m.p. 288°, from the bark of Myrica gale. The structure 13 was assigned to it. Myriconal gave an acetate $C_{32}H_{50}O_3$ m.p. 304-5° and a 2:4 dinitrophenyl hydrazone m.p. 250°. On lithium aluminium hydride reduction it furnished myricadiol 12, showing thereby that it must be represented either as Δ14-taraxerene-28-ol-3 one or Δ-14-taraxerene-3 ol-28-al 13. The decision in favour of structure 13 was made on the basis of the I.R. spectrum which showed clearly an aldehyde peak.

4. Sawamilletin 14

Sawamilletin (taraxeryl methyl ether) 14 has been isolated by H. Ito et al.[26a] and J.A. Bryce et al.[26b] Both from chemical studies and spectral properties its structure has been assigned as 14. It has been synthesised by the reaction of taraxerol with methyl iodide.

5. Epi-taraxerol 15

Epi-taraxerol 15 $C_{30}H_{50}O$ m.p. 261-2°, $(\alpha)_D$ - 22.6° has recently been isolated by the present author[27] from the neutral fraction of the stem-bark of Macaranga Denticulata. The details regarding its chemistry is reported in the next chapter (Chapter III).

Chapter III

Investigation of Macaranga Denticulata, Muell Arg.

Isolation of taraxerone, β-sitosterol and a new triterpene, 3-epitaraxerol from the stem-bark of M. Denticulata.

Section A : Extraction

Dried and powdered stem bark ~~and~~ of M. Denticulata was extracted with benzene. The benzene solution was concentrated by distilling off benzene when a gummy residue was obtained. The residue was extracted with ether, washed with aqueous NaOH solution and then with water till neutral. The ether solution was dried over sodium sulphate and ether evaporated when a gummy residue was obtained. The gummy residue, thus obtained was chromatographed and the following fractions were isolated.

Section B : Chromatography of the neutral part

The above gummy neutral part was chromatographed over deactivated alumina and the following fractions were collected.

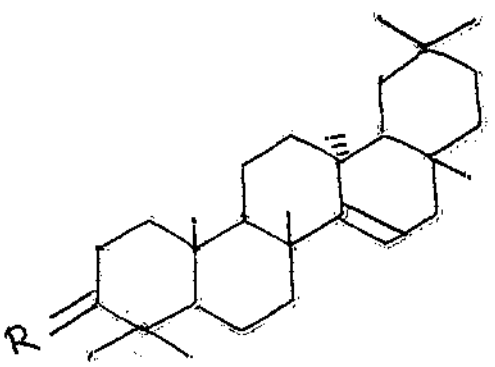
Fraction No.	Eluent	Eluate	M.p. of the residue on evaporation
1	Petroleum ether	Solid with oil	230-35°
2	Petroleum ether: benzene (4:1)	Solid	249-55°
3	Petroleum ether: benzene (3:2)	Solid	128-32°

Section C : Examination of fraction No. 1 and isolation of taraxerone 16

Fraction No. 1 on rechromatography over alumina and several crystallization from chloroform-methanol mixture furnished crystals having constant m.p., 237-39°, $(\alpha)_D$ + 9°. Elemental analysis coupled with mass spectrometric determination of molecular weight established the molecular formula of the compound to be $C_{30}H_{48}O$ (M^+ 424). It developed a yellow colour with tetranitro methane indicating unsaturation in the compound. It gave a violet coloration in Liebermann-Burchard reaction and gave a positive test in Zimmermann colour reaction showing the compound is a triterpene ketone, the keto group being at C-3 position. The I.R. spectrum of the compound showed absorption peaks at 1705 (6 membered ring ketone) and 822 cm^{-1} (trisubstituted double bond, $c = c\langle_H$). The compound showed UV absorption at λ_{max}^{EtOH} 282 mμ (ϵ 64) showing that the keto group and the double bond were unconjugated.

On LAH reduction it gave an alcohol 16a, $C_{30}H_{50}O$ m.p. 278-80°, $(\alpha)_D$ + 4.4°, which on acetylation furnished an acetate 16b, $C_{32}H_{52}O_2$, m.p. 294-6°, $(\alpha)_D$ + 9.16°. The physical constants of the ketone 16, the derived alcohol 16a and its acetate 16b are very close to the reported[28] physical constants of triterpene ketone taraxerone, m.p. 240°, $(\alpha)_D$ + 12°, taraxerol, m.p. 282-3°, $(\alpha)_D$ + 0° and taraxeryl acetate, m.p. 304-5°, $(\alpha)_D$ + 9° respectively.

The identity of the isolated ketone with taraxerone 16 was confirmed by m.m.p. determination and I.R. comparison with an authentic sample of taraxerone.



16, R = O

16a, R = H (OHβ)

16b, R = H (OAcβ)

Section D : Examination of fraction No. 2 and isolation of the new triterpene - 3 epi-taraxerol

Fraction No. 2 on rechromatography over alumina and several crystallization from a mixture of chloroform and methanol furnished needle shaped crystals 17 having a constant melting point, 261-2°, $(\alpha)_D$ - 22.6°. Elemental analysis coupled with mass spectroscopic determination established the molecular formula to be $C_{30}H_{50}O$ (M^+ 426). I.R. spectrum of the compound (Fig. 33) showed absorption peaks at 3420 (hydroxyl) and 825 cm^{-1} (trisubstituted double bond, $C = C\langle_H$). The compound was transparent in the UV region 220 to 300 mμ. The NMR spectrum besides 8 methyl groups in the region δ0.85 to δ1.05 , showed peaks at δ5.38 (1H, multiplet) attributed to vinylic proton. A signal located at δ3.45 (width at half wave, $W_{1/2}$ 7Hz) showed the proton at C-3 to be equatorial.

On acetylation with acetic anhydride and pyridine the compound afforded an acetate 17a, m.p., 161-2°, $(\alpha)_D$ - 41°. Elemental analysis coupled with mass spectrometric determination established its molecular formula to be $C_{32}H_{52}O_2$ (M^+ 468). IR spectrum (Fig.34) showed absorption peaks at 1730 and 1240 (acetate), and 830 ($C=C\langle_H$) $cm.^{-1}$. The NMR spectrum besides signals for 8 methyl groups at saturated carbon in the region 0.82 to 1.08 δ, showed peaks at 2.07δ (3H, singlet, -O.CO.$C\underline{H}_3$), 4.64 δ (1H, multiplet, proton of the carbon, bearing the acetoxy group, $\underline{H}$-C-O.$COCH_3$) and 5.3 δ (C = $C\langle_{\underline{H}}$). The downfield shift of the C-3 proton is consistent with its equatorial orientation and hence the axial orientation of C-3 acetoxy group. The similarity of the NMR spectra of the alcohol and its derived acetate 17a to that of taraxerol and its acetate suggested that the alcohol 17 might be 3-epimer of taraxerol.

Chromium trioxide-pyridine oxidation on the alcohol gave a ketone $C_{30}H_{48}O$, m.p. 238-40°, $(\alpha)_D$ + 10° which was found to be identical with taraxerone 16, by m.m.p. and comparison of IR spectra with an authentic sample. Since the 3β-alcohol 16a, taraxerol, prepared by LAH reduction of taraxerone was found to be different in every respect from the alcohol 17, the hydroxyl group at C-3 must be α-oriented. Thus the new triterpene was established as 3-epi-taraxerol 17.

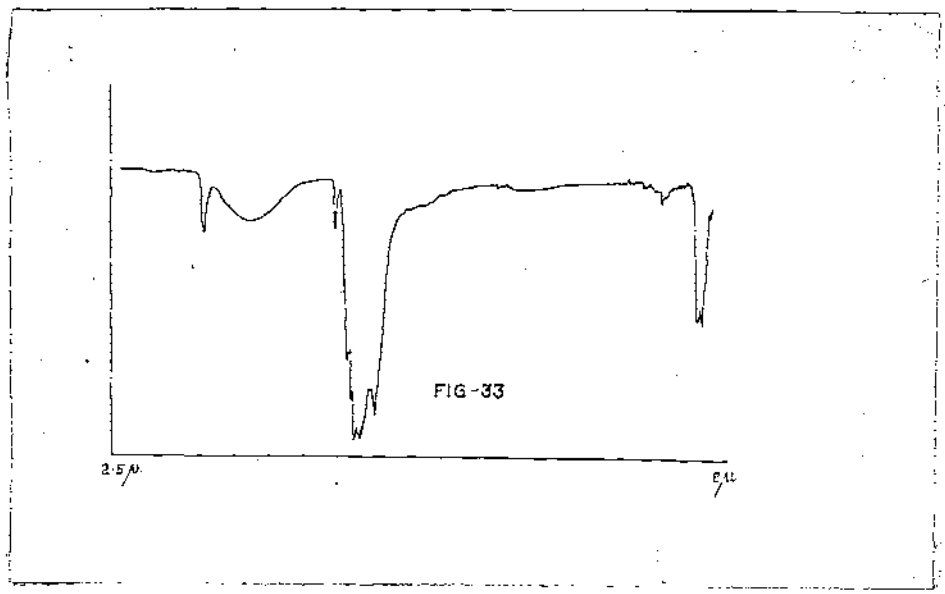


FIG-33

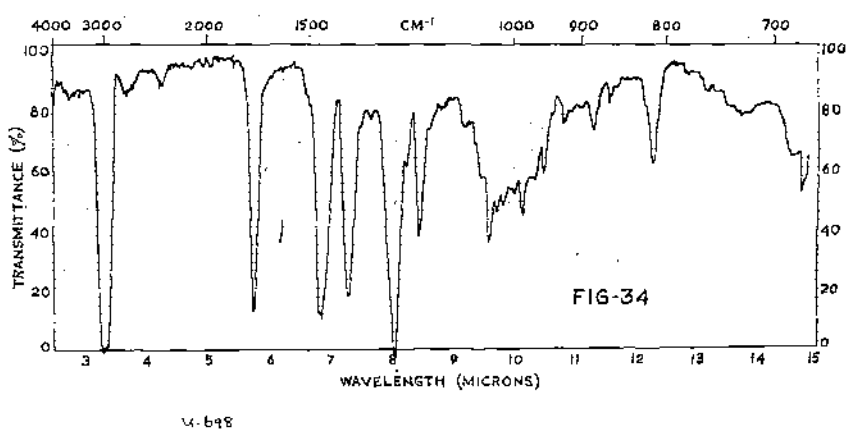
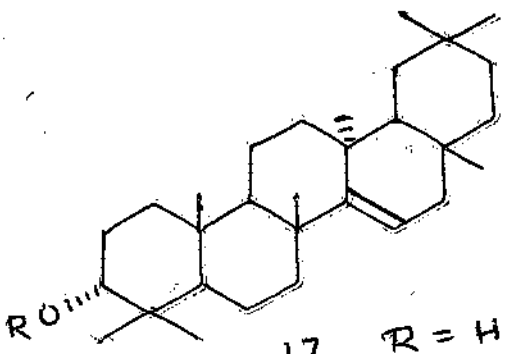


FIG-34

M-698



17 , R = H

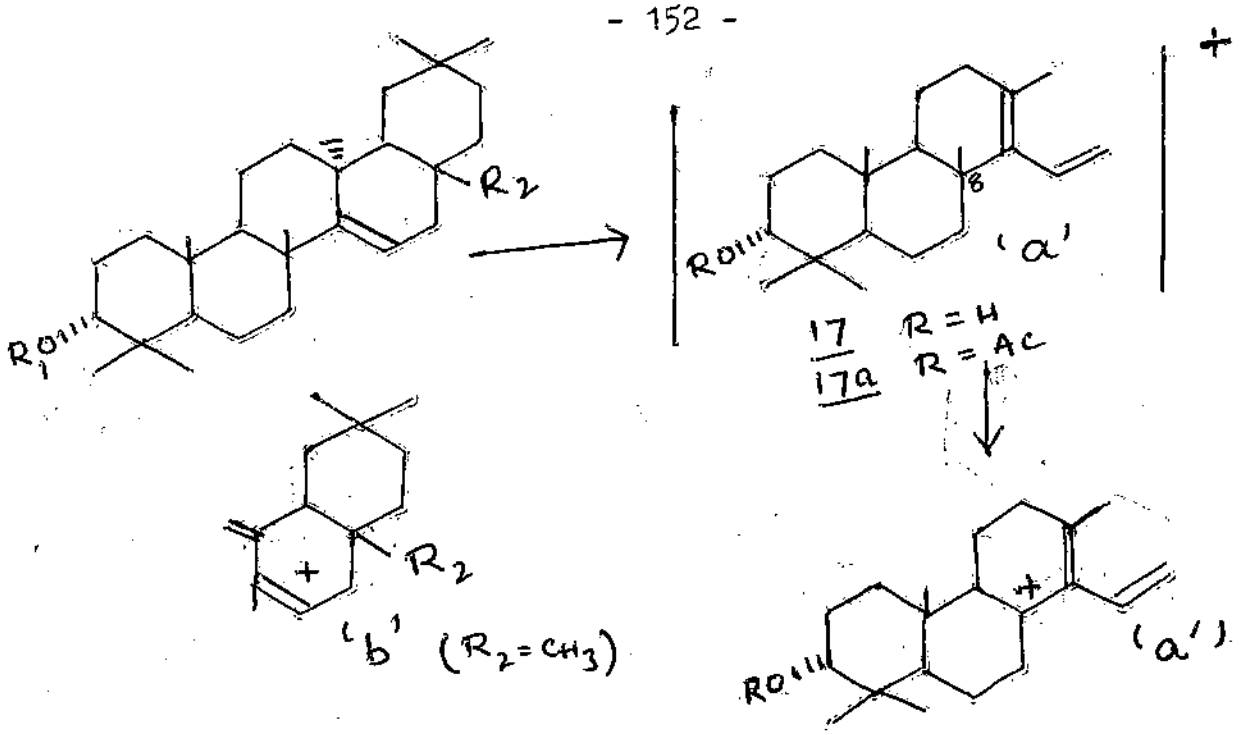
17a , R = Ac

Application of Mass spectrometry

Djerassi and his coworkers[29] measured the mass spectrometric fragmentations of taraxerol and taraxeryl acetate. The mass spectrometric fragmentations of epi-taraxerol 17 and its acetate 17a are in complete agreement with that of taraxerol and its acetate. This clearly demonstrated that carbon skeleton of both the alcohols must be same.

In these molecules a similar retro-Diels-Alder decomposition was observed as was in the case of Δ^{12}-unsaturated derivative, except that the collapse of ring D rather than ring C occurred. The resulting fragment 'a' exhibits a mass of m/e 302 for 17 and 344 for 17a depending on the C_3-substituent 'a'. Ion 'a' is accompanied by a satellite peak 15 mass unit lower, which is formed by the loss of methyl group, probably the allylically activated one at C-8(α').

The spectrum of 17 exhibited additional peaks at 284, 269 m/e due to the loss of H_2O and (H_2O + CH_3) respectively from the species 'a', while that of 17a showed ('a'-CH_3COOH) and ('a'-CH_3COOH) ion peaks at 284 and 269 m/e.



In addition to species 'a' and its further decomposition products, the spectra of 17 and 17a showed a very abundant fragment at m/e 204 ('b'). This arises out of the cleavage of ring C and comprises ring D and E. Furthermore, the fragment 'b' losses the substituent at C-17 giving rise to a fragment 'b'-CH_3 in both cases. Nothing specifically could be said regarding the formation of the species 'b', as no experimental proof was available.

The structure 17 was further confirmed by its partial synthesis from taraxerone 16 by Meerwein-Pondorf reduction according to the method of Paton et al.[30] when 3-epi-taraxerol and taraxerol were produced and separated by chromatography.

Takeda and his coworkers[10,11] carried out reduction of taraxerone with sodium and iso-amyl alcohol and reported the isolation of an alcohol m.p. 267-69°, $(\alpha)_D$ + 11.9°, acetate m.p. 205-7°, $(\alpha)_D$ - 21.8°. The alcohol, thus obtained was thought to be the 3'-

epimer of taraxerol and was named iso-taraxerol. But the physical constants recorded by them are not in agreement with those of our compound 3 epi-taraxerol and its acetate. To clarify this sodium and iso amyl alcohol reduction of taraxerone was repeated. Two products were obtained and were separated by chromatography. The less polar material eluted in petroleum:benzene (4:1) was found to be identical with epi-taraxerol, isolated from the plant as well as with the one synthesised from taraxerone by Paton's method, in all respects. The more polar material, eluted in petroleum:benzene (3:2) was found to be identical with taraxerol. This is also in conformity with the chromatographic behaviour of the equatorial and axial isomers of triterpene 3-alcohols.

Section E:

Examination of fraction 3 : Isolation and identification of β-sitosterol

Fraction 3 on crystallization from chloroform and methanol mixture had m.p. 136-7°, $(\alpha)_D$ - 32°. Elemental analysis showed the molecular formula as $C_{29}H_{50}O$. On treatment with acetic anhydride and pyridine it afforded an acetate, $C_{31}H_{52}O_2$, m.p. 127-8°, $(\alpha)_D$ -40°. The acetate was identified as β-sitosterol acetate by direct comparison with an authentic specimen of β-sitosterol acetate. Hence the parent alcohol was identified as β-sitosterol.

CHAPTER IV

Experimental

Isolation of the neutral material

Dried and powdered stem bark of Macaranga denticulata, Muell. Arg. (2 kg.) was extracted with benzene in a Soxhlet apparatus for twenty hours. Benzene was distilled off and the residual gummy solid (20 gm.) was taken up in ether (2 liter). The ether solution was washed with 10% sodium hydroxide solution (2 x 200 ml). The ether layer was washed with cold water till the washings were neutral, dried over anhydrous sodium sulphate and evaporated, when the neutral material (9 gm.) was obtained as a yellow gummy solid.

Chromatography of the above gummy solid

The above gummy material (9 gm) dissolved in benzene (25 ml) was placed on a column of alumina (400 gm.) deactivated with 16 ml. of 10% aqueous acetic acid. The chromatogram was developed with petroleum and eluted with the following solvents (Table I).

Table I

Eluent	Fractions (50 ml each)	Residue on evaporation	Melting point
Petroleum (200 ml)	1-4	Oil (3.gm)	-
Petroleum (300 ml)	5-10	Solid (1.2 g)	230-5°
Petroleum:benzene (9:1) (200 ml)	11-14	Oil (0.6 g.)	-
Petroleum:benzene (4:1) (350 ml)	15-21	Solid (.55 g)	249-55°
Petroleum:benzene (3:2) (250 ml)	22-26	Solid (1.1 g)	128-32°

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

Examination of Fractions 5-10 (Table I) : Isolation of taraxerone 16

The solids from fractions 5-10 (Table I) were combined (1.2 gm), m.p. 230-5° and was rechromatographed over a column of active alumina (60 gm). The solid dissolved in benzene (5 ml) was placed on the column. The chromatogram was developed with petroleum and was eluted with the following solvents (table II).

Table II

Chromatography of the above material (1.2 g.)

Eluent	Fractions (50 ml each)	Residue on evaporation	Melting point
Petroleum	1-4	Trace oil	-
Petroleum:benzene (4:1)	5-10	Crystalline solid	235-7°

Further elution with more polar solvents did not afford any material

The fractions 5-10 (Table II) were combined (0.85 gm) and crystallised from a mixture of chloroform and methanol. After three crystallization it afforded shining crystals (0.5 gm) m.p. 237-9°; $(\alpha)_D$ +9°. Its melting point was not depressed when mixed with an authentic sample of taraxerone. It also showed identical IR spectra when compared with that of authentic specimen of taraxerone.

Found : C, 84.84; H, 11.02%

Calculated for $C_{30}H_{48}O$: C, 84.84; H, 11.39%

U.V. (95% ethanol) : λ max 282 mμ (ϵ 64)

I.R. (KBr disc) : ν max 1705 (carbonyl), 822 (C=C$\langle_H$) cm^{-1}

Colour reaction tests

(a) Tetranitromethane displayed a yellow colour.

(b) Libermann-Burchardt : The compound developed a violet coloration with a mixture of acetic anhydride and conc. sulphuric acid.

(c) Zimmermann colour test was positive.

Lithium aluminium hydride reduction of taraxerone 16 :
Preparation of taraxerol 16a

To the ketone (200 mg) dissolved in dry ether (25 ml) was added LAH (25 mg) and the mixture was refluxed on the water bath for four hours. The reaction mixture was then cooled and to this 15 ml of saturated solution of sodium sulphate was added. The mixture was extracted with ether, washed to neutral with water and dried (Na_2SO_4). Removal of the ether gave a solid (190 mg) which was chromatographed over alumina. A column of alumina (10 gm. deactivated with 0.4 ml of 10% aqueous acetic acid) was developed with petroleum and the above residue dissolved in benzene (4 ml) was added to it. The following solvents were used for elution (Table III).

Table III

Eluent	Fractions 50 ml each	Residue
Petroleum	1-2	Nil
Petroleum:benzene (4:1)	3-4	Nil
Petroleum:benzene (3:2)	5-10	Crystalline solid 170 mg m.p. 268-70°

Further elution with more polar solvents did not yield any material

Fractions 5-10 (table III) were combined and the solid (170 mg) was crystallised from chloroform and methanol mixture when a constant melting solid, m.p. 278-80°, $(\alpha)_D$ + 4.4° was obtained. Its melting point was not depressed when mixed with an authentic specimen of taraxerol.

Found : C, 84.14; H, 11.69%

Calculated for $C_{30}H_{50}O$: C, 84.44; H, 11.81%

Preparation of taraxeryl acetate 16b

To the alcohol 17a (200 mg.) dissolved in pyridine (5 ml) was added acetic anhydride (5 ml) and the solution was heated on a water bath for five hours. The solution was cooled and then poured into ice cold water when a crystalline solid separated out. The solid collected by filtration was recrystallised from a mixture of chloroform and methanol to afford crystals of taraxeryl acetate 16b, (120 mg) m.p. 294-6°, $(\alpha)_D$ + 9.16°.

Found : C, 81.72; H, 11.52%

Calculated for $C_{32}H_{52}O_2$: C, 82.05; H, 11.11%

Examination of fractions 15-21 (Table I) : Isolation of epi-taraxerol 17

The fractions 15-21 (Table I) were combined (0.55 gm.), m.p. 249-55° and was rechromatographed over a column of active alumina (30 g.). The solid dissolved in benzene (6 ml) was placed on the column. The chromatogram was developed in petroleum and was eluted

with the following solvents (Table IV).

Table IV

Eluent	Fractions 50 ml each	Residue
Petroleum	1-3	Oil
Petroleum:benzene (4:1)	4-6	Nil
Petroleum:benzene (3:2)	7-10	Solid (0.45 g) m.p. 257-9°

More polar solvent did not elute any further material

Fractions 7-10 (Table IV) were combined and crystallised from chloroform-methanol mixture to afford needle shaped crystals of 3-epi-taraxerol 17 m.p. 261-2°, $(\alpha)_D$ - 22.6°.

Found : C, 84.60; H, 11.93%

$C_{30}H_{50}O$ requires : C, 84.44; H, 11.87%

UV (ethanol) : No absorption within the range 220-300 mμ

IR (KBr) : ν_{max} 3420 (hydroxyl), 825 cm^{-1} (C=C$\diagdown_H$)

NMR (60 Mc) : δ.85 to δ1.05 (24H, 8 CH_3), 5.3 δ (1H, multiplet) C=C$\underset{H}{\diagdown}$), 3.45 δ (1H, W 1/2 7HZ)

Mass : m/e 426, 302, 284, 269, 204, 189

Acetylation of 3-epitaraxerol : Preparation of 3-epi-taraxeryl acetate 17a:

3-epi-taraxerol (0.2 g) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The solution was then poured on ice cold water. The crude acetate was crystallised from a mixture of chloroform and methanol to afford a pure sample of 3-epi-taraxeryl acetate 17a, m.p. 161-2°, $(\alpha)_D$ - 41°.

Found :		C, 81.68; H, 11.34%
$C_{32}H_{52}O_2$ requires :		C, 82.05; H, 11.11%
IR (Nujol)	:	1730, 1240 (acetate), 830 (C = C$\swarrow_H$) cm^{-1}
NMR (60 Mc)	:	0.82-1.08 δ (24H, 8 CH_3), 2.07 δ (3H, singlet, $C\underline{H}_3$-C-O-), 4.64 δ (1H, multiplet $\underline{H}$-C-OAc), 5.3 δ (C=C$\swarrow\underline{H}$, 1H, multiplet)
Mass	:	m/e 468, 344, 284, 269, 204, 189

Preparation of taraxerone 16

3-epi-taraxerol (0.2 g) was oxidised with CrO_3-Py complex prepared from pyridine (2 ml) and CrO_3 (0.2 g) at 15°C. The crude product (0.12 g) obtained by working up in the usual manner was chromatographed over a column of active alumina (6 g). The chromatogram was prepared with petroleum and the product dissolved in benzene (4 ml) was poured on the column. It was eluted with the following solvents (Table V).

Table V

Eluent	Fractions 50 ml each	Residue
Petroleum	1-2	Nil
Petroleum:benzene (9:1)	3-4	Nil
Petroleum:benzene (4:1)	5-6	Solid m.p. 236-8° (0.1 g)

Further elution with more polar solvents did not yield any material

Fractions 5-6 (Table V, 0.1 g) on recrystallisation from a mixture of chloroform and methanol furnished pure crystals of taraxerone 16, m.p. 237-9°, $(\alpha)_D$ + 10°, which was found to be identical with authentic sample of taraxerone (m.m.p. and I.R. comparison).

Found: C, 84.69; H, 11.08%

Calculated for $C_{30}H_{48}O$: C, 84.84; H, 11.23%

Meerwein Pondorf reduction of taraxerone : Preparation of 3-epi-taraxerol 17 and taraxerol 16a

A mixture of taraxerone (1.0 g) and Al-isopropoxide (1.3 g) in absolute isopropanol (12.5 ml) was distilled slowly with the addition of isopropanol to maintain constant volume. After 5 hours the distillate no longer contained acetone and the solution was evaporated to dryness. The product isolated in the usual way with ether was dissolved in benzene (10 ml) and poured on a column of

alumina (60 g., deactivated with 2.2 ml of 10% aqueous acetic acid) developed with petroleum. The following solvents were used for elution (Table VI).

Table VI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1-4	Nil
Petroleum:benzene (9:1)	5-8	Nil
Petroleum:benzene (4:1)	9-16	Solid (0.38), m.p. 256-8°
Petroleum:benzene (3:2)	20-28	Solid (0.6 g) m.p. 275-7°
Further elution with more polar solvents did not yield any material		

Fractions 9-16 (Table VI) were combined and crystallised from chloroform-methanol mixture to give 3-epi-taraxerol, m.p. 260-2° which did not depress the melting point when mixed with an authentic sample of 3-epi-taraxerol. The acetate prepared in the usual manner had melting point 161-2°, identical with 3-epi-taraxeryl acetate (m.m.p.).

Fractions 20-28 (Table VI) were combined and crystallised from a mixture of chloroform and methanol to give crystals, m.p. 277-8°, acetylation of which afforded an acetate 295-6°, identical with an authentic specimen of taraxeryl acetate (m.m.p.).

Sodium and isoamyl alcohol reduction of taraxerone : Preparation 3-epi-taraxerol 17 and taraxerol 16a

Sodium (2 gm) was added slowly to a refluxing solution of taraxerone (500 mg) in isoamyl alcohol (25 ml) and refluxing continued until all the sodium had dissolved. After steam distillation the solid precipitate was collected by filtration. The crude solid (450 mg) was chromatographed. It was dissolved in benzene (6 ml) and poured on a column of alumina (30 g, deactivated by 1.1 ml of 10% aqueous acetic acid). The chromatogram was developed in petroleum and was eluted with the following solvents (Table VII).

Table VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1-2	Nil
Petroleum:benzene (9:1)	3-4	Nil
Petroleum:benzene (4:1)	5-7	Solid (0.13 g) m.p. 256-7°
Petroleum:benzene (3:2)	9-12	Solid (0.28 g) m.p. 276-8°

Further elution with more polar solvents did not afford any material

Fractions 5-7 (Table VII) were combined and crystallised from chloroform-methanol mixture to afford needle shaped crystals of 3-epi-taraxerol, m.p. 260-1° which did not depress the melting point when mixed with an authentic samples of 3-epi-taraxerol. The

acetate prepared in the usual way had melting point 160-2°, identical with 3-epi-taraxeryl acetate (m.m.p.).

Fractions 9-12 (Table VII) were combined and crystallised from a mixture of chloroform and methanol to afford crystals m.p. 277-9°, which on acetylation afforded an acetate m.p. 296-7°, identical (m.m.p.) with an authentic specimen of taraxeryl acetate.

Examination of Fractions 22-26 (Table I) : Isolation of β-sitosterol

Fractions 22-26 (table I) were combined and the solid crystallised from chloroform and methanol mixture when fine needle shaped crystals of β-sitosterol was obtained, m.p. 136-7°, $(\alpha)_D$ -32°.

Found : C, 83.34; H, 11.62%

Calculated for $C_{29}H_{50}O$: C, 83.98; H, 12.15%

Preparation of β-sitosterol acetate

β-sitosterol (0.2 g) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The product, isolated in the usual way with ether was crystallised from chloroform and methanol mixture when crystals of the acetate m.p. 127-8°, $(\alpha)_D$-40° were obtained, identified as β-sitosterol acetate by comparing with an authentic specimen of β-sitosterol acetate (m.m.p. and I.R. comparison).

Found : C, 81.15; H, 11.35%

Calculated for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48%.

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PART V

Chemical Investigations on the bark of Bischofia Javanica Blume

Chapter I

Morphological feature of Bischofia (Euphorbiaceae) species and Bischofia javanica Blume

Bischofia, Blume[1]

Bischofia species are usually glabrous trees. Leaves alternate, 3-foliolate, leaflets often crenate. Flowers in axillary or lateral panicled racemes, minute, dioceious, apetalous, males scattered or clustered, females longer pedicelled. Male Fl. sepals 5', concave, obtuse, imbricate, concealing the anthers. Disk 0, stamens 5, filaments short; anthers large, cells parallel. Pistillode short, broad. Fem Fl. sepals ovate, caducous. Staminodes 5, small or 0. Ovary exserted, 3-4 celled, styles long, linear, stout, entire, ovules 2 in eact cell. Fruit globose, fleshy, with 3-4 cells lined with a parchement-like 2-valved endocarp. Seeds turgidly oblong, testa fibro-crustaceous, albumen fleshy; Cotyledons broad flat, radicle straight elongate.

Bischofia javanica Blume., which is called Kainjal in Bengali[2,3] a round-headed more or less deciduous quite glabrous tree 30-40 feet, bark smooth. Leaves very variable; petiole 1-6 in. leaflets 3-5 in. from ovate to oblong lanceolate, acuminate, repand-toothed, petiolules $\frac{1}{2}$-$\frac{3}{4}$ in. Panicles very slender, flowers green, males minute on short slender pedicels, fem 1/6 in. diam. on stout pedicels. Fruit fleshy, on long thickened pedicels, smooth, size

of a pea, blue black. Seeds smooth, shining, testa splitting longitudinally, dark brown.

The plant[2] is available in sub-Himalayan forests from the Ravi eastwards through Oudh and Gorakhpur to Bihar, Bengal and Assam; specially in Orisa and on the West coast from konkan southwards to the Nilgiris in India and in Malay and Pacific Islands. It is an excellent planking timber[3], flowering in March-April and fruiting in December-March.

Juice of the leaves is considered cure for sores[2].

Chapter II

Isolation of Betulic acid as acidic constituent and epi-friedelanol acetate, friedelin and β-sitosterol as neutral constituents from benzene extract of the bark of Bischofia javanica Blume.

Section A : Extraction

Dried and powdered bark of Bischofia javanica was extracted with benzene in a Soxhlet apparatus. Distillation of the solvent gave a gummy residue, which was taken up in ether. The ether solution was treated with aqueous sodium hydroxide solution and the alkali layer separated. The ether solution was washed with water and dried over anhydrous sodium sulphate. Evaporation of ether furnished a gummy residue.

Section B : Chromatography of the neutral part :

(Table I)

The above gummy neutral part was chromatographed over alumina and the following fractions were collected.

Fractions no.	Eluent	Eluate	M.p. of the residue
1	Petroleum ether	Solid with oil	280-285°
2	Petroleum ether: benzene (4:1)	Solid	252-4°
3	" (3:2)	Nil	[illegible]°
4	" (2:3)	Solid	128-33°

Section C : Examination of Fraction No. 1 and isolation of epi-friedelanol acetate 1

Fraction 1 (Table I) on rechromatography over active alumina followed by several crystallization from chloroform and methanol mixture furnished crystals of 1, m.p. 289-92°, $(\alpha)_D$ + 40°. Elemental analysis and mass spectrum established the molecular formula of the compound as $C_{32}H_{54}O_2$ (M^+ 470). It gave no coloration with tetra nitromethane indicating that it was a saturated compound. I.R. spectrum showed absorption peaks at 1736 and 1239 cm^{-1} (acetate). The acetate on alkaline hydrolysis and subsequent chromatography of the reaction product afforded a crystalline solid 2, m.p. 277-9°, $(\alpha)_D$+9°. Elemental analysis corresponded to the molecular formula $C_{30}H_{52}O$. In I.R. spectrum it showed absorption peak at 3420 cm^{-1} (OH). UV spectrum of the compound did not show any absorption in the region 215 to 300 mμ. The above physical and chemical data of the acetate 1 and alcohol 2 closely corresponded to that of epi-friedelanol acetate and epi-friedelanol respectively[4-6]. The acetate 1 was found to be identical with an authentic sample of epi-friedelanol acetate by m.m.p. determination and I.R. comparison.

Section D : Examination of the Fraction No. 2 and isolation of Friedelin 3

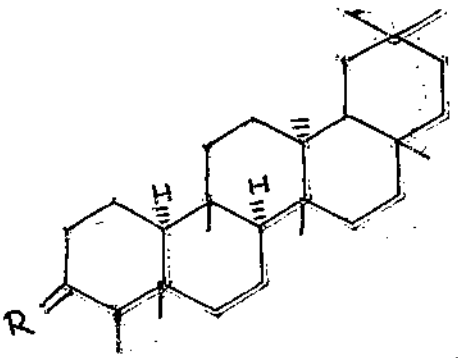
Fraction 2 (Table 1) on rechromatography over alumina followe by several crystallization from a mixture of chloroform and methano furnished fine needle shaped crystals m.p. 256-8°, $(\alpha)_D$ - 32°.

Elemental analysis and mass spectrometric determination showed the molecular formula of the compound to be $C_{30}H_{50}O$ (M^+ 426). It developed no coloration with tetranitromethane indicating that it was a saturated compound. It gave a violet coloration in Libermann Burchardt reaction and a positive Liebermann colour test indicating that the compound is a triterpene ketone, the keto group being at the customary C-3 position[7].

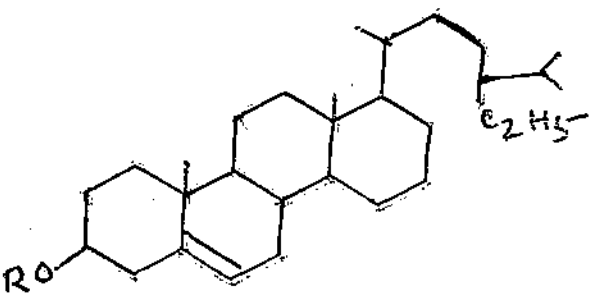
The compound 3 gave an oxime $C_{30}H_{51}NO$, m.p. 294-6°, showing that oxygen atom was present as a carbonyl group. The I.R. spectrum of the compound showed a peak at 1705 cm^{-1} (six membered ring ketone). The compound exhibited an UV absorption at λmax 255 mμ (ϵ=71). The above chemical and physical data closely corresponded to that of friedelin and the compound 3 was found to be identical with an authentic sample of friedelin by m.m.p. determination and I.R. comparison.

Section E : Examination of fraction No. 4 : Isolation and identification of β-sitosterol 4

Fraction 4 (Table I) on crystallisation from chloroform and methanol mixture had m.p. 136-7°, $(\alpha)_D$ - 32°. Elemental analysis showed the molecular formula as $C_{29}H_{50}O$. On treatment with acetic anhydride and pyridine it afforded an acetate 5, $C_{31}H_{52}O_2$, m.p. 126-7°, $(\alpha)_D$ - 37°. The acetate was identified as β-sitosterol acetate by direct comparison with an authentic specimen of β-sitosterol acetate. Hence the parent alcohol was identified as β-sitosterol.



1 , R = H(OAcα)
2 ; R = H(OHα)
3 , R = O



4 , R = H
5 , R = Ac

Section F : Examination of Acid fraction : Isolation and identification of betulinic acid 6

The alkali washed portion of the benzene extract, on acidification with dilute hydrochloric acid yielded a solid which was extracted with ether. The ethereal solution containing the acid fraction was esterified with diazomethane. The crude methyl ester obtained after evaporation of ether was chromatographed over deactivated alumina to afford a solid 7, which after crystallisation from $CHCl_3$-MeOH mixture had m.p. 222-4°, $(\alpha)_D$ + 5°. Elemental analysis showed the molecular formula to be $C_{31}H_{50}O_3$. It did not show any absorption in UV spectrum in the region 220-300 mμ, I.R. spectrum showed peaks at 3520 (-OH), 1735 ($-COOCH_3$), 1660 and 876 cm^{-1} ($=CH_2$). NMR spectrum of the compound showed signals:

(a) Olefinic proton signals for = $C\underline{H}_2$; two doublets in the region 4.8-4.9 ppm.

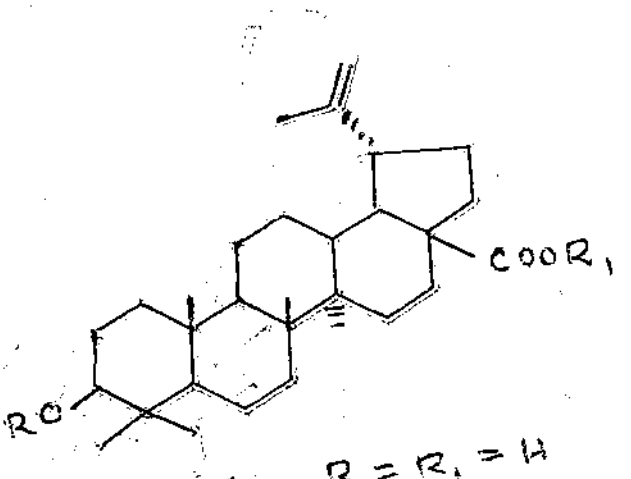
(b) An intense signal at 3.75 ppm for -COOC$\underline{H}_3$ group.

(c) A singlet for -C$\underline{H}$OH at 2.01 ppm.

(d) A sharp peak for -CH_3 occurring as -CH=C(-$\underline{CH}_3$)-C at 1.75 ppm.

(e) A tall singlet corresponding to 5-CH_3 group at 1.00 ppm.

The physical constants of the compound 7 are very close to that of methyl betulinate[8] and was found to be identical with an authentic sample of methyl betulinate by m.m.p. determination and I.R. comparison. The characterization was further confirmed by the preparation of its acetyl derivative when acetyl-methyl betulinate 8, $C_{33}H_{52}O_4$, m.p. 200-1°, $(\alpha)_D$ + 4° was obtained. It was identical with an authentic sample of acetyl methyl betulinate. Hydrolysis of the methyl betulinate 7 with potassium tertiary butoxide in dimethyl sulfoxide gave betulic acid 6, $C_{30}H_{48}O_3$ m.p. 299-302° identical with an authentic sample (IR comparison).



6, R = R, = H

7, R = H, R, = CH3

8, R = AC, R, = CH3

CHAPTER III

Experimental

Extraction : Isolation of the neutral fraction

Dried and powdered trunk bark of Bischofia javanica (2 kg) was extracted with benzene in a Soxhlet apparatus for twenty hours. Benzene was distilled off and the gummy residue (9 gm.) was taken up in ether (1 liter). The ether solution was washed with 10% aqueous sodium hydroxide solution (3 x 300 ml) and then with water till neutral. The ether solution was dried over anhydrous sodium sulphate and the ether evaporated, when a gummy material (4.6 gm.) was obtained.

Chromatography of the above gummy material

The above gummy material (4.6 gm) was dissolved in benzene (6 ml) and was placed on a column of alumina (300 gm) deactivated with 6 ml of 10% aqueous acetic acid. The chromatogram was developed with petroleum and was eluted with the following solvents (table II).

Table II

Chromatography of the above gummy material

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (300 ml)	1-6	Solid contaminated with ~~with~~ oil m.p. 280-285° (0.750 gm.)
Petroleum:benzene (4:1) (400 ml)	7-14	Solid (1.2 gm.) m.p. 252-4°
Petroleum:benzene (3:2) (200 ml)	15-18	Nil
Petroleum:benzene (2:3) (300 ml)	19-24	Solid m.p. 128-33° (2 gm.)

Further elution with more polar solvent did not afford any crystalline material

Isolation of epi-friedelinol acetate 1

Fractions 1-6 (table II) were combined (0.750 gm), dissolved in 3 ml of benzene and was rechromatographed over a column of active alumina (40 gm). The chromatogram was developed with petroleum (Table III).

Table III

Chromatography of the residue of fractions 1-8 (Table II)

Eluent	Fractions 50 50 ml each	Residue on evaporation
Petroleum (150 ml)	1-3	Oil
Petroleum (200 ml)	4-7	Solid (0.2 g.) m.p. 282-6°

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

The above solid (fractions 4-7, Table III) were combined (0.2 g) and was crystallised from a mixture of chloroform and methanol to afford crystals of 1, m.p. 289-92°, $(\alpha)_D$ + 40°. Its melting point was not depressed when mixed with an authentic sample of epi-friedelanol acetate. I.R. spectra of the two were also superimposable.

Found :	C, 81.37; H, 11.25%
Calculated for $C_{32}H_{54}O_2$:	C, 81.70; H, 11.48%
I.R. (Nujol) :	1736 and 1239 cm^{-1}
U.V. (95% ethanol) :	No absorption in the region 220-300 mμ.

Hydrolysis of the acetate 1 : Preparation of epi-friedelanol 2

Epi-friedelanol acetate (0.1 g.) was dissolved in benzene (5 ml) and was refluxed with methanolic potassium hydroxide solution (10 ml, 10%) for 3 hours. The reaction mixture was concentrated on water

bath and then diluted with water. The precipitated solid (0.08 gr.) was collected by filtration and crystallised from a mixture of chloroform and methanol to afford crystals of 2, m.p. 277-9°, $(\alpha)_D$ + 9°. Its melting point was not depressed when mixed with an authentic sample of epi-friedelanol. I.R. spectra of the two compounds were also superimposable.

Found : C, 84.32; H, 12.06%

$C_{30}H_{52}O$ requires : C, 84.11; H, 12.14%

I.R. (Nujol) : 3420 cm^{-1} (-OH)

U.V. (95% ethanol): No absorption in the region 215-300 mμ.

Isolation of friedelin 3

Fractions 7-14 (Table II) were combined (1.2 gm), dissolved in benzene (5 ml) and was chromatographed over a column of active alumina (60 gm.). The chromatogram was developed with petroleum (Table IV).

Table IV

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum 100 ml	1-2	Oil
Petroleum (300 ml)	3-8	Solid (.9 gr.), m.p. 255-7°

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

The fractions 3-8 (Table IV) were combined and crystallised from a mixture of chloroform and methanol to afford fine needles shaped crystals 3, m.p. 256-8°, $(\alpha)_D$ - 32°. Its melting point was not depressed when mixed with an authentic specimen of friedelin. I.R. spectra of the two compounds were superimposable.

Found :	C, 84.18; H, 11.76%
Calculated for $C_{30}H_{50}O$:	C, 84.44; H, 11.81%
I.R. (Nujol) :	1705 cm^{-1} (six membered ring ketone)
U.V. (95% ethanol) :	λmax 255 mμ. (ϵ = 71).

Isolation of β-sitosterol 4

The solid fractions 19-24 (Table II) were combined (2 gm) and crystallised from chloroform and methanol mixture when fine needle shaped crystals of β-sitosterol 4 were obtained m.p. 136-7°, $(\alpha)_D$ - 36°.

Found :	C, 83.34; H, 11.62%
Calculated for $C_{29}H_{50}O$:	C, 89.98; H, 12.15%

Preparation of β-sitosterol acetate 5

β-sitosterol 4 (0.5 g.) was acetylated with pyridine (5 ml) and acetic anhydride (5 ml) in the usual way. The solid (0.45 g), thus obtained, was crystallised from chloroform and methanol mixture when crystals of the acetate 5, m.p. 126-7°, $(\alpha)_D$ - 37° were obtained. It was identified as β-sitosterol acetate by comparing with an authentic specimen of β-sitosterol acetate (m.m.p. and I.R. romparison).

Found : C, 81.15; H, 11.35%

Calculated for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48%

Isolation of Methyl betulinate 7

The aqueous alkaline layer was throughly shaken with ether to remove any neutral material that might be present. The aqueous layer was acidified with cold and dilute 10% hydrochloric acid (1 litre) when some insoluble solids separated out. The acidified portion was extracted with ether, washed with water till neutral and then dried (Na_2SO_4). Ether was removed when a gummy residue (2 gm) was obtained. To the latter dissolved in ether (250 ml) was added a solution of diazomethane in ether prepared from nitrosomethyl urea (1.4 g.) and was kept overnight. Next 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 then dried (Na_2SO_4). Evaporation of the ether yielded a gummy residue (1.5 g.)

Chromatography of the above gummy material (1.5 g) : Isolation of methyl betulinate 7

The above crude ester (1.5 g.) dissolved in benzene (12 ml) was placed over a column of alumina (100 gm. deactivated with 4 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum and was eluted with following solvents (Table V).

Table V

Chromatography of the above gummy material (1.5 g.)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (200 ml)	1-4	Oil
Petroleum:benzene (4:1) (200 ml)	5-8	Nil
Petroleum:benzene (3:2) (300 ml)	9-14	Solid (1.2 g), m.p. 216-8°

Further elution with more polar solvents did not yield any crystalline solid

Solids obtained from the fractions 9-14, m.p. 216-18° (Table V) were combined (1.2 g), and crystallised from a mixture of chloroform and methanol to afford colourless needles of methyl betulinate 7, m.p. 222-4°, $(\alpha)_D$ + 5°, identical with authentic sample (m.m.p. and I.R.).

Found : C, 78.79; H, 10.52;

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

UV : No absorption in the region 220-300 mμ.

I.R. (Nujol): 3520 (-OH), 1735 ($-COOCH_3$), 1660 and 876 cm^{-1} (= CH_2)

NMR (60 Mc) : 4.8-4.9 δ (two doublets; = CH_2), 3.75 δ (singlet, $-COOCH_3$), 2.01 δ (singlet, -CHOH), 1.75 δ (sharp singlet $H_2C=C-$) and 1.00 δ (a tall singlet accounting for 15 protons, 5 CH_3).

Preparation of acetyl methyl betulinate 8

Methyl betulinate 7 (200 mg) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The crude acetate (160 mg) thus obtained was crystallised from a mixture of chloroform and methanol to give crystals of acetyl methyl betulinate 8, m.p. 200-1°, found to be identical with an authentic sample of acetyl methyl betulinate (m.m.p.).

Found : C, 77.31; H, 10.38%

Calculated for $C_{33}H_{52}O_4$: C, 77.34; H, 10.15%

Preparation of betulinic acid 6

To a normal solution of potassium tertiary butoxide in tertiary butanol (10 ml), a solution of methyl betulinate 7 (150 mg) in dimethyl sulfoxide (10 ml) was added and the reaction mixture was heated at 100° for 4 hours in an oil bath. After working up in the usual manner it afforded betulinic acid, m.p. 299-302°, identical with an authentic sample of betulinic acid (m.m.p. and I.R. comparison).

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STEREOCHEMISTRY OF THE C-19 ISOPROPENYL SUBSTITUENT IN THE MERCURIC ACETATE OXIDATION PRODUCT OF THE TRITERPENES OF LUPANE SERIES

H.N. Khastgir and S.N.Bose
Department of Chemistry, University of North Bengal, Darjeeling.

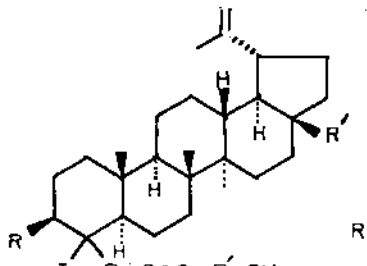
(Received in UK 19 September 1967)

Allison and coworkers [1,2] carried out mercuric acetate oxidation on lupeol acetate (Ia), betulindiacetate (Ib) and methyl acetyl betulinate (Ic) and assigned structures (IIa), (IIb) and (IIc) to the respective products. They also performed the same oxidation on betulin (IIIa) and acetyl betulinic acid (IIIb) and formulated the products as (IV) and (V) respectively. The stereochemistry of the isopropenyl group at C-19 in the products of oxidation was not established by them. Recently Chopra et al[3] repeated this oxidation on methyl acetyl betulinate and established structure (VI) for the product on the basis of UV absorption NMR spectrum and osmic acid hydroxylation of the tetrasubstituted double bond but did not assign the stereochemistry of the C-19 isopropenyl group.

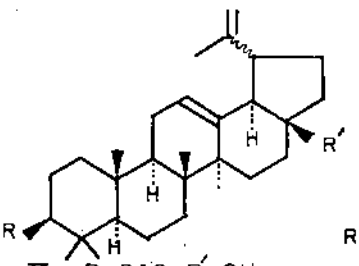
In the present communication we report the evidences which indicated that the stereochemical assignment of the isopropenyl group should be cis in relation to the β-substituent at C-17. On mechanistic grounds we thought it probable that the dehydrogenation may lead to cis orientation of the isopropenyl group with respect to the C-17 substituent. Most probably the initial product of dehydrogenation is (VII) which then isomerizes to the thermodynamically more stable (VIII), the addition of proton at C-19 taking place from rearside.

To provide reasonable evidences regarding the stereochemical assignment of the isopropenyl group we chose the lactone 3β-acetoxy-lup-20 (30)-en-28,13β-olide (V*), m.p. 300-2°, $[\alpha]_D$ + 58°

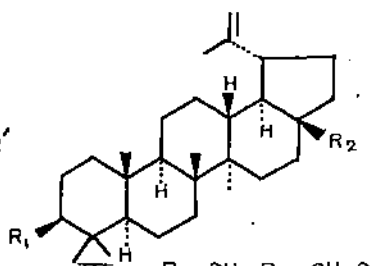
* In the lactone (V) the C-18 H is necessarily α-oriented.



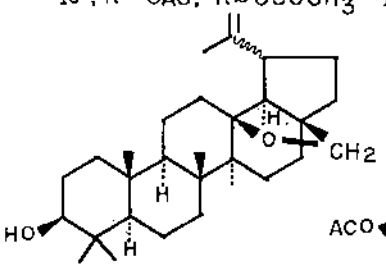
Ia; R=OAC, R'=CH_3
Ib; R=OAC, R'=CH_2OAC
Ic; R OAC, R'=$COOCH_3$



IIa; R=OAC, R'=CH_3
IIb; R=OAC, R'=CH_2OAC
IIc; R=OAC, R'=$COOCH_3$



IIIa; R_1=OH, R_2=CH_2OH
IIIb; R_1= OAC, R_2=COOH



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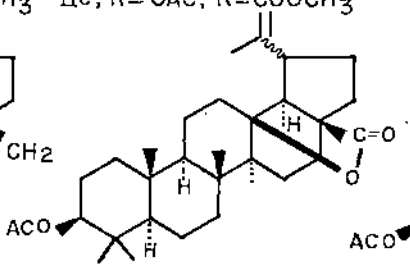

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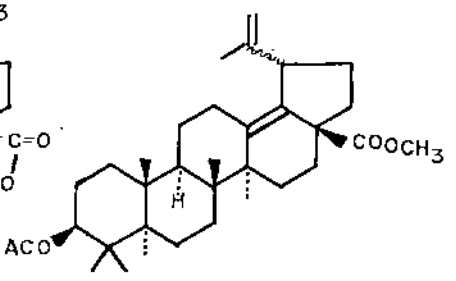
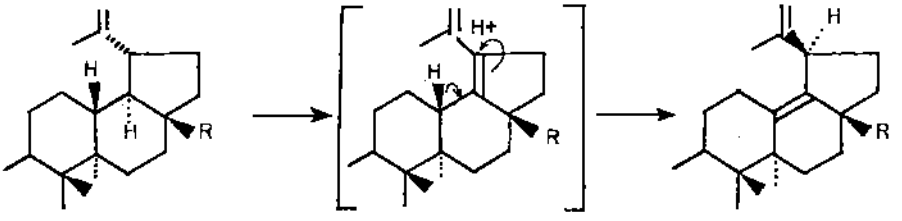
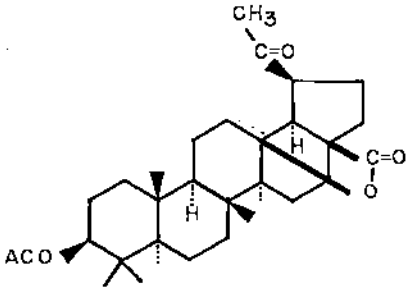


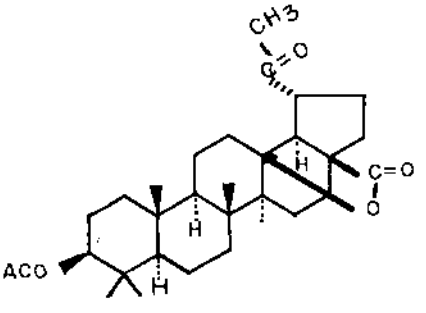
VI



VII VIII



IX



X

. $\nu_{max}^{CHCl_3}$ 1730, 1775 cm^{-1} (reported[1] m.p. 315-17°, $[\alpha]_D$ + 60°) as our starting material. The presence of terminal methylene protons was indicated by an nmr signal at 4.9 ppm. Examination of the Dreiding model of this compound clearly indicated that if the C-19 substituent were trans to the C-17 group as in lupeol and betulinic acid then it should be amenable to acid induced isomerization[4a, b] as observed in the lupeol series with expansion to six membered ring E with chair conformation. But if the original orientation were cis no skeletal rearrangement would occur. Accordingly, we exposed the lactone (V) to the action of HCl-$CHCl_3$[5,6], 98% formic acid[7,8] HCl-acetic acid [1,2] and in each case the starting material was recovered in good yield (mixed melting point and comparison of infrared spectra) and no isomerized product could be detected. These experiments suggested that the skeleton of the lactone and the stereochemistry of the substituents in the compounds is preserved unchanged. The lactone was ozonised at 0°C in $CHCl_3$ solution and the ozonide on decomposition under neutral conditions furnished the ketone (IX), melting point 301-3°, $[\alpha]_D$ - 9°, $\nu_{max}^{CHCl_3}$ 1715, 1780 cm^{-1} (reported[1] m.p 317°, $[\alpha]_D$ - 2°). The latter on equilibration with potassium tertiary butoxide in benzene solution, reacetylation and chromatography furnished a new ketone (X), m.p. 300-2°, $[\alpha]_D$ - 24° $\nu_{max}^{CHCl_3}$ 1714, 1780 cm^{-1} in 60% yield thus demonstrating that the cis ketone (IX) has isomerized to the more stable trans ketone (X). The mixture melting point of the two ketones showed considerable depression. The epimerization of the ketone (IX) by base is an expected process. Thus the stereochemistry of (IX) requires that $-CO-CH_3$ group at C-19 be cis to the C-17 lactone.

Satisfactory analytical data have been secured for the compounds reported here. Further studies are in progress and more complete details will be reported later.

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Melting points are uncorrected. Optical rotations were determined in chloroform solution unless stated otherwise.