CHAPTER—II

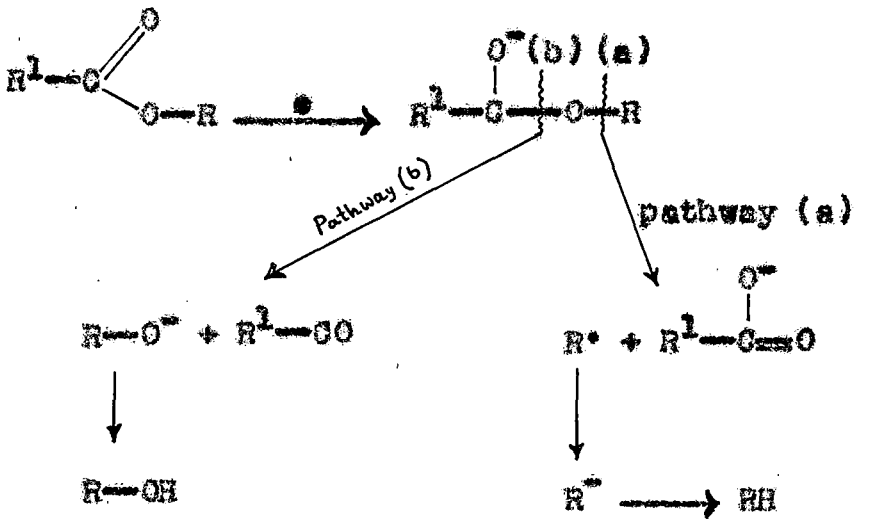
RESULTS AND DISCUSSION

SECTION—A

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

INTRODUCTION:

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



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

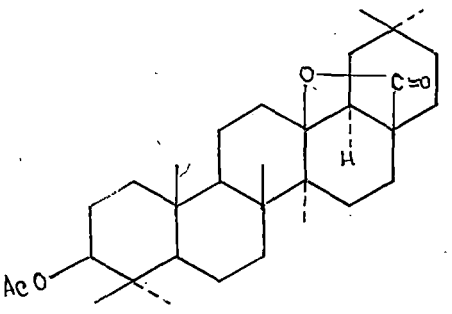
Interested by these results we tried to apply this type of reactions on triterpenoids. Our first attempt was on triterpenoid lactones. Since lactones can be considered as the intramolecular esters, we applied the reduction reaction on them. We took secondary and tertiary lactones of different sterical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

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

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

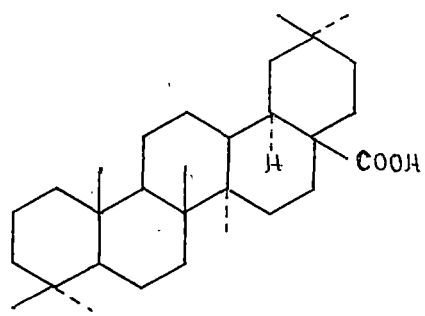
(a) <u>Treatment of lithium-ethylenediamine on triterpene tertiary lactones:</u>

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



<u>67</u>

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



68

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

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

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

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

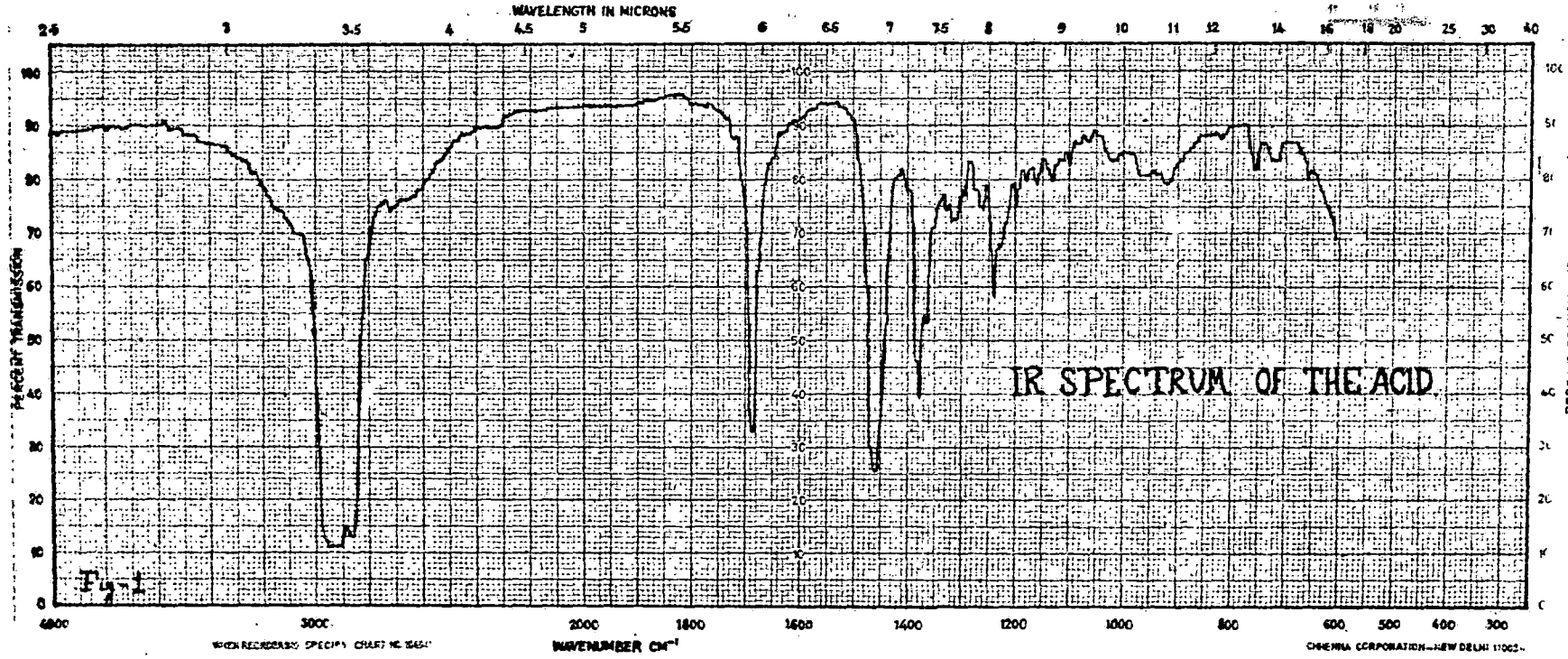


Fig.1: IR spectrum of oleanan-18∝-28-oic acid, 68.

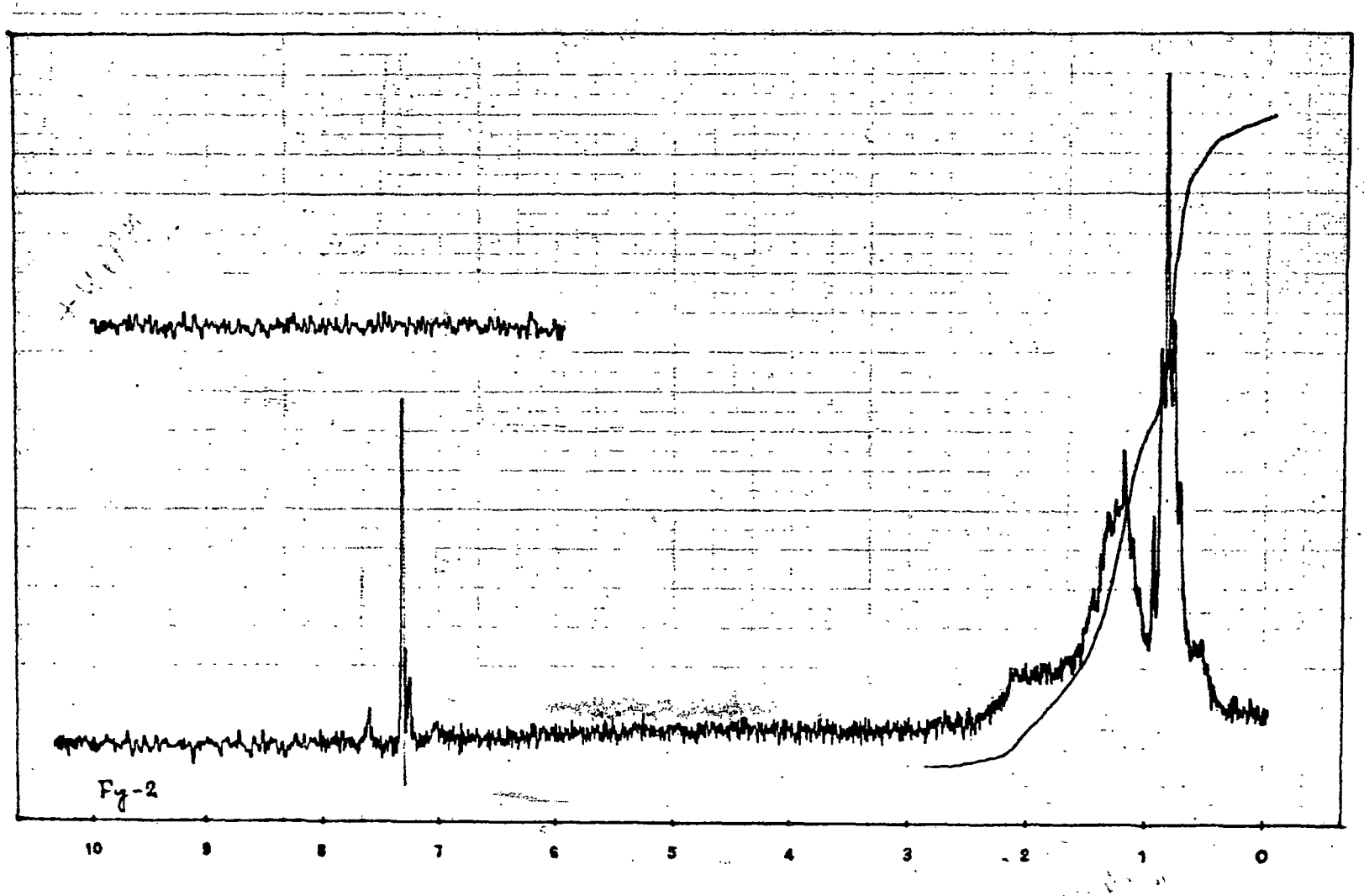


Fig.2: PMR spectrum of oleanan-18∝-28-oic acid, 68.

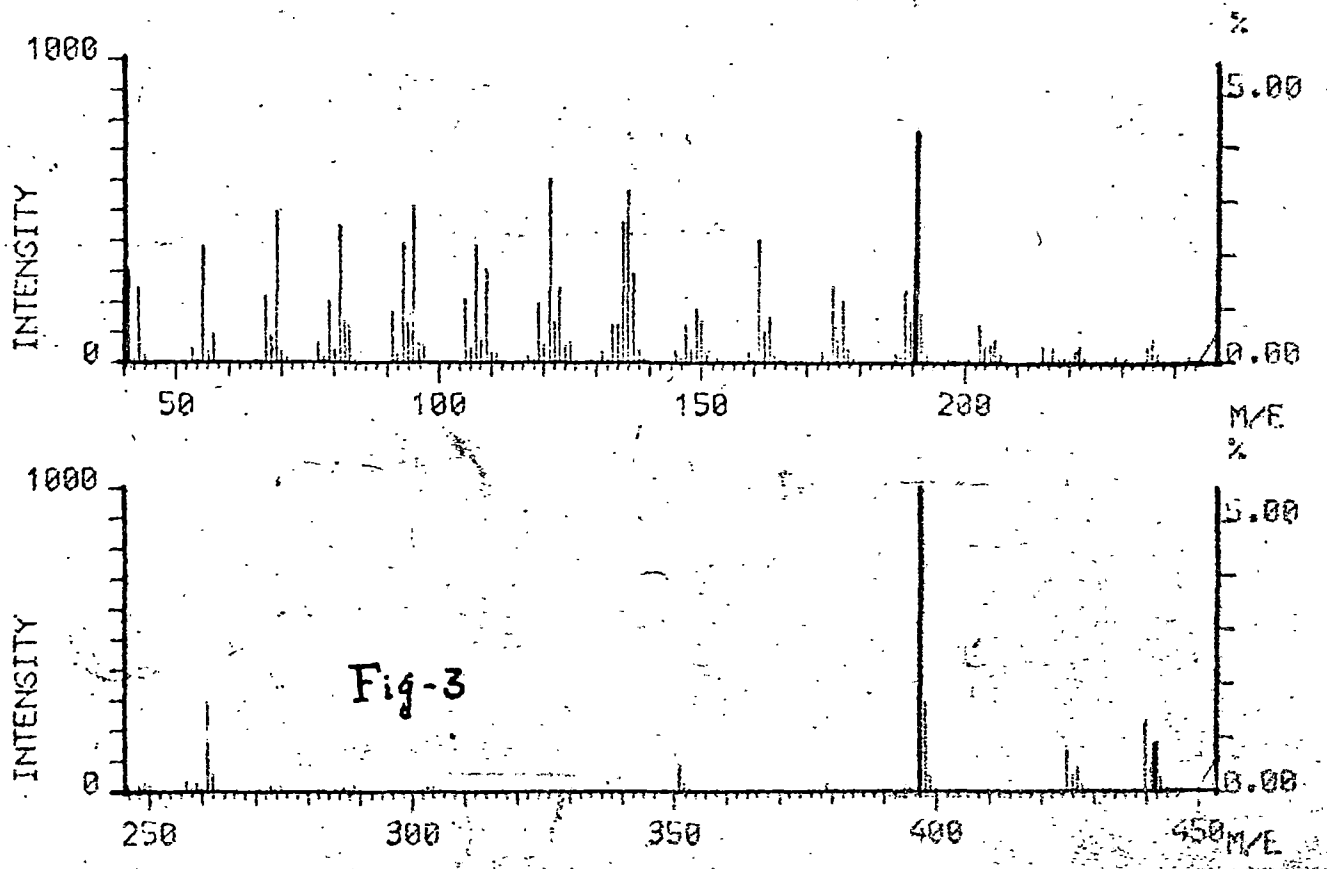


Fig-3

Fig.3: Mass spectrum of oleanen-18∝-28-oic acid, 68.

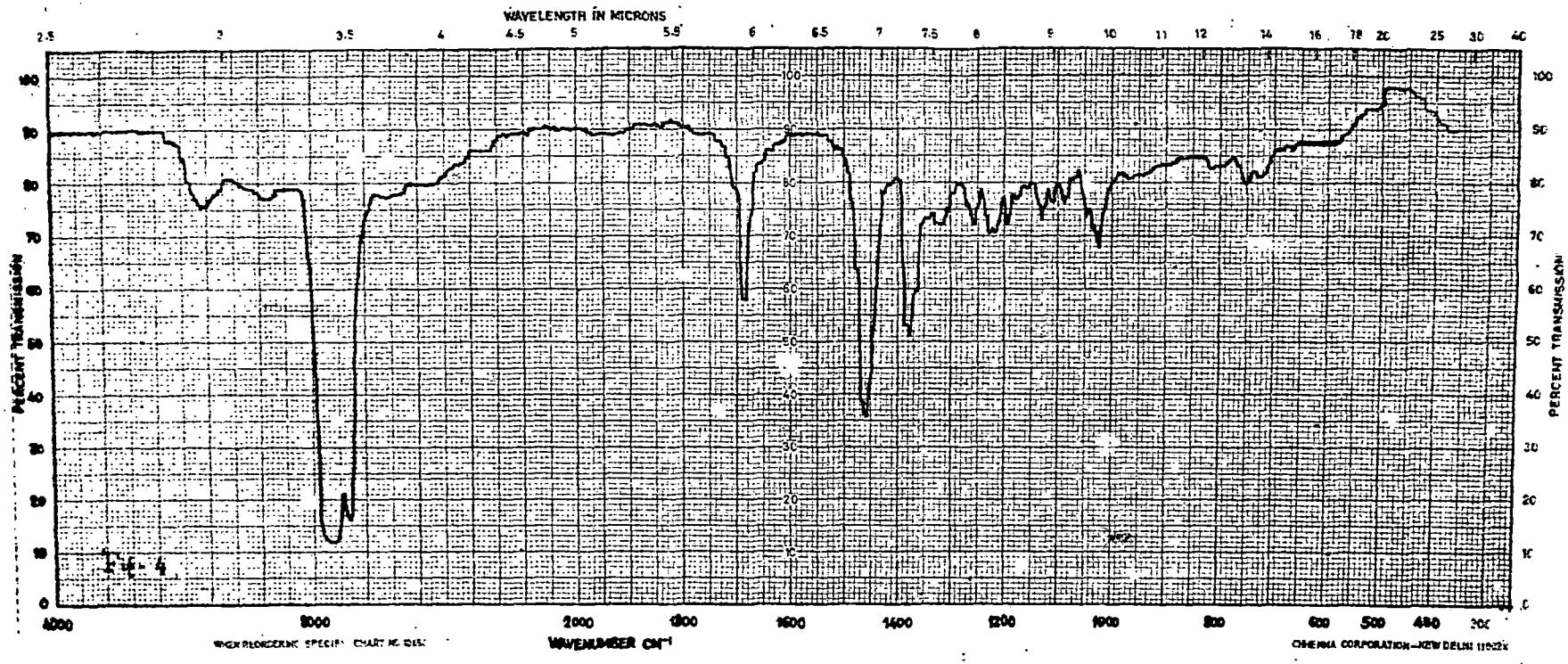
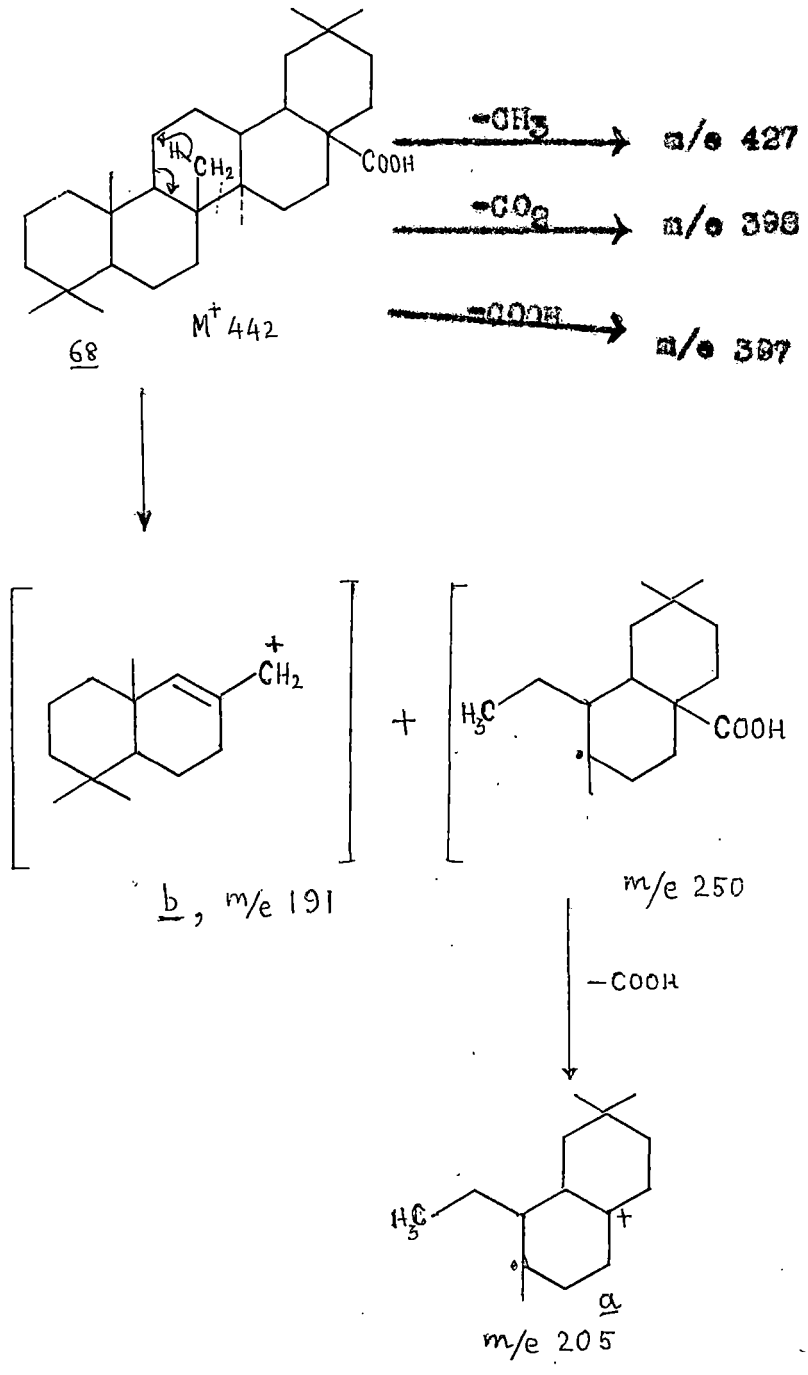
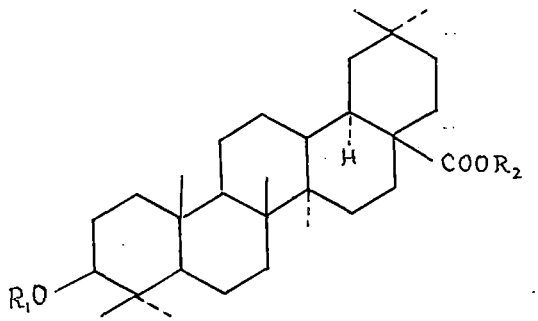


Fig.4: IR spectrum of 3-hydroxy-oleanan-18∝-H-28-oic acid, 69a.

Chart—1


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

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



69a, R_1=H, R_2=H

69b, R_1=H, R_2=CH_3

69c, R_1=AC, R_2=H

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

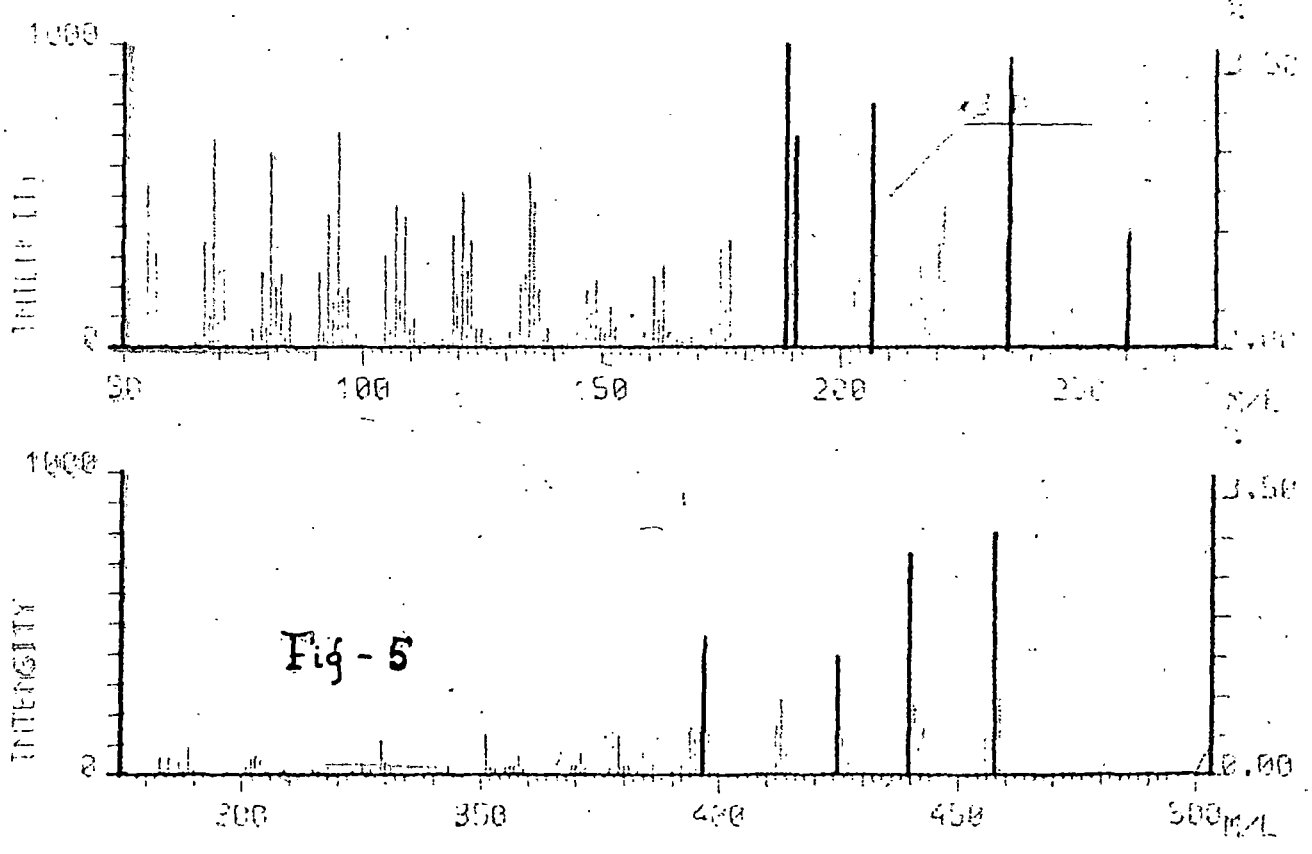
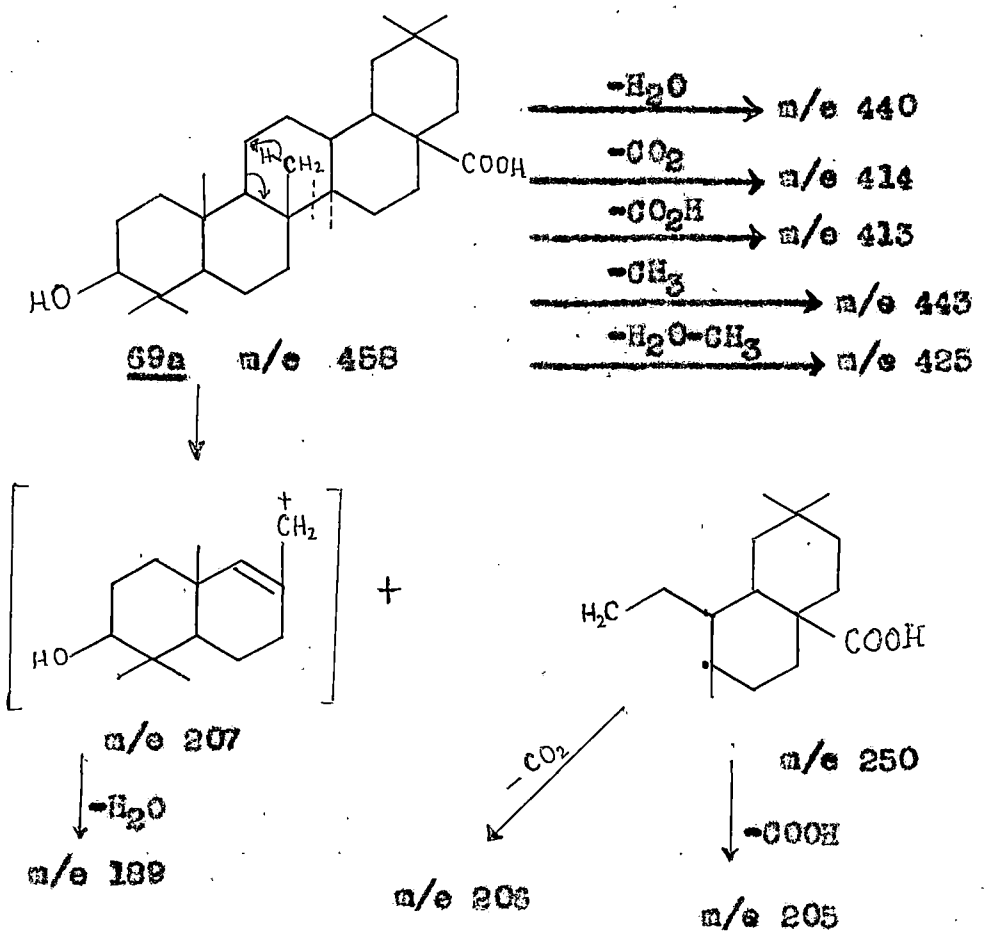


Fig - 5

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

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

Chart—2



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

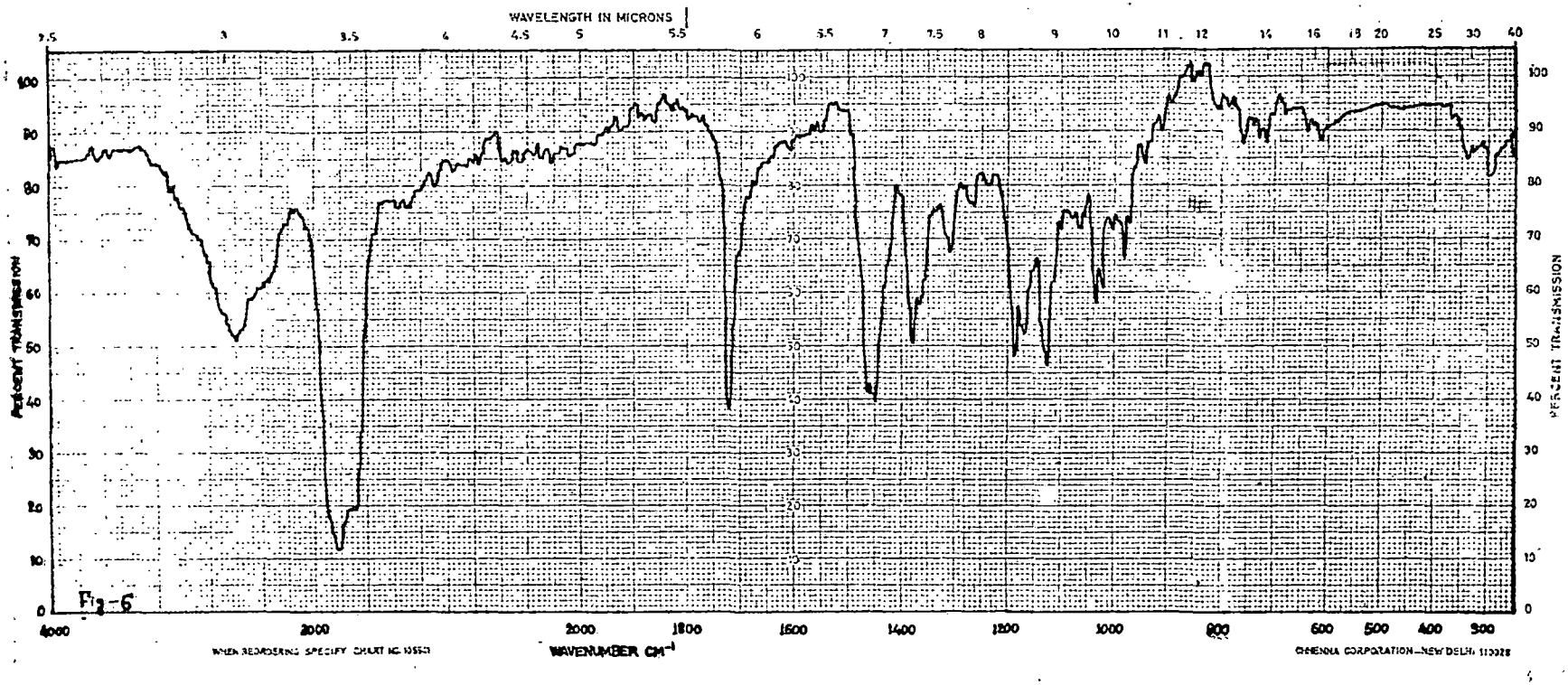


Fig.6: IR spectrum of methyl-3-hydroxy-oleanan-18∝-H-28-oate, 69b.

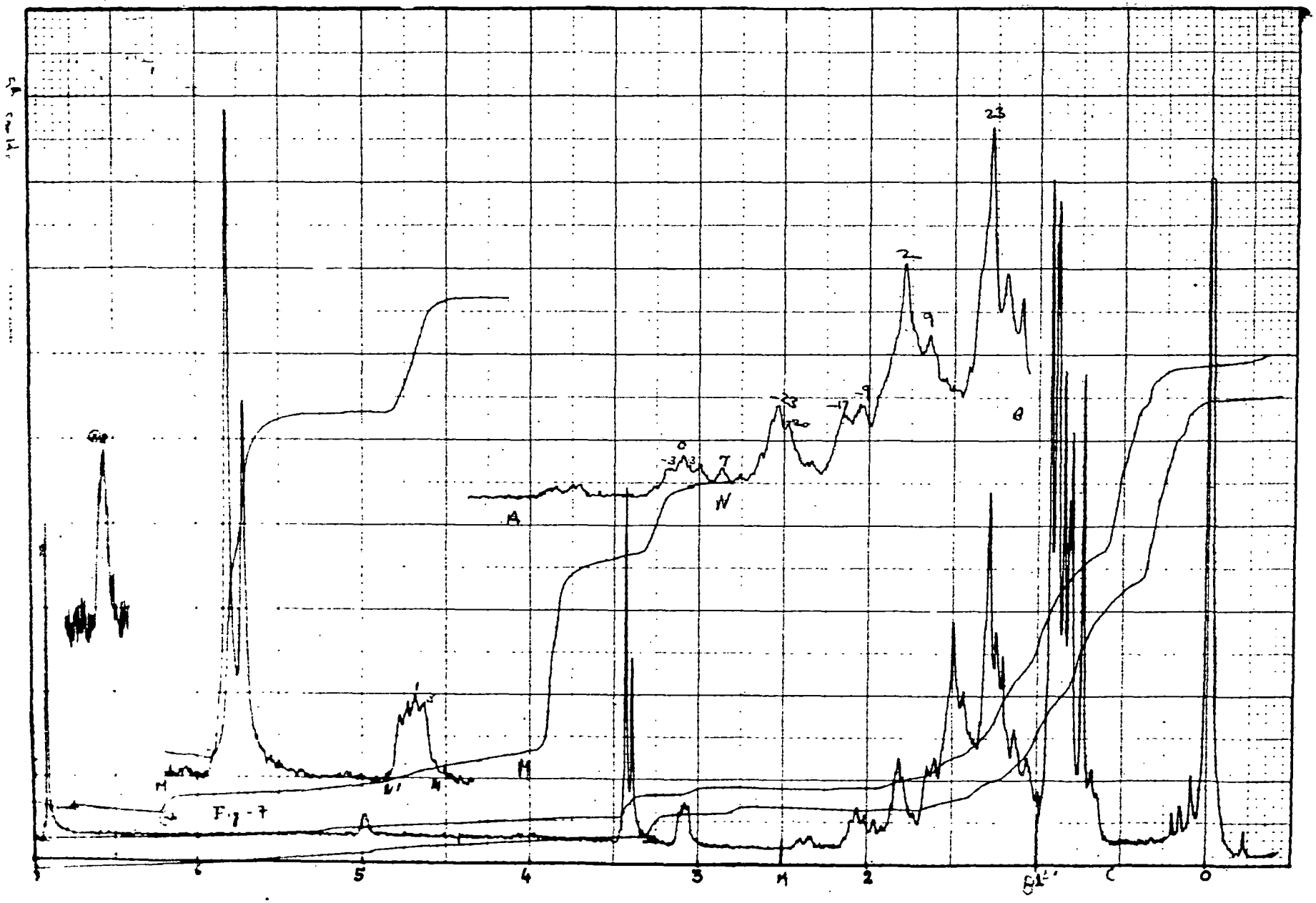


Fig.7: PMR spectrum of methyl-3-hydroxy-olean-18 α-H-28-oate, 69b.

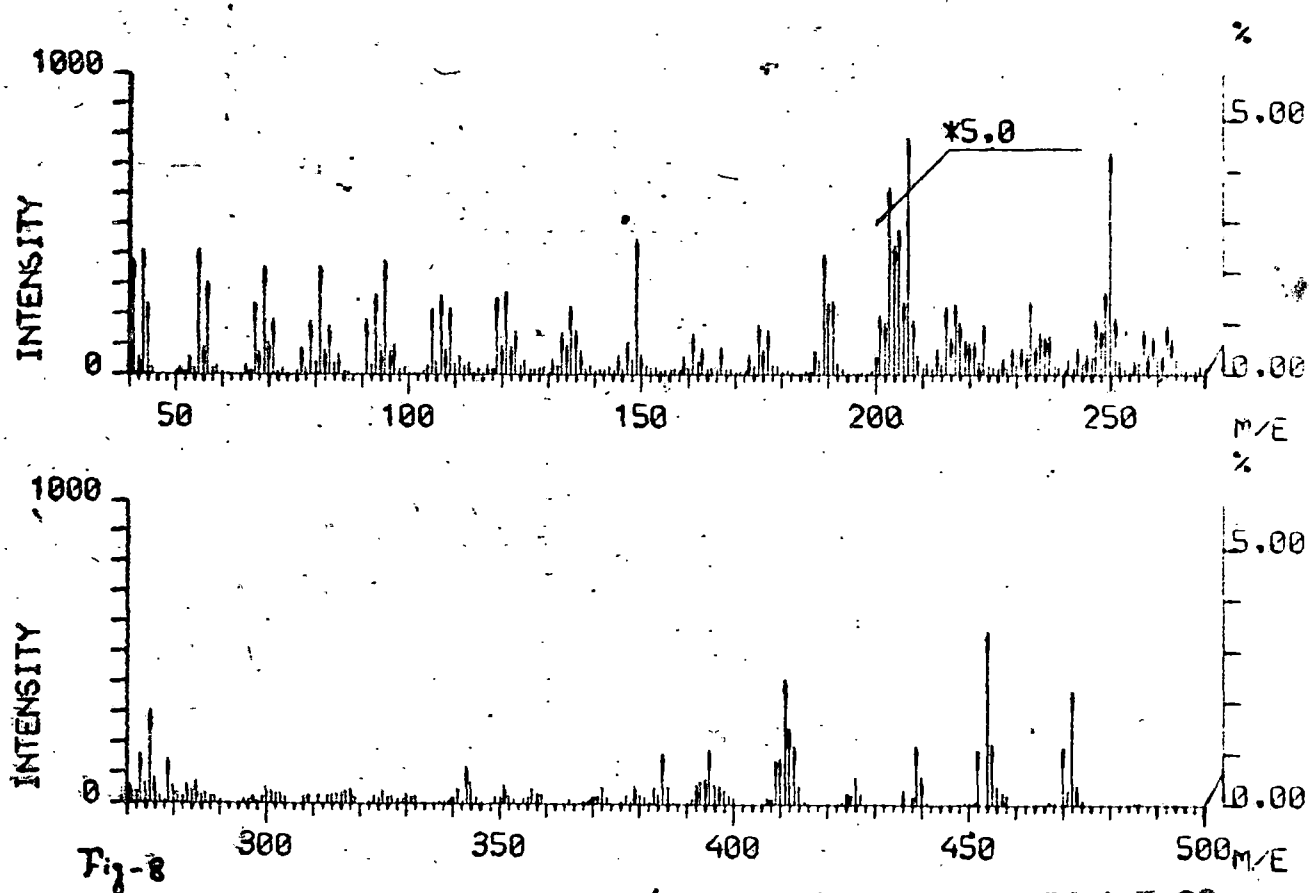
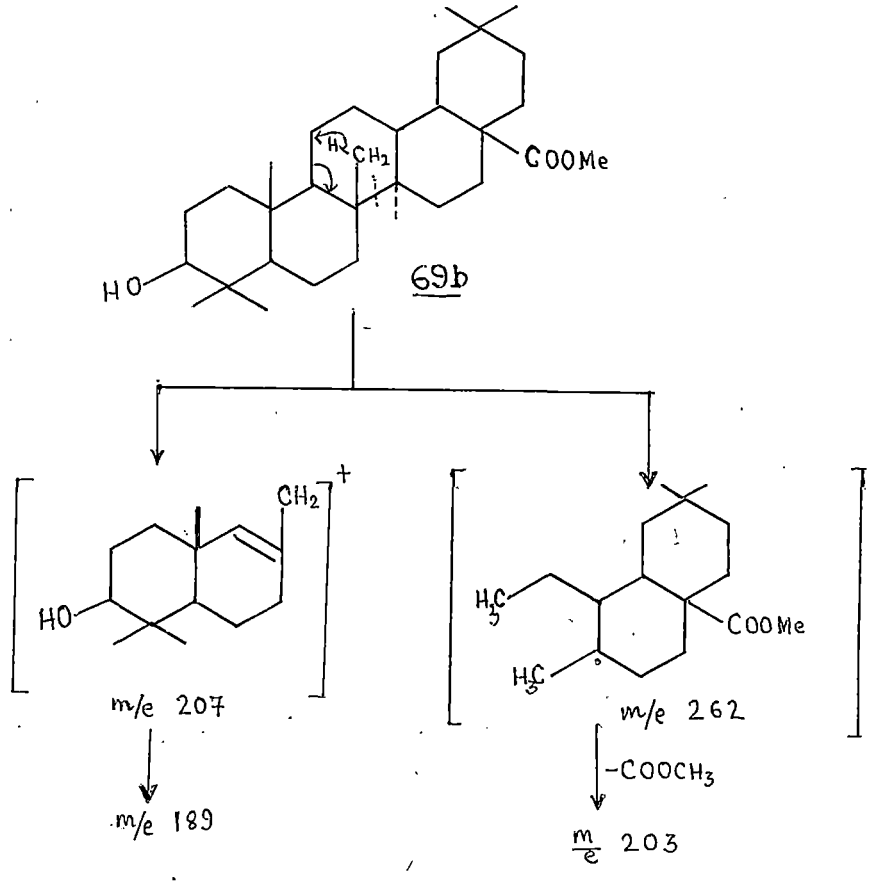


Fig-8

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

Chart—3



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

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

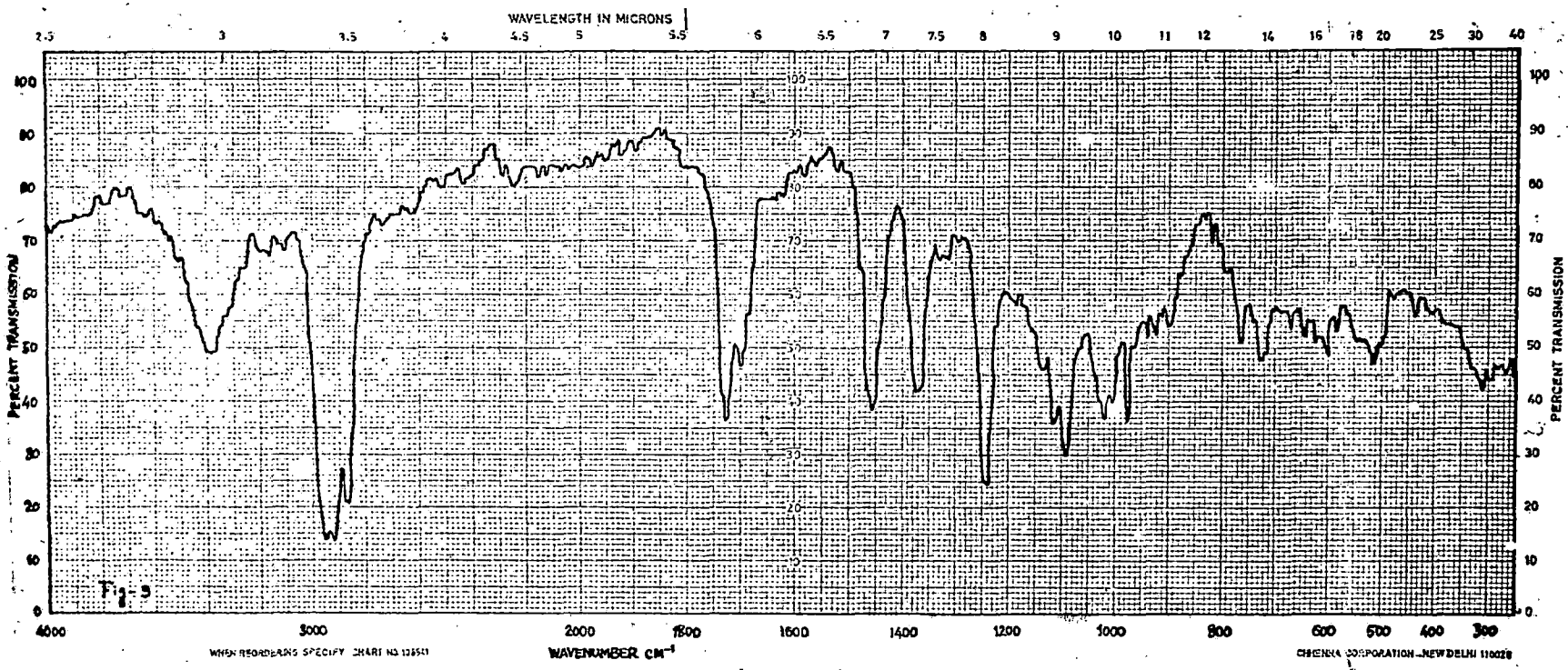


Fig.9: IR spectrum of 3-acetyl-oleanan-18 ∝-H-28-oic acid.

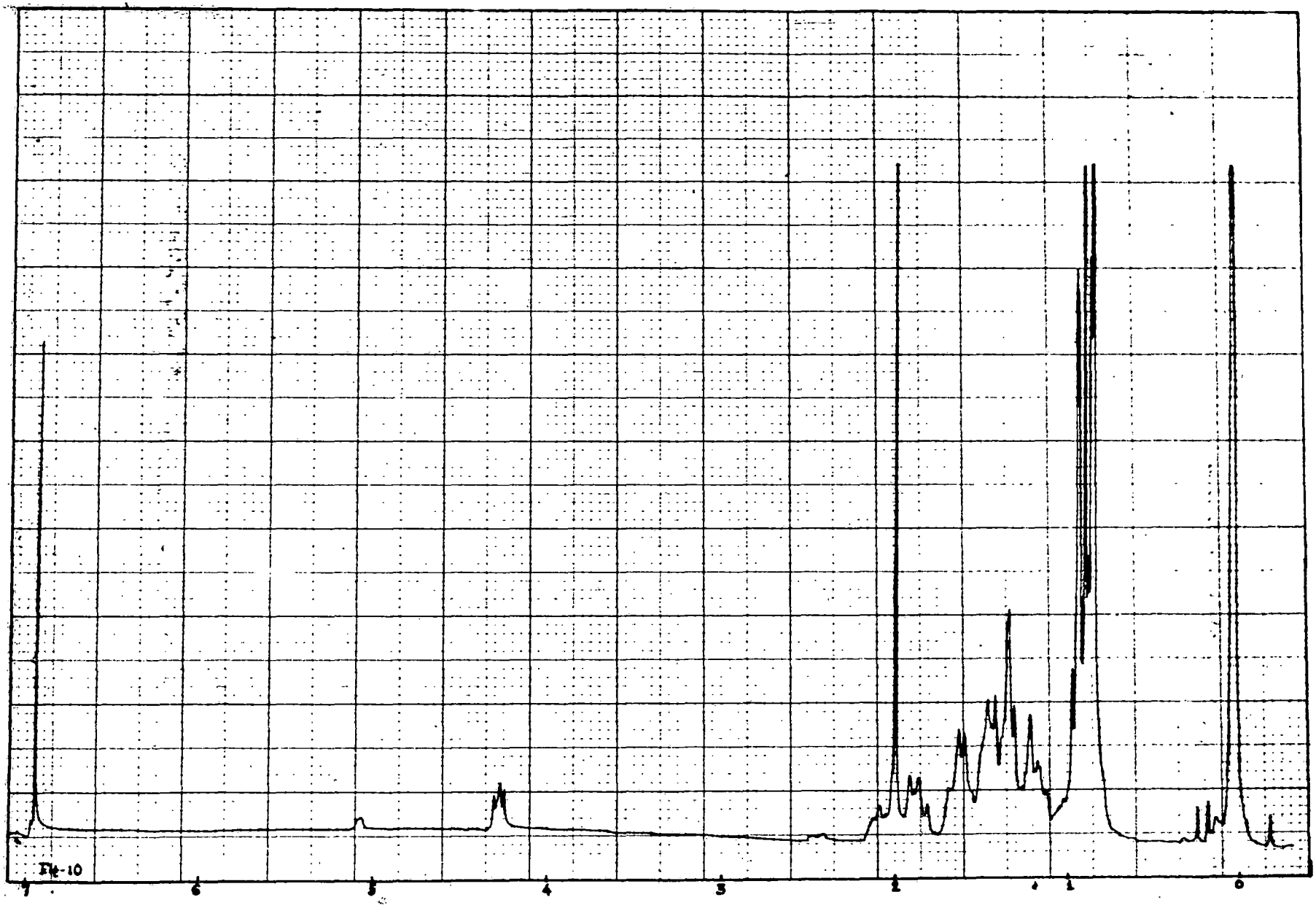


Fig.10: PMR spectrum of 3-acetyl-oleanan-18α-H-28-oic acid, 69c.

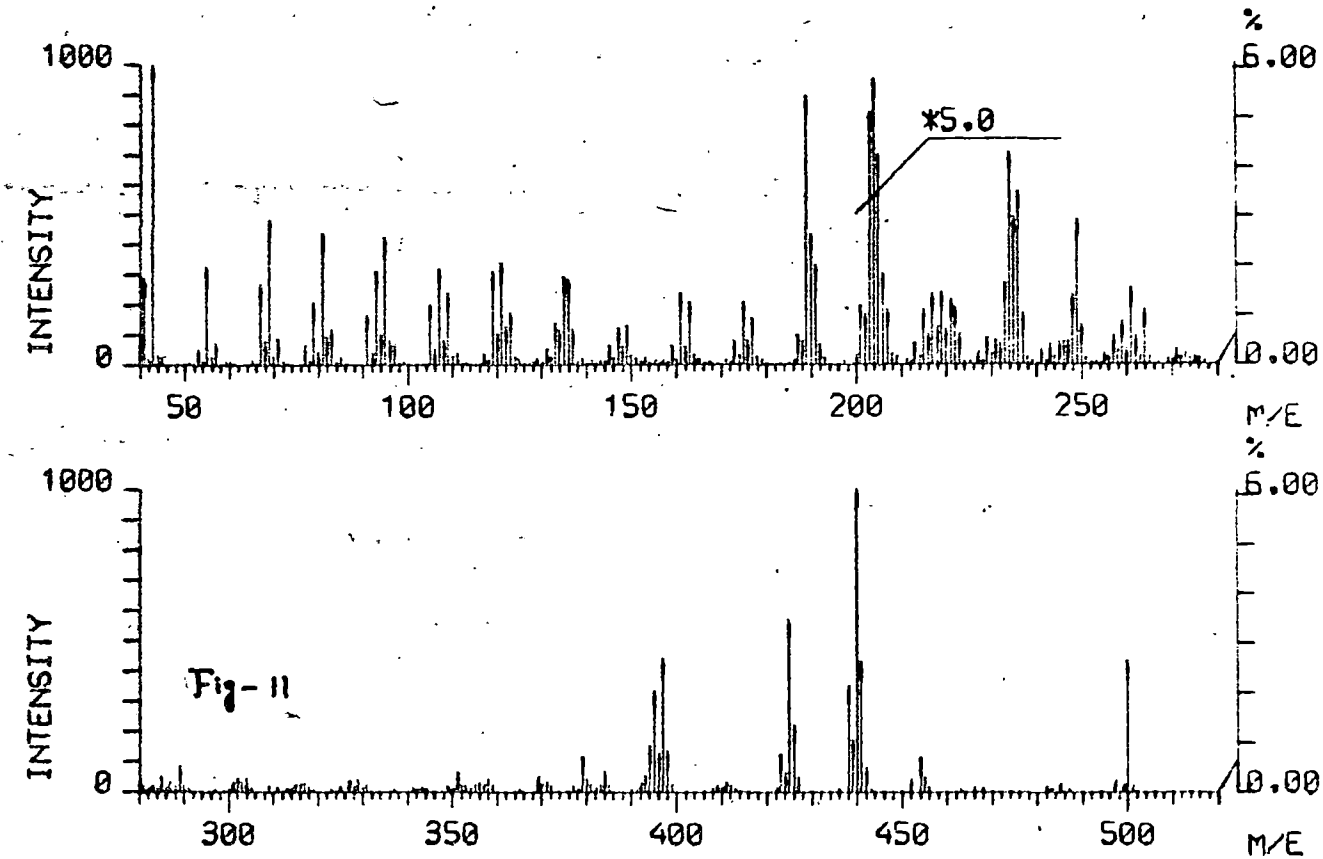
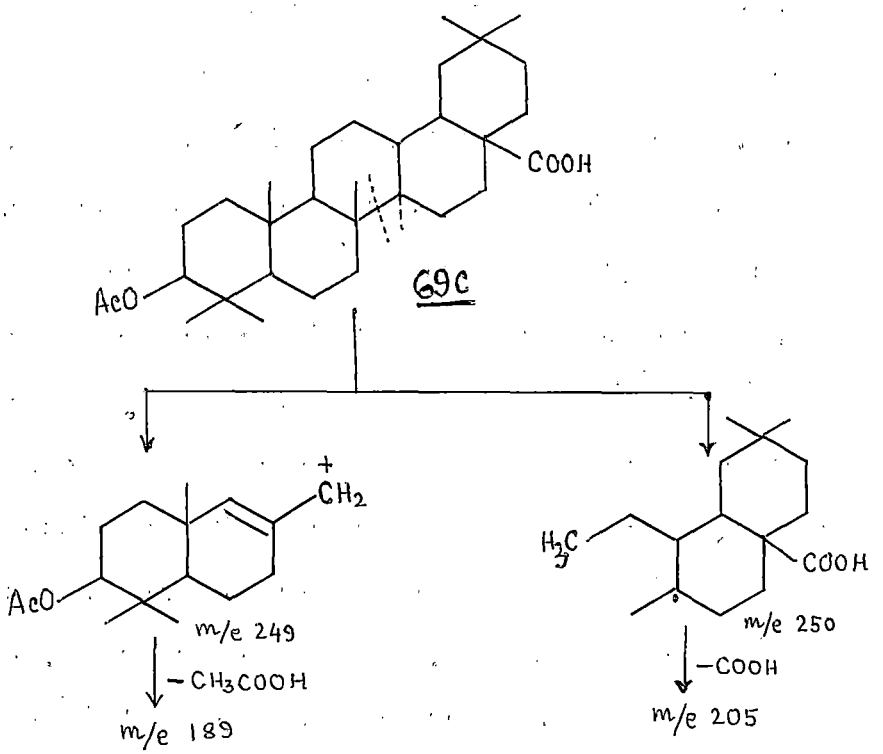


Fig-11

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

Chart—4



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

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

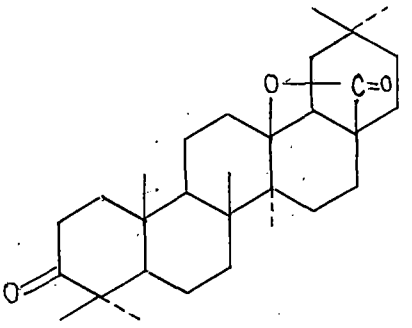
attached in 13 position was tertiary. The cleavage of C—13—O bond is in accord with the mechanism given by Barton and coworkers[21,22 and 24]. Since the lactyl oxygen at C—13 is attached to the tertiary carbon it was assumed that complete deoxygenation at C—13 would give the saturated acid without introduction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

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

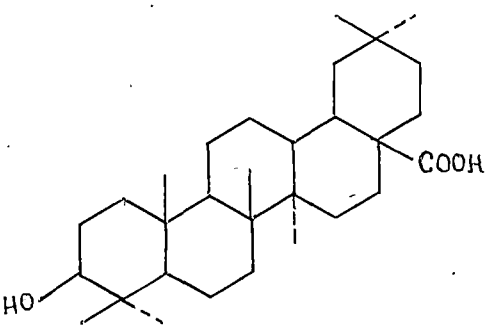
Treatment of lithium in presence of ethylenediamine on 3-oxo-oleanan-18α-H-28⟶13β-olide[29] 70.

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

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



70

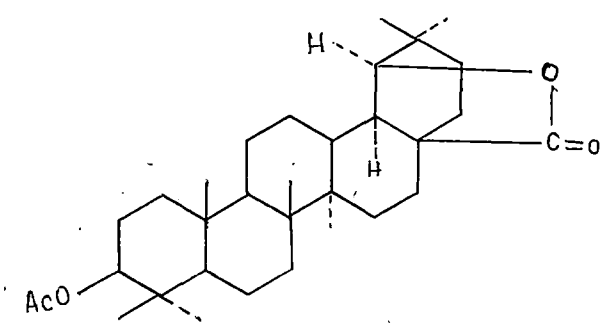


69a

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

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

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



71

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

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

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

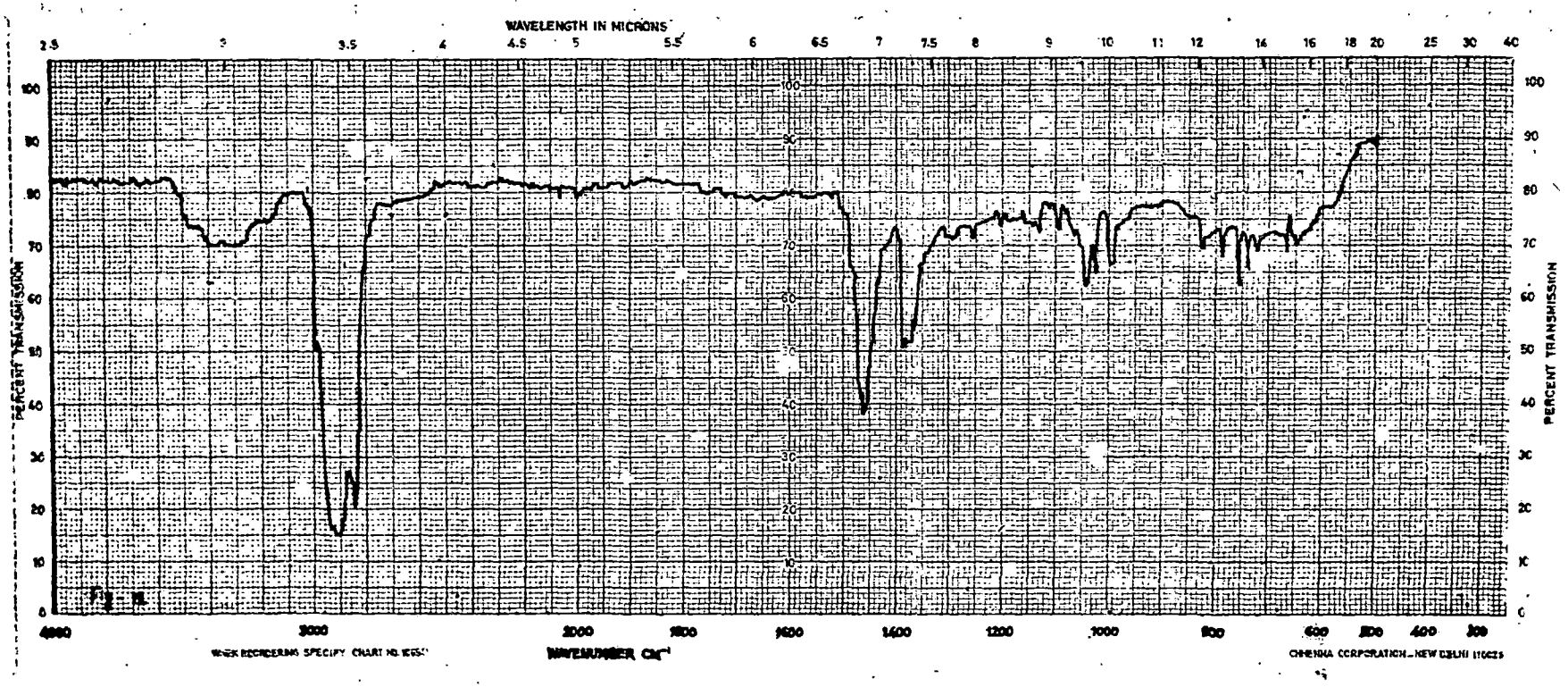
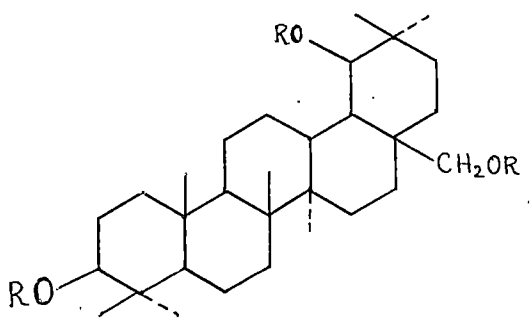


Fig.12: IR spectrum of oleanan-18α-H-3β,19β,28-triol, 72a.

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



72a, R = H

72b, R = OAc.

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

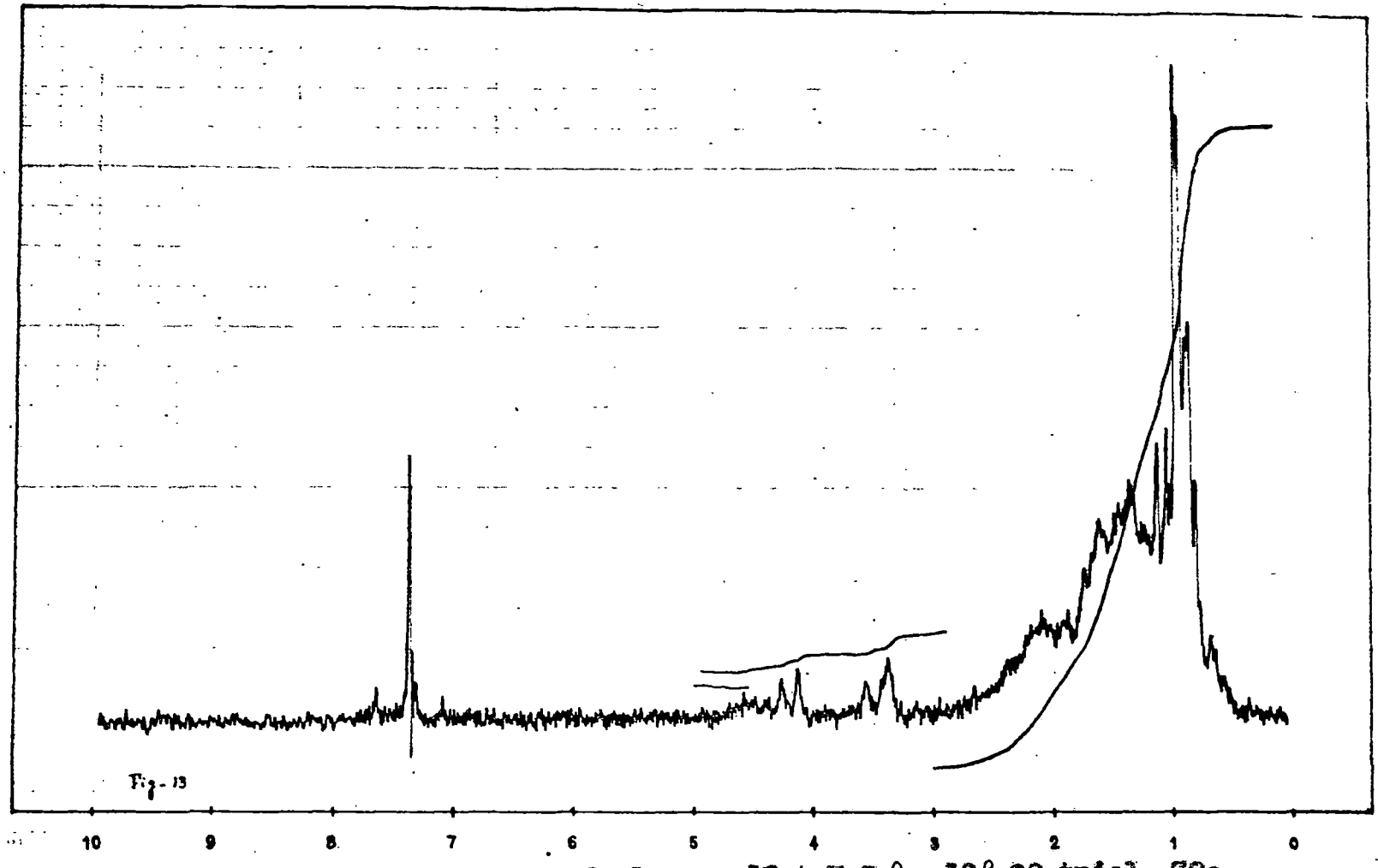


Fig.13: PMR spectrum of oleanan-18α-H,3β, 19β,28-triol, 72a.

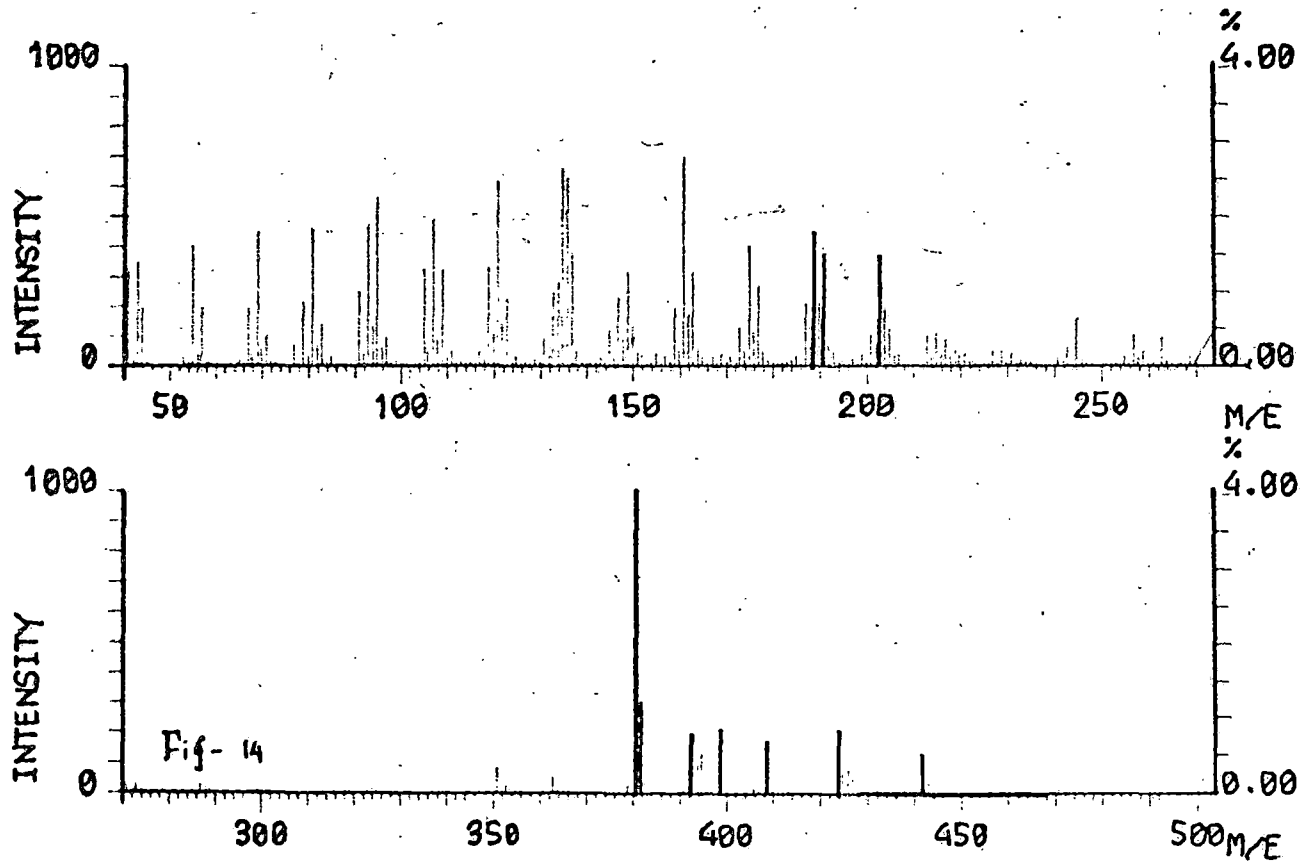
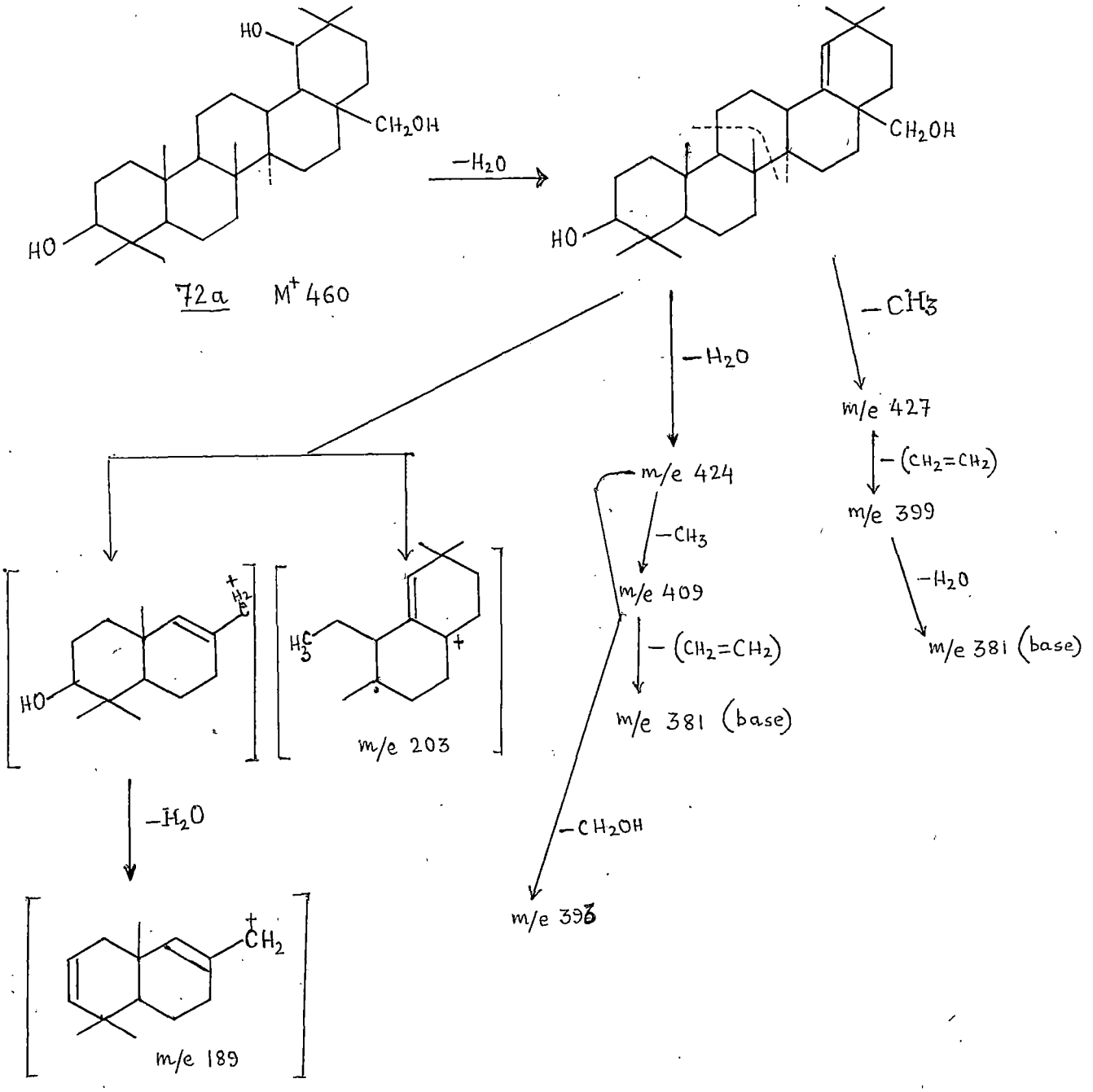


Fig- 14

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

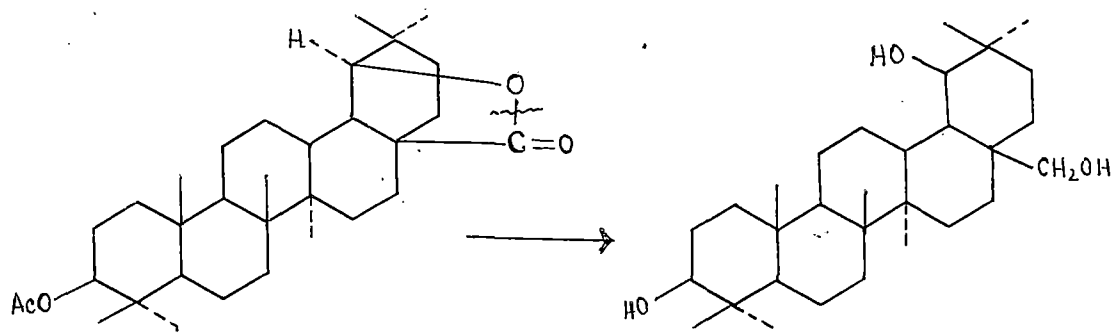
explained by the cleavage of ring C of a saturated oleanan skeleton[28], as shown in Chart—5.

Chart—5



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

Scheme—7

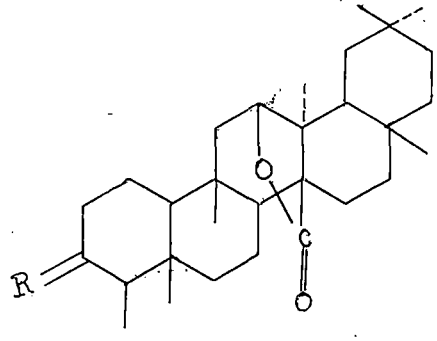


71 72a

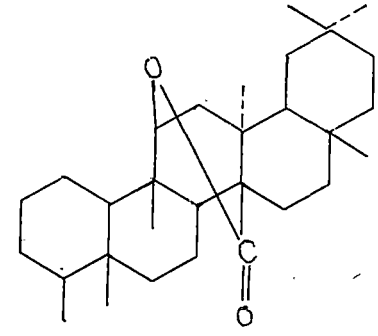
(c) Lithium-ethylenediamine reaction on sterically hindered triterpene lactones:

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

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



73, R=O
74a, R=H_2



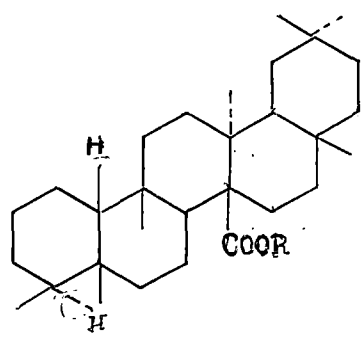
74b

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

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

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

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



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

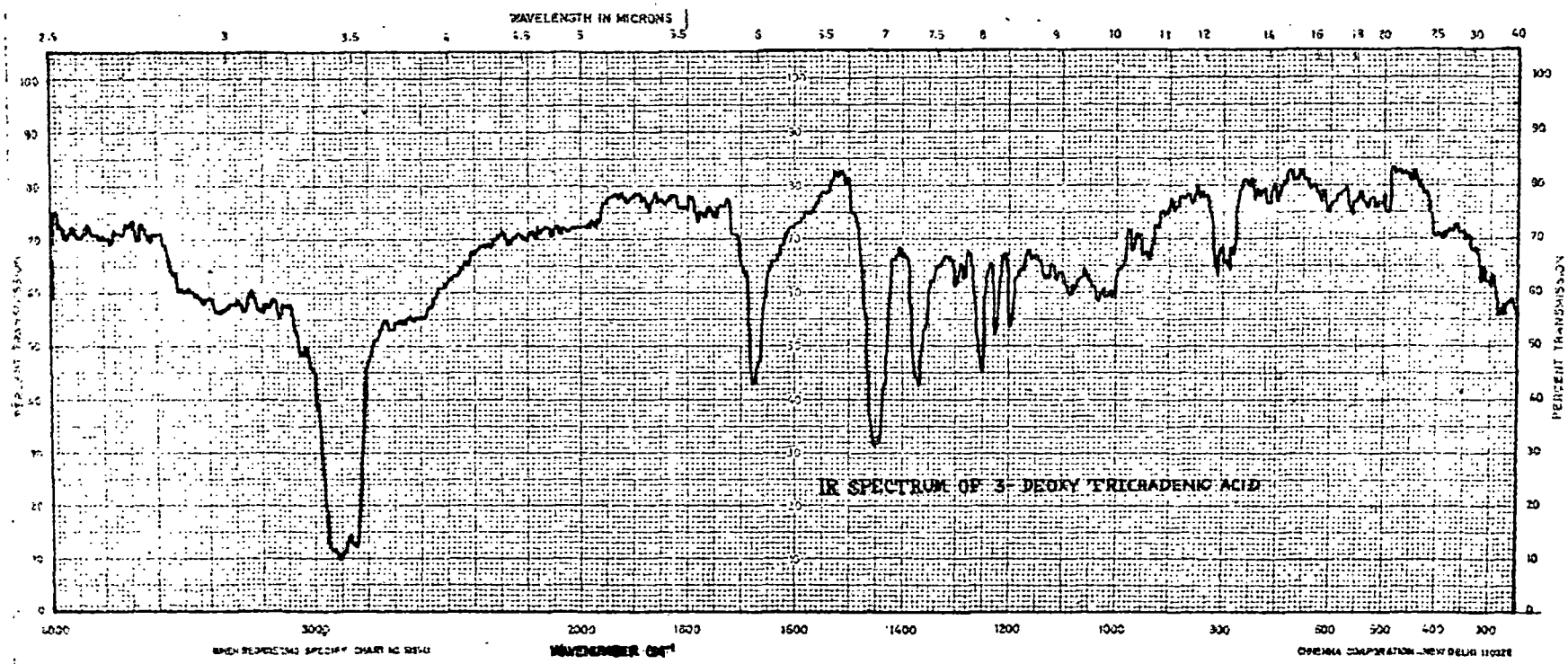


Fig.15: IR spectrum of 3-deoxy-trichadenic acid, 75a.

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

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

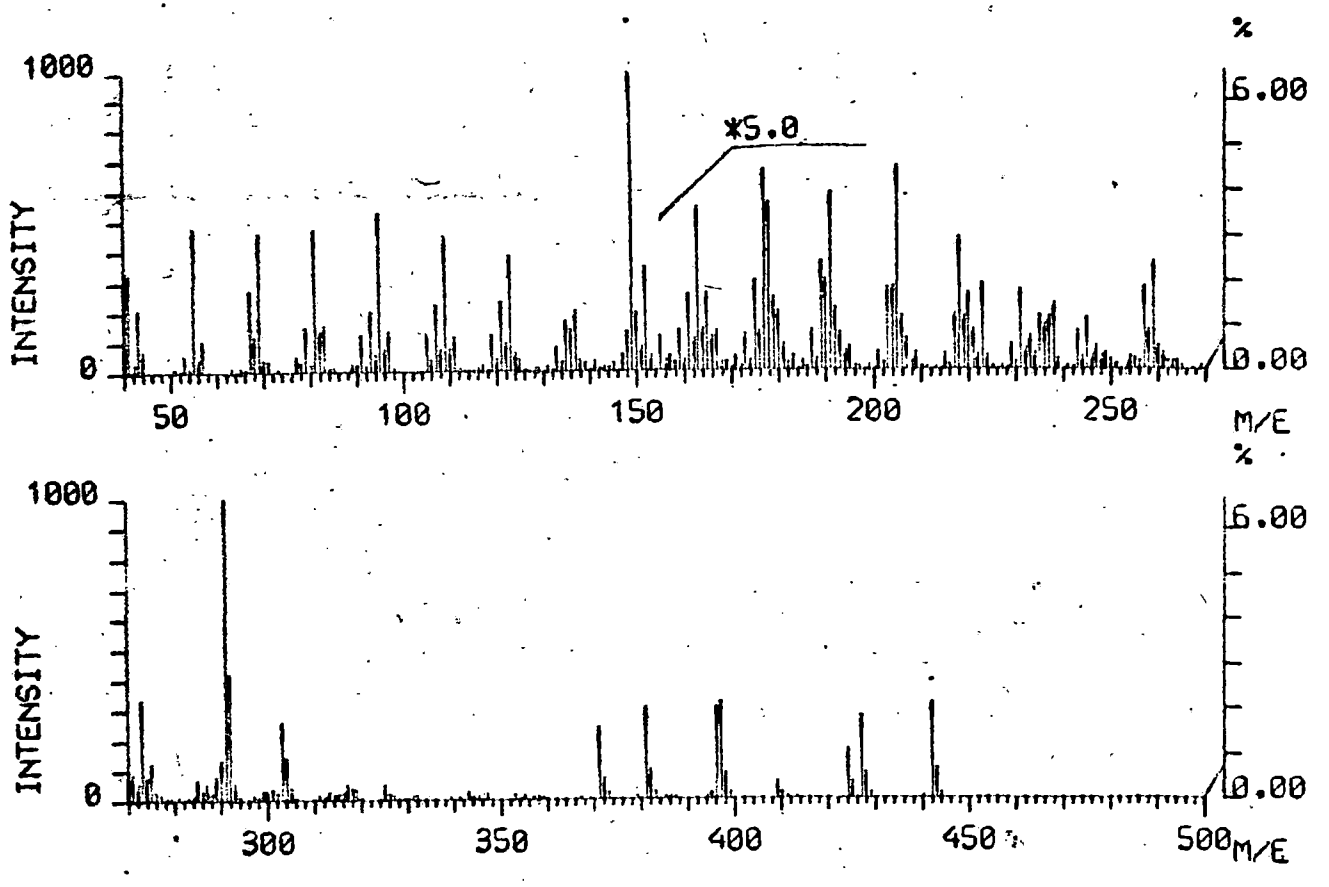


Fig.16: Mass spectrum of 3-deoxy-trichadenic acid, 75a.

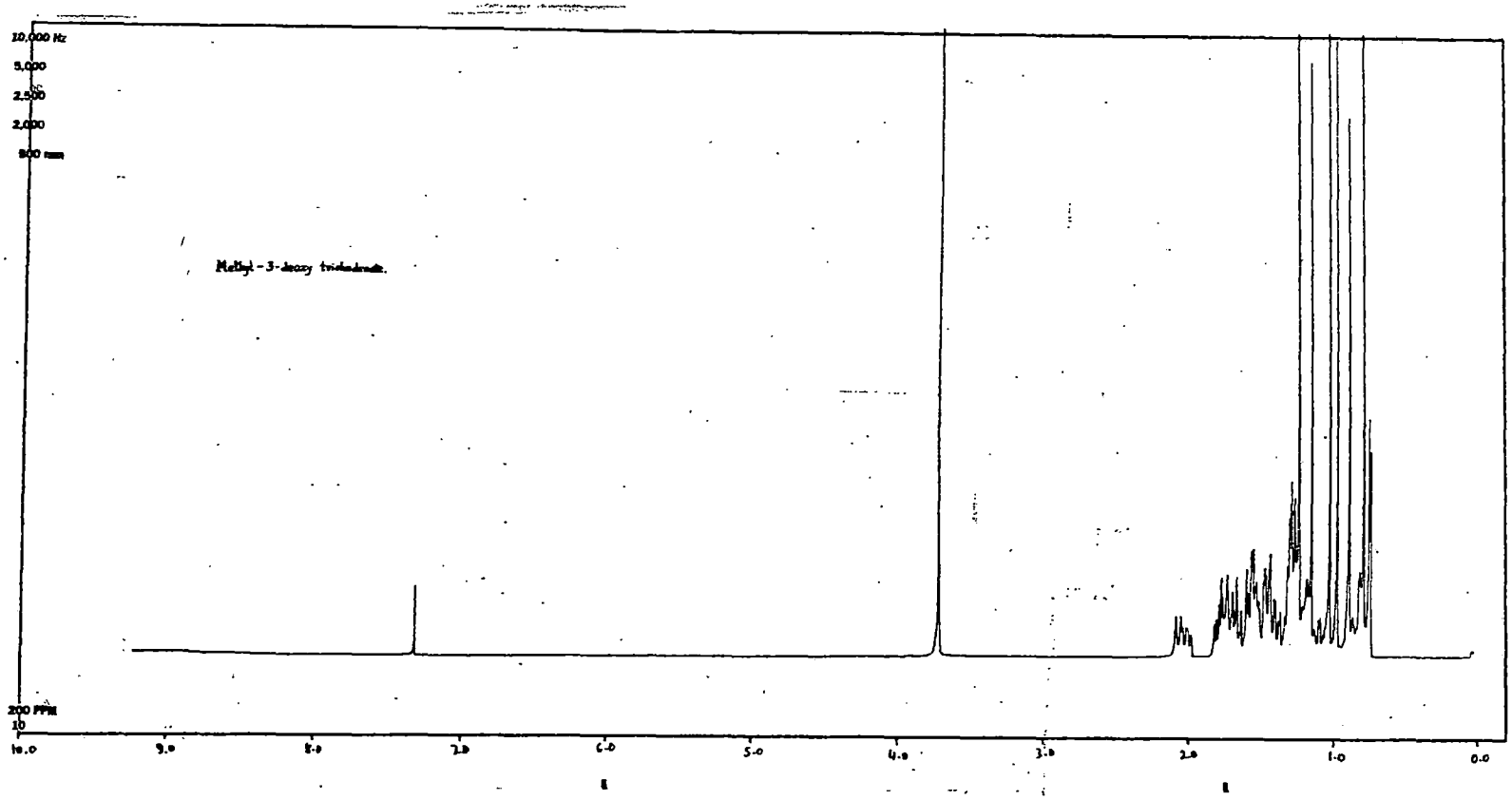


Fig.17: PMR spectrum of methyl-3-deoxy-trichadenate, 75b.

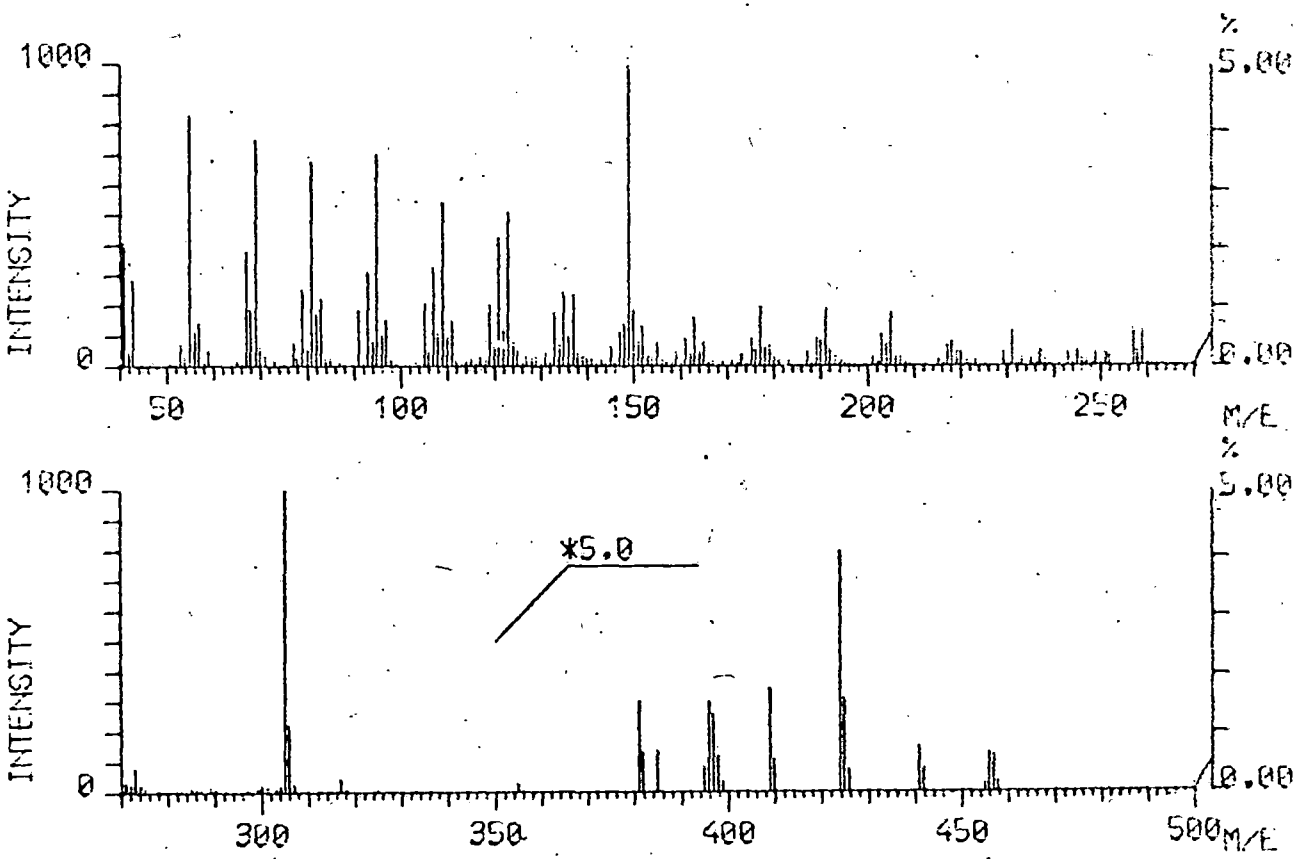
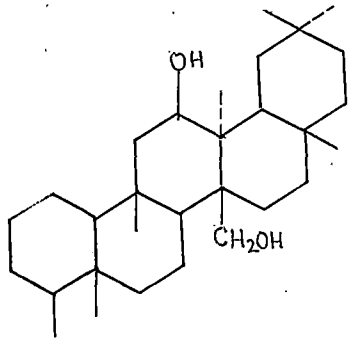


Fig.18: Mass spectrum of methyl-3-deoxy-trichadonate, 75b.

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



76

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

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

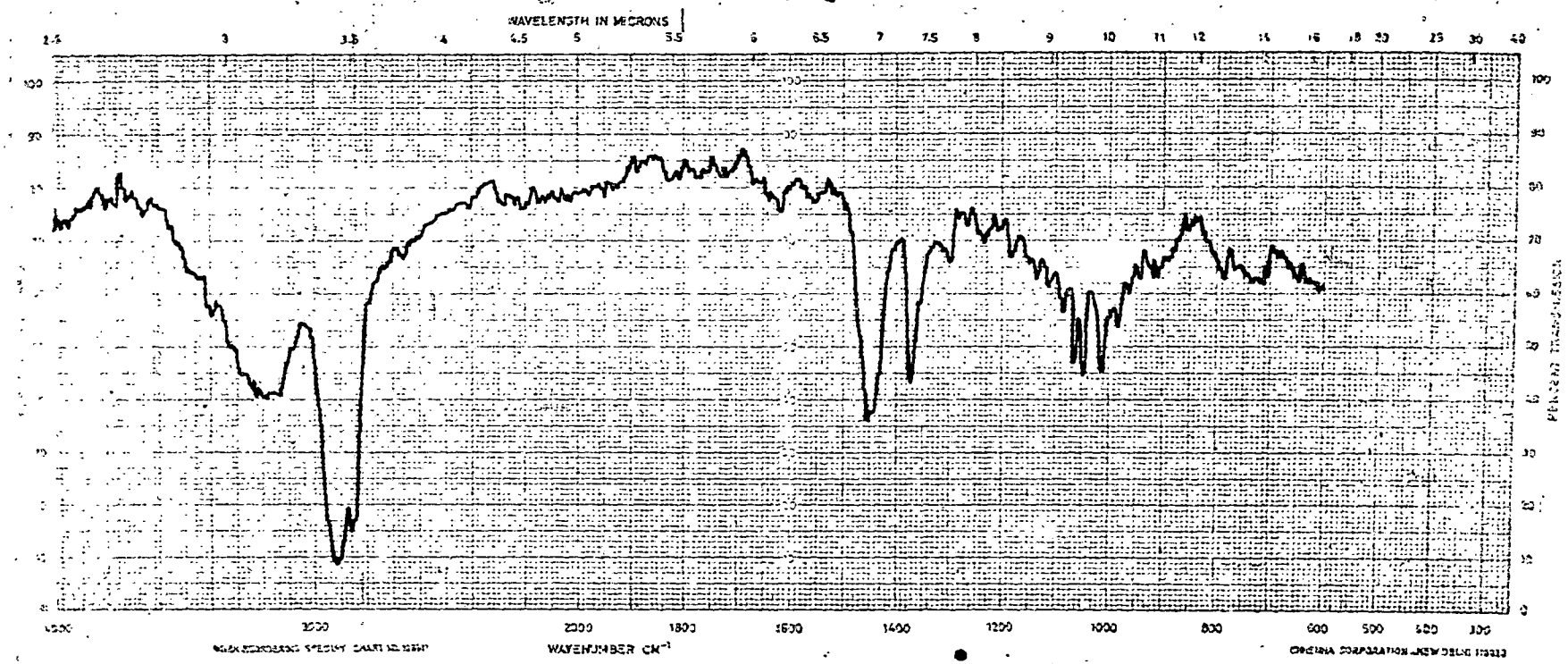


Fig.19: IR spectrum of friedelan-12β,28-diol, 76.

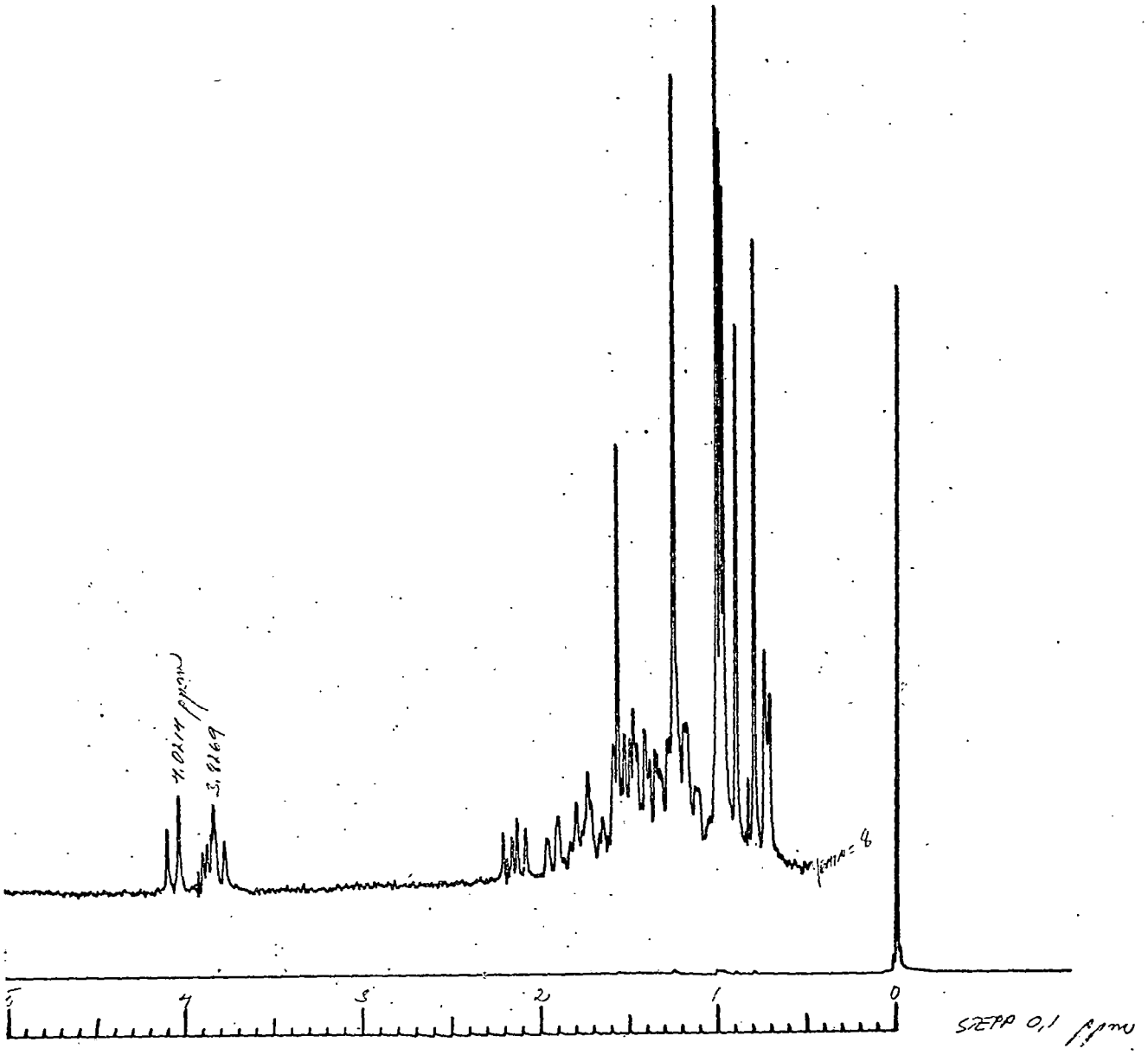
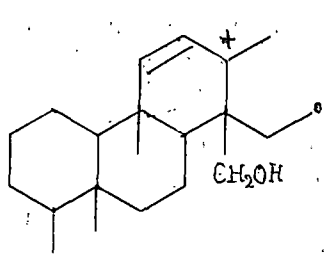


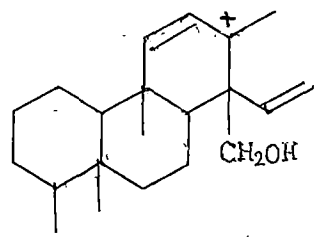
Fig.20: PMR spectrum of friedelan-12β,26-diol, 76.

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



m/e 302

a'



m/e 301

b'

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

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

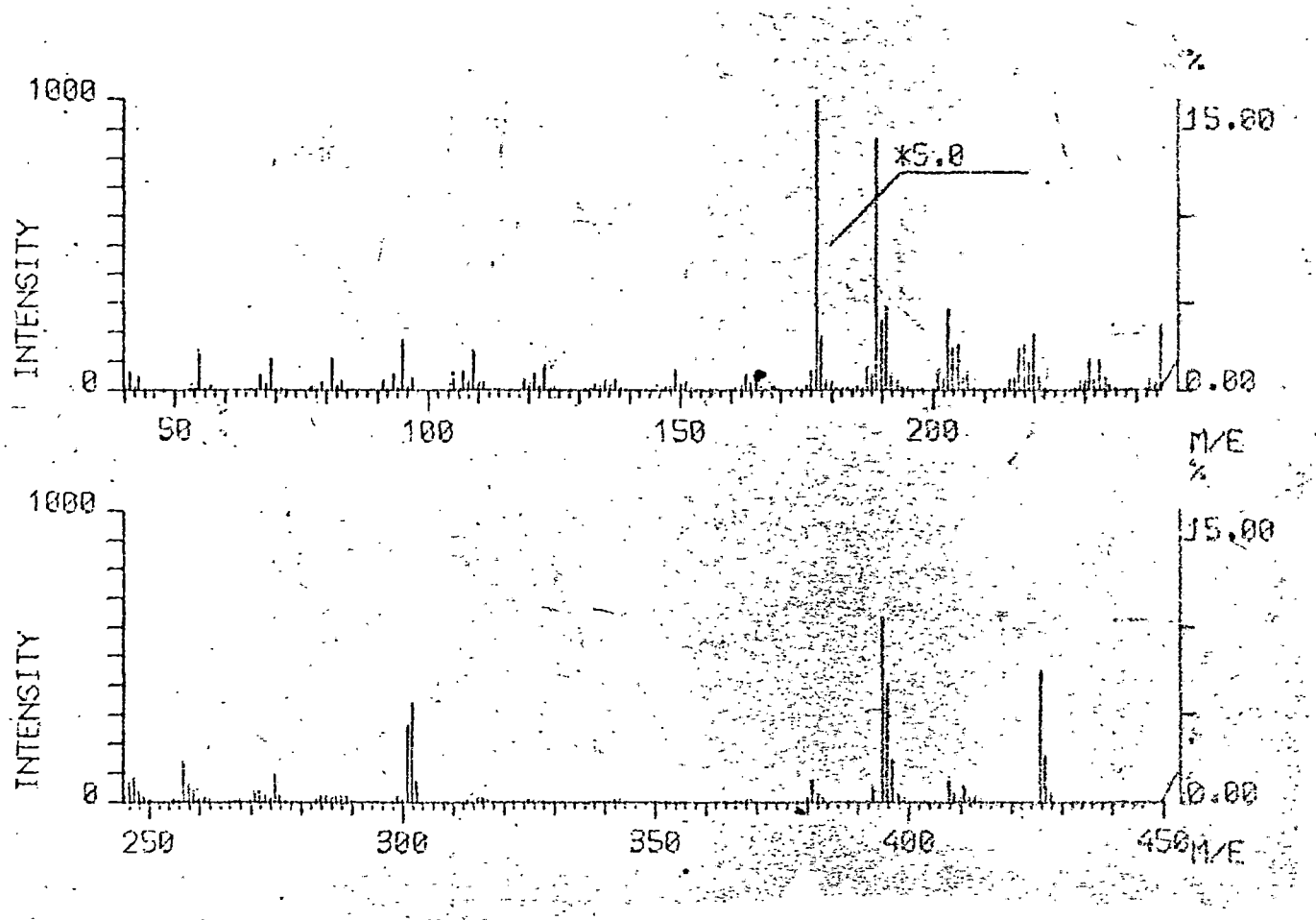


Fig.21: Mass spectrum of friedelan-12β,28-diol 76.

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

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

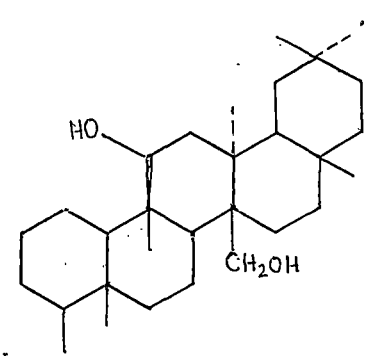
The lactone 74b in ethylenediamine was refluxed with lithium in an atmosphere of nitrogen gas was followed by usual work up and separation into acid and neutral fractions by 10% sodium hydroxide.

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

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

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

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



76a

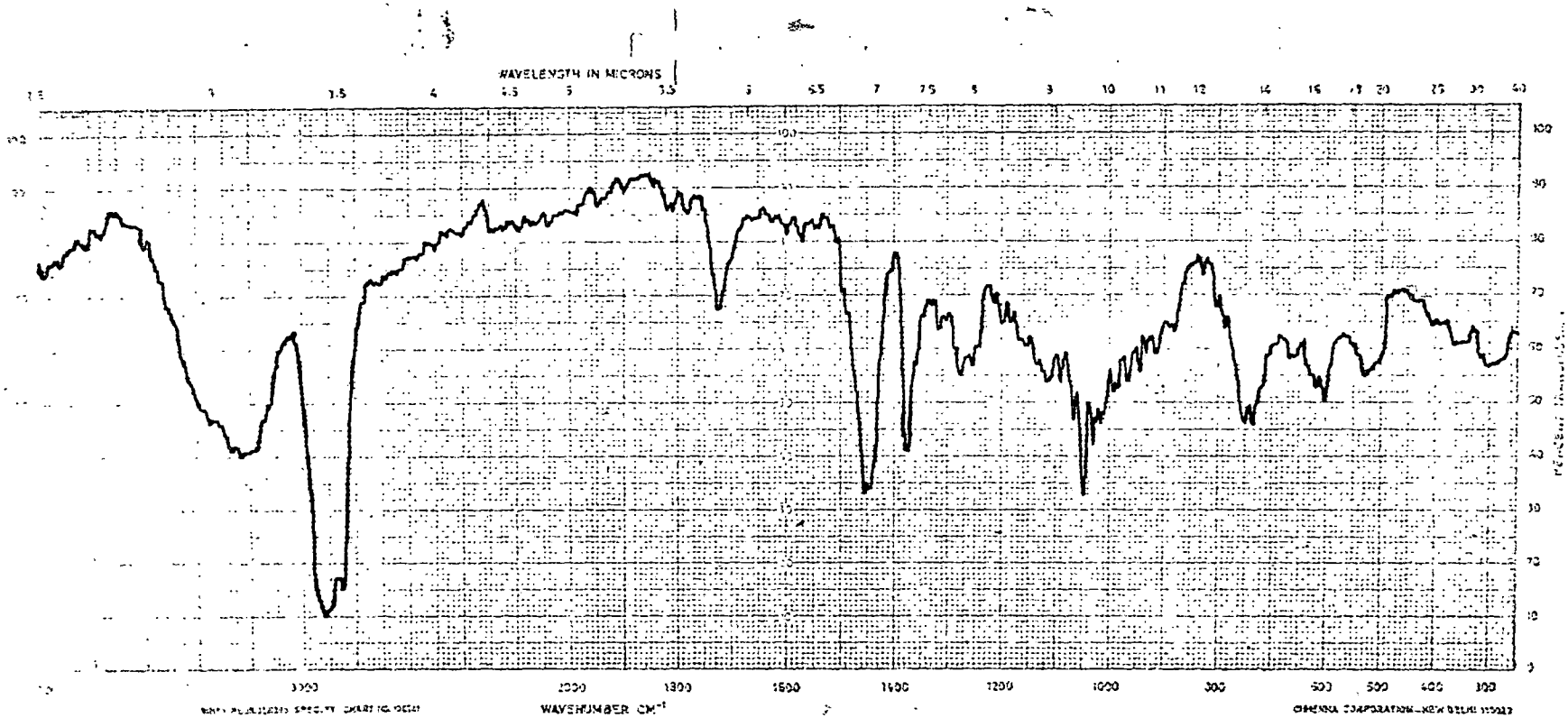


Fig.22: IR spectrum of friedelan-11β,26-diol, 76a.

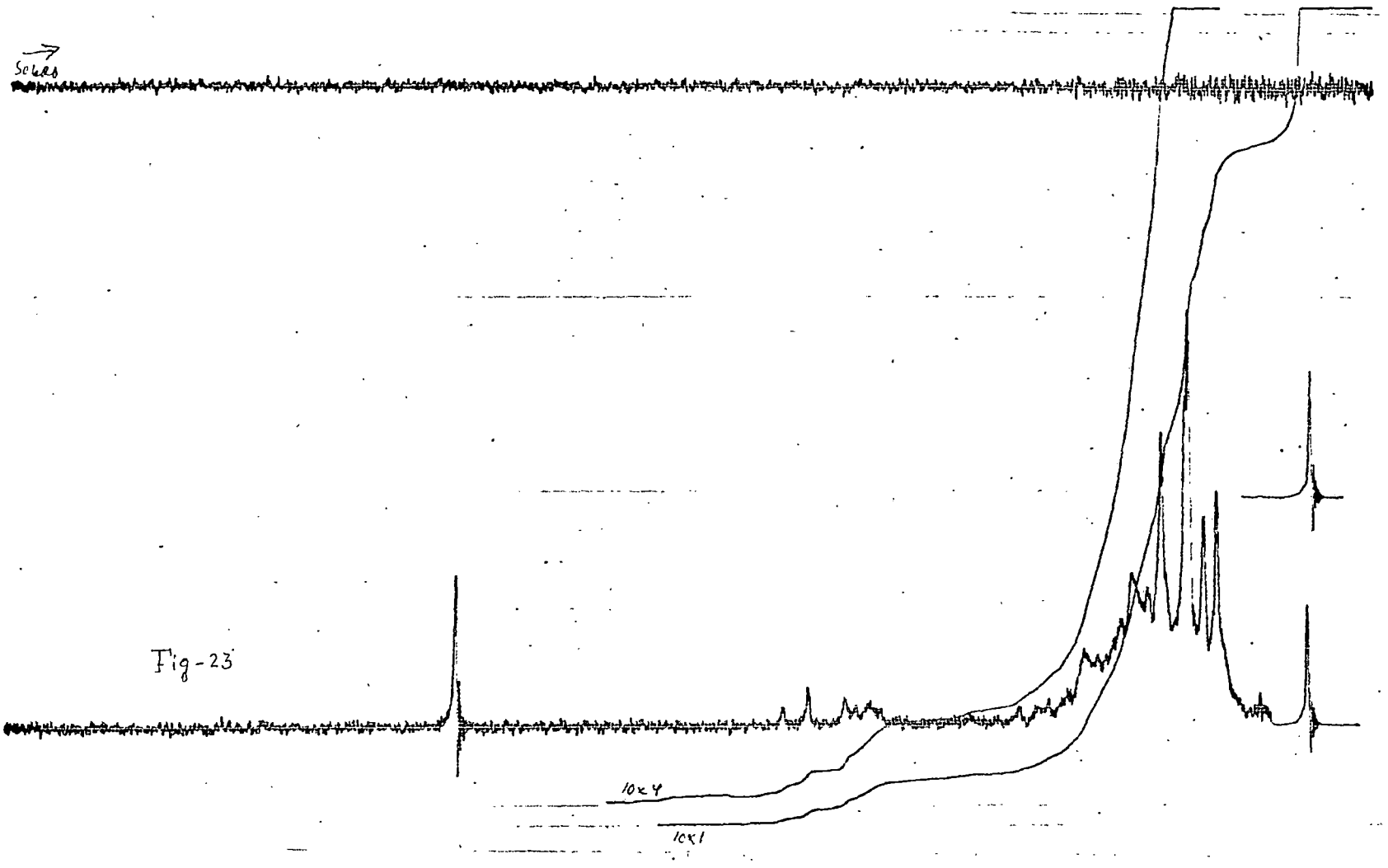


Fig.23: PMR spectrum of friedelan-11β ,26-diol, 76a.

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

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

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

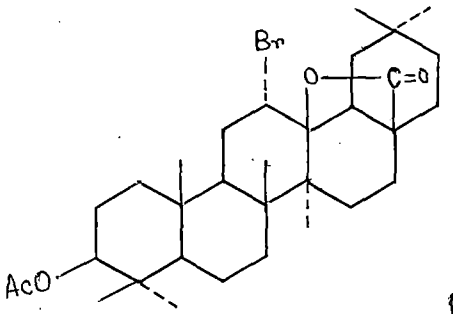
So from above findings it is clear that the ring opening of lactones, in different sterical environments, gives products in differents yields. The more hindered lactones give the corresponding saturated acids in higher yield by deoxygenation at carbon where lactyl oxygen is attached and the less hindered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

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

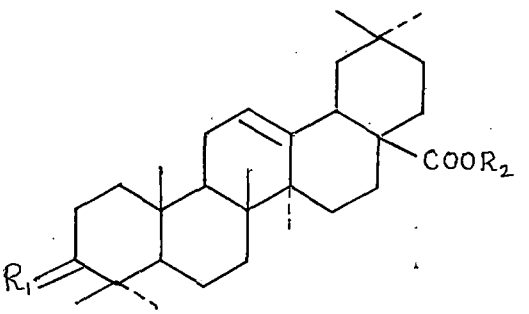
In order to examine the effect of bromine on the nature and yields of products formed on Li-ethylenediamine reduction the following compounds have been selected and studied for the purpose:

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

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



77



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

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

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

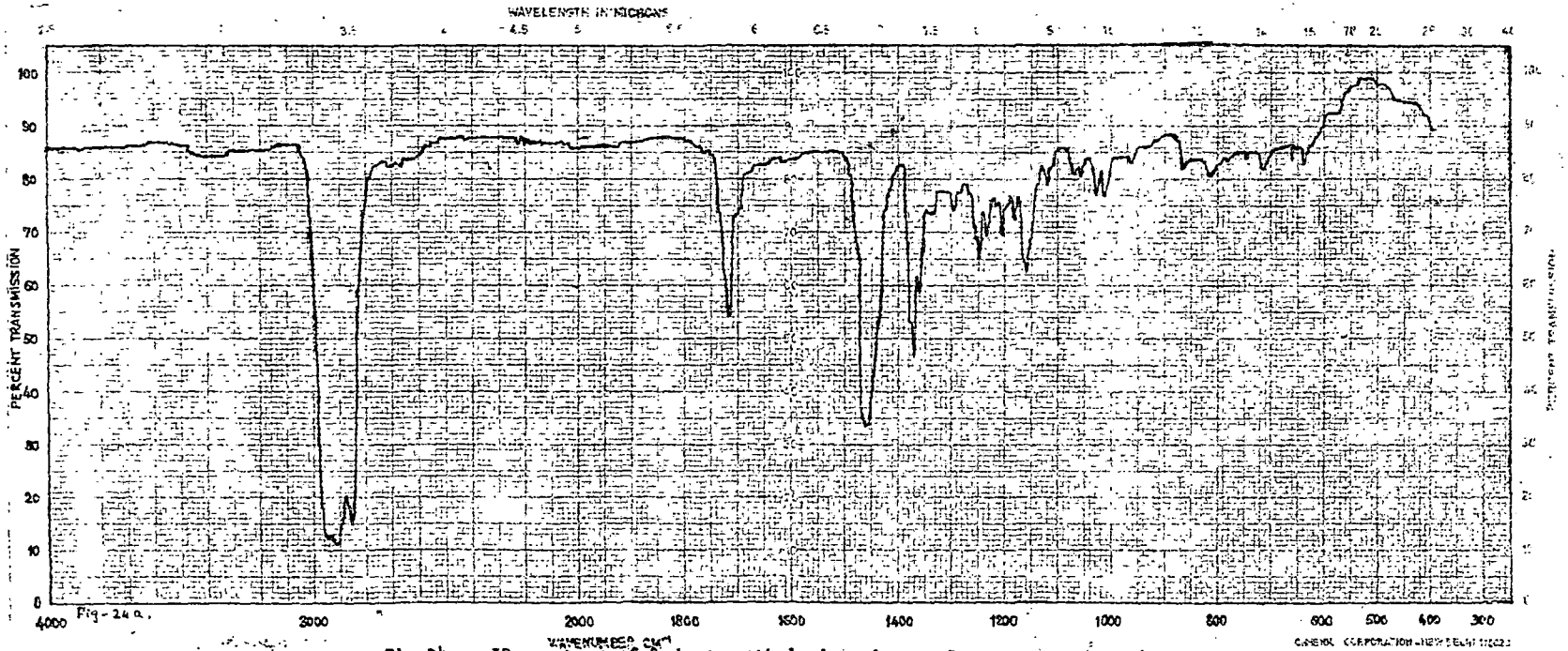


Fig 24a : IR spectrum of 3-deoxy-methyl-olesnolate, 78b.

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

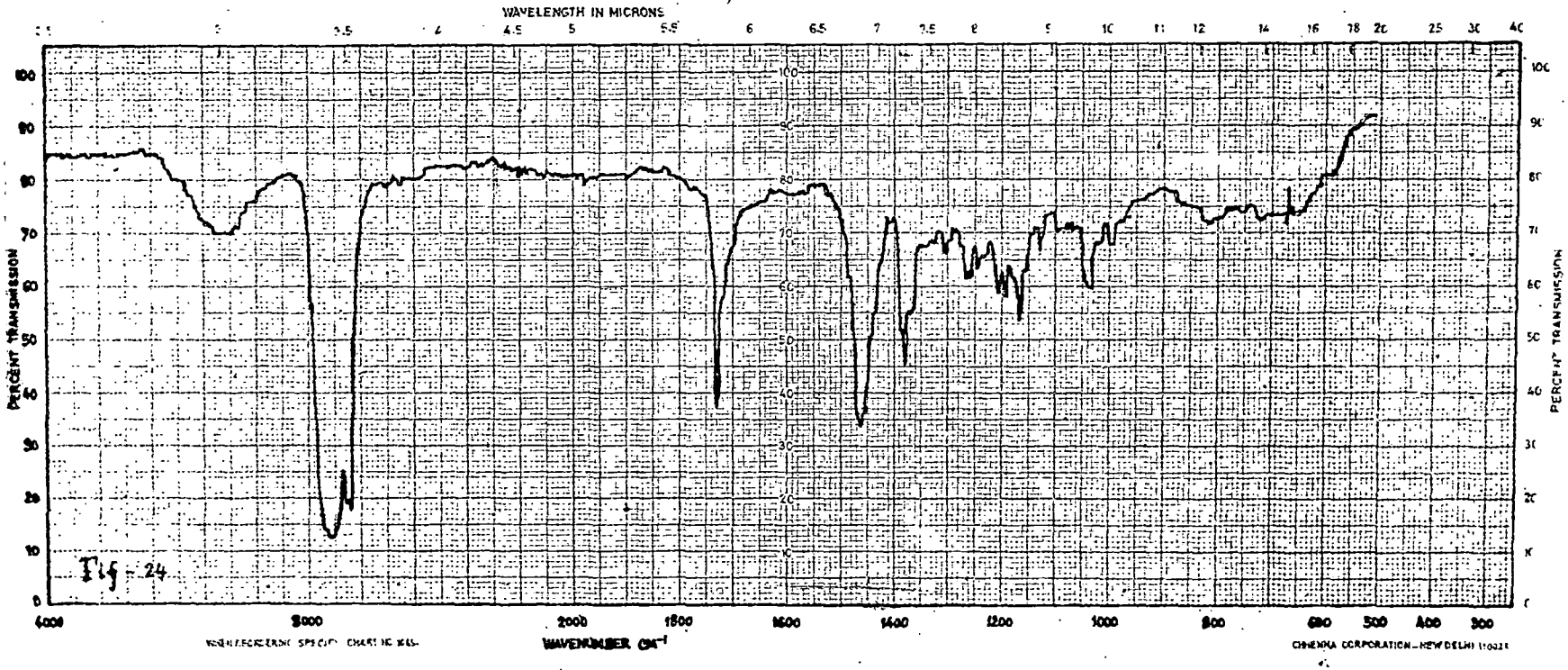


Fig.24: IR spectrum of methyl oleanolate, 78d.

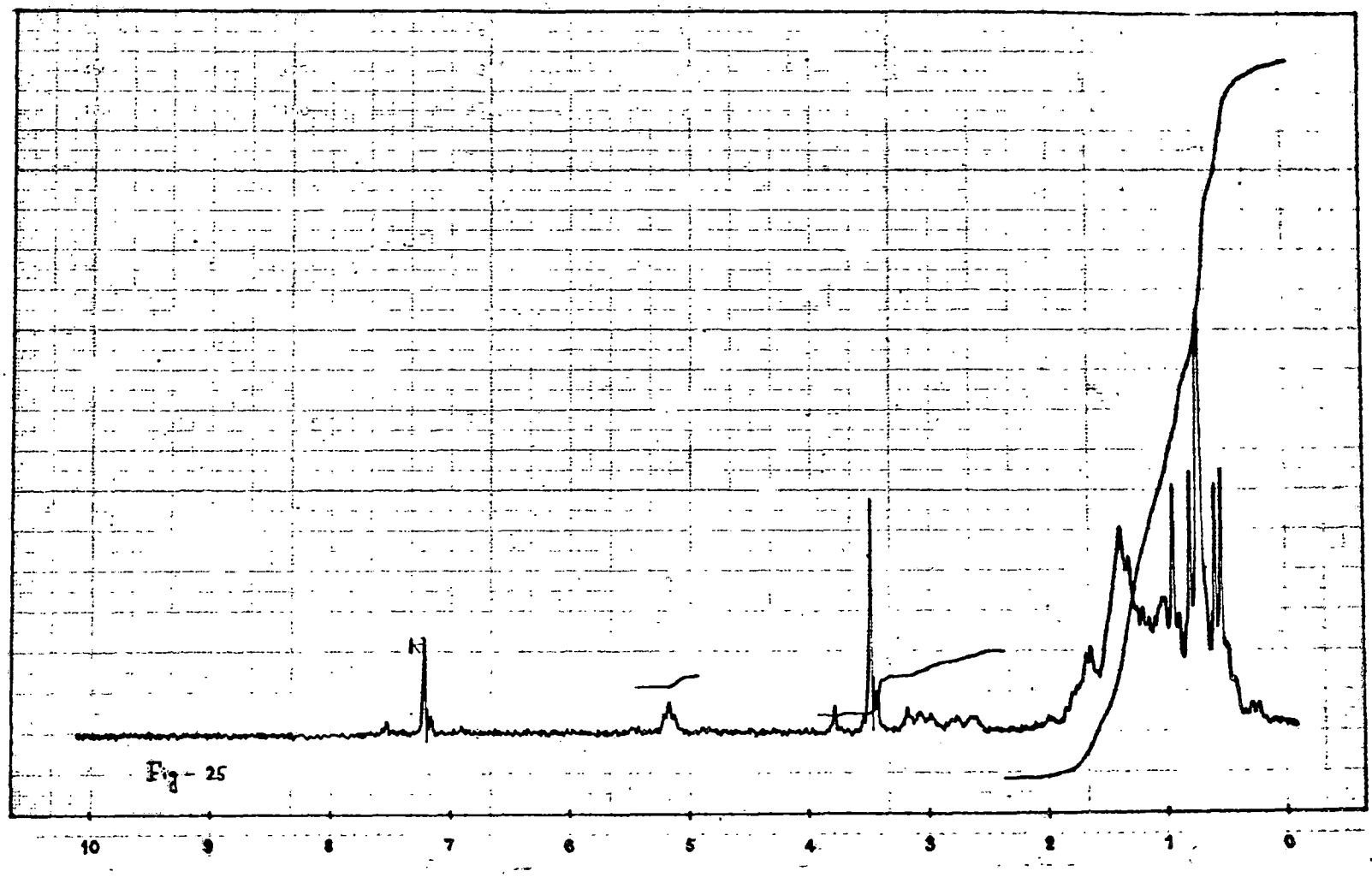


Fig.25: PMR spectrum of methyl oleanolate, 78d.

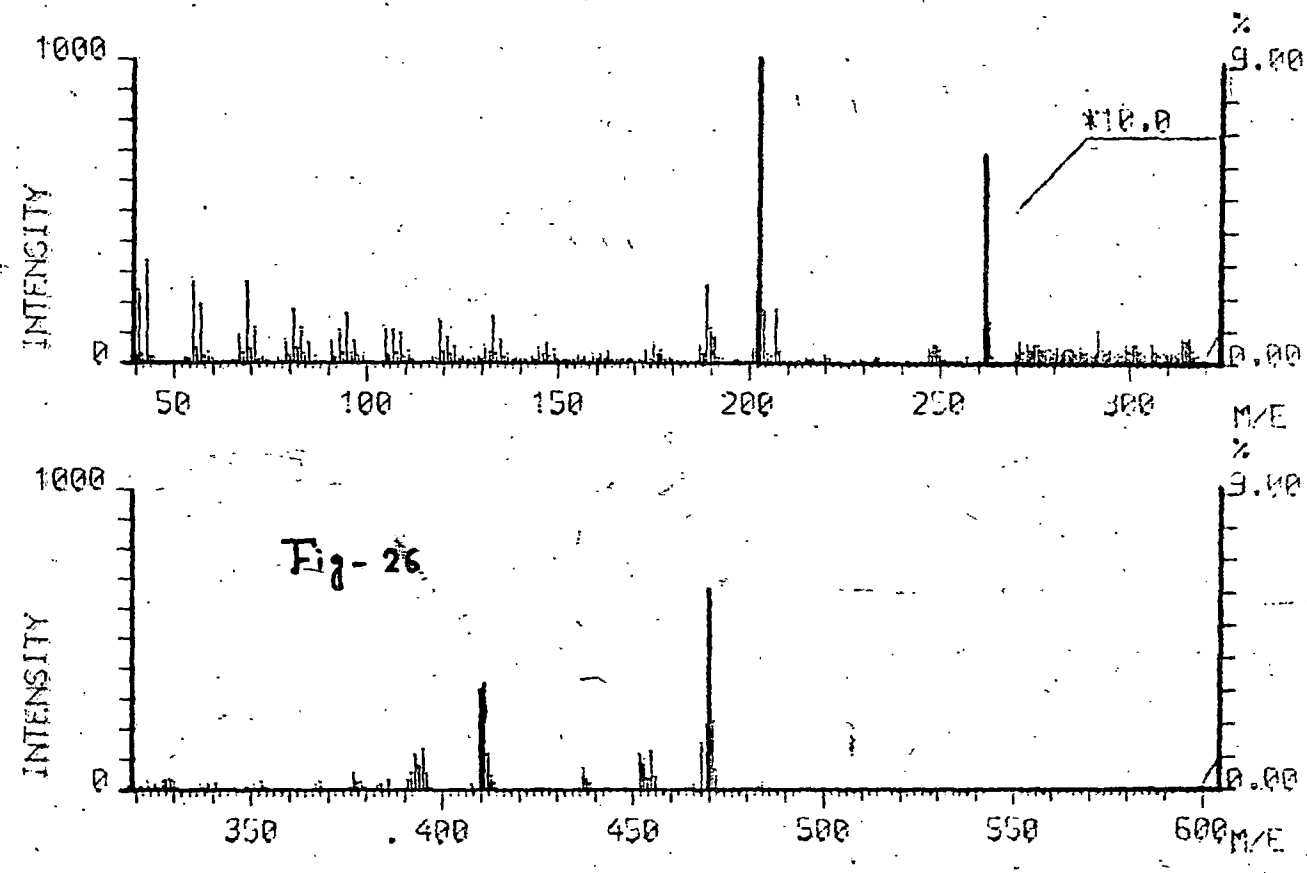
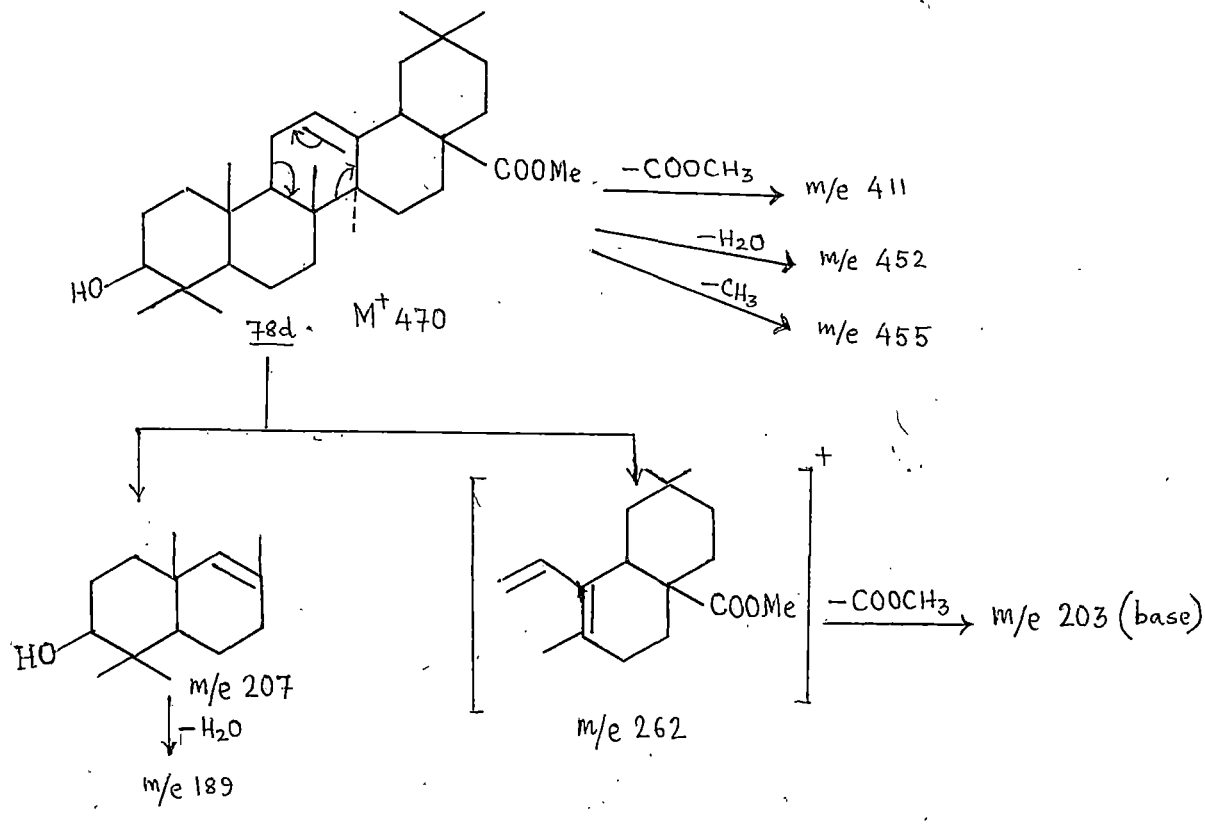


Fig- 26

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

Chart—6



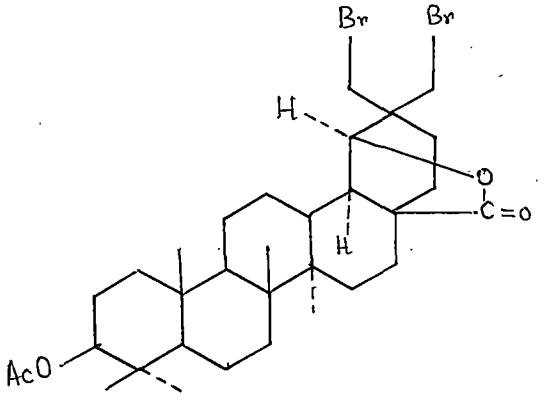
From the above data the compound was proved as methyl oleanolate[36] which was confirmed by comparison with an authentic specimen (m.m.p and GC—IR comparison). So the compound which was obtained originally from the reaction of Li-ethylenediamine on 3-acetate of 12α-bromo-oleanan-28⟶ 13β-olide was confirmed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

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

From neutral fraction no compound was isolated.

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

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



79

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

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

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

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

SECTION—B

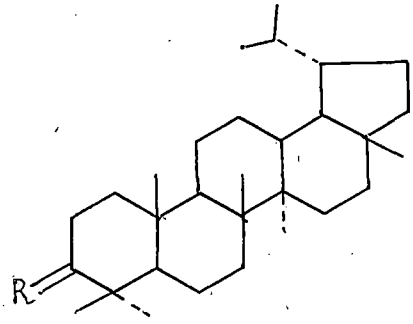
STUDIES ON THE ACTION OF LITHIUM ON TRITERPENOIDS CONTAINING (a) 3-KETO (b) STERICALLY HINDERED ESTERS WITH OR WITHOUT ISOPROPENYL GROUP IN ETHYLENEDIAMINE

(a) Studies on 3-keto triterpenoids:

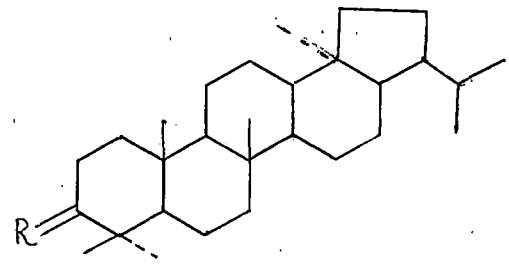
Reactions of Li-ethylenediamine on 3-keto compounds were studied taking lupanone 80a and moretanone 81a as model compounds.

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

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



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



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

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

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

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

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

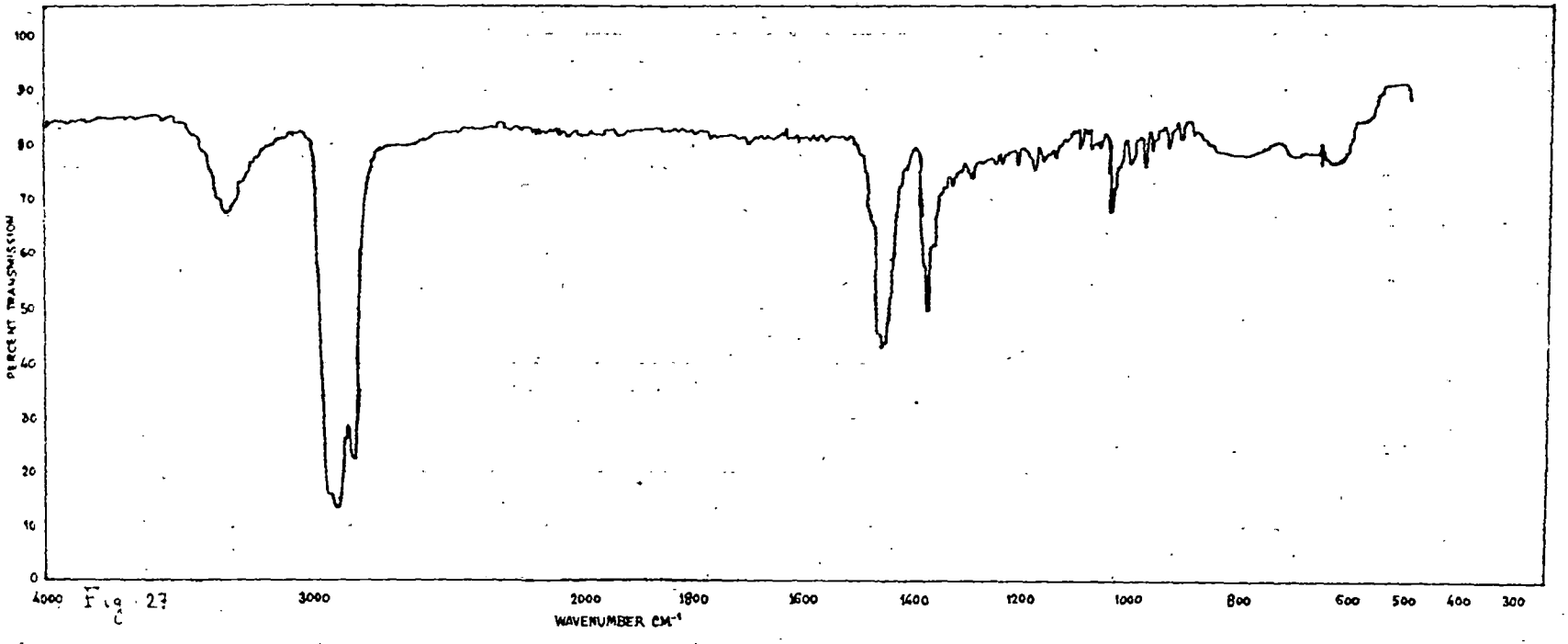


Fig.27: IR spectrum of lupanol, 80b.

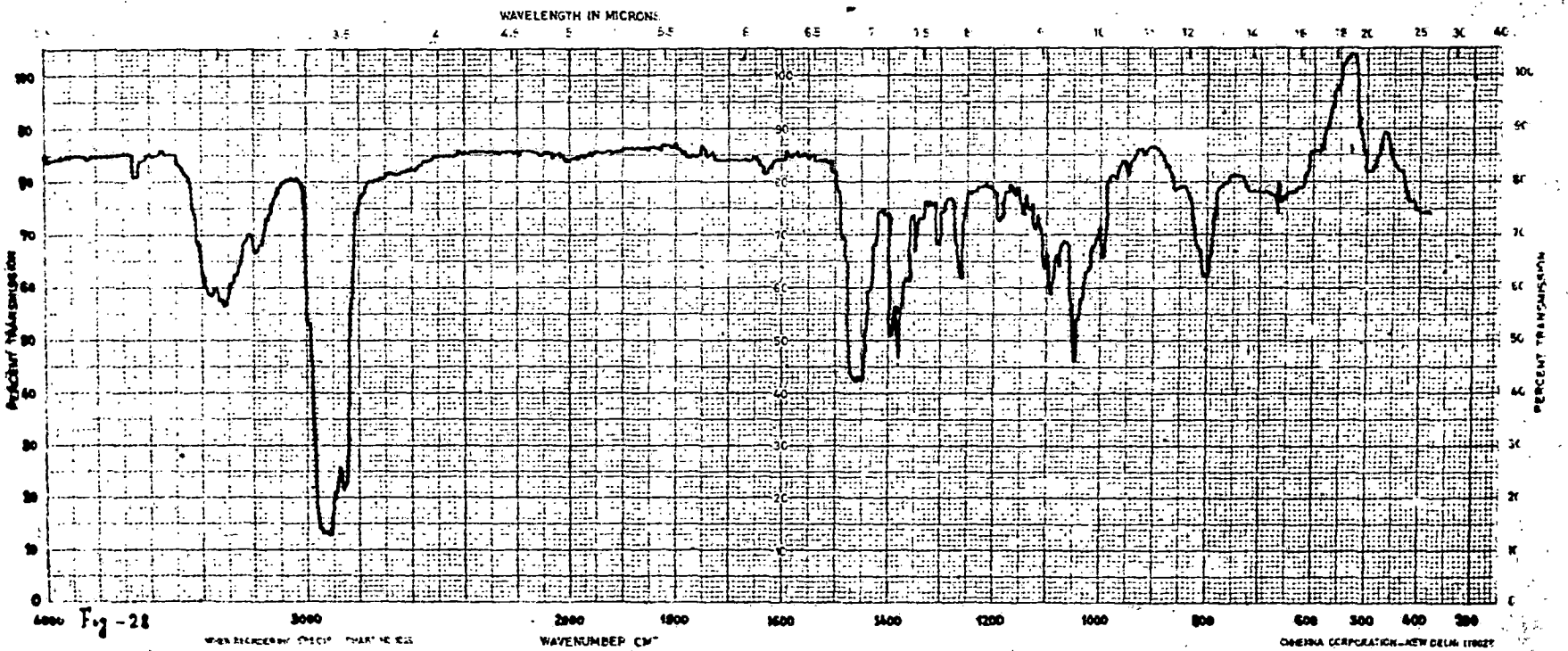


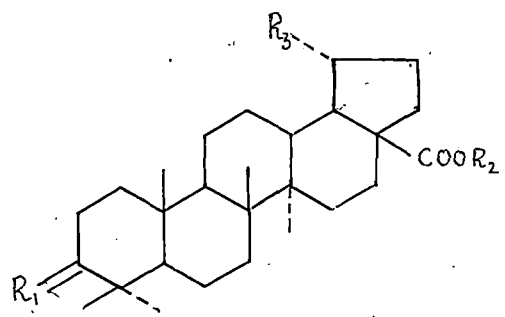
Fig.28: IR spectrum of moretanol, 81b.

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

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

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

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



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

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

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

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

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

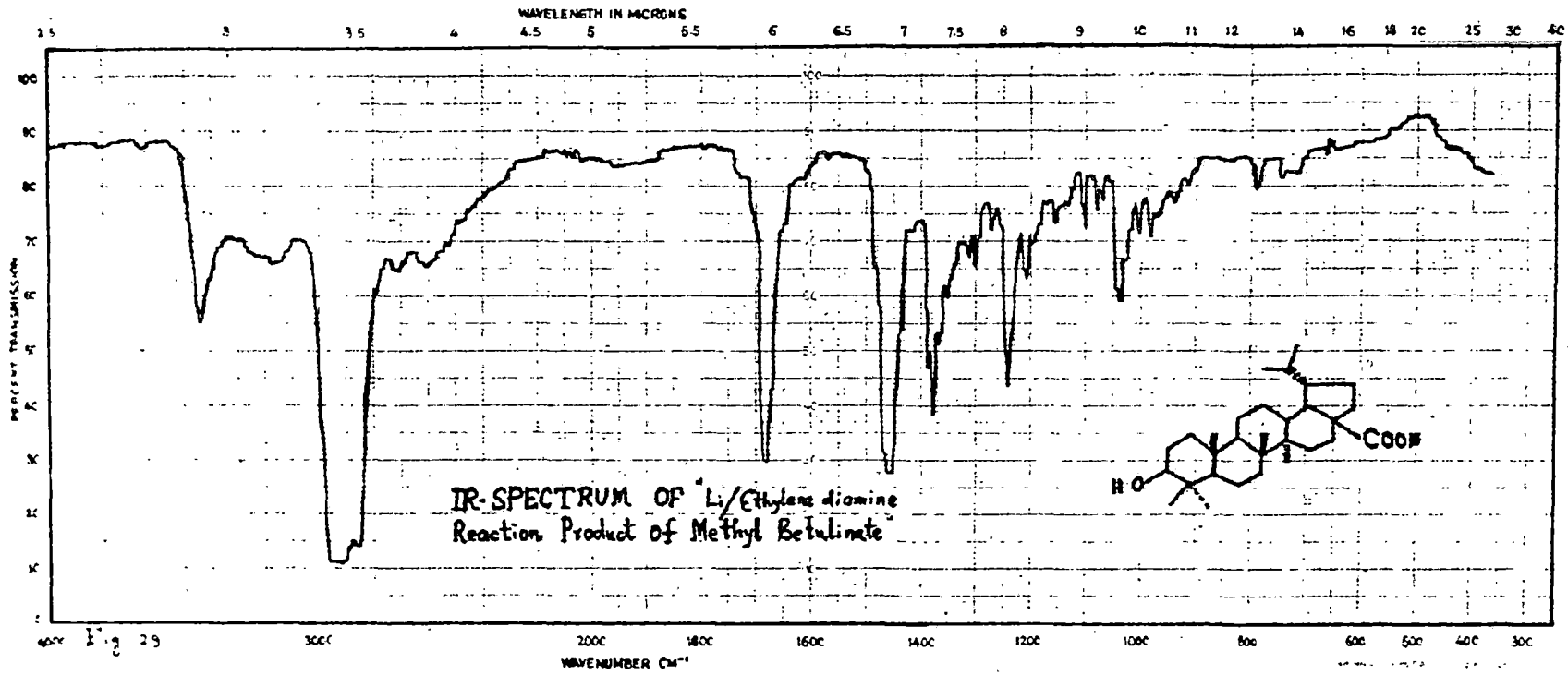


Fig.29: IR spectrum of dihydro betulenic acid, 82b.

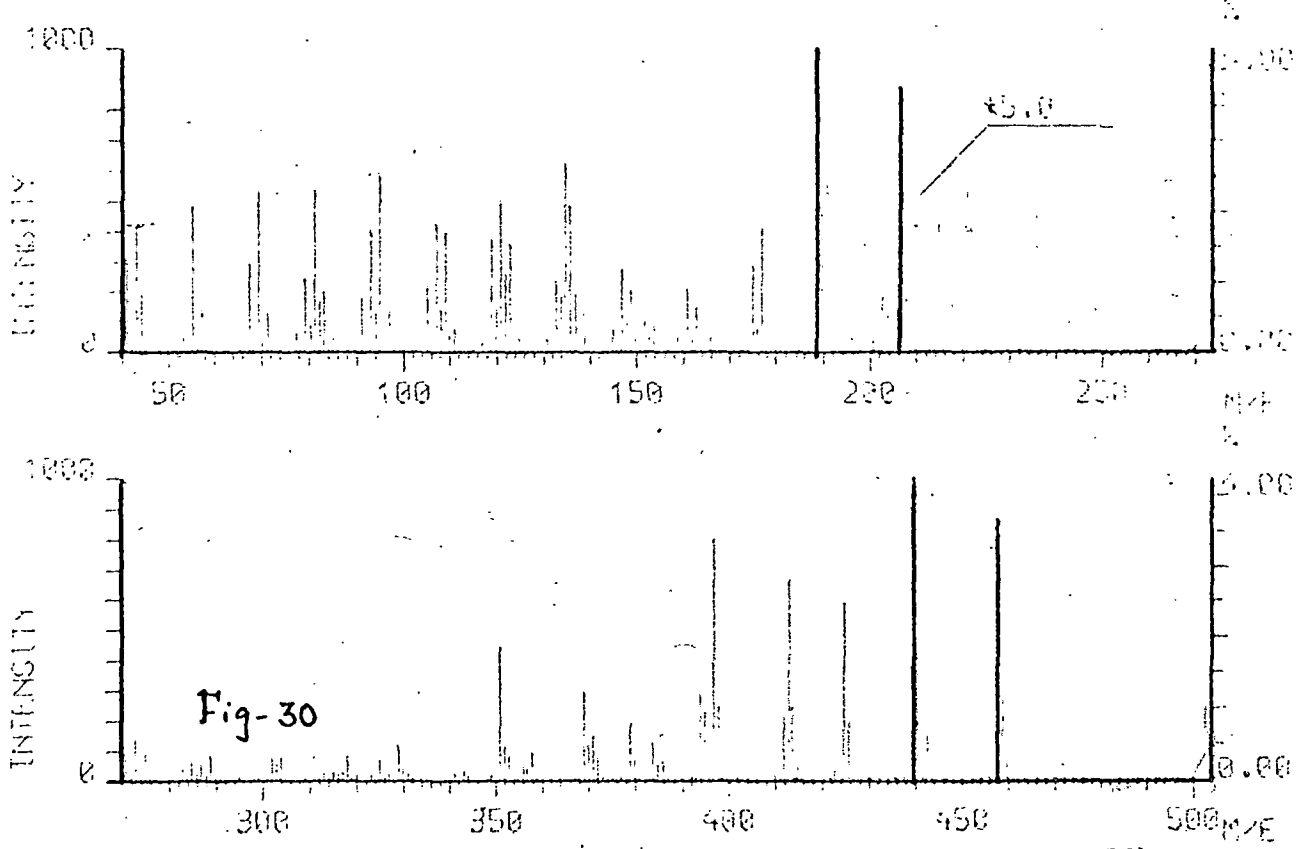


Fig-30

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

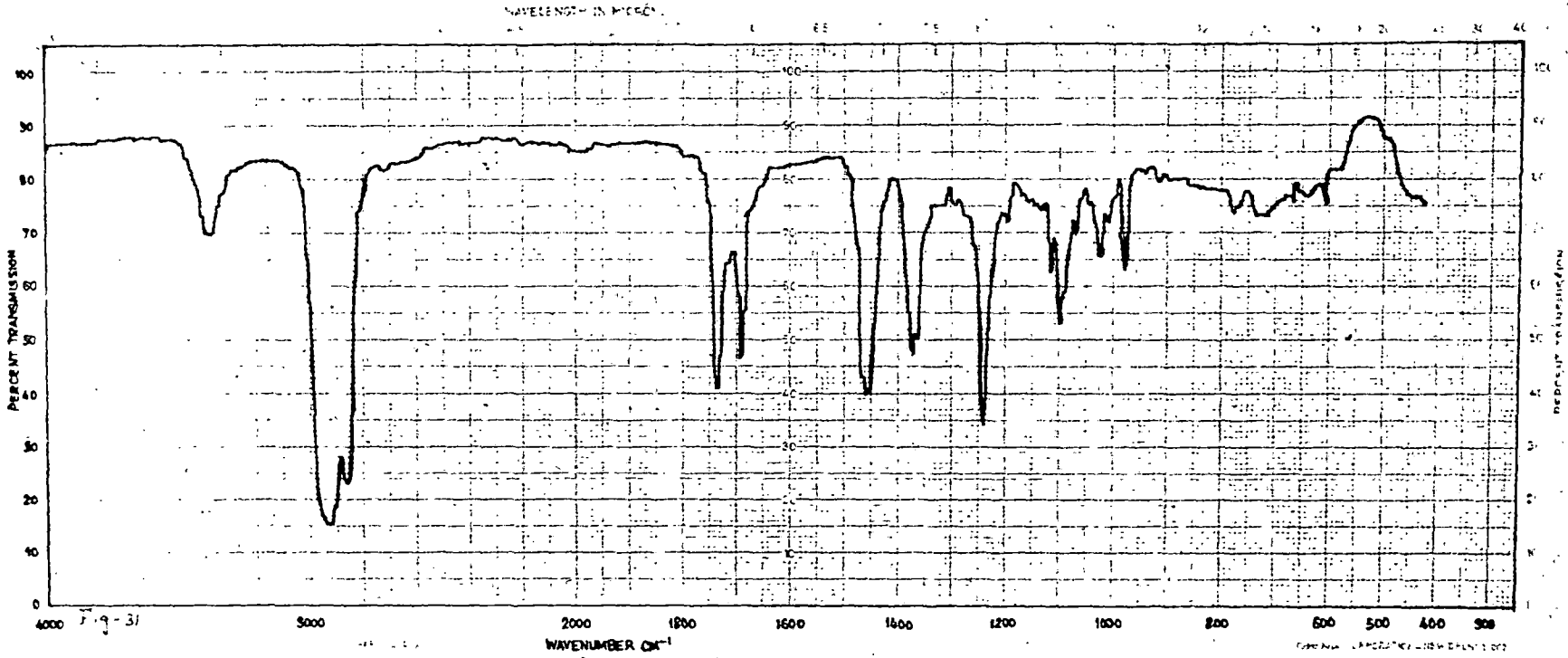


Fig.31: IR spectrum of acetyl betulanic acid, 82c.

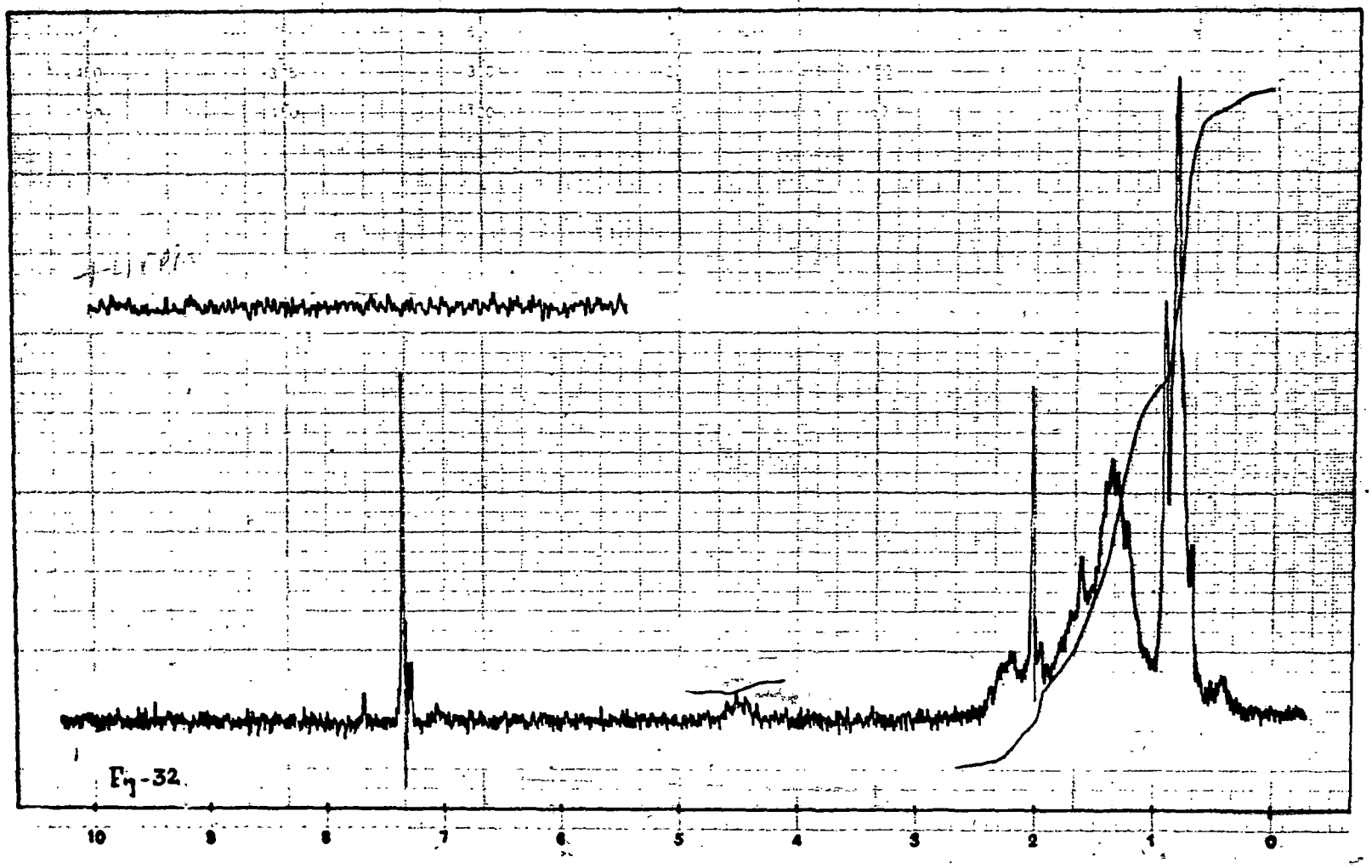


Fig.32: PMR spectrum of acetyl betulanic acid, 82c.

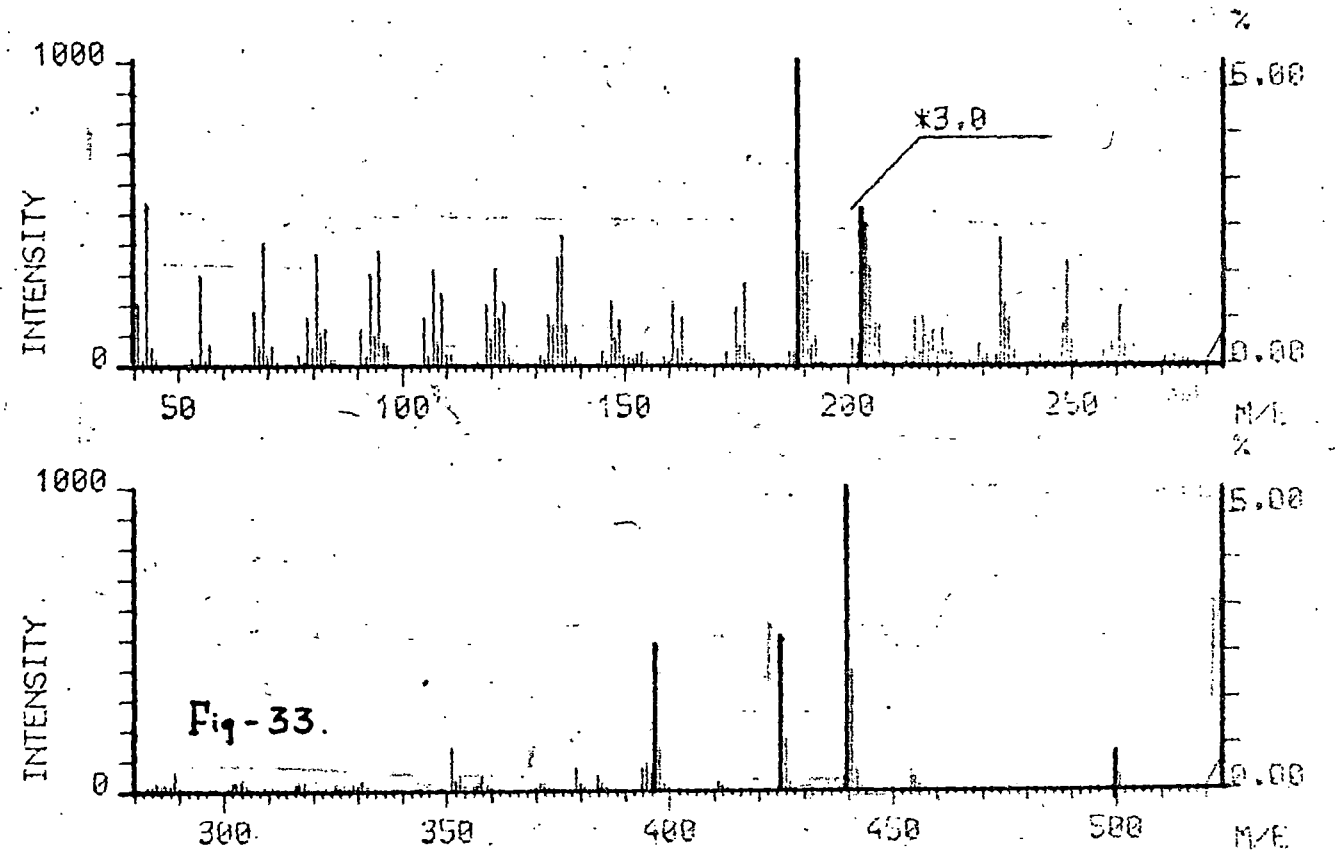


Fig-33.

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

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

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

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

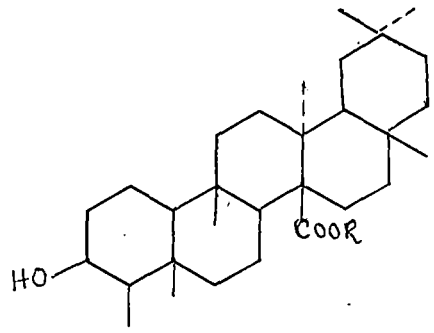
The model compound taken for the reaction of Li-ethylenediamine was methyl oleanonate[56] 78e. The compound was treated with lithium in ethylenediamine resulted a mass which was separated into acid and neutral fractions.

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

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

(iii) Sterically hindered esters:

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



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

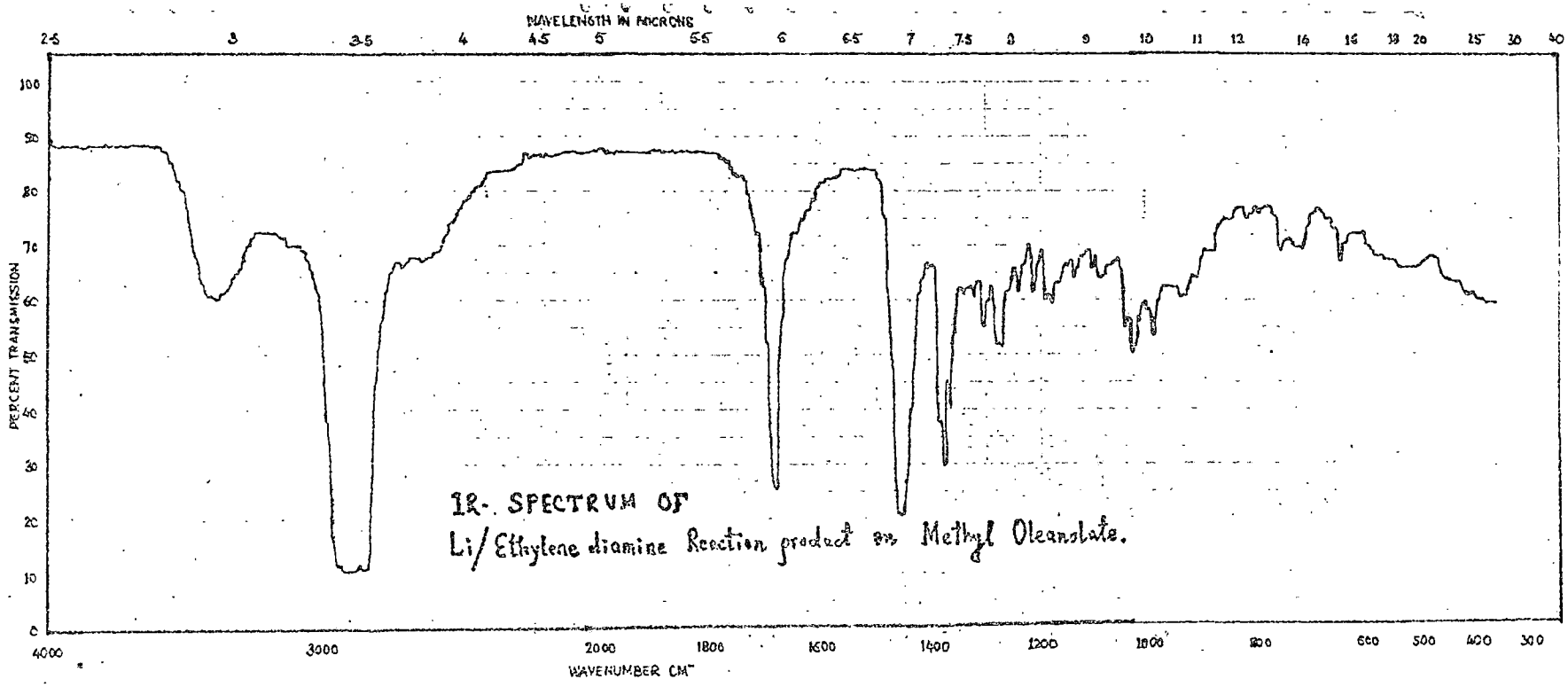


Fig.54: IR spectrum of the compound, 78.

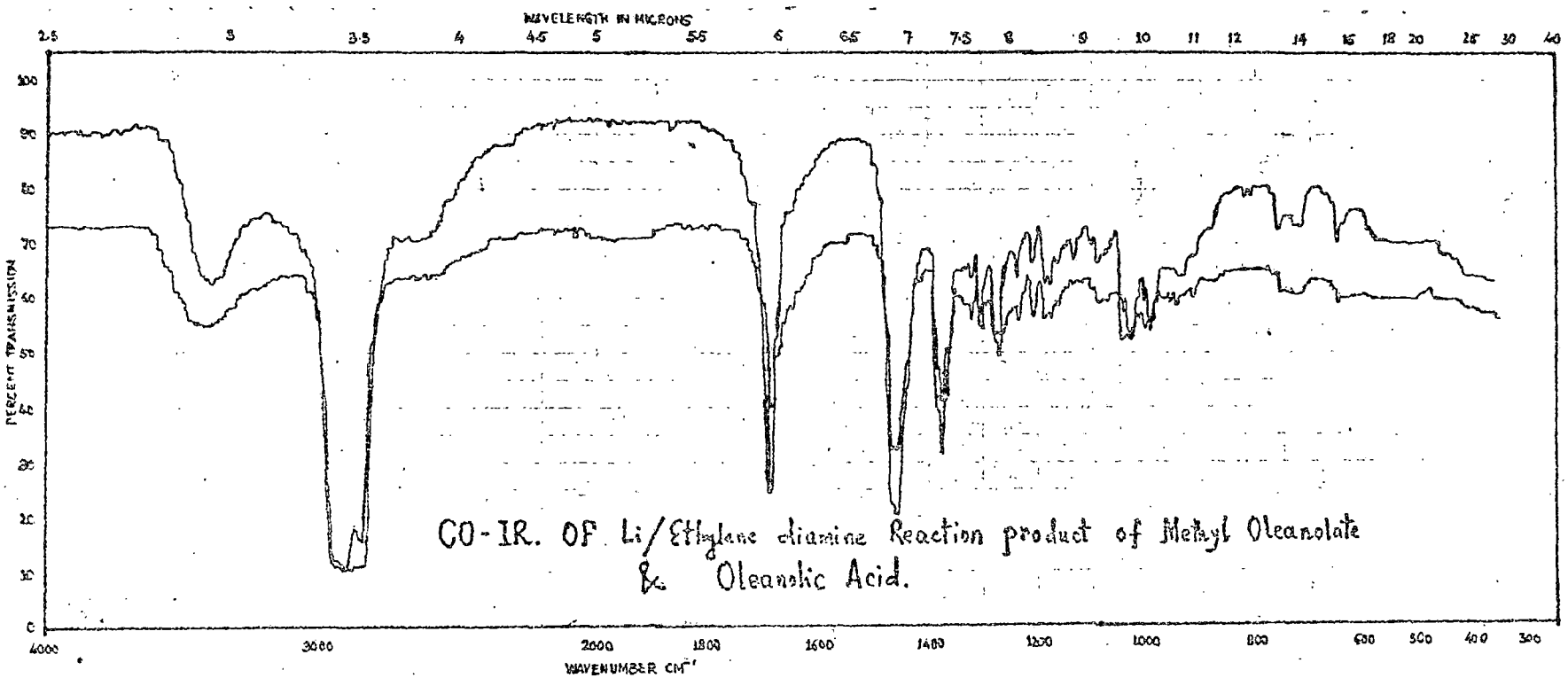


Fig.35: CO—IR spectrum of compound 78e and oleanolic acid.

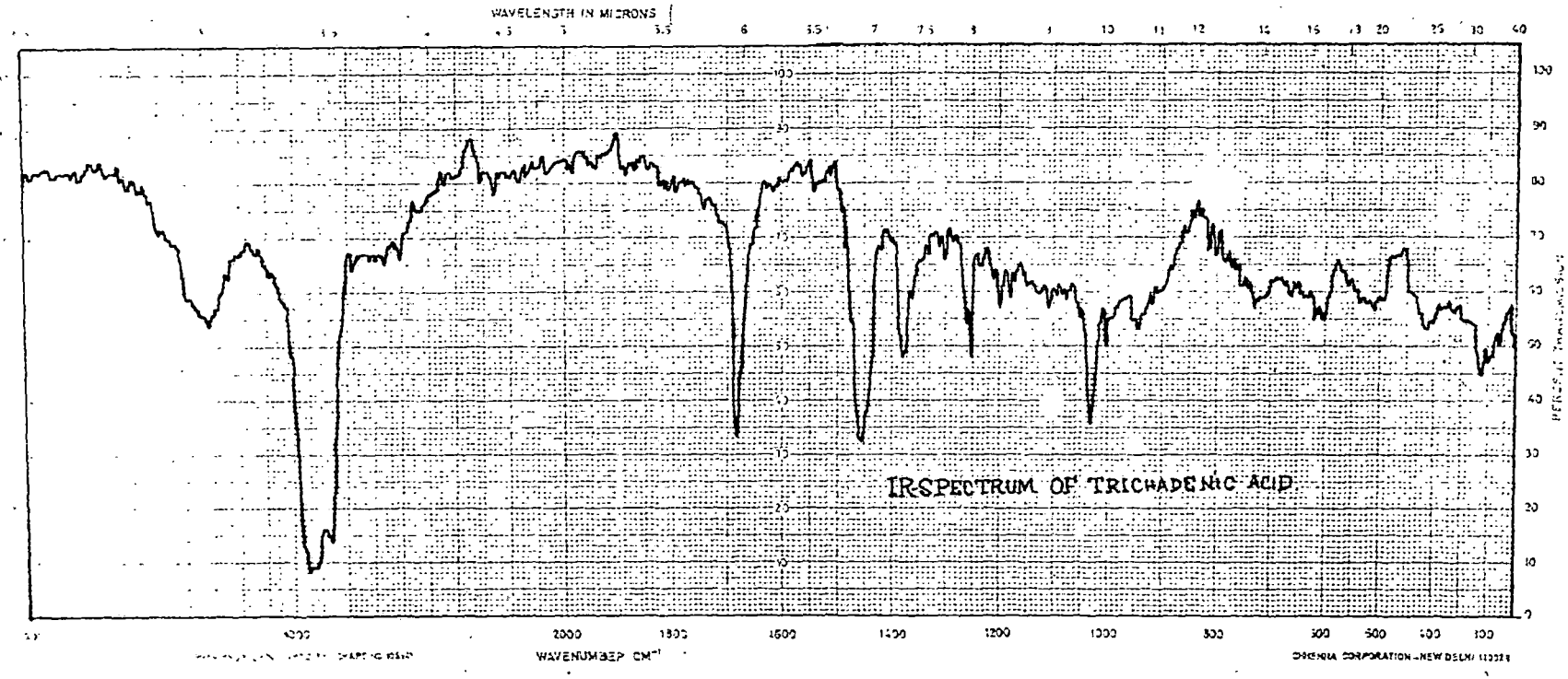


Fig.36: IR spectrum of trichadenic acid A.

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

SECTION—C:

Conclusions:

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