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).

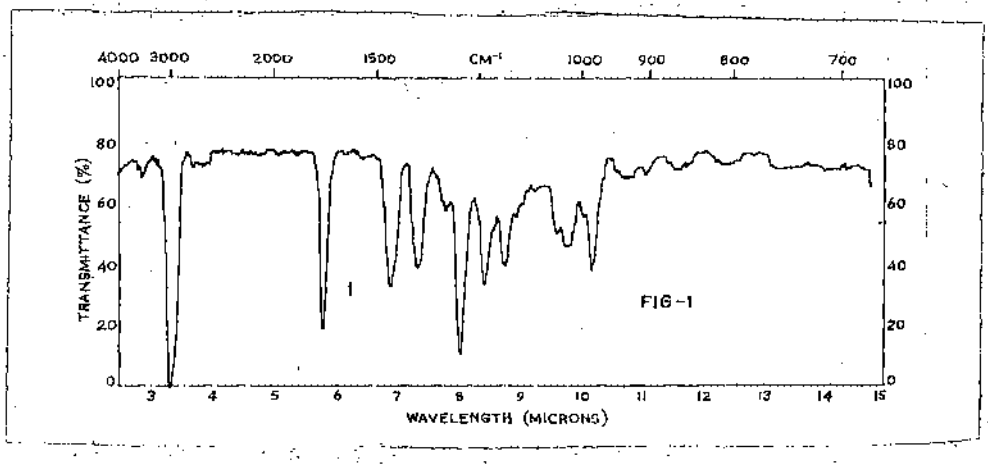

4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
TRANSMITTANCE (%)
100
80
60
40
20
0
1
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

FIG-1

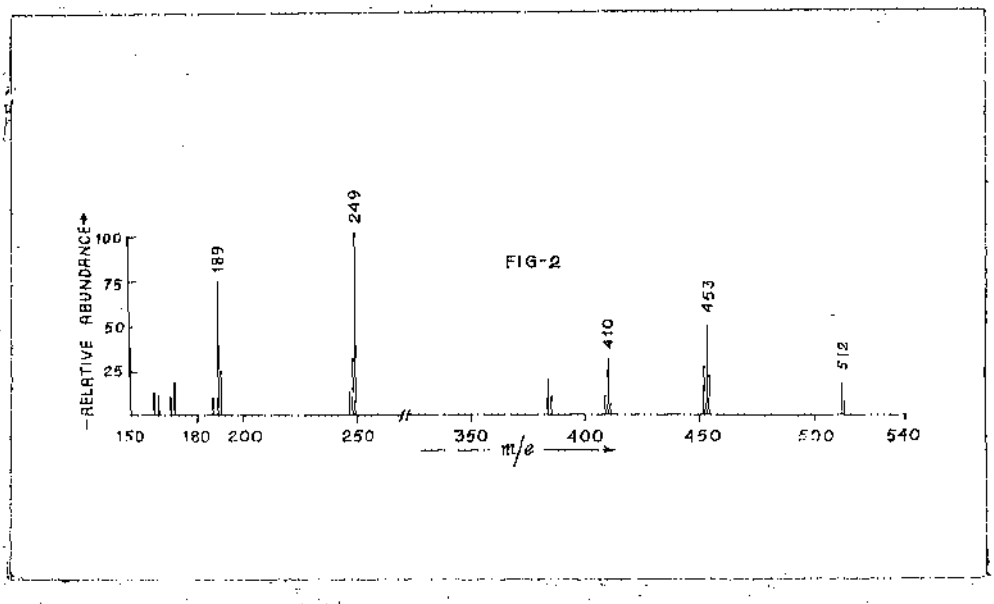
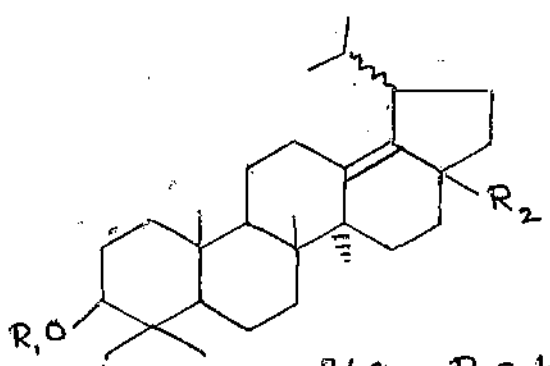

RELATIVE ABUNDANCE
100
75
50
25
189
249
410
453
512
150 180 200 250 350 400 450 500 540
m/e

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

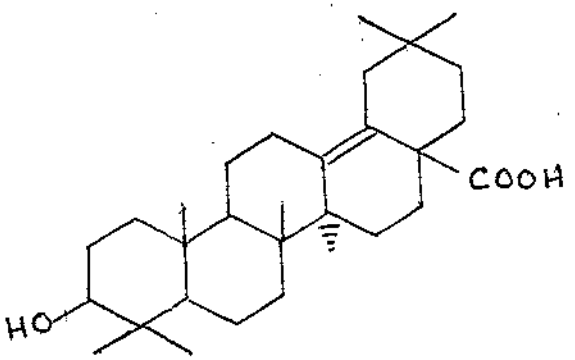
was isolated in a very poor yield. The I.R. spectrum of the hydroxy acid 34b showed absorption bands at 3390 (OH), 1700 cm^{-1} (-COOH). The latter was esterified with diazomethane followed by acetylation whereby the parent ester acetate 21b was regenerated (m.p. and m.m.p.) in good yield. This clearly 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

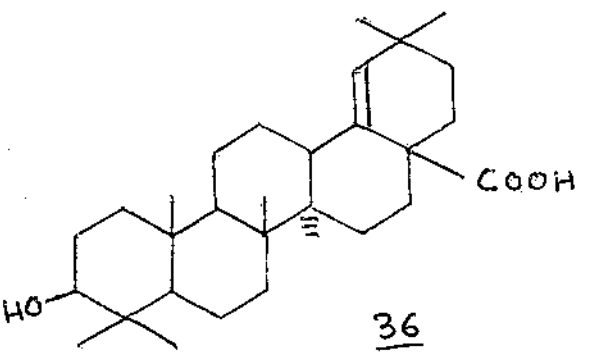
It has been observed[12,22] that β-γ unsaturated acids are generally decarboxylated with ease on pyrolysis, carbondioxide being eliminated accompanied by a shift of the double bond to the αβ-position. Although many αβ-unsaturated acids are readily decarboxylated but in the case of tertiary β-γ -unsaturated 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.



35



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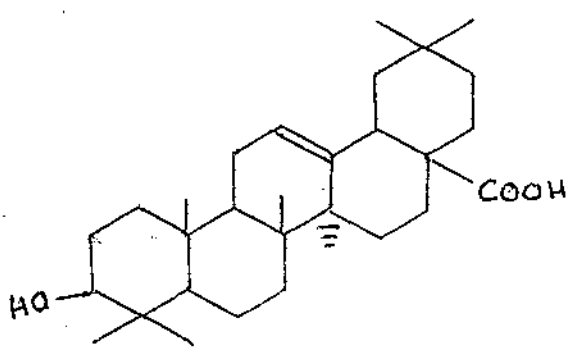
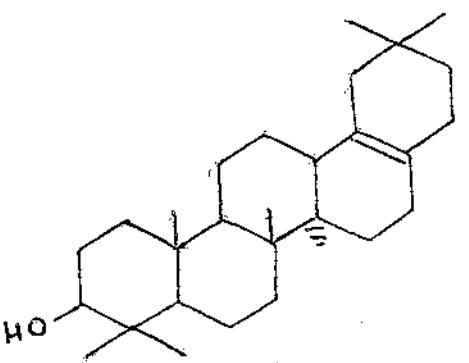


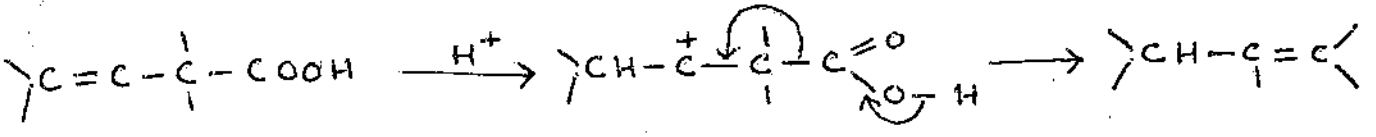
38



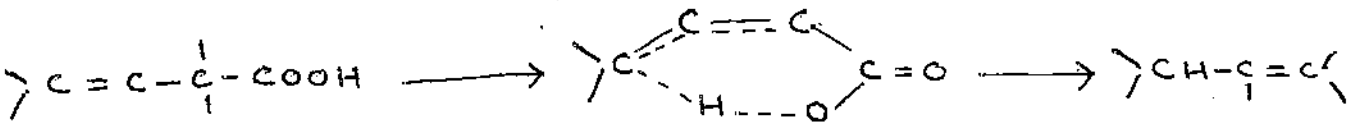
37

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.

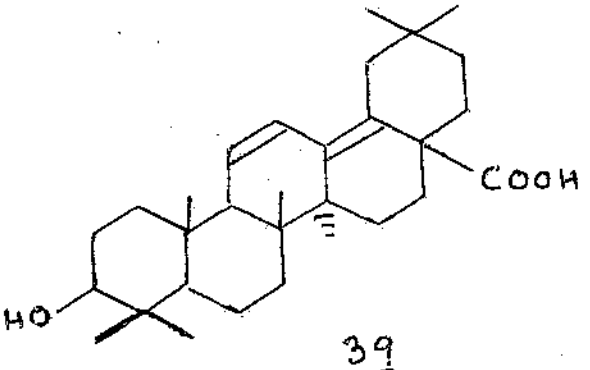


[A]

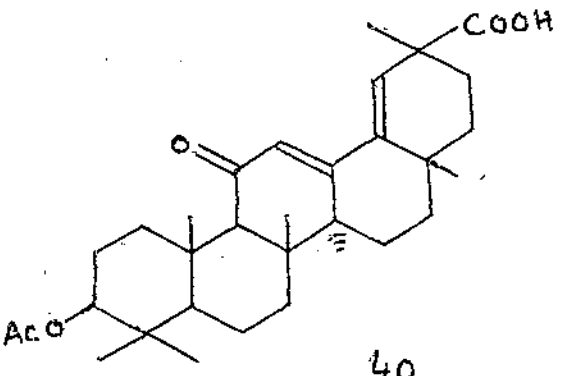


[B]

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

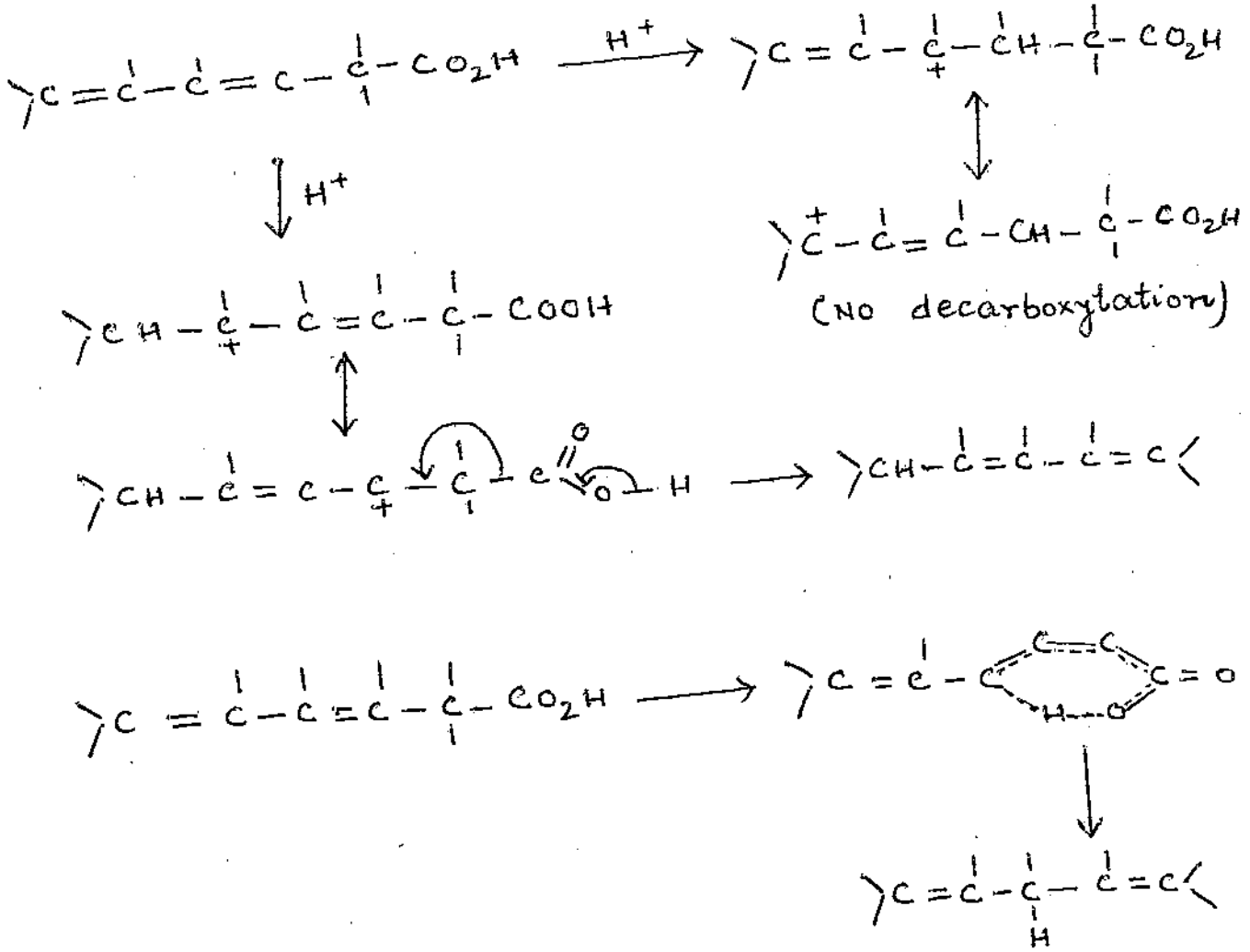


39



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 βɣ- 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)

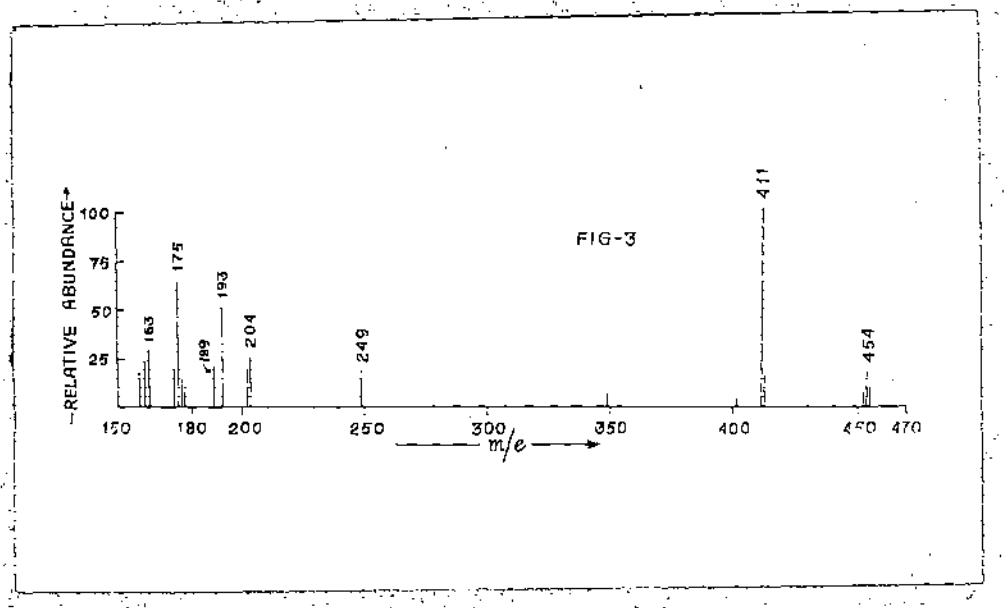


FIG-3

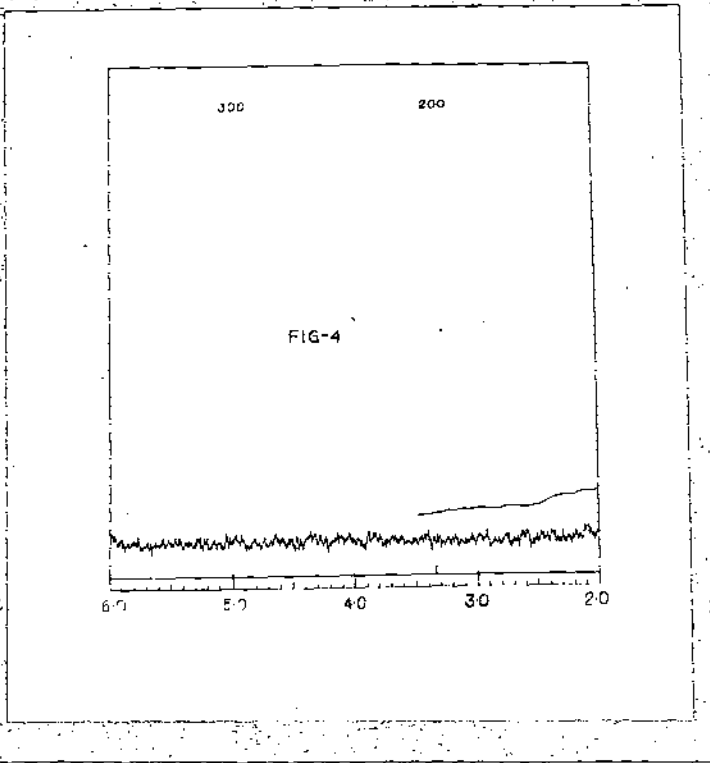


FIG-4

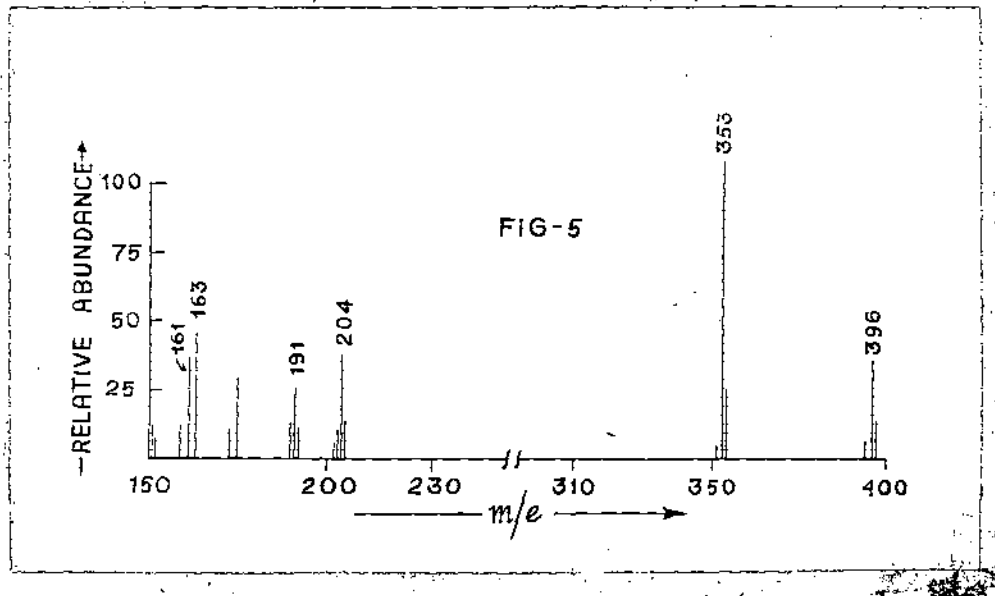
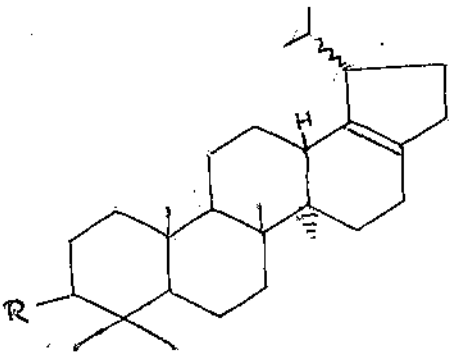
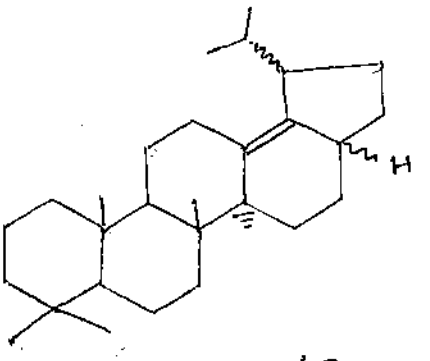


FIG-5

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

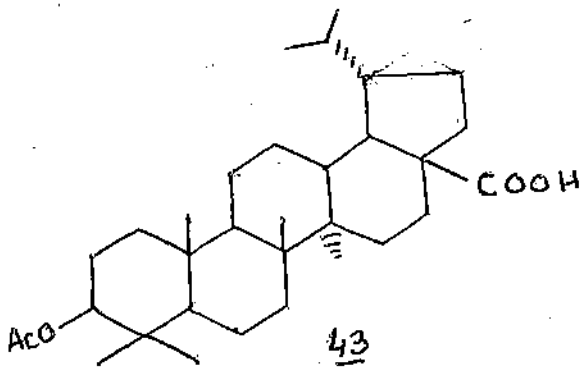


42

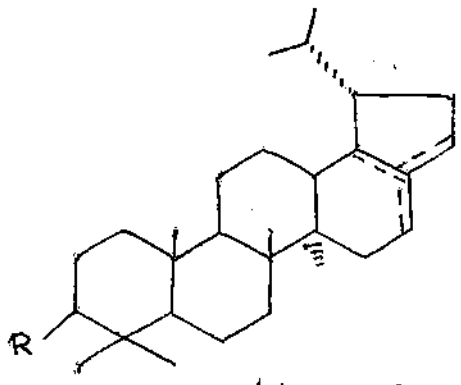
Isomerisation of the hydrocarbon 41d to the new hydrocarbon 42

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-

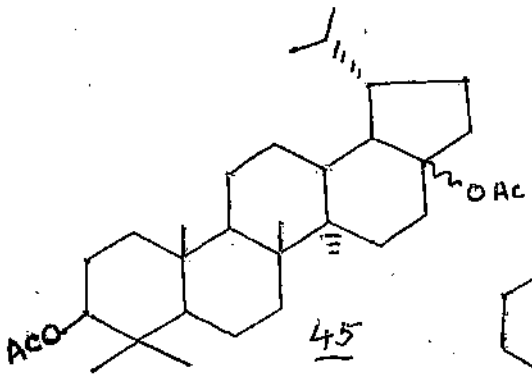
cussion see page 29). In order to establish the stereochemistry of the isopropyl group in 42, the following sequence of reactions were also carried out.



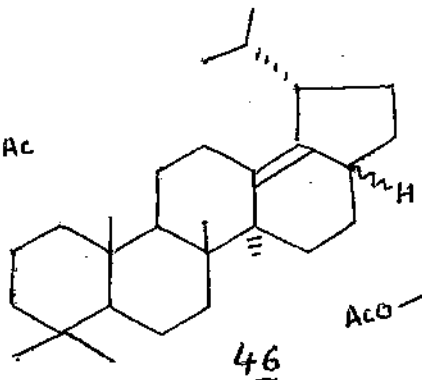
43



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

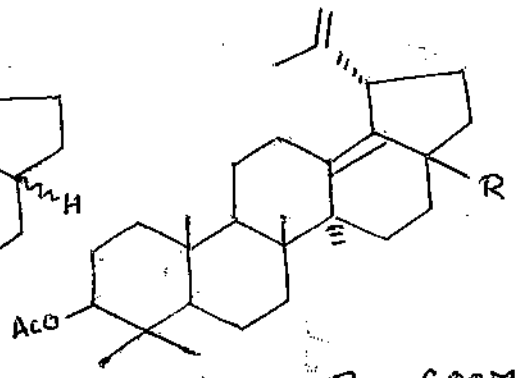


45



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

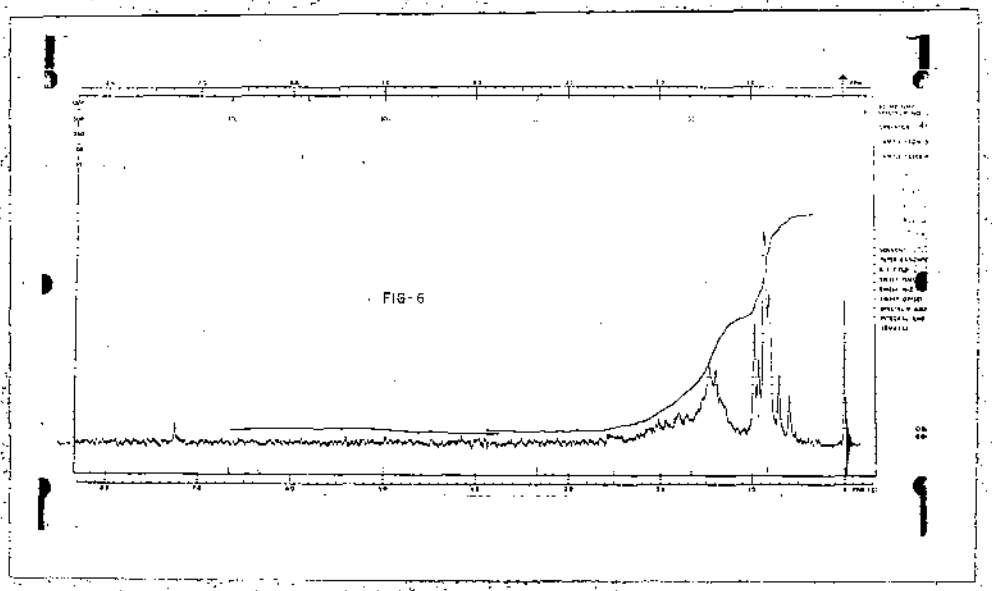


FIG-6

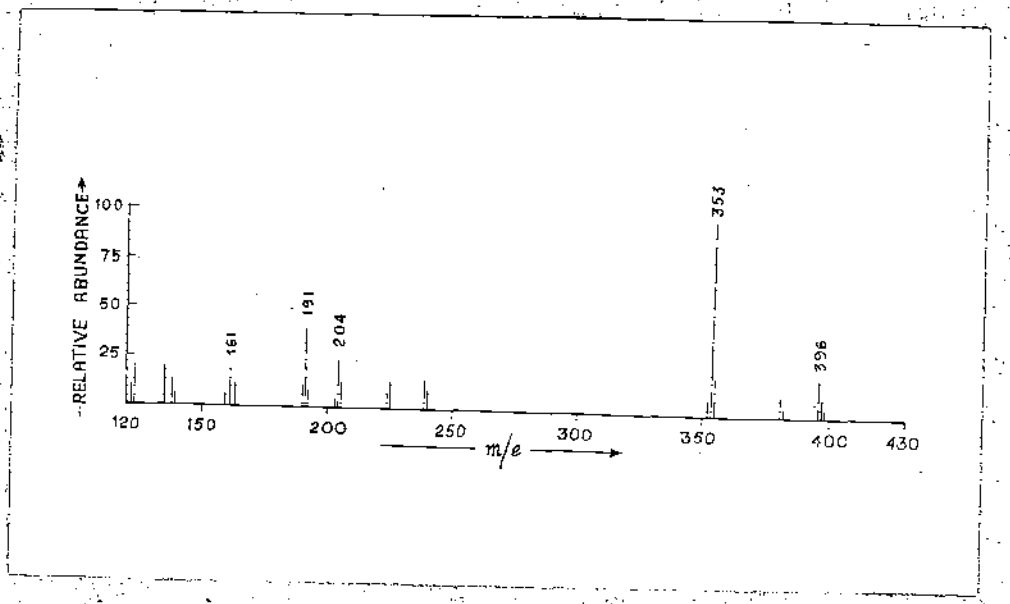
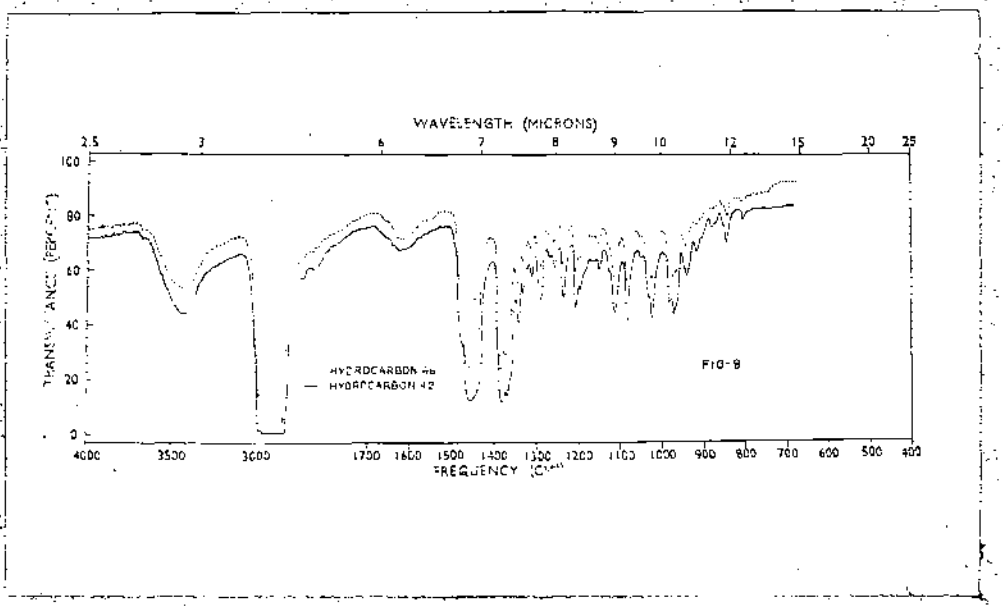
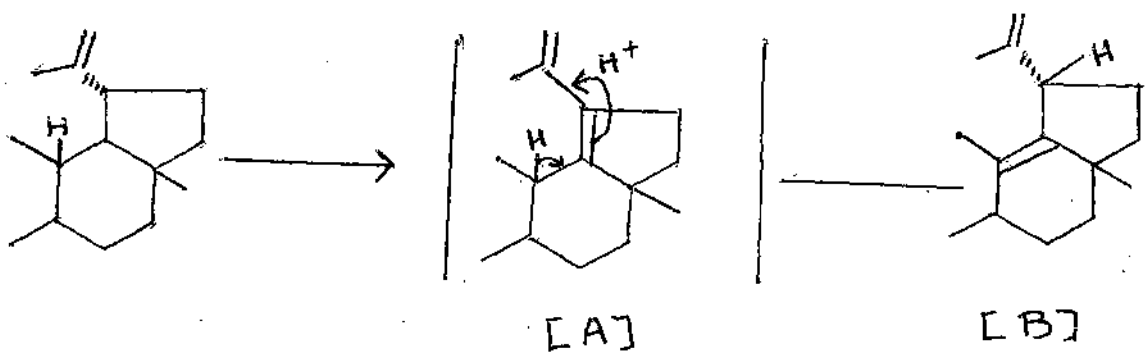

RELATIVE ABUNDANCE
100
75
50
25
120
150
200
250
300
350
400
430
m/e
161
191
204
353
396

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]

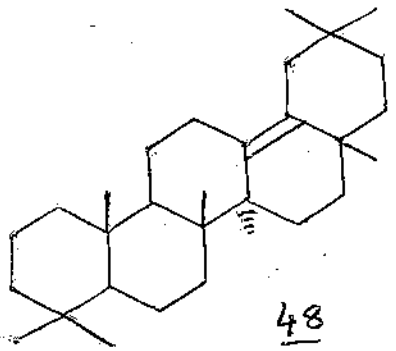
Application of mass spectrometry

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

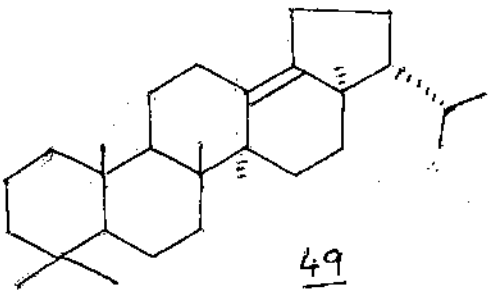
spectra of various triterpenoids and suggested mechanisms for the fragmentation patterns recorded. They also recorded the mass spectra of $\Delta^{13(18)}$-oleanene 48 and suggested the mechanism for the genesis of the various fragments formed from the molecular ion. Pandey et al.[27,28] applied mass 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

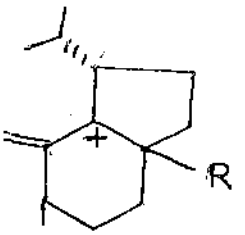
(m/e 204), 52a (m/e 191) and the fragment due to the ion 50a (m/e 191) originating from the left hand portion of the molecule (A/B ring).



48

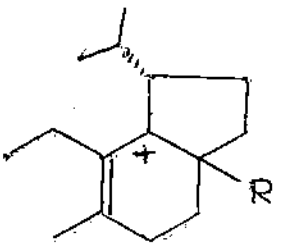


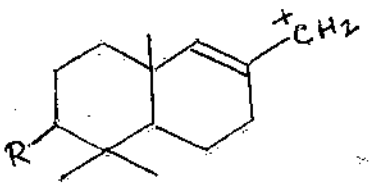
49



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

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





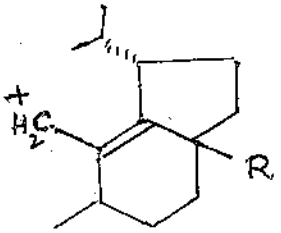
50a, R = H (m/e 191)

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

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

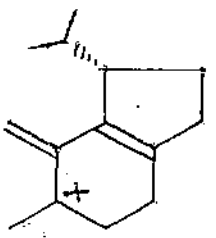
51b, R = COOMe (m/e 262)

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)

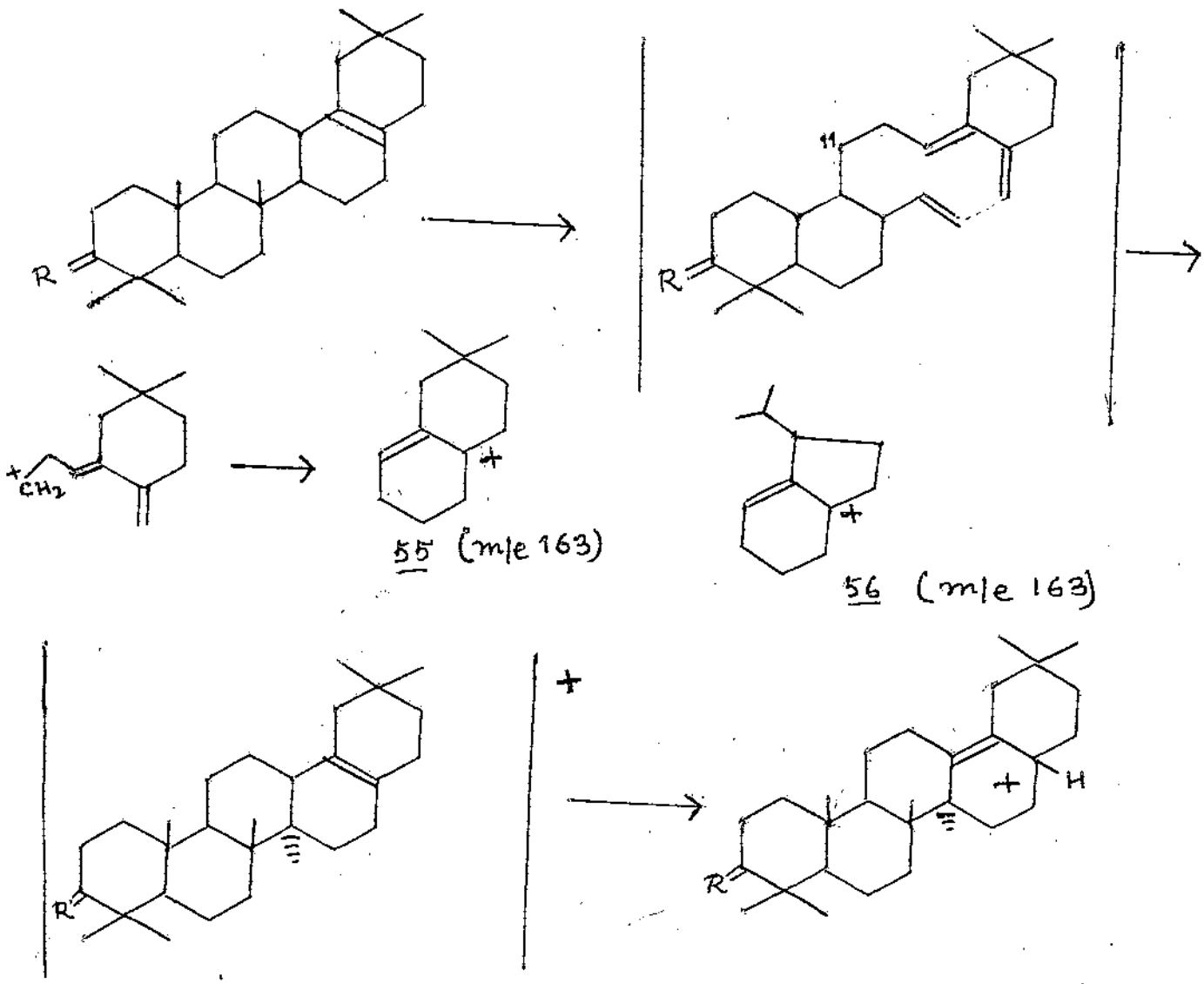


54 (m/e), 189

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.