CHAPTER III

Section A

Isolation of Aleuritolic acid and Betulinic acid

Extraction: Dried and powdered trunk bark and stem of Aleurites montana, 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 the ether furnished a gummy residue which constituted the neutral portion (A) of the extract (the work on the neutral portion A is dealt in Chapter V).

The alkali washed portion on acidification with dilute hydrochloric acid yielded a solid which was extracted with ether. The ethereal solution containing the acid portion was esterified with diazomethane. The crude methyl ester obtained after evaporation of ether was chromatographed over deactivated alumina. Petroleum ether eluate on concentration gave a solid which after repeated chromatography (three times) afforded a solid m.p. 238-40°, which after several crystallisation from a mixture of chloroform and methanol gave needle shaped crystals, m.p. 241-3°, $(\alpha)_D$ + 23.08°. This compound did not show any absorption in the region 220-300 mμ. Infrared spectrum showed peaks at 1735 cm^{-1} (broad, -O-$COCH_3$ and -$COOCH_3$), 1245 cm^{-1} (O-CO-CH_3) and at 820 cm^{-1} (trisubstituted double bond). Hydrolysis

of this compound with 5% methanolic potassium hydroxide furnished a neutral solid m.p. 208-10°, $(\alpha)_D$ + 11.11° which still showed the ester peak at 1735 cm^{-1} ($-COOCH_3$) and a new peak at 3480 cm^{-1} (-OH). Hydrolysis with 10% and 15% methanolic potassium hydroxide was attempted but in each case the starting material could be recovered, indicating that the ester group was probably situated at a tertiary position. A more drastic condition was necessary to effect this hydrolysis. Hydrolysis of the methyl ester was accomplished by heating with potassium tertiary butoxide in dimethyl sulfoxide[49] at 105° for four hours. The reaction mixture was cooled and acidified with dilute hydrochloric acid. A solid separated out which was extracted with chloroform. The chloroform layer was washed with water till neutral and then dried over anhydrous sodium sulphate. Distillation of the chloroform afforded a solid which after repeated crystallisation from chloroform-methanol mixture gave an amorphous solid having m.p. 300-302°. The acid appeared to be a new one and has been named Aleuritolic acid after the name of the species from which it has been isolated for the first time.

The acid was amorphous, gave sparingly soluble potassium salt. It was soluble in ether, pyridine, hot methanol and sparingly soluble in chloroform. The acid did not show any UV absorption in the region 220-300 mμ. Titration of the pure acid with standard sodium hydroxide solution showed that it was a monobasic triterpene acid. Elemental analysis and equivalent weight determination was consistent with the molecular formula $C_{30}H_{48}O_3$. Infrared spectrum of the acid showed peaks at 3340 cm^{-1} (-OH), 3070 cm^{-1}, 1700 cm^{-1} (-COOH) and at 820 cm^{-1}

(trisubstituted double bond). On acetylation of the methyl ester of aleuritolic acid, a crystalline solid m.p. 240-2°, $(\alpha)_D$ + 23.08° was obtained and was found to be identical with the solid m.p. 241-43°, $(\alpha)_D$ + 23.08° isolated from the plant. This fact establishes that the acid is present in the plant as its acetate.

The second solid which came out from the chromatogram had m.p. 220-22°, $(\alpha)_D$ + 1.4° and has been found to be identical with methyl betulinate 41, $C_{31}H_{50}O_3$. It did not show any absorption in UV spectra in the region 220-300 mμ, infrared spectrum showed peaks at 3520 cm^{-1} (-OH), 1735 cm^{-1} ($-COOCH_3$), 1660 (unsaturation) and at 876 cm^{-1} ($=CH_2$). NMR spectrum of the compound showed signals;

(a) Olefinic proton signals for $= CH_2$; two doublets in the region 4.6-4.8 ppm.

(b) an intense signal at 3.75 ppm for -COOMe group.

(c) a singlet for -CHOH at 2.01 ppm.

(d) a sharp peak for $-CH_3$ occurring as $HC = \overset{CH_3}{\underset{C}{|}}\!\!-C$ at 1.75 ppm

and (e) a tall singlet corresponding to 5 CH_3 group at 1.00 ppm.

The reported physical constants of methyl betulinate and its derivatives agree well with our observed values. On acetylation it gave acetoxy methyl betulinate $C_{33}H_{35}O$, m.p. 200-202°, $(\alpha)_D$ + 5° identical with an authentic sample[50] (m.m.p., I.R. and T.L.C.) and on oxidation with cromium trioxide pyridine complex it afforded methyl betulonate 42, $C_{31}H_{48}O_3$, m.p. 163.5°, $(\alpha)_D$ + 42°. Hydrolysis of the methyl ester with potassium tertiary butoxide in dimethyl

sulfoxide[49] gave betulinic acid, $C_{30}H_{48}O_3$, m.p. 299-302° identical with an authentic sample (IR).

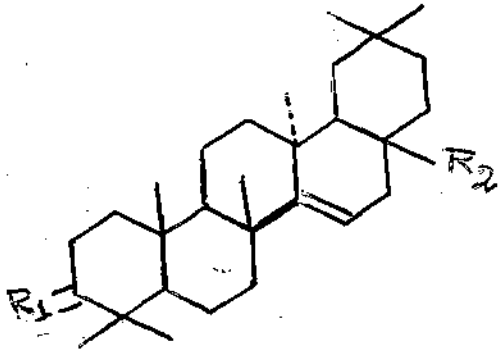
Structure of Aleuritolic acid, $C_{30}H_{48}O_3$.

Section B

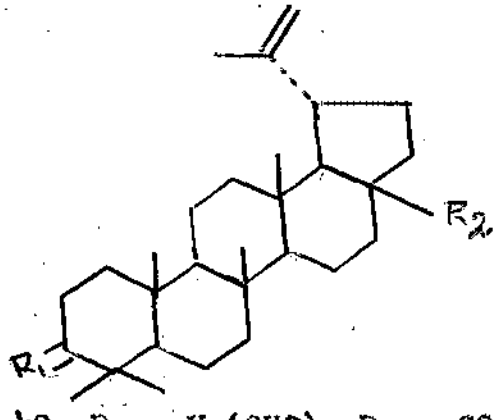
(a) Nature of the oxygen atoms of aleuritolic acid: Elemental analysis and molecular weight determination (mass) showed the presence of three oxygen atoms in the aleuritolic acid. The formation of the sodium salt with sodium hydroxide solution and a infrared band at 3070 cm^{-1} and 1700 cm^{-1} strongly indicated the presence of a carboxyl group in aleuritolic acid. Furthermore, a strong infrared band at 3480 cm^{-1} indicated the presence of a hydroxyl group in aleuritolic acid.

(b) Nature of the carboxyl group: On esterification with diazomethane aleuritolic acid 34 yielded a methyl ester $C_{31}H_{50}O_3$, m.p. 208-10°, $(\alpha)_D$ + 11.11°, infrared spectrum of which showed peaks at 3480 cm^{-1} (-OH), 1735 cm^{-1} ($-COOCH_3$) and at 820 cm^{-1} (trisubstituted double bond). NMR spectrum of the methyl ester exhibited peaks at 5.50δ(1H, vinyl proton, trisubstituted double bond), 3.54δ (singlet 3H, $-COOCH_3$), and several sharp signals between 0.8 to 1.65δ (total 21H, 7 x CH_3 seven methyl groups). Hydrolysis of the methyl ester of aleuritolic acid was attempted with 5%, 10% and 15% methanolic potassium hydroxide solutions respectively. But in each case the starting material was recovered unchanged. It is well known that the triterpenoid acids that are generally hindered cannot be hydrolysed

by the above method. For the hydrolysis of hindered tertiary ester group, a recent method developed by Chang and Wood[49] was employed. By heating at 105° for four hours with potassium tertiary butoxide in dimethyl sulfoxide, the ester group of methyl aleuritolate 35 could be hydrolysed. This observation coupled with the NMR data of methyl aleuritolate (δ 3.54, for 3H, -COOC$\underline{H}_3$) suggested that the carboxyl group in the aleuritolic acid is probably present in a tertiary position as C - $\overset{C}{\underset{C}{\overset{|}{C}}}$ - COOH.



34. R_1 = H (OH,β), R_2 = COOH
35. R_1 = H (OH,β), R_2 = COOMe
36. R_1 = H (OAc, β), R_2 = COOMe
37. R_1 = O, R_2 = COOMe
38. R_1 = H (OAc,β), R_2 = COOH
39. R_1 = O; R_2 = COOH



40. R_1 = H (OHβ), R_2 = COOH
41. R_1 = H (OH,β), R_2 = COOMe
42. R_2 = O, R_2 = COOMe

(c) Nature of the third oxygen atom: The strong infrared band at 3480 cm^{-1} indicated the presence of a hydroxyl group in aleuritolic acid. This is further confirmed by the preparation of acetyl aleuritolic acid 38 m.p. 279-81° and acetyl methyl aleuritolate 36

m.p. 241-3°, $(\alpha)_D$ + 23.08°, whose infrared spectra showed the absence of the peak in the hydroxyl region but instead showed new peaks at 1735 and 1235 cm^{-1} ($-O-COCH_3$). The secondary nature of the hydroxyl group has been proved by the oxidation of methyl aleuritolate 35 with chromium-trioxide-pyridium complex to methyl aleuritolonate 37, $C_{31}H_{48}O_3$, m.p. 174-6°, $(\alpha)_D$ + 11.76, infrared spectra of which showed a sharp peak at 1705 cm^{-1} (six membered ring ketone). This compound 37, also showed a positive Zimmermann test. These facts indicate the presence of the hydroxyl the group as a $-CH(OH)-CH_2-$ group.

(d) Nature of other functional groups:

Methyl aleuritolate 35 showed yellow coloration with tetranitromethane indicating the presence of unsaturation in the compound. Methyl aleutitolate consumed one mole equivalent of perbenzoic acid showing the presence of one double bond. However, methyl aleuritolate did not take up hydrogen, when it was shaken in an atmosphere of hydrogen in ethyl acetate solution in the presence of 10% palladium-on-charcoal catalyst and was quantitatively recovered unchanged. This experiment indicated that the double bond was most probably present in a hindered position. The above facts coupled with the NMR peat peak at δ 5.50 (vinyl proton, 1H) showed that the double bond must be a trisubstituted one.

(e) Pentacyclic nature of aleuritolic acid

On the basis of the molecular formula $C_{30}H_{48}O_3$, the nature of the oxygen functions and the presence of a trisubstituted double

bond, it may be deduced that aleuritolic acid must be pentacyclic in nature.

Section C

Discussion of the IR and NMR spectra of aleuritolic acid and its derivatives

The proposal 34 for aleuritolic acid has been deduced from its chemical behaviour, analysis of infrared spectra and NMR spectra and from the fragmentation pattern of mass spectra of acetyl methyl aleuritolate 36 and methyl aleuritolonate 37.

The infrared spectra of aleuritolic acid showed peaks at 3480 cm^{-1} indicating the presence of hydroxyl group; peaks at 3070 cm^{-1} and at 1700 cm^{-1} are indicative of carboxyl group and peak at 820 cm^{-1} is indicative of trisubstituted double bond. The infrared spectra of methyl aleuritolate 35 showed peaks at 3480 cm^{-1} for the hydroxyl group, at 1735 cm^{-1} for the carbomethoxy group and at 820 cm^{-1} for the trisubstituted double bond. The infrared spectra of acetyl methyl aleuritolate 36 (fig. 1) showed a broad composite peak at 1735 cm^{-1} for both acetyl group and carbomethoxy group and at 1245 cm^{-1} for acetyl group and at 820 cm^{-1} for trisubstituted double bond. NMR spectra of acetyl methyl aleuritolate (fig. 2) showed a peak at δ 5.50 (multiplet accounting for one proton, vinyl proton, trisubstituted double bond), at δ 4.46 (multiplet, accounting for one proton H-C-OCOCH$_3$), at δ 3.5 (a sharp singlet accounting for three protons --COOCH$_3$), at δ2.04 (a sharp singlet accounting for three protons,

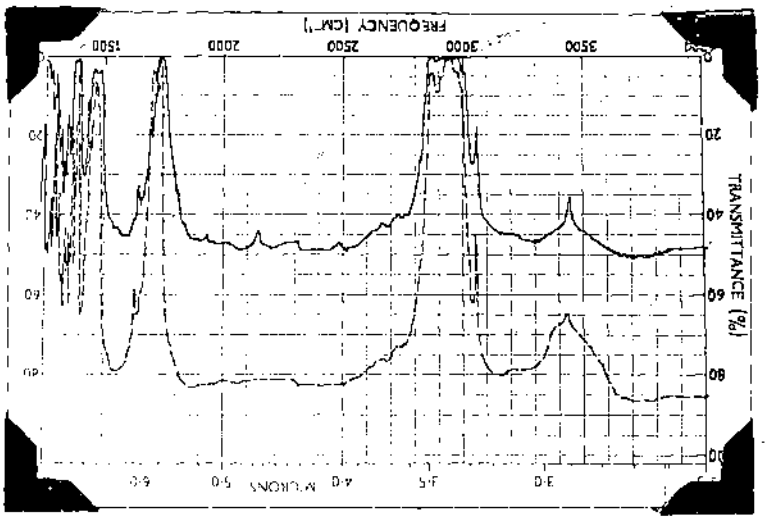


Fig. 1 : I.R. of Acetyl methyl aleuritolate(solid line)

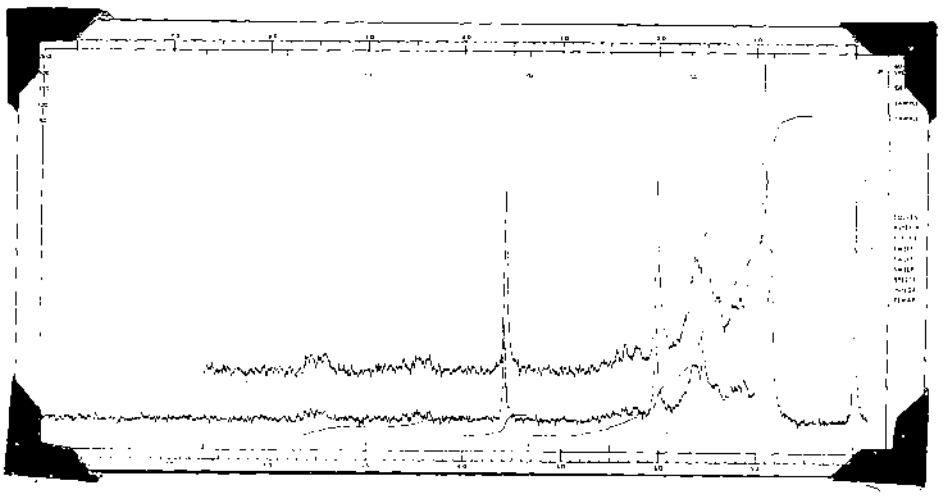


Fig. 2 : NMR spectra of acetyl methyl aleuritolate.

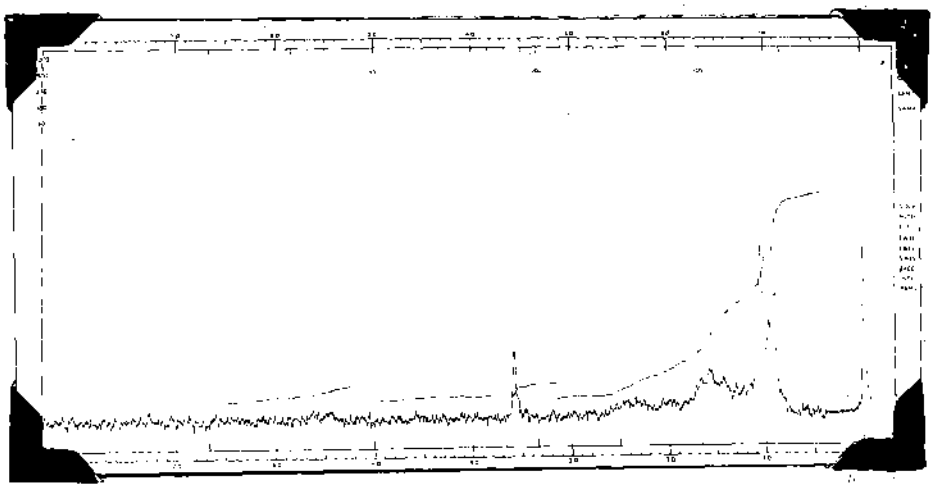


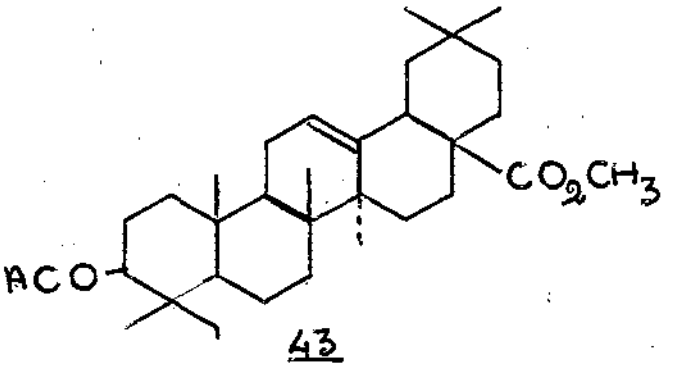
Fig. 3 : NMR spectra of methyl aleuritolonate.

-OCOC$\underline{H}_3$) and several sharp signals between 0.8 to 1.65δaccounting for twenty one protons suggesting the presence of seven methyl groups. NMR spectra of methyl aleuritolate 35 (fig. 3) showed a multiplet at 5.50δ (accounting for one proton, trisubstituted double bond), ~~at 3.54δ (a sharp singlet accounting for one proton, trisubstituted double bond)~~, at 3.54δ (a sharp singlet accounting for three protons, -COOC$\underline{H}_3$) and signals between 0.8 to 1.65$\delta$for 21 protons suggesting the presence of seven methyl groups.

Oxidation of methyl aleuritolate 35 with Chromium trioxide-pyridine complex furnished the ketone, methyl aleuritolonate 37 $C_{31}H_{48}O_3$, mol. wt. 468 (mass), m.p. 174-6°, $(\alpha)_D$ 11.76°. Infrared spectra of 37 showed peaks at 1705 cm^{-1} (six membered ring ketone), at 1735 cm^{-1} (for carbomethoxy group) and at 820 cm^{-1} for trisubstituted double bond. NMR spectra of methyl aleuritolonate 37 showed a multiplet at 5.58δ accounting for one proton (vinyl proton, trisubstituted double bond), a sharp singlet at 3.58δ accounting for three protons (-COOC$\underline{H}_3$) and signals between 0.8 to 1.68δ accounting for twenty one protons (seven methyl groups). The keto ester showed a positive Zimmermann test, indicating the location of the carbonyl group at C-3 position[51].

The acetoxy methyl aleuritolate 36 showed yellow coloration with tetranitromethane, indicating the presence of ethylenic linkage in the compound. This has been confirmed by titrating the acetoxy methyl aleuritolate 36 with perbenzoic acid. The compound consumed one mole of perbenzoic acid indicating the presence of one double bond in the

compound. Acetoxy methyl aleuritolate 37 on heating with HCl- acetic acid mixture on a water bath for fifteen minutes[52] gave a crystalline solid m.p. 219-20°, $(\alpha)_D$ + 58°. This compound, as indicated by its melting point and specific rotation values, has been found to be identical with an authentic specimen of acetyl methyl oleanolate[53].



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This fact coupled with the NMR data establishes that aleuritolic acid (34) contains a modified oleanane skeleton with a trisubstituted double bond. The identity of the rearranged product with acetyl methyl oleanolate 43 once again establishes the pentacyclic nature of the acid 34 with a secondary hydroxy group at 3 position, a -COOH group at 17-position and a double bond.

Section D

The position of the double bond : Application of mass spectrometry

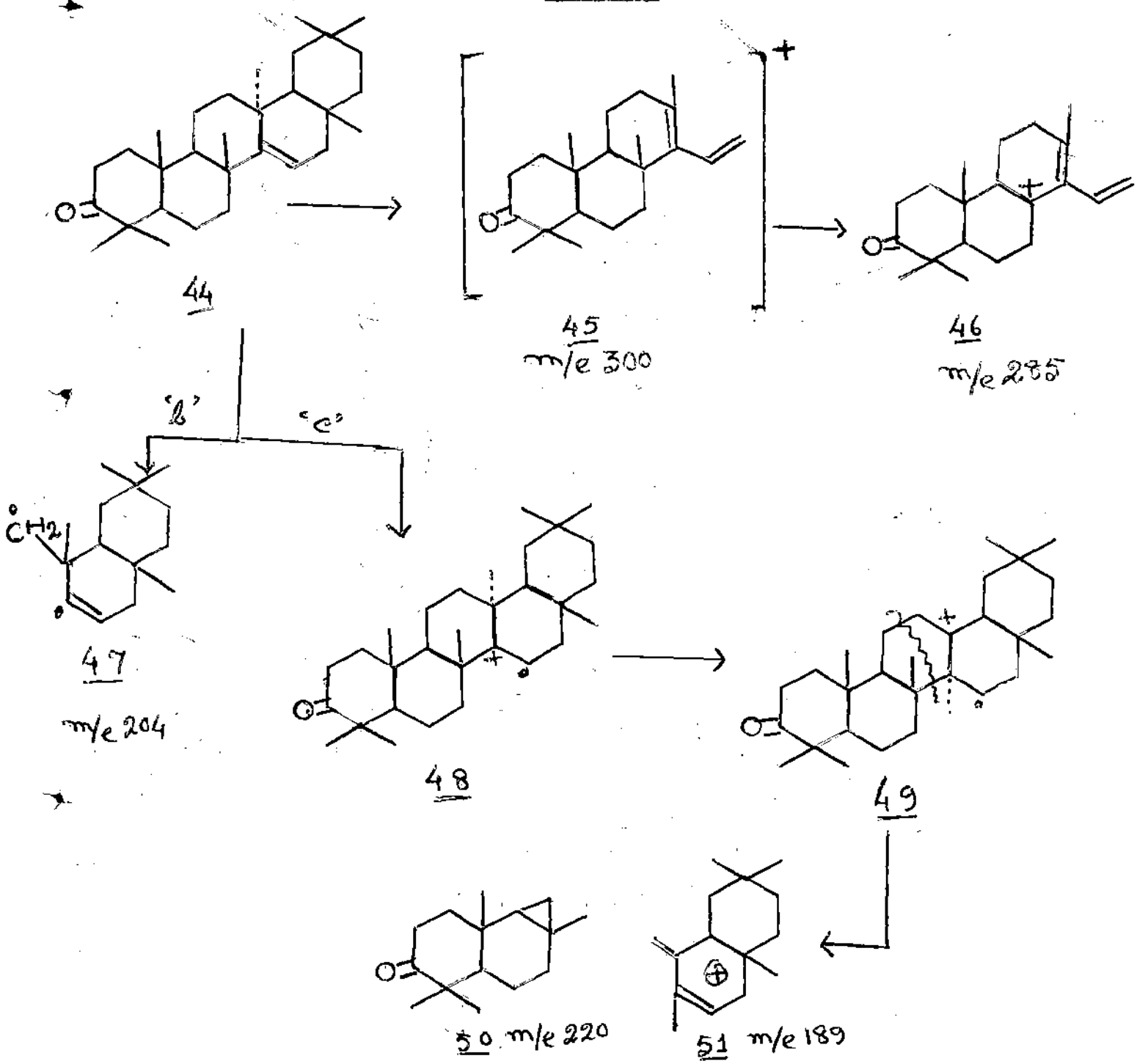
Recent papers have demonstrated the application of mass spectrometry in the structural determination of pentacyclic saturated[54,55] and unsaturated triterpenoids[56-60]. The present study provides an additional example where the technique was used to confirm the

pentacyclic nature of aleuritolic acid and to provide additional evidence for the location and identity of its functional groups.

Following a survey of the mass spectra of a series of triterpenoid compounds, it was discovered that the mass spectra of acetyl methyl aleuritolate 36 (fig. 4) and methyl aleuretolonate 37 (fig. 5) were very similar to that of Δ^{14}-taraxerene skeleton, which suggested that they had similar pentacyclic structures. The main ion reactions of acetyl methyl aleuritolate 36 and methyl aleuritolonate 37 include the expected retro-Diels-Alder type of cleavages through ring D^{61} and cleavages through ring C as shown in Chart IV and V below.

The fragmentation pattern of Δ^{14}-taraxerene can be explained by three reactions, 'a', 'b' and 'c'. In the case of taraxerone 44 reaction 'a' by the retro-Diels-Alder decomposition and collapse of the ring D leads to the resonance stabilized even electron ion on the diene at m/e 285, 46 (Chart IV) comprising the ring A, B and C. Reaction 'b' is the fission of 11-12 and 8-14 bonds to produce the resonance stabilised radical ion at m/e 204, 47. Reaction 'c' involves the removal of missing electron from carbon-carbon double bond 48, migration of the C-13 methyl group, yielding the radical ion 49. Fission of the 11-12 and 8-14 bonds give the stable diene 51 ion and a fragment 50 at m/e 220.

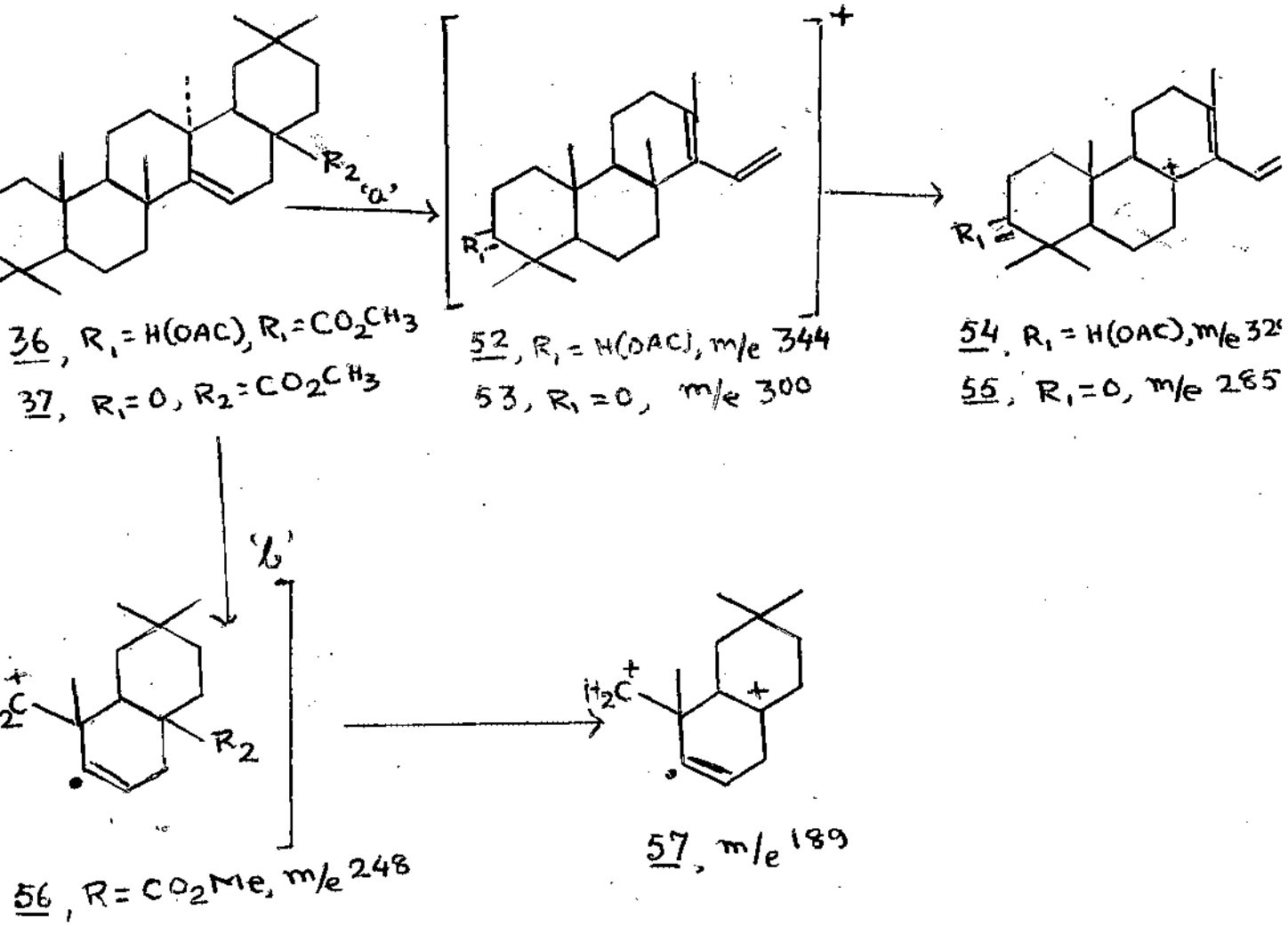
Chart IV

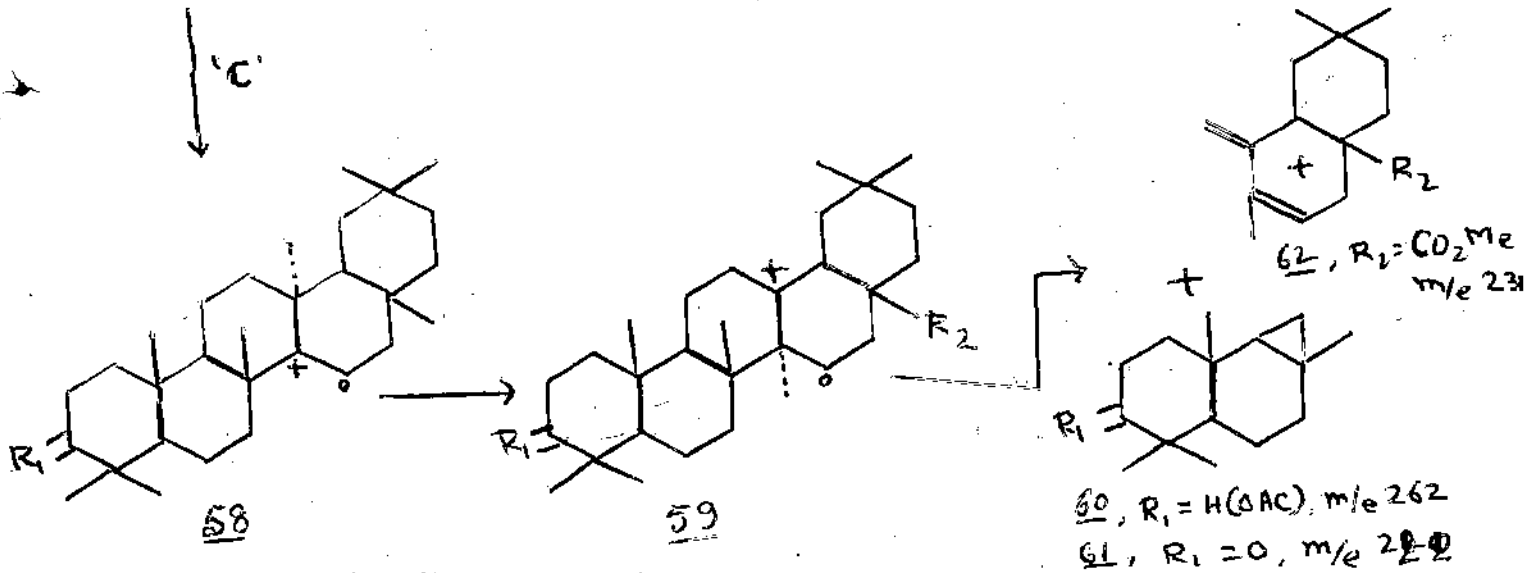


The mass spectra of acetylmethyl aleuritolate 18 (fig. 4) and methyl aleuritolonate 37 (fig. 5) had all the characteristic peaks

due to reactions 'a', 'b' and 'c' which proved that the functional groups R_1 and R_2 were attached to different halves of the molecule. Due to reaction 'a' it gave a sharp peak at m/e 344, 52 and at m/e 300, 53 which was accompanied by a peak 15 mass unit lower m/e 329, 54 and m/e 285, 55, which is formed by the loss of the methyl group, probably the allylically activated one at C_8. Furthermore, the spectra of acetyl methyl aleuritolate 36 (fig. 4) exhibited a sharp

Chart V





peak at m/e 284 and at m/e 269. These two peaks are associated with the loss of one mole of acetic acid from diene ion 52 (52-CH_3COOH = m/e 284) and 54 (54 -CH_3COOH = m/e 269). Since the reaction 'a' comprises the ring A, B and C, the acetoxy group and hence the hydroxy group in aleuritolic acid is in ring A. Due to reaction 'b' the spectra of acetyl methyl aleuritolate 36 (fig. 4) and methyl aleuritolonate 37 (fig. 5) gave a very abundant fragment at m/e 248, 56 and this has been derived from rings D and E. Furthermore, the fragment 56 loses the substituent at C-17 giving rise to a fragment 57, m/e 189 (56-COOMe). A small but a prominent peak at m/e 262, 60, at m/e 220, 61 and at m/e 233, 62 was found in the spectra of acetyl methyl aleuritolate (fig. 4) and methyl aleuritolonate (fig. 5) respectively. The peak at m/e 262, 60 is accompanied by a peak at m/e 202, (60-CH_3COOH) and the peak at m/e 233, 62, is accompanied by a peak at m/e 174, (62-COOMe). This fact establishes that the peak due to 60 comprises the ring A and B and the peak due to 62 comprises the ring D and E. This type of fragmentation can be formed due to reaction 'C' (Chart V).

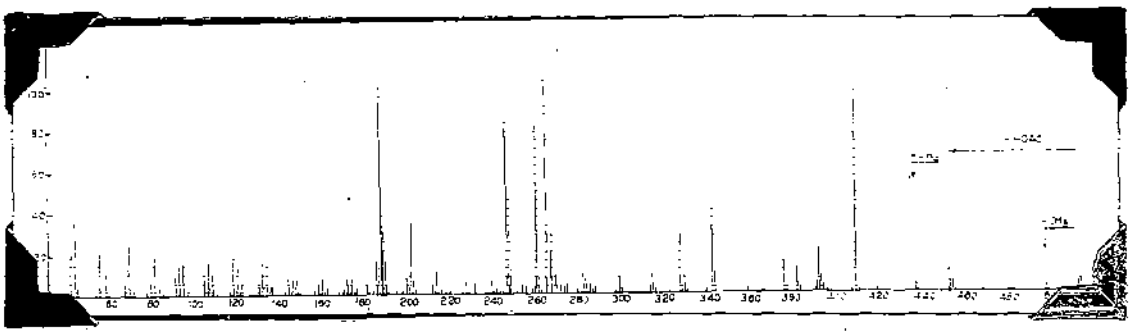


Fig. 4 : Mass spectra of acetyl methyl aleuritolate.

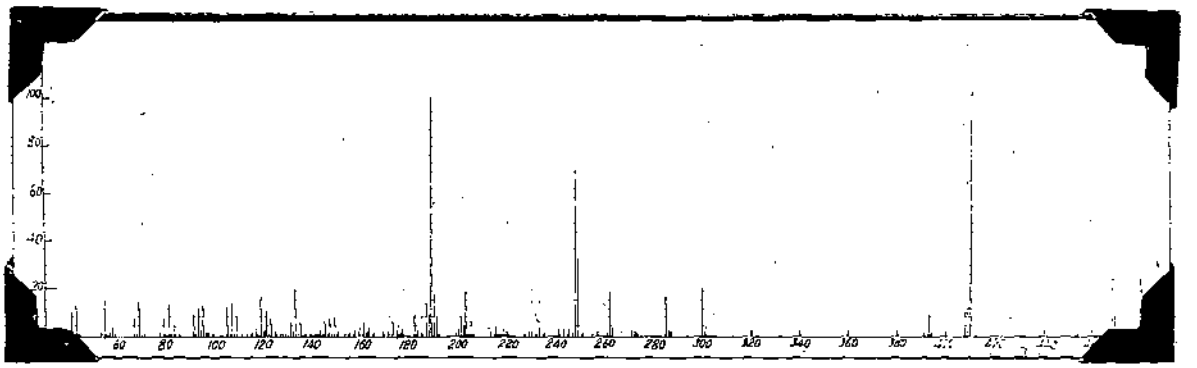


Fig. 5 : Mass spectra of methyl aleuritolonate.

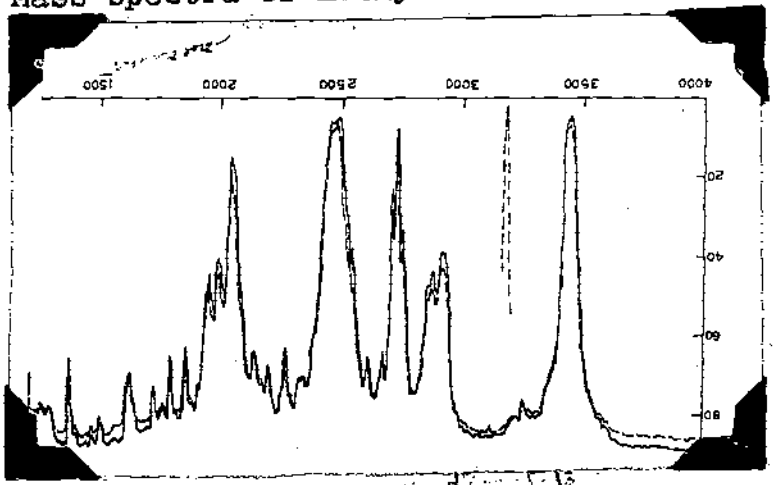
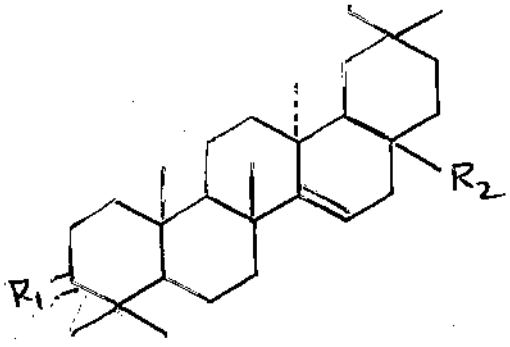


Fig. 6 : IR comparison
Solid line - myricadiol prepared from aleuritolic acid.
Dotted line - authentic sample of myricadiol.

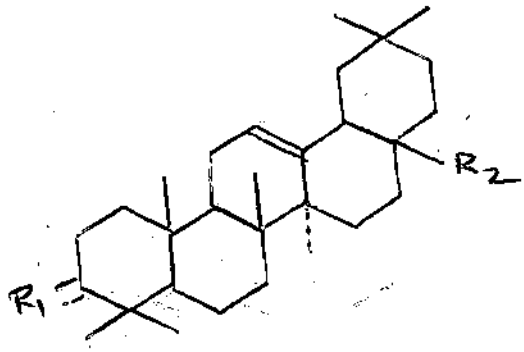
All these types of fragmentation can be explained by assuming the 14:15 double bond in oleanane skeleton and has been found to be consistent with the mass spectral data of Taraxerene derivatives reported by Djerassi et al.

The full structure and absolute configuration

The structure 34 for aleuritolic acid was further confirmed along with its stereochemistry by correlating it with a suitable member of oleanane series. Lithium aluminium hydride reduction of methyl aleuritolate afforded a crystalline diol, $C_{30}H_{50}O_2$, m.p. 265-67° which has been identified, as indicated by its melting point and specific rotation values as myricadiol[21-25] 63. Acetylation of the diol gave the corresponding diacetate 64, $C_{34}H_{54}O_4$, m.p. 251-2°, $(\alpha)_D$-3°. This has been found to be identical with an authentic sample of myricadiol diacetate 64 (m.m.p., IR and T.L.C.) (for IR comparison see fig. 6). The above myricadiol diacetate on acid isomerisation[52] gave a diacetate, $C_{34}H_{54}O_4$, m.p. 184-6°, $(\alpha)_D$ + 56.2°, identical with an authentic sample of erythrodiol diacetate 66 (m.m.p.).

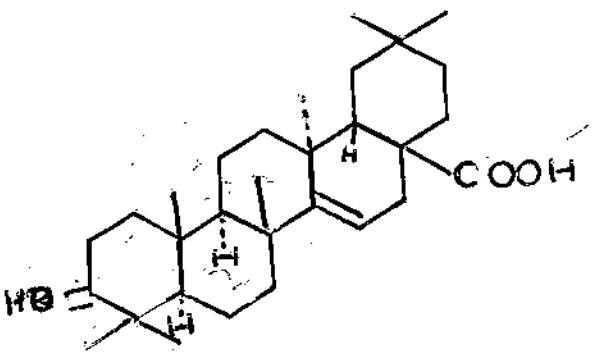


63, R_1 = H(OH, β), R_2 = CH_2OH
64, R_1 = H(OAc, β), R_2 = CH_2OAc



65, R_1 = H(OH, β), R_2 = CH_2OH
66, R_1 = H(OAc, β), R_2 = CH_2OAc

On the basis of these results, IR, UV, NMR and mass spectral datas and the chemical evidences shown above, it may be concluded that aleuritolic acid must have a pentacyclic skeleton with one tri-substituted double bond at C_{14}-C_{15}, a secondary hydroxyl group at C_3 and a tertiary -COOH group at C_{17}. Hence, aleuritolic acid is olean-14 (15)-en-3β-ol-28-oic acid and can be represented by structure 67.



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