CHAPTER -III

Section A: Reinvestigation on the acid part of Sapium sebiferum Roxb.

Isolation of a new triterpenic acid, Sebiferenic acid, (a 2α hydroxy aleuritolic acid) along with sebiferic acid and aleuritolic acid.

Extraction:

Dried and powdered trunk bark of Sapium sebiferum Roxb. was extracted with benzene in a Soxhlet apparatus. On cooling at room temperature a yellow insoluble solid separated out (identified previously as 3,4-Di-O-methyl ellagic acid by Pradhan[46a]) from the benzene extract and was collected by filtration. Distillation of the clear filtrate 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 till neutral and dried over anhydrous sodium sulphate. Evaporation of ether furnished a gummy residue which constituted the neutral portion B of the extract (the work on the neutral portion B 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 washed with water till neutral and finally dried over anhydrous sodium sulphate. Evaporation of ether yielded a gummy brown residue. This was chromatographed over silica gel. The column was developed in petrol and eluated with the following solvents:

Table - I

Chromatography of the above gummy material (10 g)

Eluent	Fraction 250 ml each	Residue on evaporation	m.p. ^{o}C
Petrol	1-5	Oil	
	6-7	Nil	
Petrol:benzene (1:1)	8-13	Oil	
	14-15	Nil	
Petrol:benzene (2:3)	16-21	Nil	
Petrol:benzene (1:4)	22-27	Solid	160-175^{o}
Benzene	28-33	Nil	
Benzene:ether (9:1)	34-40	Solid	290-300^{o}

(Contd..)

Table - I (Contd..)

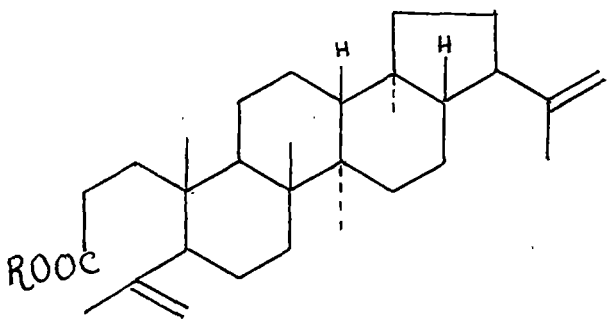
Eluent	Fractions 250 ml each	Residue on evaporation	m.p. °C
Benzene:ether (4:1)	41-46	Oil	
	47-48	Nil	
Benzene:ether (3:2)	49-58	Gummy solid (200mg)	310-320° digestion with methanol

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

TLC of Fractions 22-27 (Table-I) showed the homogeneity. Therefore fractions 22-27 were combined.

This was esterified with ethereal solution of diazomethane and after usual workup and column chromatography the methyl ester 20 m.p. 128-30° was obtained. This was crystallised from chloroform and methanol to give crystals m.p. 134-6°, $[\alpha]_D$ 24.4°. IR spectra of this compound 20 showed peaks at 1737 cm^{-1} for the carbomethoxy group and at 1640, 895, 875 cm^{-1} which can be assigned to two asymmetrically disubstituted ethylenic functions. These last three bands are completely absent in the spectra of tetrahydro methyl ester obtained by hydrogenation and this showed band at 1737 cm^{-1} for the methyl ester group. NMR spectrum

of the ester also showed the presence of four vinyl protons between 4.86 and 4.70 ppm, two methyl groups located on two double bonds at 1.72 and 1.68 ppm and four additional methyl groups on saturated carbons at 1.03, 0.97, 0.83 and 0.70 ppm together with the peaks for the methyl group of the carbomethoxy function at 3.67 ppm and methylene group adjacent to the carbomethoxy group ($-C\underline{H}_2COOCH_3$) is shown by the peak at 1.43 ppm. The NMR spectrum of the tetrahydromethyl ester showed no signal for the vinyl protons, but showed a complex of eight methyl groups around 0.93 to 0.65 ppm and a peak for the methyl signal (3H) of the carbomethoxy group at 3.67 ppm and a signal for the methylene group to which the carbomethoxy group is attached ($-C\underline{H}_2COOCH_3$) at 1.43 ppm suggested that tetrahydro methyl ester of the acid is a completely saturated compound. The acid contained two double bonds as the methyl ester of the acid consumed two equivalents of perbenzoic acid. The methyl ester was readily hydrolysed with 5% methanolic potassium hydroxide solution. The acid 19 has the m.p. 178-80°. IR spectrum showed peaks at 3070, 1707 (COOH group), 1640, 895, 875 cm^{-1} (two double bonds). The acid was sebiferic acid 19 which was isolated from Sapium sebiferum and its structure was established earlier by Pradhan et al[46(b)].



19) -R = H [Sebiferic acid]

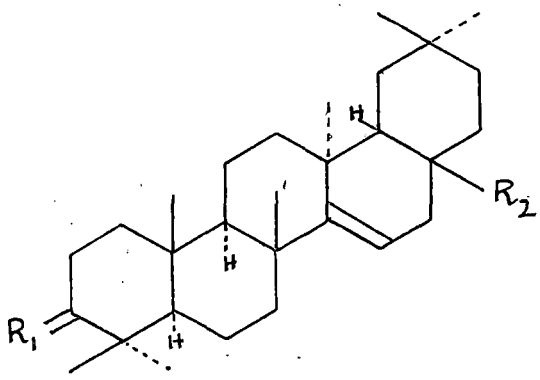
20) -R = CH_3 [Methyl sebiferate]

As TLC of fractions 34-40 (Table I) showed homogenity they were combined. This was esterified with diazomethane and the solid after chromatography and crystallisation from chloroform-methanol mixture afforded colourless crystals m.p. 208-10°, $[\alpha]_D$ 11.11° having molecular formula $C_{31}H_{50}O_3$. It did not show any absorption in UV spectra in the region 220-300 nm. Infrared spectrum of this compound 21 showed peaks at 3480 (-OH), 1735 ($-COOCH_3$) and at 820 (trisubstituted double bond) cm^{-1}. This methyl ester on chromium trioxide pyridine complex oxidation[47] afforded a compound which after chromatography and crystallisation from chloroform methanol mixture gave

fine needle shaped crystals 22b, $C_{31}H_{48}O_3$, m.p. 174-6°, $[\alpha]_D$ 11.76°. Infrared spectra of this compound showed peaks at 1705 cm^{-1} (six membered kenone), at 1735 cm^{-1} (for carbomethoxy group) and at 820 cm^{-1} for trisubstituted double bond. NMR spectra of this compound showed a multiplet at 5.58 ppm accounting for one proton (vinyl proton, trisubstituted double bond), a sharp singlet at 3.58 ppm accounting for three protons ($-COOCH_3$) and signals between 0.8 to 1.68 ppm 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.

The keto ester was hydrolysed with dimethyl sulfoxide in presence of potassium tertiary butoxide following the method of Chang and Wood[48] to furnish a solid 22c, $C_{30}H_{46}O_3$, m.p. 276-8°. On acetylation the methyl ester of the acid gave acetyl methyl ester which after chromatography and crystallisation from chloroform methanol mixture afforded colorless crystals, $C_{32}H_{52}O_3$, m.p. 241-3°, $[\alpha]_D$ 23.08°. NMR spectra of this compound showed peak at 5.50 ppm (multiplet accounting for one proton, vinyl proton, trisubstituted double bond) at 4.46 ppm (multiplet for one proton (H—C—O-$COCH_3$), at 3.58ppm (a sharp singlet accounting for three protons-

$COOCH_3$), at 2.04 ppm (a sharp singlet accounting for three protons, -OCO-CH_3) and several sharp signals between 0.8 to 1.05 ppm accounting for twenty one protons suggesting the presence of seven methyl groups). This acetyl methyl ester has been found to be identical with an authentic sample of acetyl methyl aleuritolate 22a m.p., tlc, co-IR) [Fig. 1]. Thus the reported physical constants of methyl aleuritolate[49a] 21 and its derivatives agree well with our observed values.



21) R_1 = H, OH(β), R_2 = $COOCH_3$

22a) R_1 = H, OAc(β), R_2 = $COOCH_3$

22b) R_1 = O, R_2 = $COOCH_3$

22c) R_1 = O, R_2 = COOH

As the tlc of fractions 49-58 (Table 1) showed homogenity, they were combined. This was esterified with diazomethane. The crude methyl ester was chromatographed over deactivated alumina. Benzene: ether (9:1) eluate afforded a solid 230-48° which after crystallisation from a chloroform-methanol mixture gave fine needle shaped crystals m.p. 253-54° having molecular formula $C_{31}H_{50}O_4$. This compound did not show any UV absorption in the region 220-300 nm. Infrared spectrum of this compound showed peaks at 3380 (broad-OH), 1730 ($-COOCH_3$) and at 820 (tri-substituted double bond) cm^{-1} [Fig. 2]. Hydrolysis of this methyl ester 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. Hydrolysis of the methyl ester was accomplished by heating with potassium tert. butoxide in dimethyl sulfoxide 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 chloroform afforded a solid which after repeated crystallisation from chloroform and methanol mixture gave an amorphous solid having m.p.

325° (decom.). The acid appeared to be a new one and has been shown to be 2α , 3β -dihydroxy olean-14(15)-en-28-oic acid i.e. 2α , hydroxy aleuritolic acid on the basis of chemical and physical evidences. The compound henceforth will be called sebiferenic acid after the name of the species from which it has been isolated for the first time.

Sebiferenic acid is amorphous solid. It gives sparingly soluble potassium salt and is soluble in ether, pyridine, hot methanol and sparingly soluble in chloroform. It developed a yellow colour with tetra nitro methane indicating the presence of a double bond in the compound. It gave a violet colouration in Liebermann-Burchardt reaction showing that the compound to be a triterpene. It did not show any UV absorption in the region 220-300 nm. Titration of the pure acid with standard sodium hydroxide solution showed that it was a monobasic acid. Elemental analysis and equivalent weight determination was consistent with the molecular formula $C_{30}H_{48}O_4$. Infrared spectrum of the acid showed peaks at 3350 (-OH), 3070, 1700 (-COOH) and at 820 (trisubstituted double bond) cm^{-1}. The methyl ester of this acid on acetylation with acetic anhydride-pyridine as usual and after chromatography furnished the acetate, $C_{35}H_{54}O_6$, m.p. 224-26°. The Infrared spectrum of this ester acetate showed peaks at 1725 (broad, $-OCOCH_3$ $-COOCH_3$) 1250 ($-OCOCH_3$) and at 820 (trisubstituted double bond) cm^{-1} [Fig. 3].

Section B : Structure of sebiferenic acid $C_{30}H_{48}O_4$.

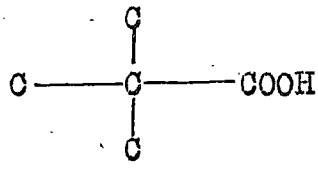
(a) Nature of the oxygen atoms in sebiferenic acid:

Elemental analysis and molecular weight determination (Mass) showed the presence of four oxygen atoms in the sebiferenic acid. The formation of the sodium salt with sodium hydroxide solution and IR bands at 3070 and 1700 cm^{-1} strongly indicated the presence of a carboxyl group in sebiferenic acid. Furthermore, a broad IR band at 3350 cm^{-1} indicated the presence of hydroxyl group in sebiferenic acid.

(b) Nature of the carboxyl group:

On esterification with diazomethane sebiferenic acid 23 yielded a methyl ester 24 m.p. 253-54°, IR spectrum of which showed peaks at 3380 (broad-OH), 1730 ($-COOCH_3$) and at 820 (trisubstituted double cond) cm^{-1} [Fig. 2]. Hydrolysis of this methyl ester 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 at C_{17} cannot be hydrolysed[48a,48b] by the above method. For the hydrolysis of hindered tertiary ester group, a method developed by Chang and Wood[48] was employed. By heating at 105° for four hours with potassium tert. butoxide in dimethyl sulfoxide, the

ester group of methyl sebiferenate could be hydrolysed. This suggested that the carboxyl group in sebiferenic acid is probably present in a tertiary position as



(c) Nature of the other two oxygen atoms:

The strong and broad IR band at 3380 cm^{-1} of methyl sebiferenate 24 indicated the presence of two hydroxyl groups in the original acid. This is further confirmed by the preparation of diacetoxy methyl sebiferenate 25, $C_{35}H_{54}O_6$, m.p. 224-26°, whose infrared spectra showed the absence of the peak in the hydroxyl region but instead showed peak at 1725 (broad, $-OCOCH_3$, $COOCH_3$), 1250 ($-O-CO-CH_3$) cm^{-1} [Fig. 3].

(d) Nature of other functional groups:

Methyl sebiferenate 24 showed yellow colouration with tetra nitromethane indicating the presence of unsaturation in the compound. It consumed one mole equivalent of perbenzoic acid showing the presence of one double bond. However, methyl sebiferenate 24 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 ^{1}H NMR peak at 5.50 ppm (multiplet) (vinyl proton, 1H) of diacetoxy methyl sebiferenate [Fig. 4] confirmed that the double bond must be trisubstituted one.

(e) Pentacyclic nature of sebiferenic acid:

On the basis of the molecular formula $C_{30}H_{48}O_4$, the nature of the oxygen functions and the presence of a trisubstituted double bond, it may be deduced that sebiferenic acid must be pentacyclic in nature.

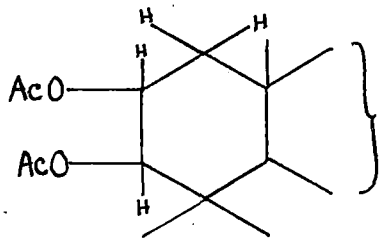
Section C: Discussion of the IR, ^{13}C NMR and H^1 NMR spectra of sebiferenic acid and its derivatives.

The IR spectra of methyl sebiferenate 24 showed peaks at 3380 cm^{-1} for the hydroxyl group, at 1730 cm^{-1} for the carbomethoxy group and at 820 cm^{-1} for the trisubstituted double bond [Fig. 2]. The IR spectra of diacetyl methyl sebiferenate showed a broad composite peak at 1725 cm^{-1} for both the acetyl groups and carbomethoxy group and at 1250 cm^{-1} for acetyl groups and at 820 cm^{-1} for trisubstituted double bond [Fig. 3].

H^1 NMR spectrum of diacetyl methyl sebiferenate 25 [Fig. 4] showed a peak at 5.50 ppm (multiplet) accounting for one proton, suggesting the presence of a trisubstituted double bond, at 1.98 ppm (a sharp singlet) accounting for three protons (-O-CO-CH_3), at 2.04 ppm (a sharp singlet) accounting for three protons (-O-CO-CH_3), at 3.58 ppm (a sharp singlet) accounting for three protons of the carbomethoxy group (-COOCH_3) and several sharp signals between 0.85 to 1.05 ppm accounting for twenty one protons suggesting the presence of seven tertiary methyl groups.

The ^1H NMR spectrum showed the methine protons at 4.7 and 5.05 ppm as a doublet and a triplet of doublets respectively. Different absorption of these two protons

(attached to acetate bearing carbons) suggested that they are situated in different chemical environments. This might arise from the coupling of one of these protons with two nearby methylene protons. This part of the spectrum corresponded to the X-part of the ABXY type of spectrum shown in case of four protons attached to adjacent carbons in the system (A).

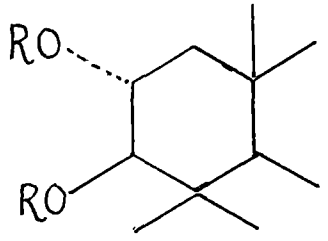


$C_{22}H_{35}O_4$

A

If we assume that one of the acetate functions is situated as in all the triterpene at C-3 of the A ring of triterpene nucleus then H^1 NMR data could easily be correlated by placing the other acetyl group at C-2. Appearance of C-3 proton with unsymmetrical doublet at 4.7 ppm having the coupling constant (J = 10 Hz) showed that the C-2 and C-3 protons are in a trans diaxial arrangement[49b] and consequently the 2α, 3β diequatorial configuration of the acetate groups could be inferred as in the case

of 2α, 3β diacetoxy methyl maslinate (crategolate)[50] and baccatin[51] diacetate methyl alphitolate diacetate[51b]. Hence the partial formula i.e. ring A of methyl sebiferenate and diacetoxy methyl sebiferenate can be written as (i) and (ii) respectively with gem dimethyl groups at C-4.



i) R = H

ii) R = Ac

<u>^{13}C NMR (CMR) spectra of acetyl methyl sebiferenate.</u>

In an effort to arrive at a conclusive evidence for assigning the structure for diacetyl methyl sebiferenate <u>25</u> we thought that ^{13}C NMR spectrum of the compound might help in solving the problem of establishing its structure. The solution of the problem depends upon three things

1) obtaining spectra with good signal-to-noise ratio
2) making correct assignment of the peaks
3) having reasonably close models to compare chemical shifts.

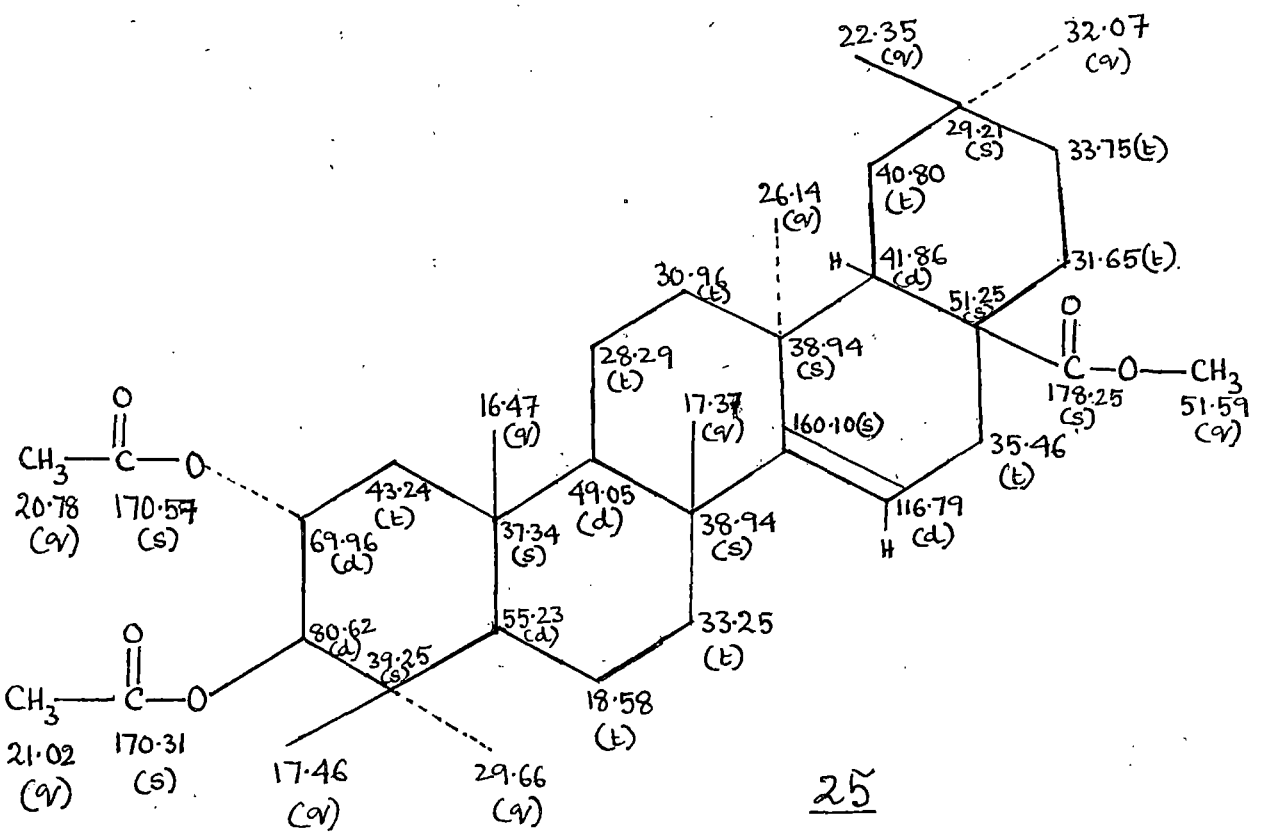
The ^{13}C NMR spectra were run at Varian-Associates, U.S.A., through the kind courtsey of Dr. J.N.Shoolery in a FT-80A Spectrometer having a microsample accessory attachement. From Fig. 5, it is clear that the peaks at 170.57(s) and 170.31(s) ppm are consistent with the presence of two acetate carbonyls. The peaks at 160.10(s) and 116.79(d) ppm are characteristic of a double bond to a ring junction with a proton on the other olefinic carbon respectively. The ^{13}C peaks at 80.62(d) and 69.96(d) ppm are due to the CH groups to which the acetates are attached.

From Fig. 5 with better signal -to- noise ratio it is clear that the peak at 178.25 ppm is the carbonyl of a carbomethoxy group. There are 35 carbons in the molecule.

Fig. 6 is the off-resonance CW decoupled spectrum. Plot expansions (Fig. 7) and (Fig. 8) can be compared to give the multiplicities of each line in the spectrum. The two methyls at 17.37 and 17.46ppm are not resolved, but two distinct chemical shifts may be printed out. This point was checked by integrating the 6 lines at the right hand end of the spectrum. Fig. 9 shows that the area of the peak at 17.4 ppm corresponds to two methyl carbons. The total number of methyl groups is found to be ten, so there are seven tertiary methyl groups, two acetate methyls and one carbomethoxy.

Fig. 10 is an off resonance noise decoupled spectrum which gives sharp lines only for non-protonated (singlet) carbons. From this chart we can see that there are six quaternary carbons (two coincide at 38.94 ppm) in addition to the three carbonyl carbons and the doubly bonded carbon at ring junction.

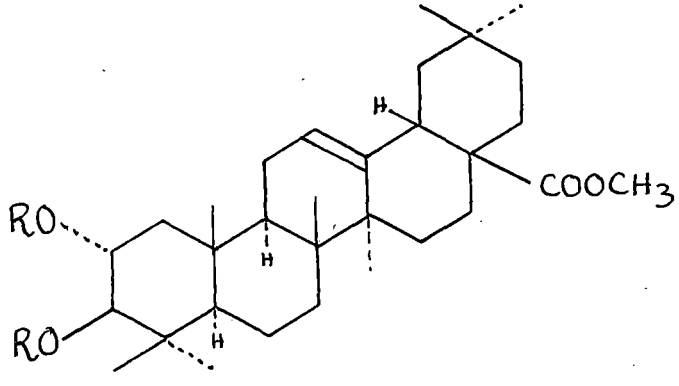
The total carbon shift assignment[52] 25 is portrayed in the formula given below.



25

s = singlet
d = doublet
q = quartet
t = triplet

Diacetyl methyl sebiferenate 25 on heating with HCl-acetic acid mixture on a water bath for fifteen minutes[53] gave a solid which after chromatography and crystallisation from dilute methanol furnished colourless crystals m.p. 166-68° [α]$_D$ 24°. IR spectra showed bands at 1750 (-CO_2Me), 1730 (acetate, 1250 (acetate), 825 (trisubstituted double bond) cm^{-1} [Fig. 11]. This 27 on hydrolysis with methanolic potassium hydroxide afforded a solid which after chromatography and crystallisation from benzene and petrol mixture afforded colourless crystals m.p. 220-22°, [α]$_D$ 36°. This compound was found to be identical with that of authentic methyl crategolate 26 (maslinate) [Fig. 12](supplied by Prof. P.Sengupta by IR comparison and m.m.p.).



26) R = H

27) R = Ac

This fact coupled with the NMR data establish that sebiferenic acid 23 contains a modified oleanane skeleton with a trisubstituted double bond. The identity of the rearranged product followed by hydrolysis with methyl crategolate (maslinate) 26 once again establishes the pentacyclic nature i.e. modified oleanane skeleton of the acid with the position and stereochemistry of hydroxyl groups (secondary) at C-2 (OH, α) and at C-3 (-OH, β) and the position of carboxyl group at C-17.

Section D : The position of the double bond: Application of mass spectrometry.

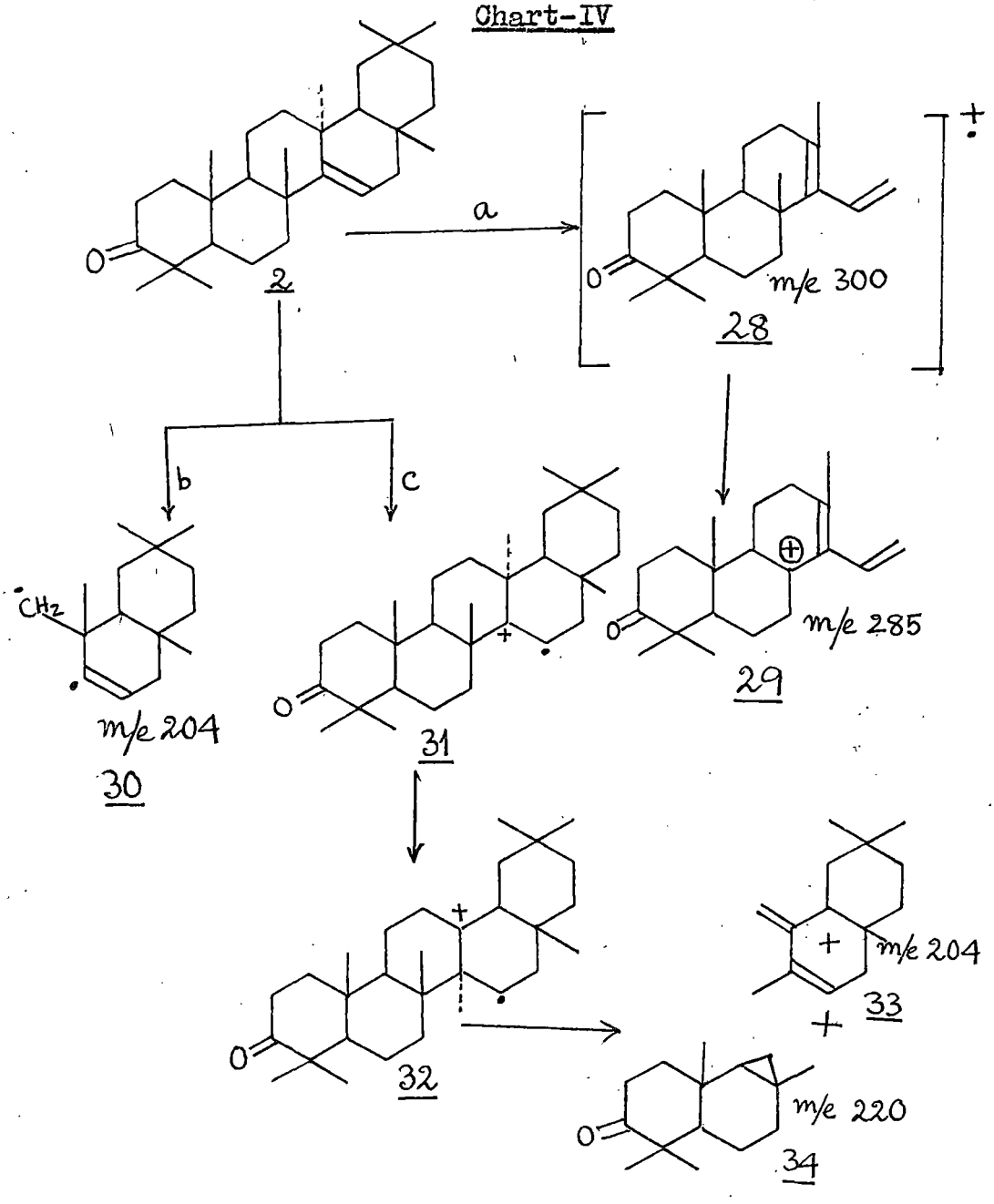
Recent papers have demonstrated the application of mass spectrometry in the structural determination of pentacyclic saturated[54,55] and unsaturated triterpenoids[56-60]. The present study provides an additional example where the technique was used to confirm the pentacyclic nature of sebiferenic acid 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 methyl sebiferenate [Fig. 13] and diacetoxy methyl sebiferenate [Fig. 14] were very similar to that of Δ^{14}-taraxerene skeleton, which suggested that they had similar pentacyclic structures. The main ion fragments of methyl sebiferenate 24 and diacetyl methyl sebiferenate 25 include the expected retro-Diels-Alder type of cleavages through ring D[61] and cleavages through ring C similar to that of Δ^{14}-taraxerene as cited in Chart IV.

The fragmentation pattern of Δ^{14}-taraxerene can be explained by three reactions, a, b and c. In the case of taraxerone 2 reaction "a" by the retro Diels-Alder decomposition and collapse of ring D leads to the resonance stabilised even electron on the diene at m/e 285 29

(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, 30. Reaction "c" involves the removal of missing electron from carbon-carbon double bond 31, migration of the C-13 methyl group yielding the radical ion 32. Fission of the 11-12 and 8-14 bonds give the stable diene-ion 33 m/e 204 and a fragment 34 at m/e 220.

Chart-IV



(a) Discussion on the mass spectrum of methyl sebiferenate 24

The molecular ion of methyl sebiferenate 24 had been found to be (M^+= 486) by the chemical ionisation method (methane ionisation) [Fig. 13]. The peak at m/e 527 and at m/e 515 were due to ($M^+ + C_3H_5$) and ($M^+ + C_2H_5$) ions respectively. The peak at m/e 469 were due to the loss of hydroxyl mass unit (17) from the molecular ion. Further loss of water (mass unit 18) from ion peak at m/e 469 resulted peak at m/e 451. The peaks at m/e 471 and at m/e 427 had been attributed to the loss of methyl and carbomethoxy (i.e. 15 and 59 mass units) groups from the molecular ion. The peak at m/e 409 resulted from further loss of one molecule of water (i.e. 18 mass units) from the ion peak at m/e 427. The loss of another water molecule (i.e. 18 mass units) from ion peak at m/e 409 yielded an ion peak at m/e 391.

The ion peak at m/e 318 had been attributed due to the fragment 35 which underwent the loss of allylically activated methyl group at C-8 and thus the ion peak at m/e 303 resulted due to fragment 36. The ion peak at m/e 248 had been indicated due to the fragment 37 which lost the carbomethoxy group and as a result fragment 38 formed and was responsible for the ion peak at m/e 189, Methyl

addition to the fragment 37 resulted the formation of the fragment 39 responsible for the ion peak at m/e 263. The loss of H-COOCH$_3$ i.e. 60 mass units from the latter ion gave rise to fragment 40 responsible for ion peak at m/e 203. Hydrogen addition to fragment 37 resulted ion peak at m/e 249 responsible for fragment 41. Another ion peak at m/e 233 was due to the fragment 42 and the ion peak at m/e 238 was due to fragment 43. All the fragments from the molecular ion are shown in chart V. Loss of $HCOOCH_3$ from fragment 41 resulted also 38 responsible for ion peak at m/e 189

Chart-V

Mass fragmentation of methyl Sebiferenate 24

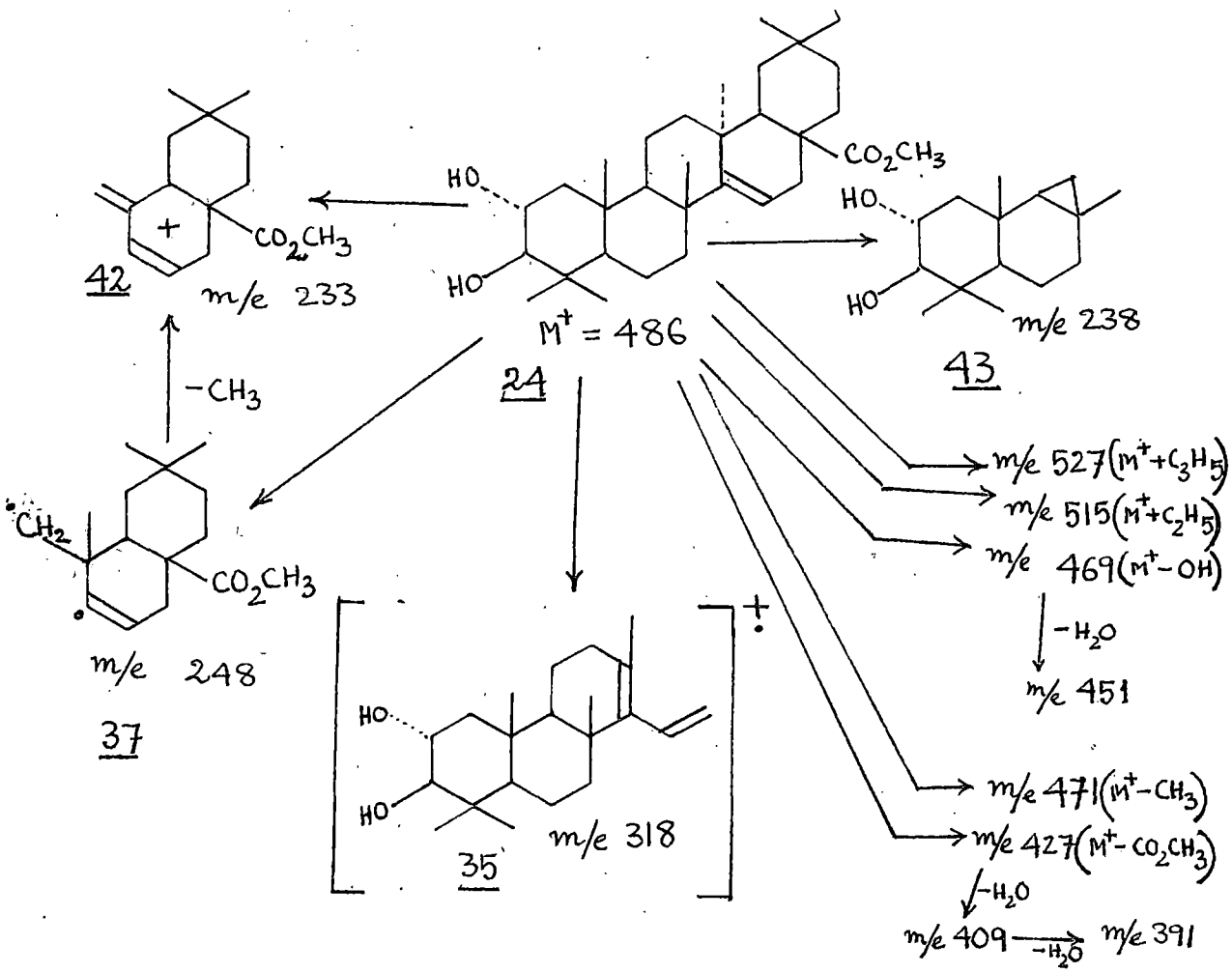
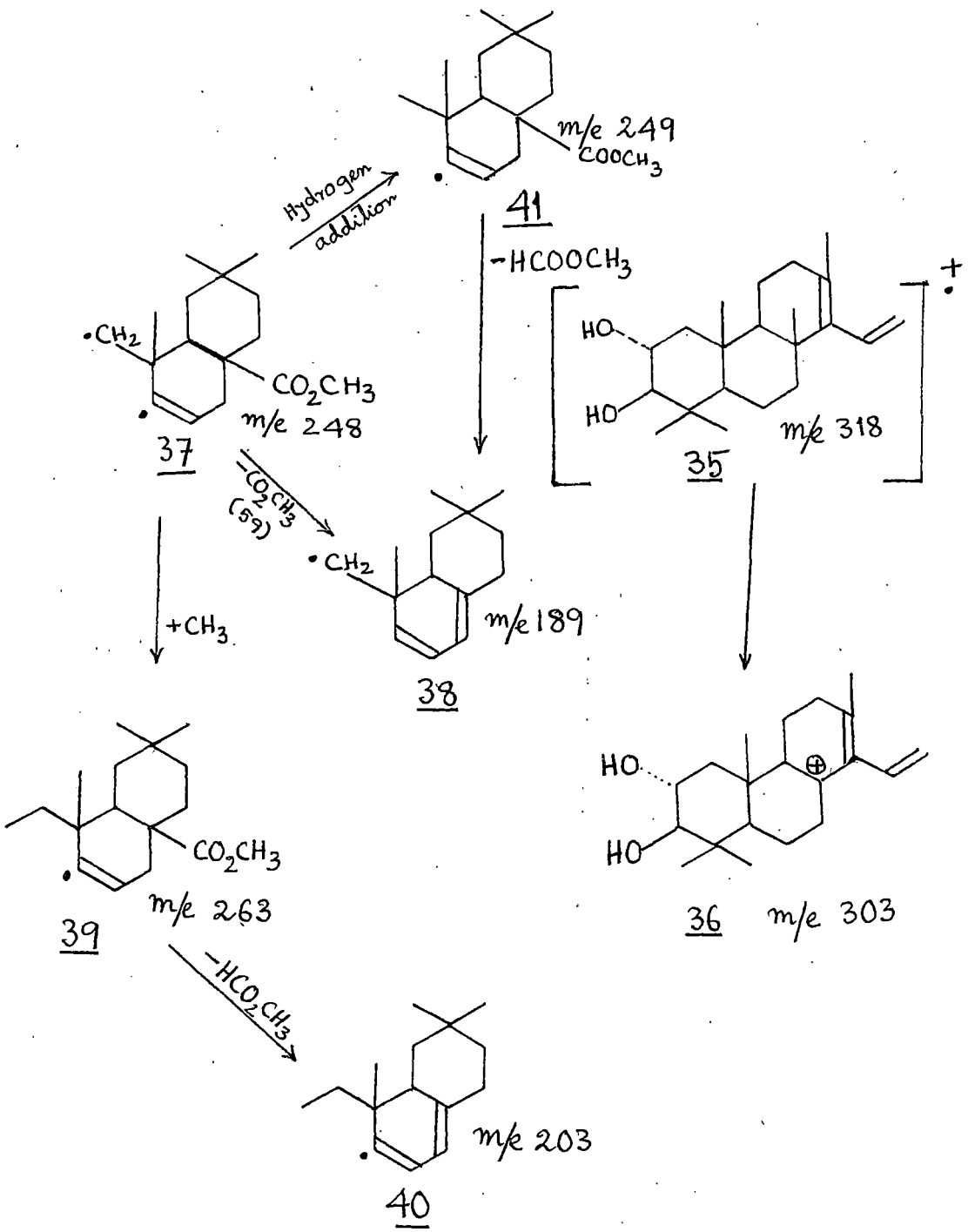


Chart - V (Contd..)

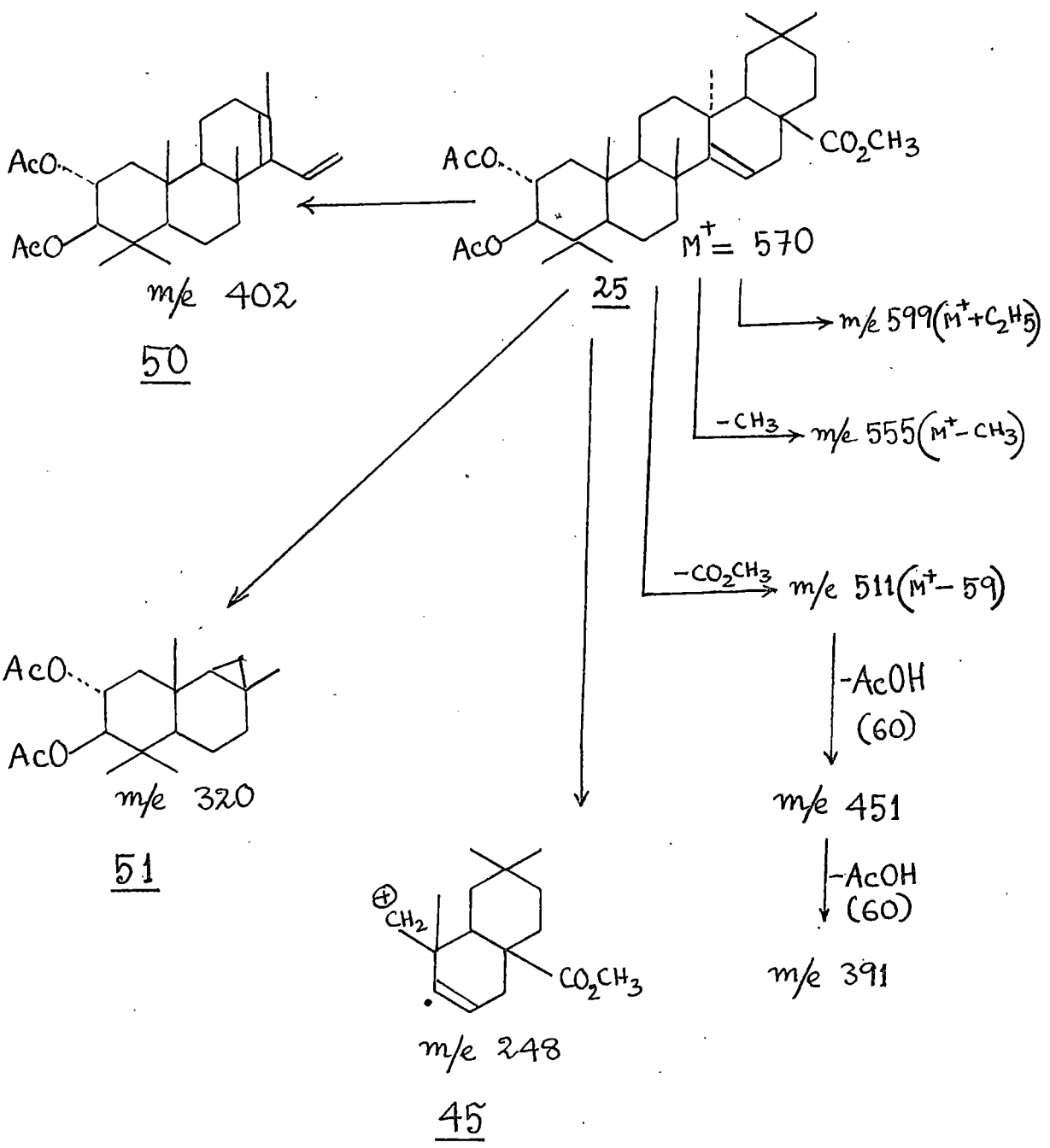


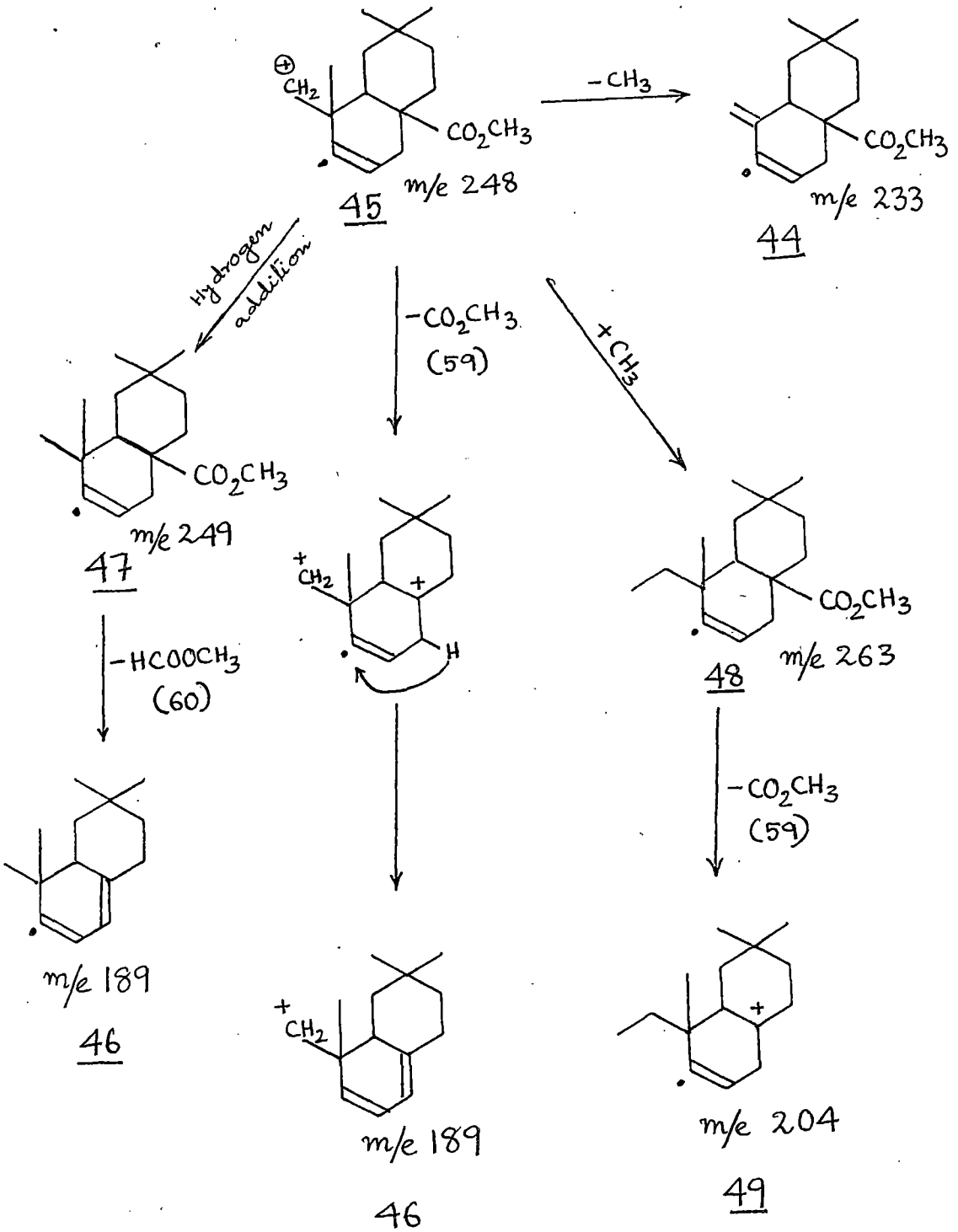
b) Discussion on the mass spectrum of acetyl methyl sebiferenate 25.

The molecular ion of acetyl methyl sebiferenate 25 had been found to be ($M^+ = 570$) by the chemical ionisation method (methane ionisation) [Fig. 14]. The ion peak at m/e 599 was due to ($M^+ + C_2H_5$). Loss of methyl (i.e. 15 mass unit) from the molecular ion resulted ion peak at m/e 555. Loss of carbomethoxy (i.e. 59 mass unit) from molecular ion provided an ion peak at m/e 511 which lost one molecule of acetic acid (i.e. 60 mass unit) and as a result the ion peak at m/e 451 was observed. This was further fragmented by the loss of another acetic acid molecule (60 mass unit) to yield ion peak at m/e 391. The ion peaks at m/e 233 and m/e 248 had been attributed due to the fragment 44 and 45 respectively. 45 by the loss of carbomethoxy i.e. 59 mass unit resulted the fragment 46 for which ion peak at m/e 189 was observed. Hydrogen and methyl addition to the fragment 45 resulted the fragments 47 and 48 for which ion peaks at m/e 249 and m/e 263 were observed respectively. Further loss of carbomethoxy from later fragment 48 yielded fragment 49 for which ion peak at m/e 204 was indicated. Ion peaks at m/e 402 and m/e 320 were due to the fragments 50 and 51 respectively. All the fragments are depicted in Chart VI. Loss of $HCOOCH_3$ from fragment 47 resulted 46 also responsible for ion peak at m/e 189.

Chart-VI

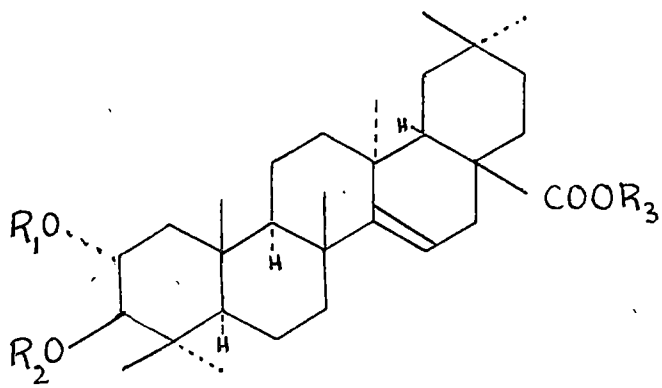
Mass fragmentation of acetyl methyl sebiferenate 25




$\oplus CH_2$
CO_2CH_3
m/e 248
45
$-CH_3$
CO_2CH_3
m/e 233
44
Hydrogen addition
$-CO_2CH_3$ (59)
$+CH_3$
CO_2CH_3
m/e 249
47
$-HCOOCH_3$ (60)
$^+CH_2$
H
CO_2CH_3
m/e 263
48
$-CO_2CH_3$ (59)
m/e 189
46
$^+CH_2$
m/e 189
46
m/e 204
49

Thus all the characteristic ion peaks of methyl sebiferenate [Fig. 13] and acetyl methyl sebiferenate [Fig. 14] were consistent with the mass fragments of Δ^{14} -taraxenene moiety as reported by Djerassi et al[37], Confirming beyond any doubt that such Δ^{14} -taraxerene moiety is present in this new sebiferenic acid.

On the basis of these results of IR, UV, ^{13}C NMR, ^{1}H NMR, mass spectral data and the chemical evidences shown above, it may be concluded beyond any doubt that sebiferenic acid 23 must have modified oleanane skeleton with a trisubstituted double bond at C_{14}-C_{15}, the secondary hydroxyl groups at C-2 and at C-3 having diequatorial conformation as 2α, 3β and a tertiary carboxyl group (-COOH) at C-17. Hence sebiferenic acid is 2α, 3β dihydroxy olean-14(15)-en-28-oic acid and can be represented by the structure 23. Thus methyl sebiferenate and acetyl methyl sebiferenate can be represented as 24 and 25 respectively.



23. $R_1 = R_2 = R_3 = H$

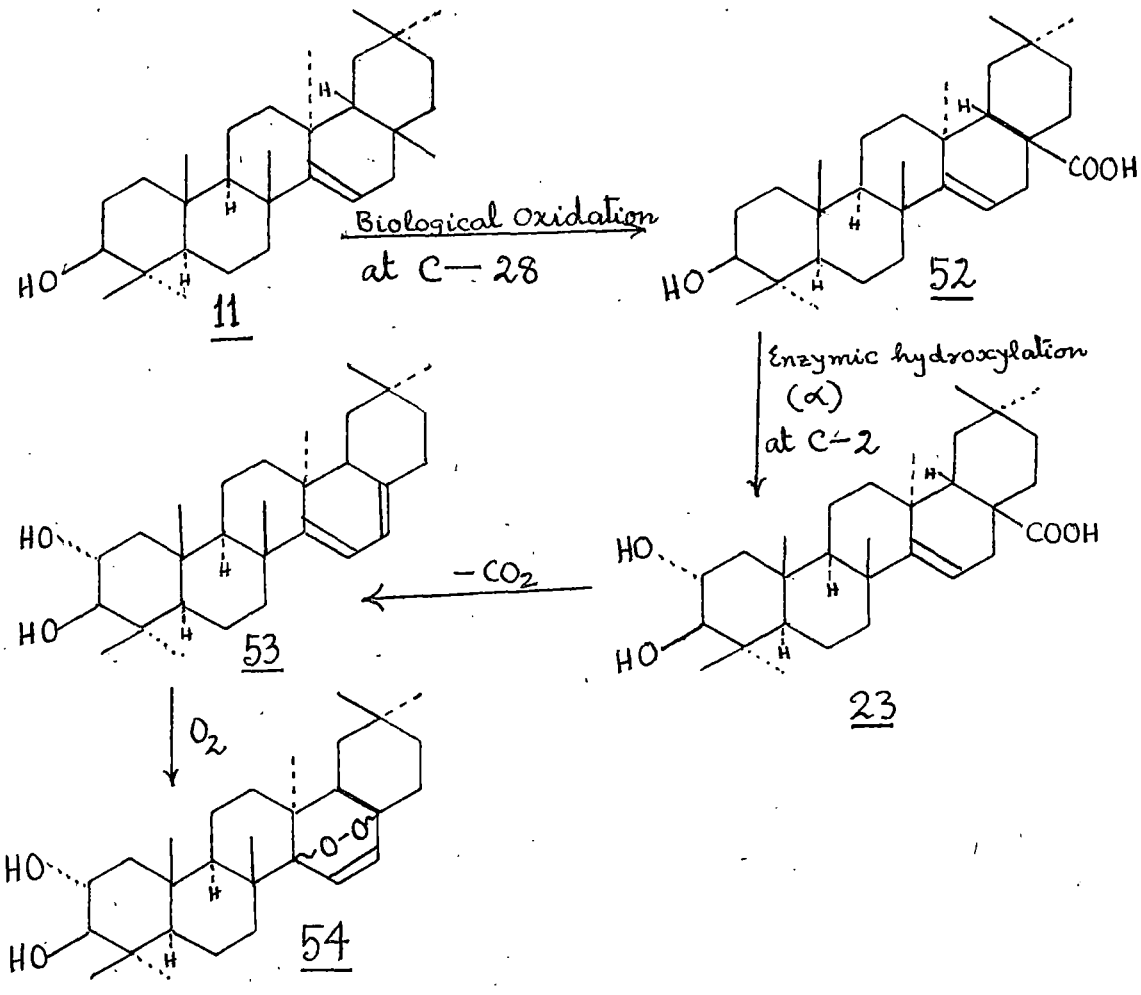
24. $R_1 = R_2 = H$, $R_3 = CH_3$

25. $R_1 = R_2 = Ac$, $R_3 = CH_3$

Section E : Biogenesis of Sebiferenic acid.

Biogenesis of taraxerol 11 had been discussed already in Chart III. Biological oxidation at C-28 of taraxerol 11 may furnish aleuritolic acid 52 which has been isolated and identified also by the author from the same plant i.e. Sapium sebiferum Roxb. Enzymic hydroxylation (α) at C-2 may furnish this new sebiferenic acid 23. This is depicted in the following Chart VII.

Chart-VII



The biogenetic formation of baccatin 54 present in Sapium baccatum Roxb. may now be explained by the enzymic oxidation of aleuritolic acid to sebiferenic acid which then undergoes decarboxylation to give the intermediate 14-15, 16-17 homoannular diene 53. The last one finally furnishes baccatin 54 by oxidation.

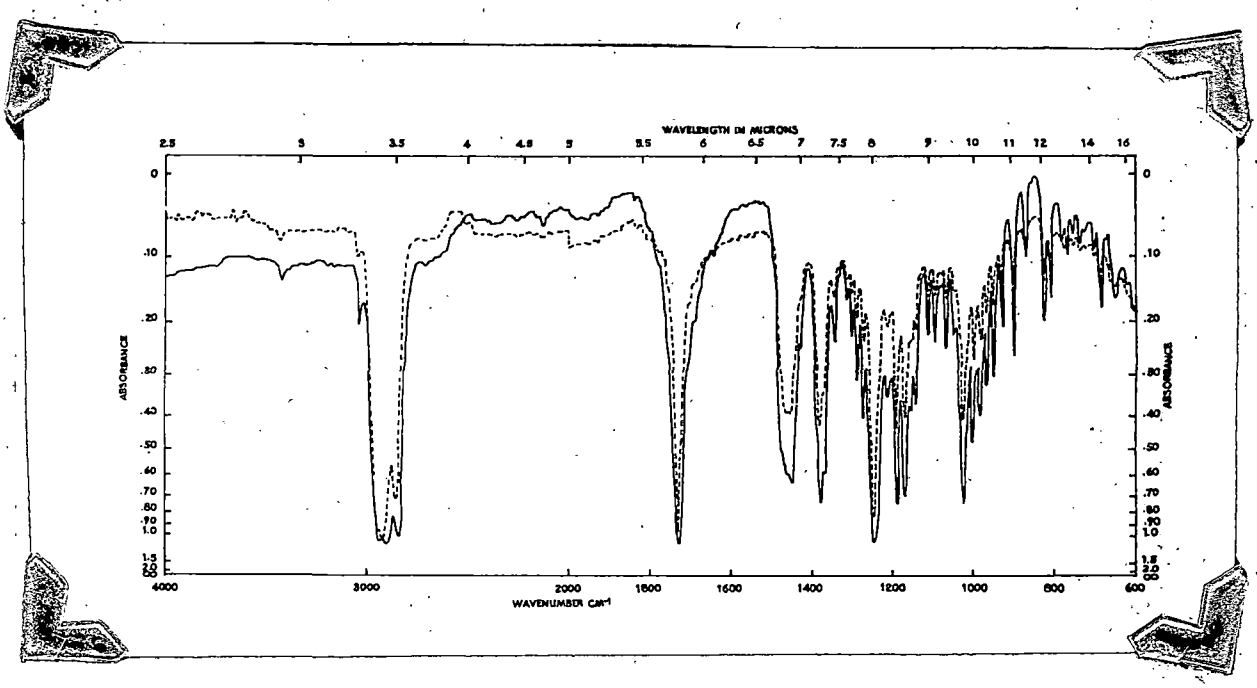


Fig. 1 IR comparison
Solid line - authentic acetyl methyl aleuritolate
Dotted line - acetyl methyl aleuritolate

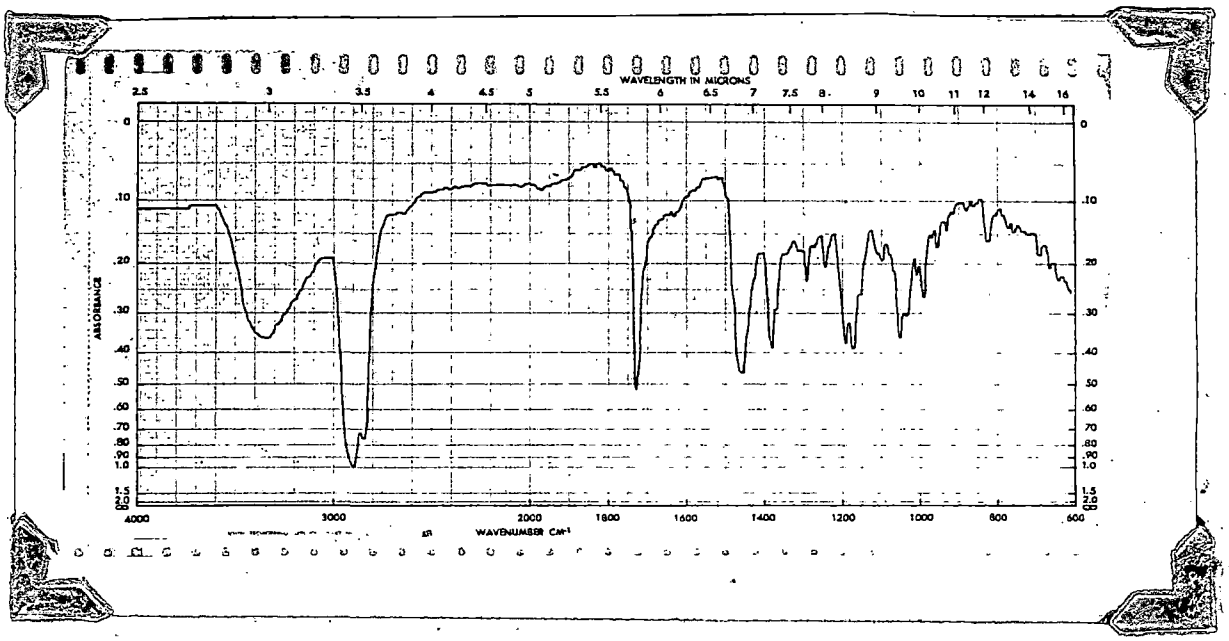


Fig. 2 IR spectrum of methyl sebiferenate.

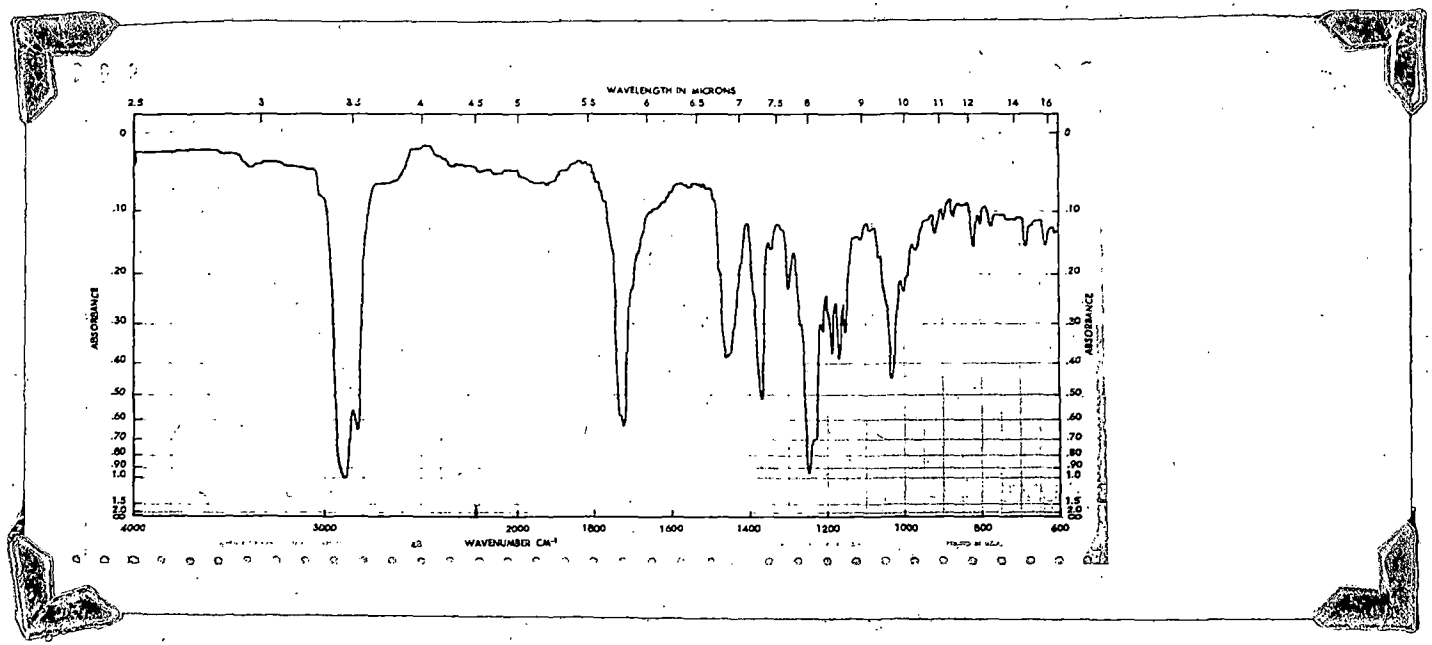


Fig. 3 IR spectrum of acetyl methyl sebiferenate.

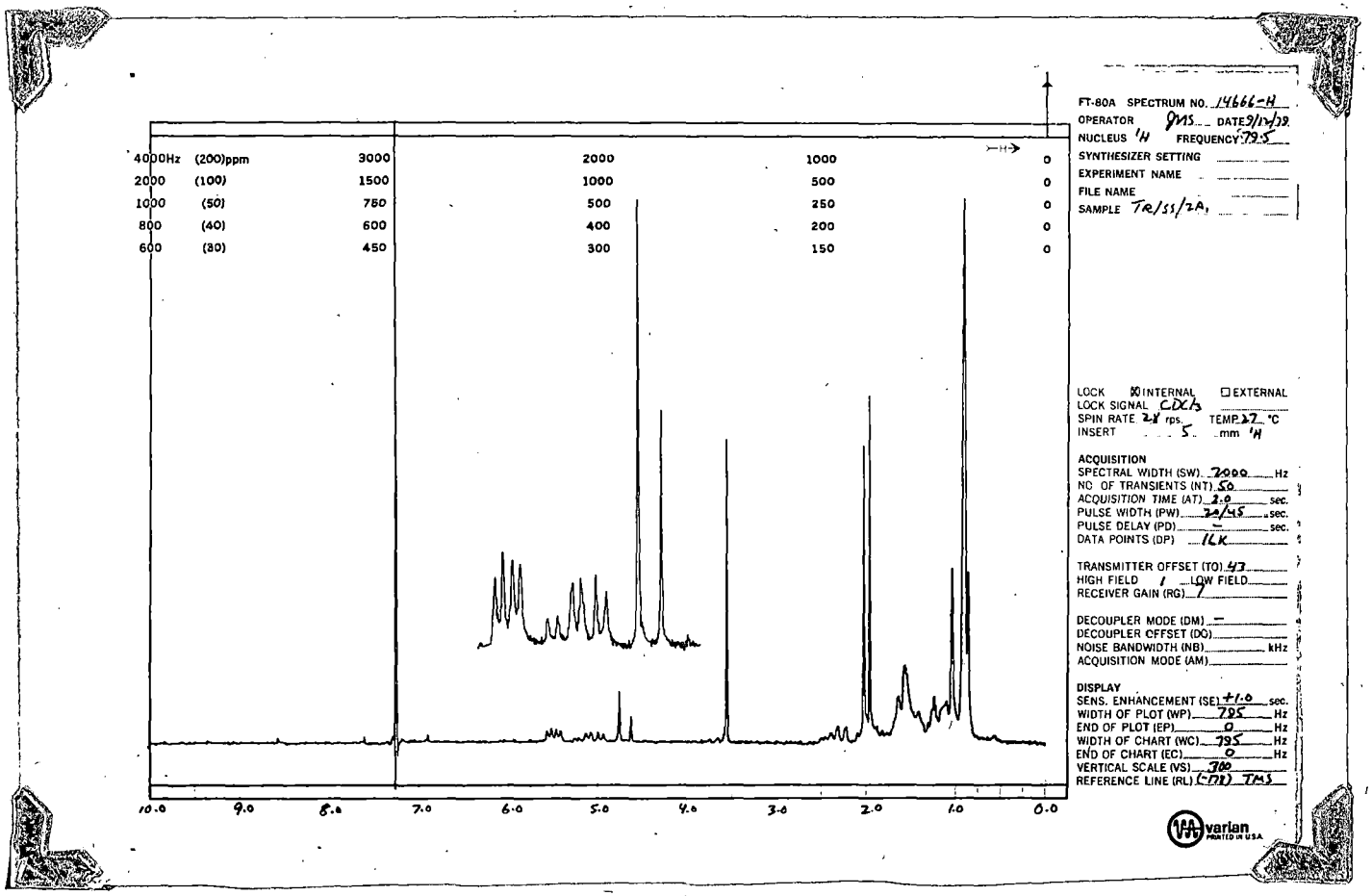
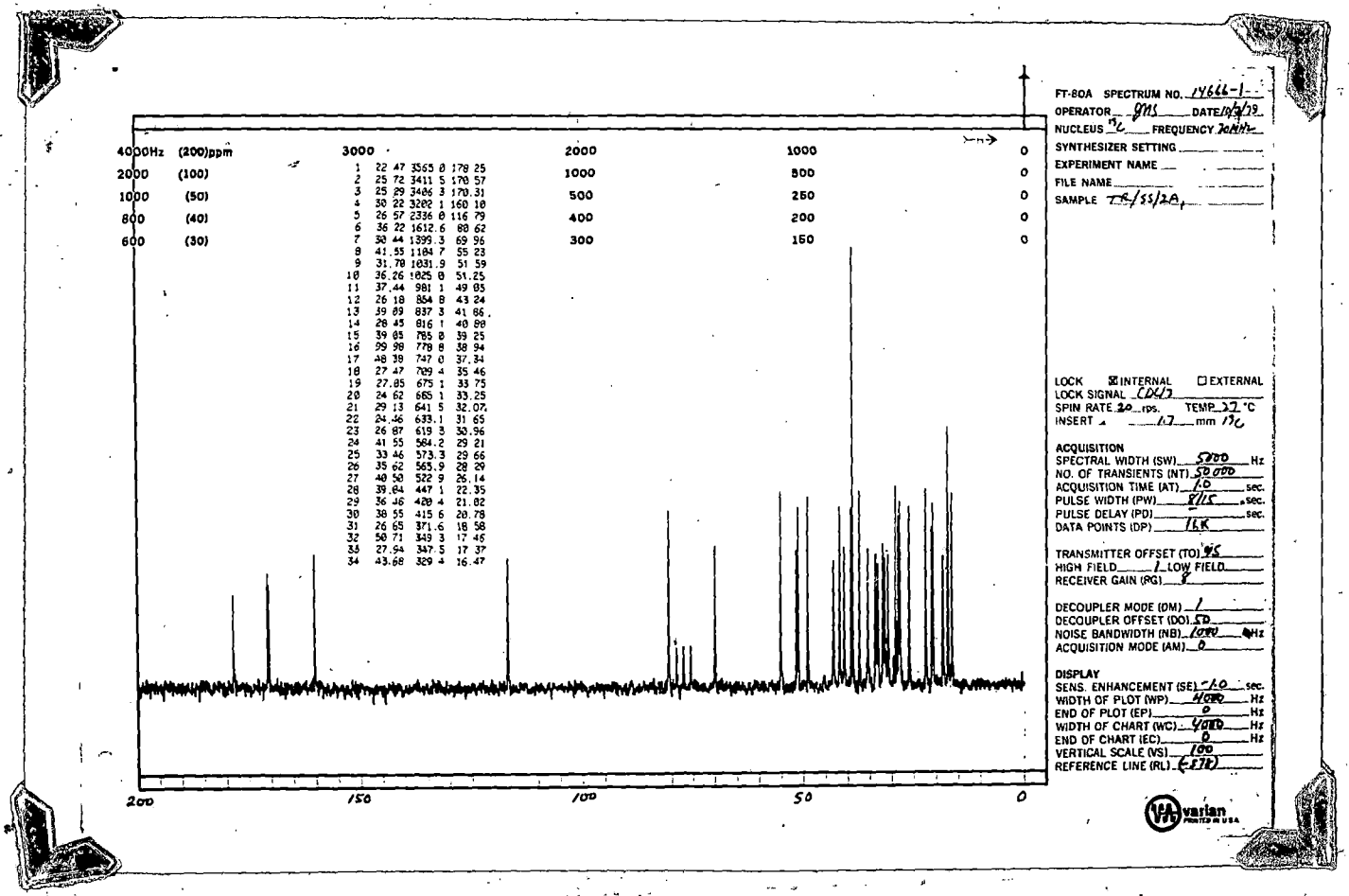


Fig. 4 ^{1}H NMR spectrum of acetyl methyl sebiferenate



1	22.47	3565.0	178.25				
2	25.72	3411.5	170.57				
3	25.29	3406.3	170.31				
4	30.22	3202.1	160.10				
5	26.57	2336.0	116.79				
6	36.22	1612.6	80.62				
7	30.44	1399.3	69.96				
8	41.55	1104.7	55.23				
9	31.78	1031.9	51.59				
10	36.26	1025.0	51.25				
11	37.44	981.1	49.05				
12	26.18	864.8	43.24				
13	39.89	837.3	41.86				
14	28.45	816.1	40.80				
15	39.05	785.0	39.25				
16	99.98	778.0	38.94				
17	48.38	747.0	37.34				
18	27.47	709.4	35.46				
19	27.85	675.1	33.75				
20	24.62	665.1	33.25				
21	29.13	641.5	32.07				
22	24.46	633.1	31.65				
23	26.87	619.3	30.96				
24	41.55	584.2	29.21				
25	33.46	573.3	28.66				
26	35.62	565.9	28.29				
27	40.50	522.9	26.14				
28	39.64	447.1	22.35				
29	36.46	420.4	21.02				
30	38.55	415.6	20.78				
31	26.65	371.6	18.58				
32	50.71	349.3	17.46				
33	27.94	347.5	17.37				
34	43.68	329.4	16.47				

Fig. 5 ^{13}C NMR spectrum of acetyl methyl sebiferenate

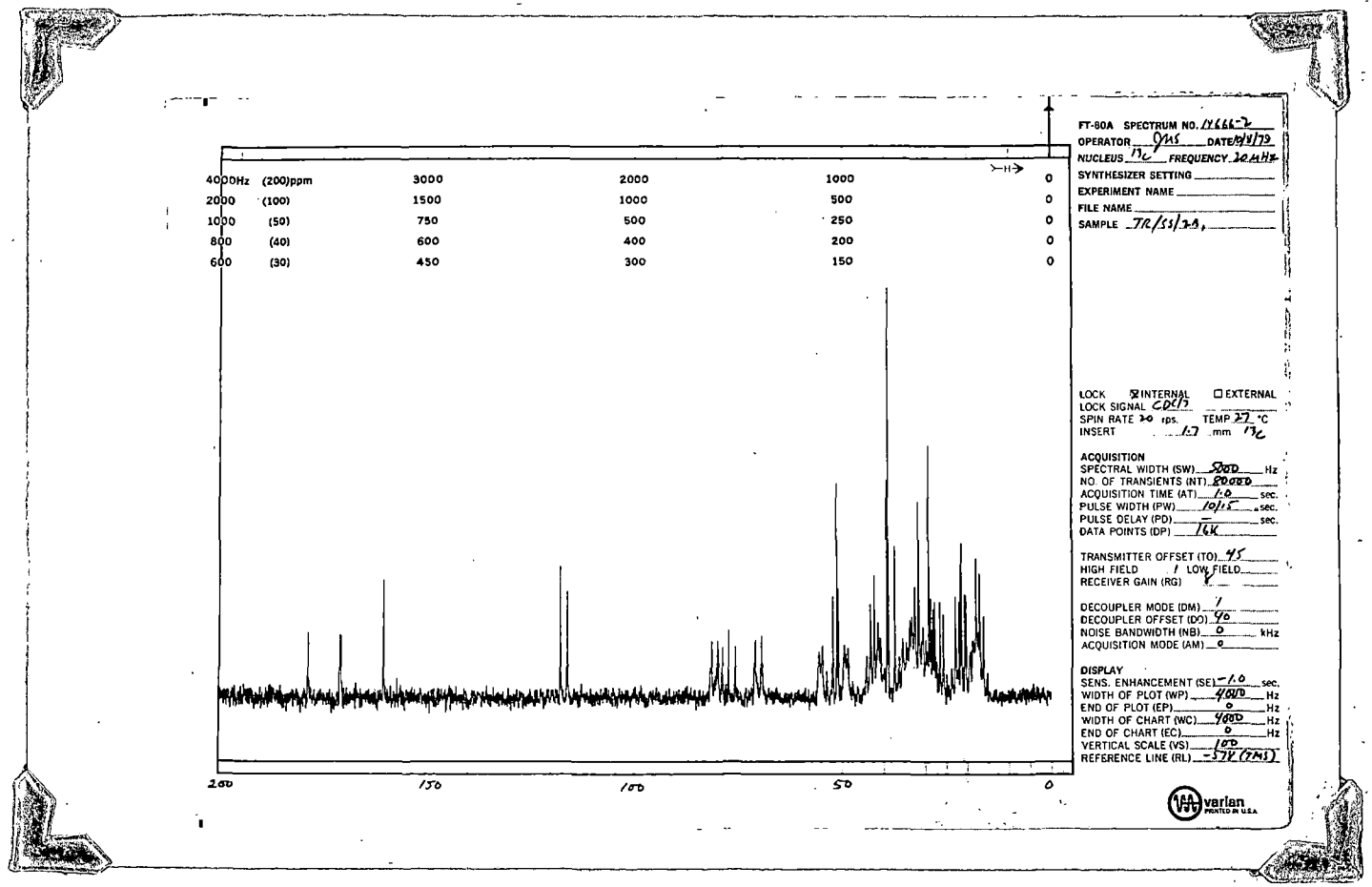


Fig. 6 (^{13}C NMR) Off resonance CW decoupled spectrum of acetyl methyl sebiferenate

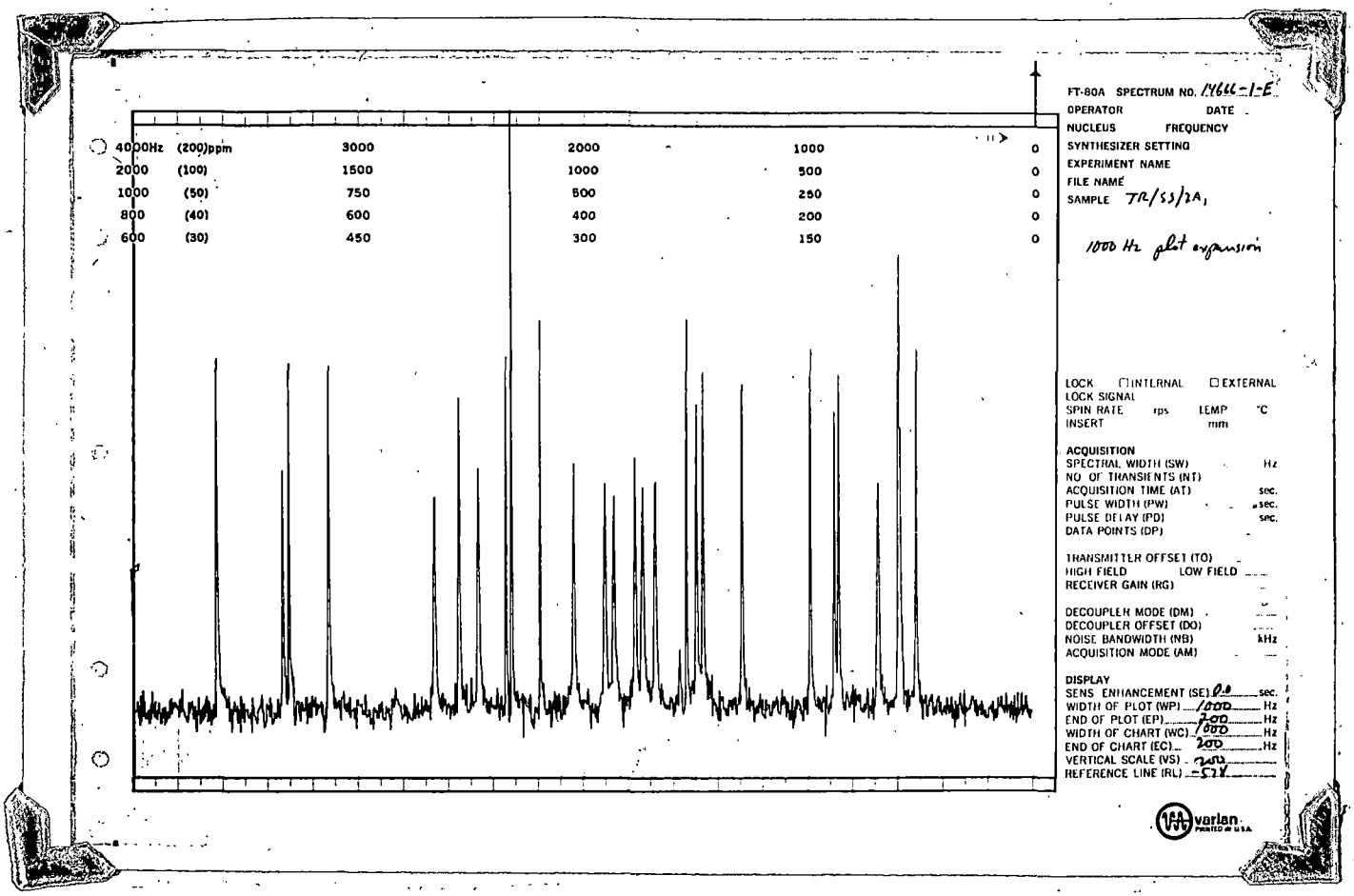


Fig. 7 Plot expansion (0-50 ppm) of (^{13}C NMR) spectra of acetyl methyl sebiferenate

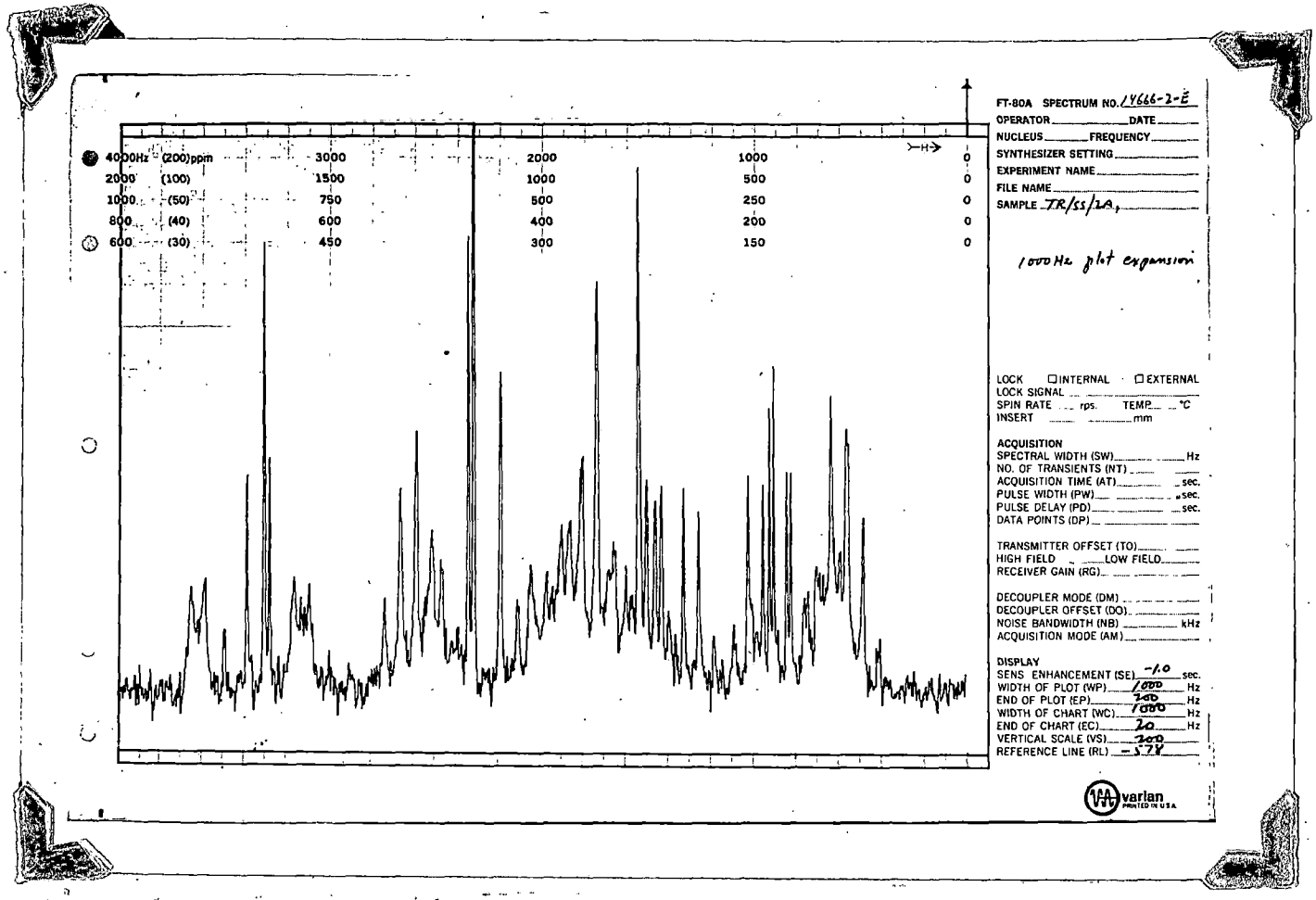


Fig. 8 Plot expansion (0-50ppm) of (^{13}C NMR) spectra of acetyl methyl sebiferenate

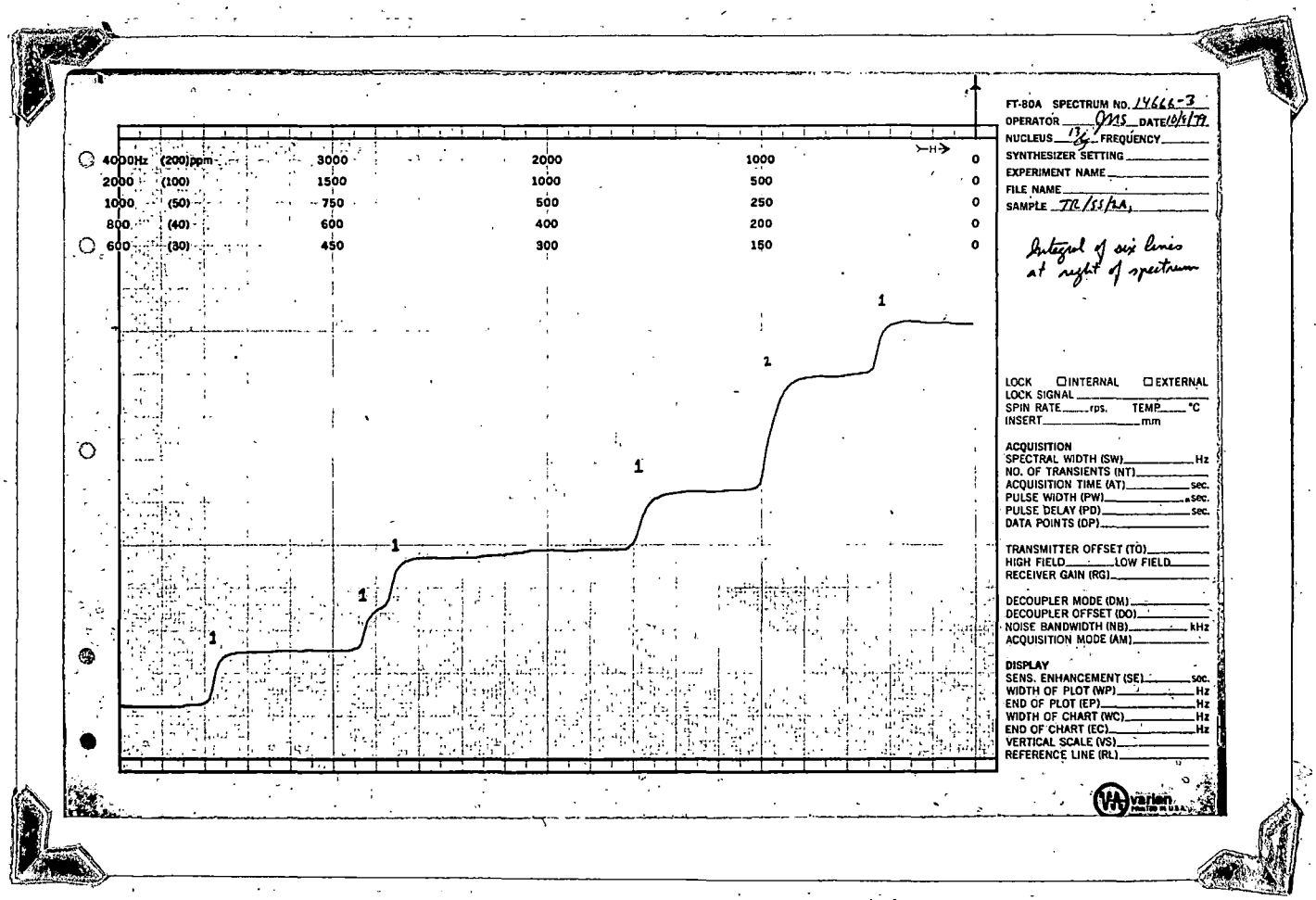


Fig. 9 Integration of the ^{13}C NMR in the tertiary C-Methyl region.

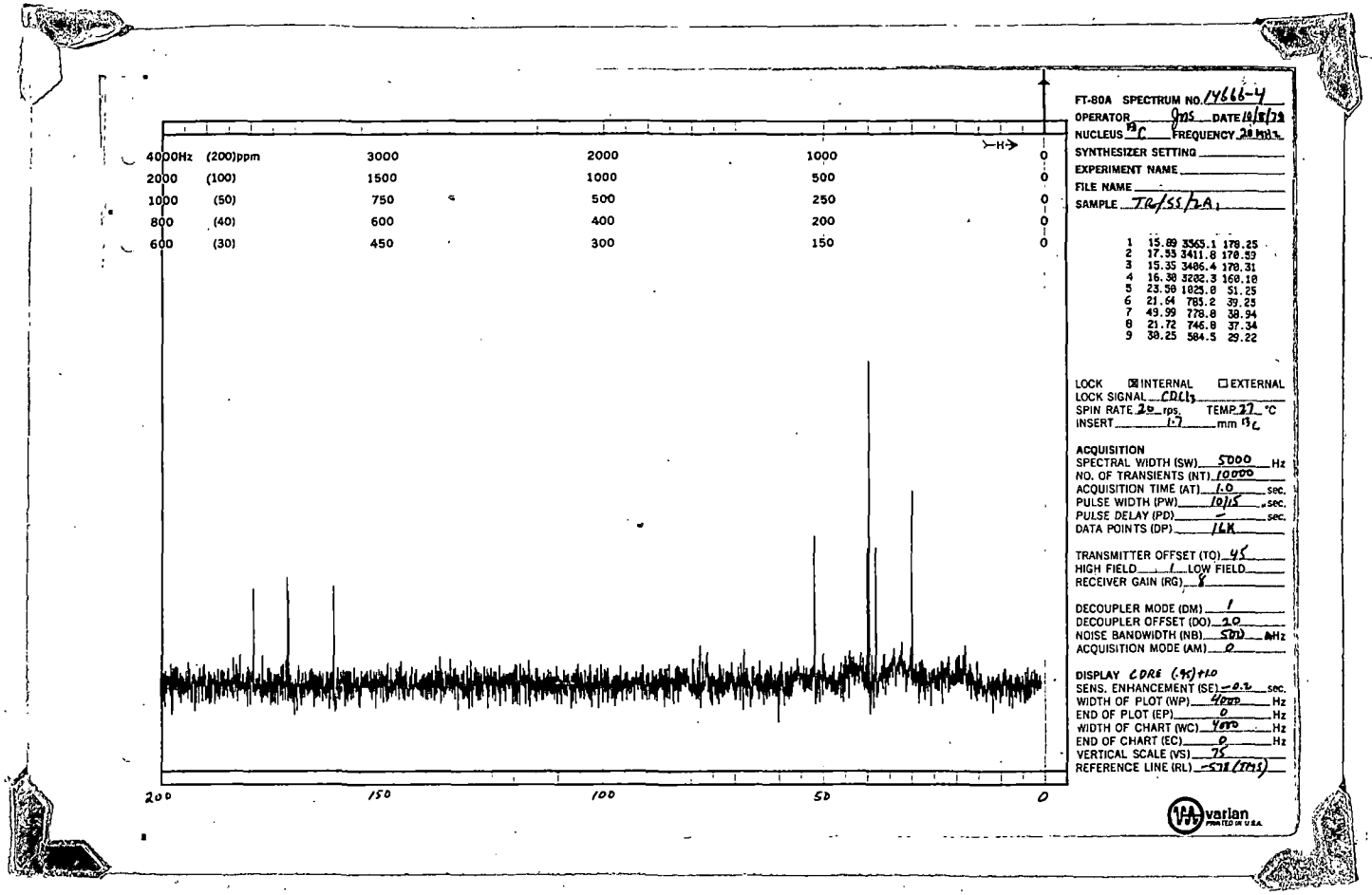


Fig. 10 (^{13}C NMR) Off resonance noise decoupled spectrum of acetyl methyl sebiferenate.

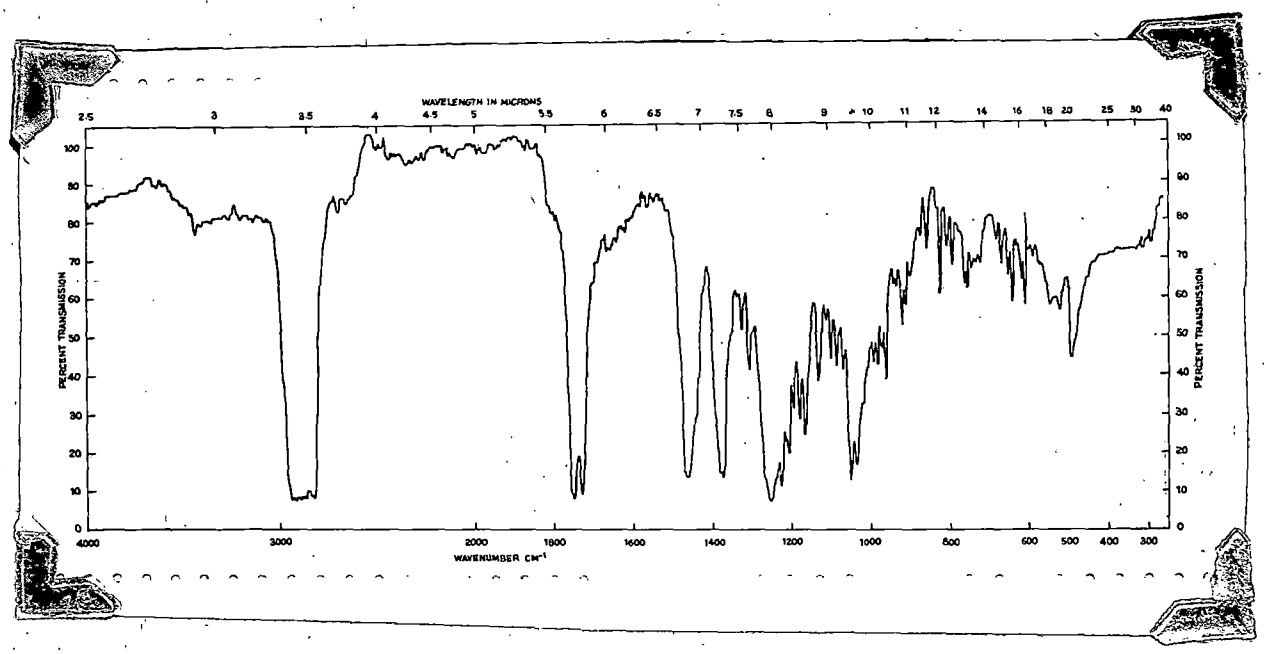


Fig. 11 IR spectrum of acetyl methyl crategolate (maslinate).

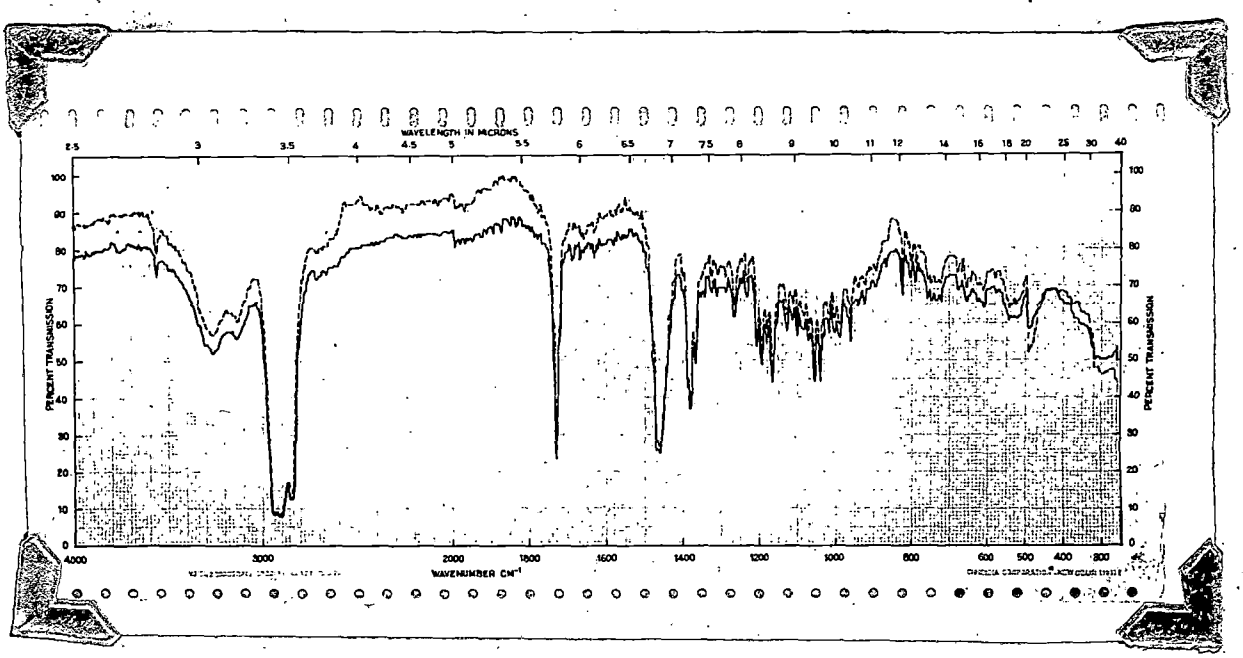


Fig. 12 IR comparison
Dotted line -methyl crategolate (maslinate) preparedfrom sebiferenic acid
Solid line - authentic sample of methyl crategolate (maslinate).

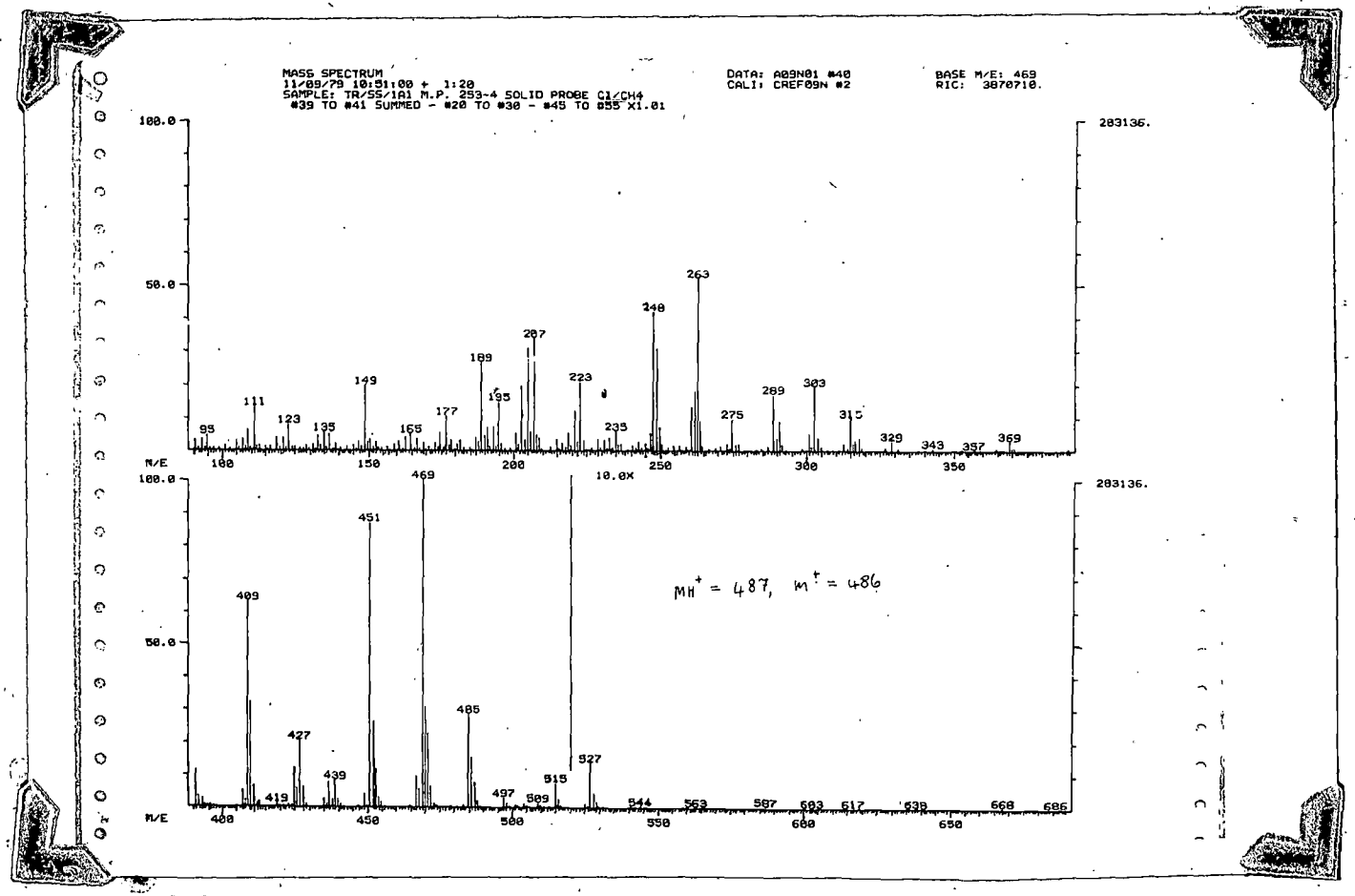


Fig. 13 Mass spectrum of methyl sebiferenate

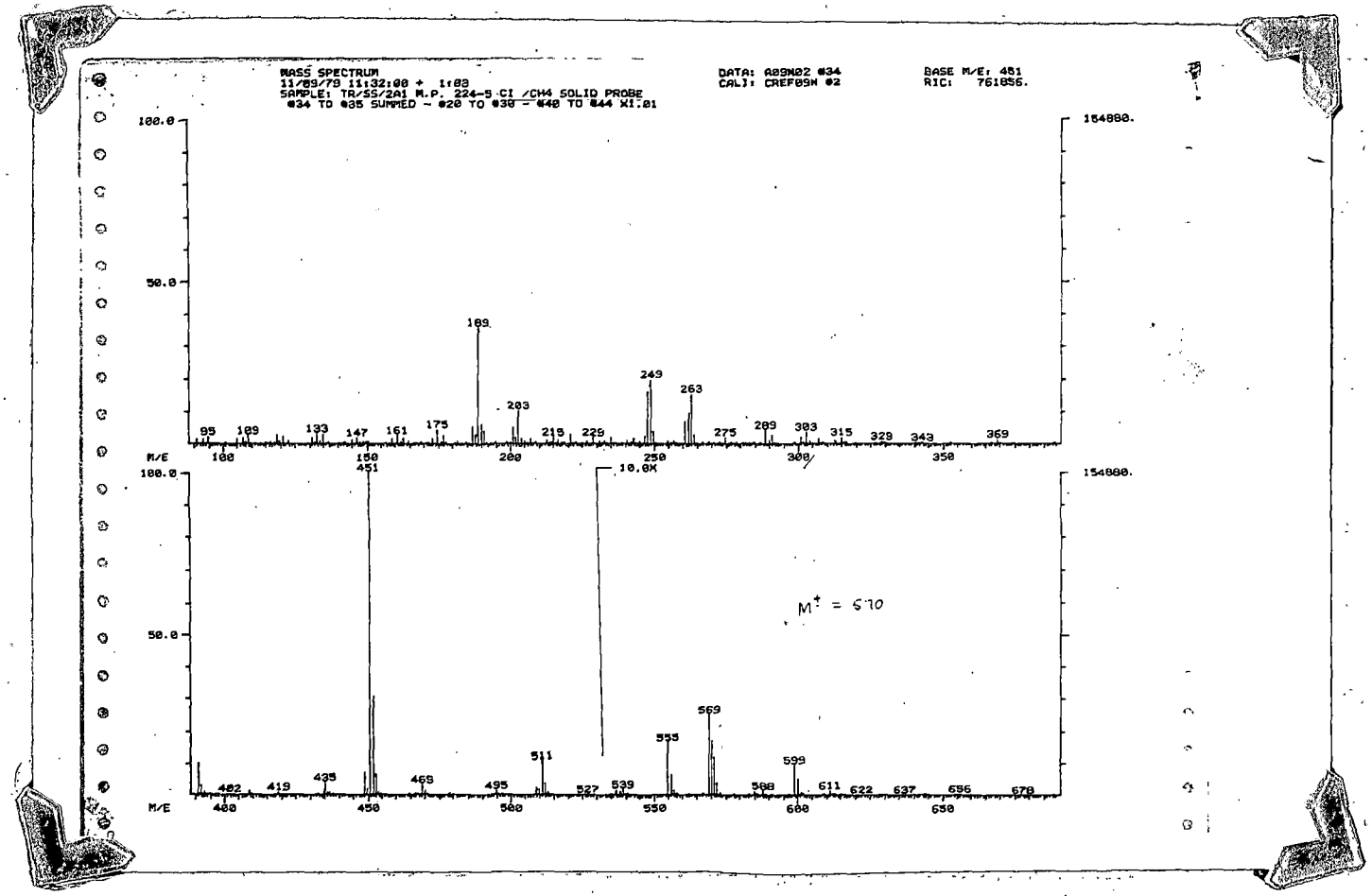


Fig. 14 Mass spectrum of acetyl methyl sebiferenate.