CHAPTER-II

INVESTIGATION ON THE NEUTRAL PART OF SAPIUM SEBIFERUM, Roxb.

Isolation of moretenone, moretenol, β-sito-sterol, an aliphatic alcohol, a new triterpenoid-3-epimoretenol and a new nor-triterpene, $C_{29}H_{46}O_4$ from the neutral part of trunk bark and stem of Sapium Sebiferum, Roxb.

Section A : Extraction

Dried and powdered trunk bark and stem of Sapium Sebiferum, Roxb. was extracted with benzene. On cooling the benzene extract to room temperature, a yellow benzene insoluble solid separated out and was collected by filtration. The chemistry of this insoluble solid C has been dealt in Part III. The clear filtrate was concentrated by distilling off benzene when a gummy residue was obtained. The residue was extracted with ether and the ether extract was washed with aqueous sodium hydroxide solution and then with water till it was neutral. The ether solution was dried over sodium sulphate and the ether evaporated when a gummy residue was obtained. The gummy residue was chromatographed and the following fractions were isolated.

Section B : Chromatography of the neutral part

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

Fraction No.	Eluent		Eluate	M.P. of the residue
1.	Petroleum ether		Solid with oil	180-90°
2.	Petroleum:benzene	(9:1)	Solid	200-215°
3.	Petroleum:benzene	(4:1)	Solid	85-6°
4.	Petroleum:benzene	(3:2)	Solid	200-20°
5.	Petroleum:benzene	(1:1)	Solid	128-32°
6.	Benzene:ether	(3:2)	Solid	210-215°

Section C : Examination of Fraction No.1 and isolation of moretenone 108

Fraction No.1. On rechromatography over alumina and several crystallisations from chloroform and methanol mixture furnished fine needle crystals, which had a constant melting point 198-99°, $[\alpha]_D$ +50°. Elemental analysis and mass spectrum showed the molecular formula of the compound to be $C_{30}H_{48}O$ (M^+ 424). It developed a yellow colour with tetranitromethane indicating the presence of a double bond in the compound. It gave a violet colouration in Liebermann-Burchard reaction[71] and gave a positive test in Zimmermann colour test[70] showing that the compound is a triterpene ketone, the keto group being at C-3 position.

I. Nature of the Oxygen function

The compound gave a reddish yellow 2,4-dinitrophenyl hydrazone derivative $C_{36}H_{52}O_4N_2$, m.p. 271-3° and an oxime $C_{30}H_{49}NO$, m.p. 272-4°, showing that the oxygen atom was present as a carbonyl group. The Infrared spectra of the compound showed peaks at 1705, 1640 and 875 cm^{-1} indicating that the carbonyl group is present as a six numbered ring ketone. The peaks at 1640, 875 cm^{-1} showed the presence of a terminal methylene group. The compound showed UV absorption λ_{max}^{MeOH} 287 mμ (ϵ, 71.5) showing that the keto group and the double bond were unconjugated.

II. Structure of the Ketone-108

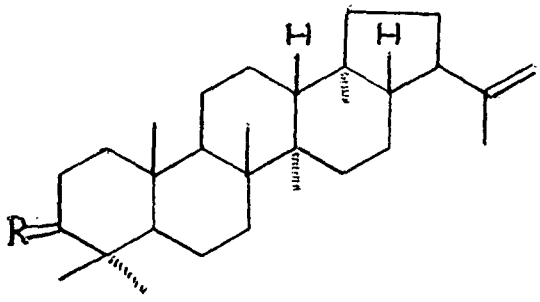
The structure of the ketone 108 followed from the chemical and physical studies of the ketone and its derivatives. NMR studies of the compound revealed the presence of six tertiary methyl groups on saturated carbons (0.70, 0.94, 0.95, 1.02 (6-H) and 1.08 ppm) with one additional methyl group on a double bond (1.68 ppm). The presence of terminal methylene protons was indicated by the signal at 4.68 ppm. Modified Wolff-Kishner reduction[72] of the ketone afforded a hydrocarbon 108a m.p. 203-5°, $[\alpha]$ +22°. Elemental analysis and

mass spectra showed the molecular formula of the hydrocarbon to be as $C_{30}H_{50}$ (M^+ 410). The hydrocarbon on hydrogenation afforded a dihydro compound 109a, $C_{30}H_{52}$, m.p. 176° and 192°, $[\alpha]_D$ +20° which was completely saturated. Since at the time of this investigation this hydrocarbon was not known in the literature it was concluded that the ketone 108 might contain a new type of carbon skeleton. The ketone being a pentacyclic triterpene with a terminal methylene group. Hence we made a thorough investigation of the ketone in order to establish its structure.

Lithium aluminium hydride reduction of the ketone yielded an alcohol 108b, m.p. 231-33°, $[\alpha]_D$ +17°. Elemental analysis and mass spectrometry indicated the molecular formula as $C_{30}H_{50}O$ (M^+ 426). The alcohol 108b had NMR signals at 0.69, 0.77, 0.83, 0.94, 0.99 (6-H) (for six methyl groups on saturated carbons), 1.67 (one methyl on a doubly bonded carbon) and 4.68 (terminal methylene protons) ppm. On hydrogenation it afforded a dihydro-alcohol 109b, $C_{30}H_{52}O$, m.p. 224-6°, $[\alpha]_D$ +11°. The alcohol 109b on PCl_5-petroleum ether treatment formed a hydrocarbon 110. This hydrocarbon on ozonolysis afforded acetone and a trisnor ketone 111, $C_{27}H_{44}O$, m.p. 193-4°, $[\alpha]_D$ +113°. IR peak at 1745 cm^{-1} showed the compound to be a five numbered ring ketone. This showed that the hydroxyl group was present in the customary C-3 position with gem dimethyl group in 4 position. Retropinacoline rearrangement on the alcohol 108b gave a diene hydrocarbon $C_{30}H_{48}$, m.p. 189-90°, $[\alpha]_D$ +14.54°. It had no UV absorption in the region 220-300 mμ showing that the hydroxyl and the double bonds are quite far apart from each other. The alcohol 108b gave a benzoate $C_{37}H_{54}O_2$, m.p. 248-9°, $[\alpha]_D$ +37° and an acetate 108c, m.p. 278-9°, $[\alpha]_D$ +23°. Analysis and mass spectrometry of the acetate proved that molecular formula to be $C_{32}H_{52}O_2$ (M^+ 468). IR spectra had bands at 3070, 1725, 1250 ($-OCOCH_3$), 1640, 885 ($>C=CH_2$) cm^{-1}. The acetate

had NMR signals for six methyl groups on saturated carbons at 0.68, 0.86 (9-H), 0.95, 0.98 ppm and methyl group on a double bond at 1.68 ppm, the methyl protons of the acetate at 2.05 ppm and the terminal methylene group at 4.73 ppm. The acetate 108c consumed one mole equivalent of perbenzoic acid proving that only one double bond was present in the acetate and in the parent compound 108. The oxide acetate $C_{32}H_{52}O_3$ isolated after perbenzoic acid treatment had m.p. 271-2°, $[\alpha]_D$ +20.45°.

The hydrocarbon 108a on ozonolysis, produced formaldehyde and a norketone 112, $C_{29}H_{46}O$, m.p. 230-31°, $[\alpha]_D$ +5°. Base isomerisation of the norketone 112 yielded the original compound (m.m.p.), indicating that the environment of the keto group had the stable orientation. A comparison of the physical constants of the norketone 112 with that of isoadiantone[32] showed close similarity. The m.m.p. and IR comparison proved the identity of the norketone 112 with a sample of isoadiantone (69) kindly supplied by Prof.Berti. This identity leads to the structure of the ketone as isohopenone represented by 108.

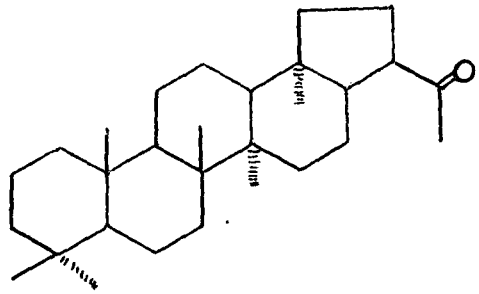


108 (R=O)

108a (R=H_2)

108b (R= )

108c (R= )



112 (=69)

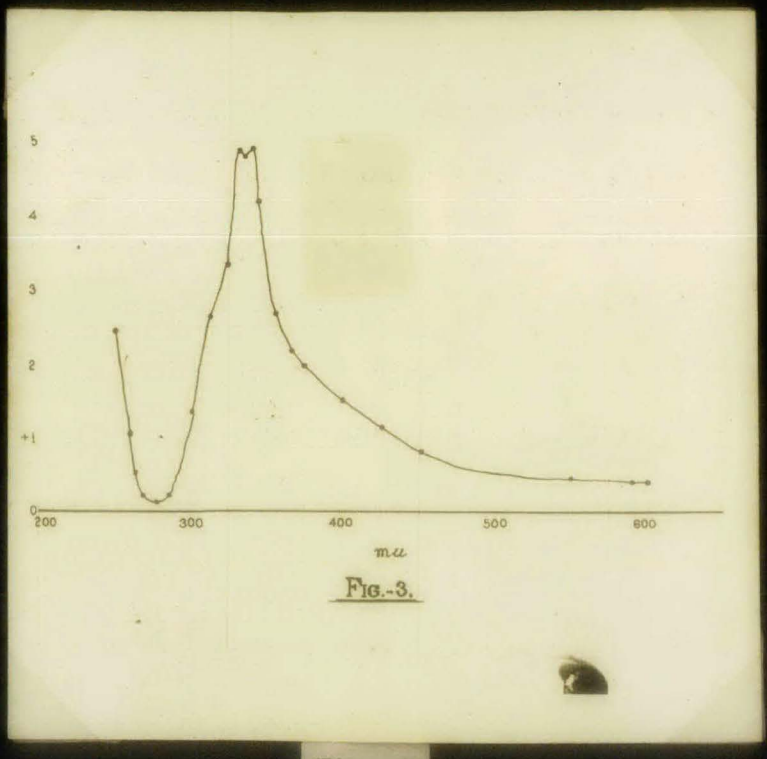


FIG.-3.

Fig. 3

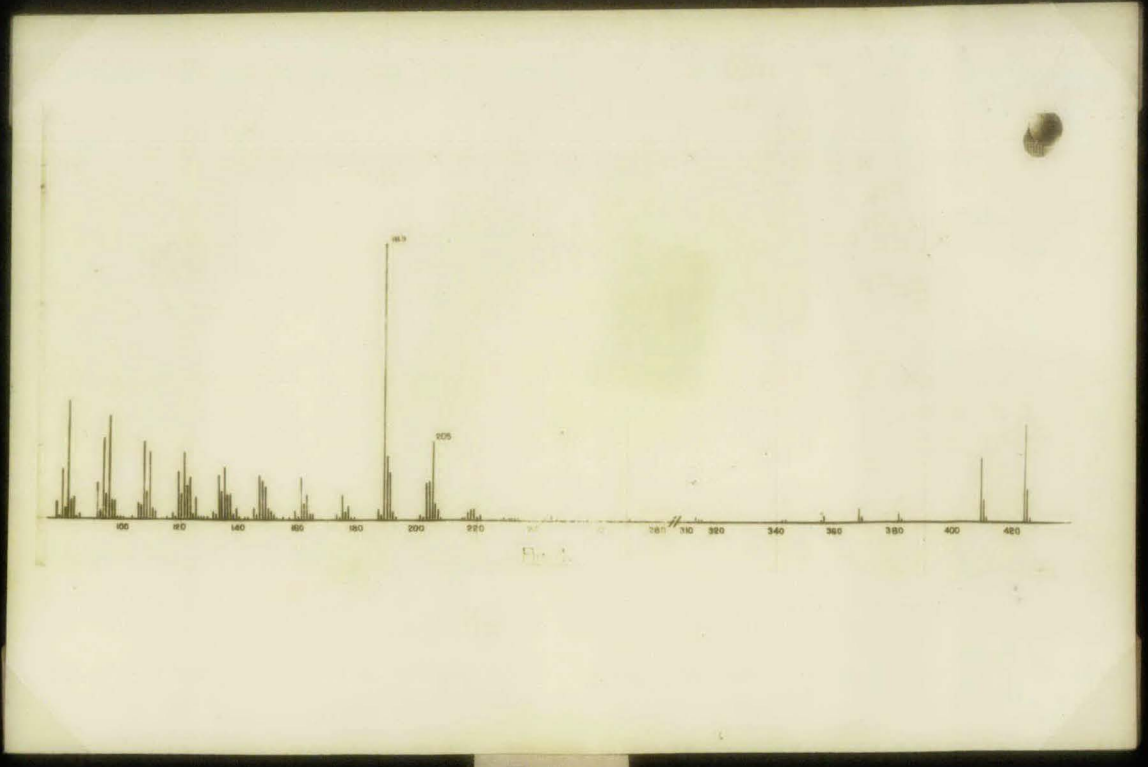
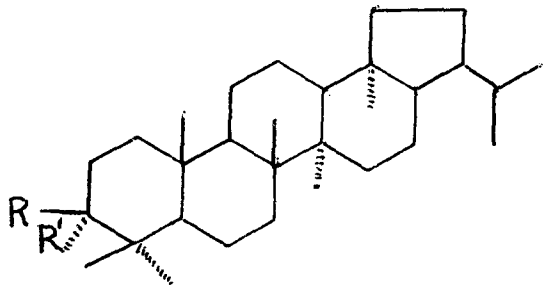
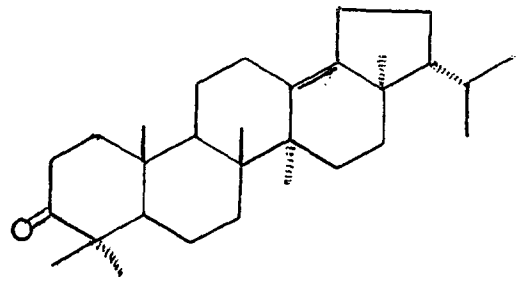


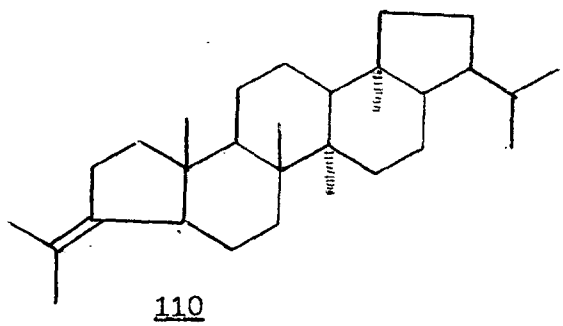
Fig. 4



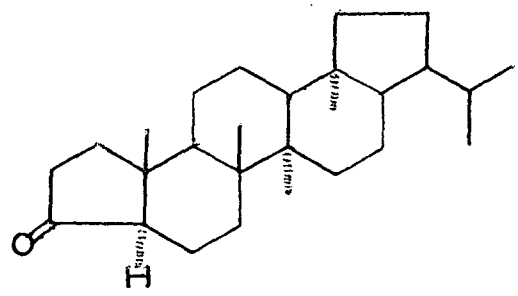
109a (R=R$_1$=H)
109b (R=OH, R$_1$=H)



113



110



111

The structure 108 was further confirmed by the acid isomerisation under mild condition to hopenone-I 42a, m.p. 196-8°, $[\alpha]_D$ +87° and hopenone-II, 113, m.p. 150-3°, $[\alpha]_D$ +52° following the experimental conditions of Fazakerley and coworkers[42]. Though the mass and NMR were similar with that of lupenone, the ORD curves were quite different. The ORD (Fig.3) curve of the ketone showed positive cotton effect as in the case other C-3 keto triterpenoids. The ORD curve of the ketone was as follows : $[\alpha]_{585}$ +42°, $[\alpha]_{342.5}$ +490° (peak), $[\alpha]_{330}$ +486° (peak), $[\alpha]_{312}$ +268° (shoulder), $[\alpha]_{280}$ +14°(trough).

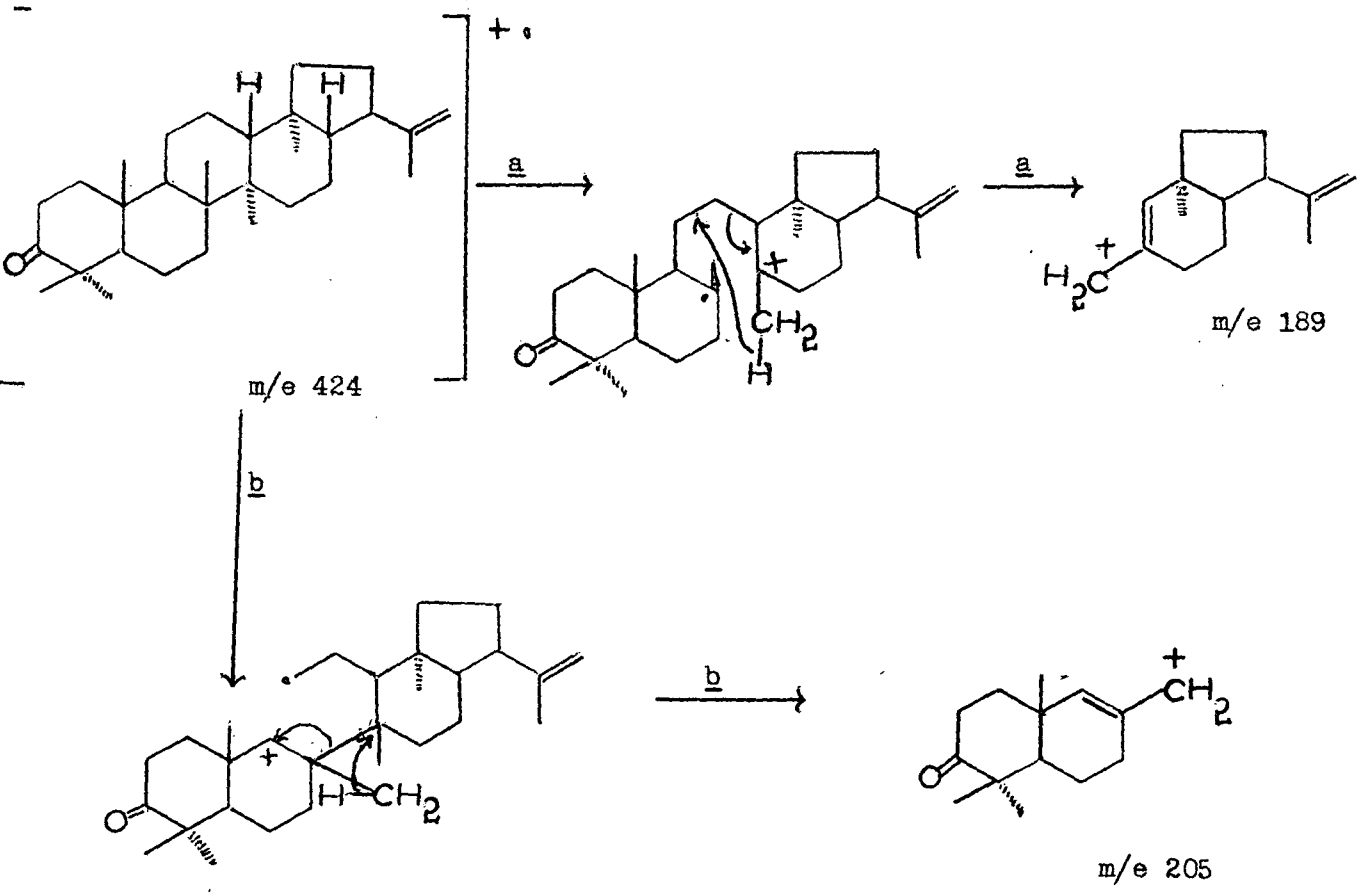
During the last decade the application of the NMR and mass spectrometries have been extensively applied in the elucidation of the structures of organic

compounds[56,73]. We have also used these tools in structural elucidation of the ketone 108.

III. Application of Mass Spectrometry

It was observed that the mass spectra of the ketone 108 (Fig.4) and its derivatives were similar to that of lupenone and its derivatives. It has been found that the ketone 108 undergoes two types of cleavages through ring C as shown in the Chart-I.

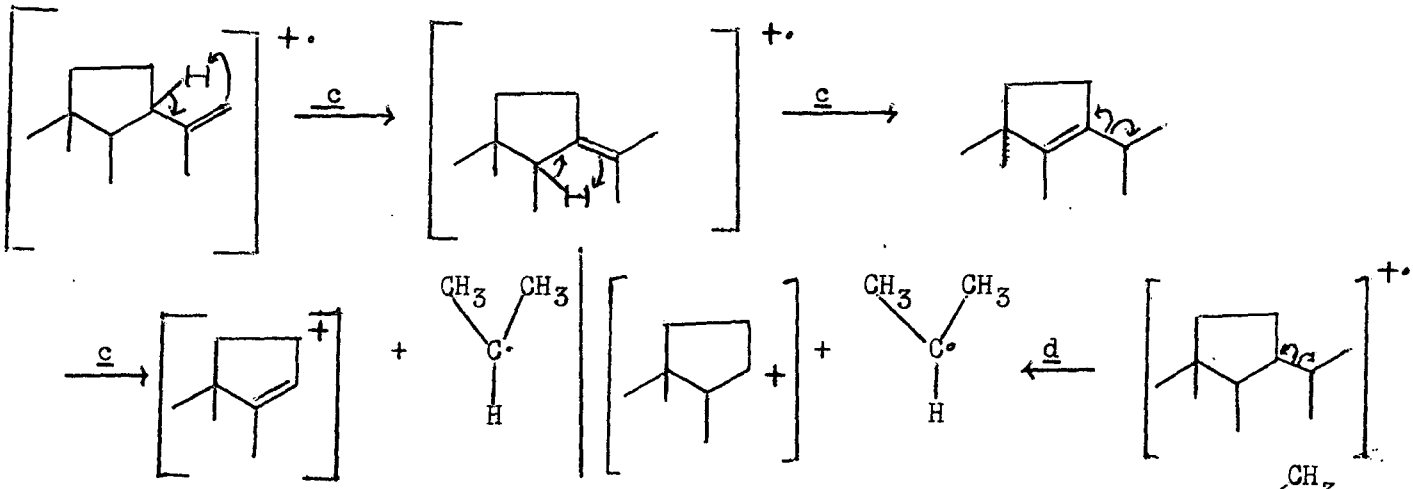
CHART-I



The appearance of mass peak at m/e (M^+ 43), which is weak but distinct in all the derivatives (in dihydro derivatives it is very strong due to homolytic fission-path d) of the ketone 108 shows that an isopropyl group is

eliminated. The formation of isopropyl ion necessitates the hydrogen transfer process, path c which may be depected as below (CHART-II).

CHART-II



The presence of the functional groups R'(R'=O; OH; OAc; H_2) and R"(=C(CH$_3$)CH$_2$; -C(CH$_3$)(H)CH$_3$) attached to two different "halves" of the molecule was obvious from the fragmentation pattern of the ketone 108 and its derivatives in which two distinct peaks due to path a and b are observed (Table I). Now, supposing that the gem dimethyl group is at position 4 in the ketone 108, then the oxygen function may be at 1,2 or 3 position. Since the ketone takes up only two deuterium atoms when treated with methanol-O-D, it excludes the presence of oxygen at C-2 position. Also because there was no significant peak in the spectrum of the ketone due to ions formed by cleavage through ring B, a reaction characteristic of 1-keto steroids, it was concluded that the keto group was attached to C-3 position.

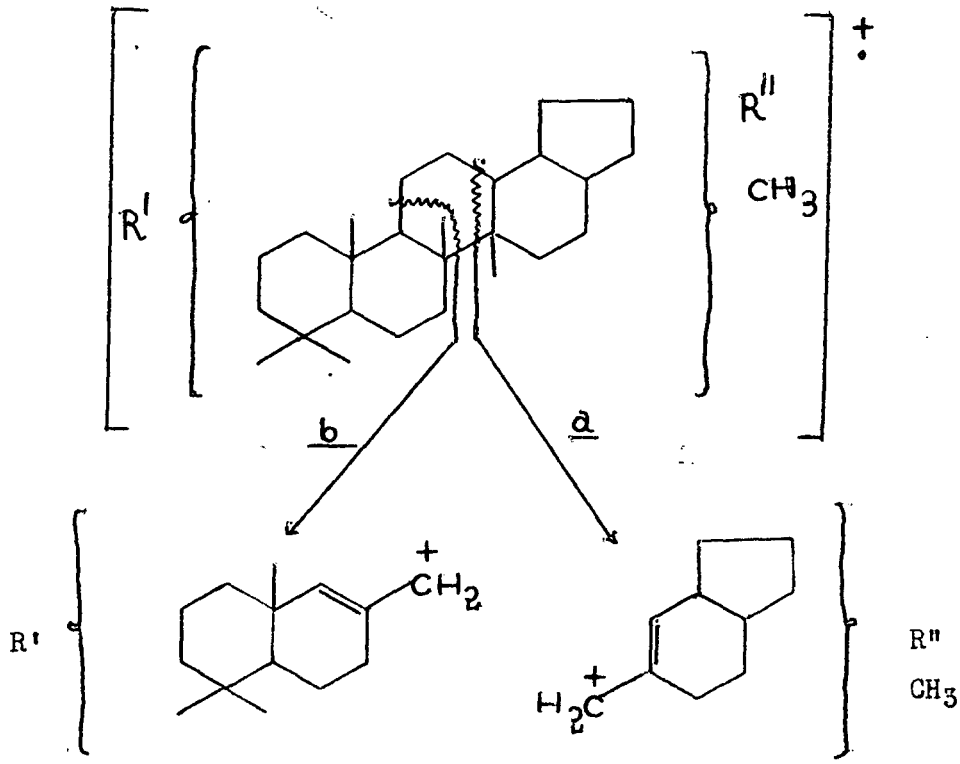


TABLE-I

Mass spectra of Ketone 108 and its derivatives

Compound	R'	R''	Peaks due to reactions				
			M^+	a	b	c	d
Ketone 108	=O	$-C(CH_3)=CH_2$	424	189	205	-	381
Alcohol 108b	-OH	$-C(CH_3)=CH_2$	426	189	207	-	383
Acetate 108c	$-OCOCH_3$	"	468	189	249	-	425
Hydrocarbon 108a	$=H_2$	"	410	189	191	-	367
Dihydrohydrocarbon 109a	$=H_2$	$-C(CH_3)_2-CH_3$	412	191	191	369	-
Deutereated ketone 108	=O	$-C(CH_3)=CH_2$	426	189	207	-	383

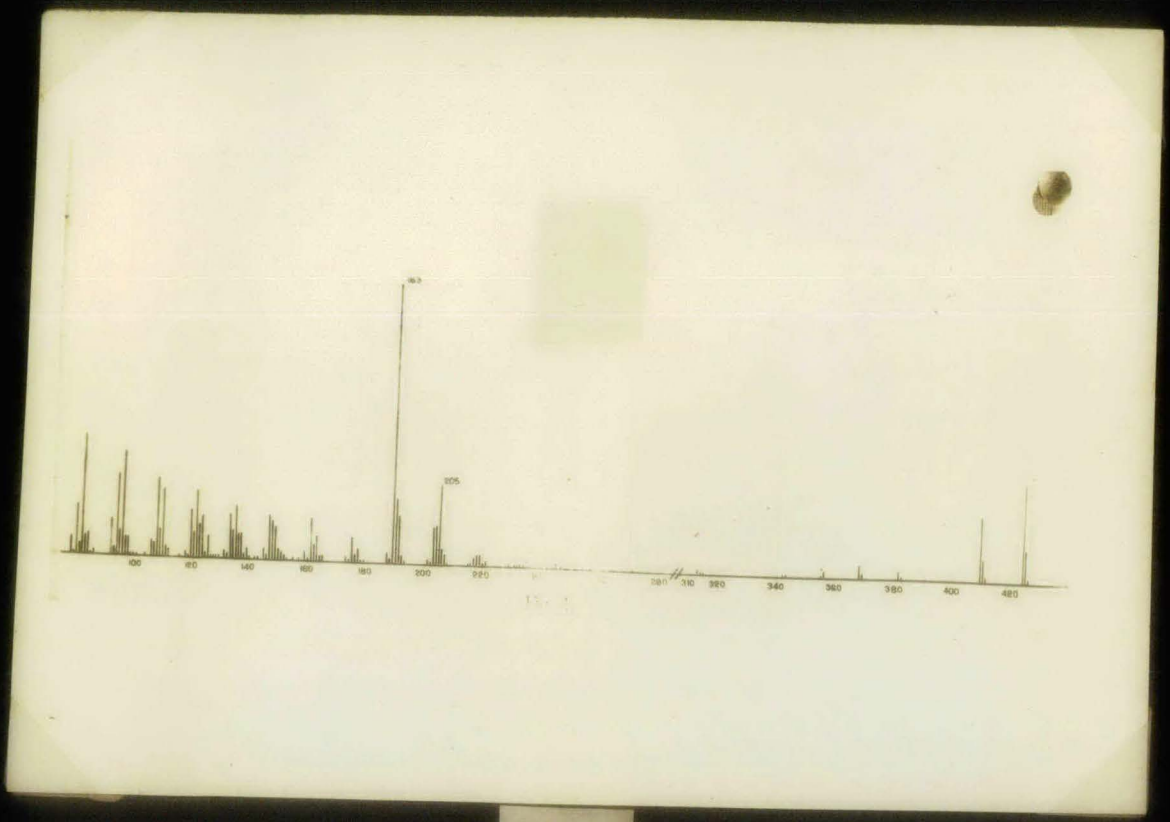

163
205
100
120
140
160
180
200
220
300
310
320
340
360
380
400
420

Fig. 4

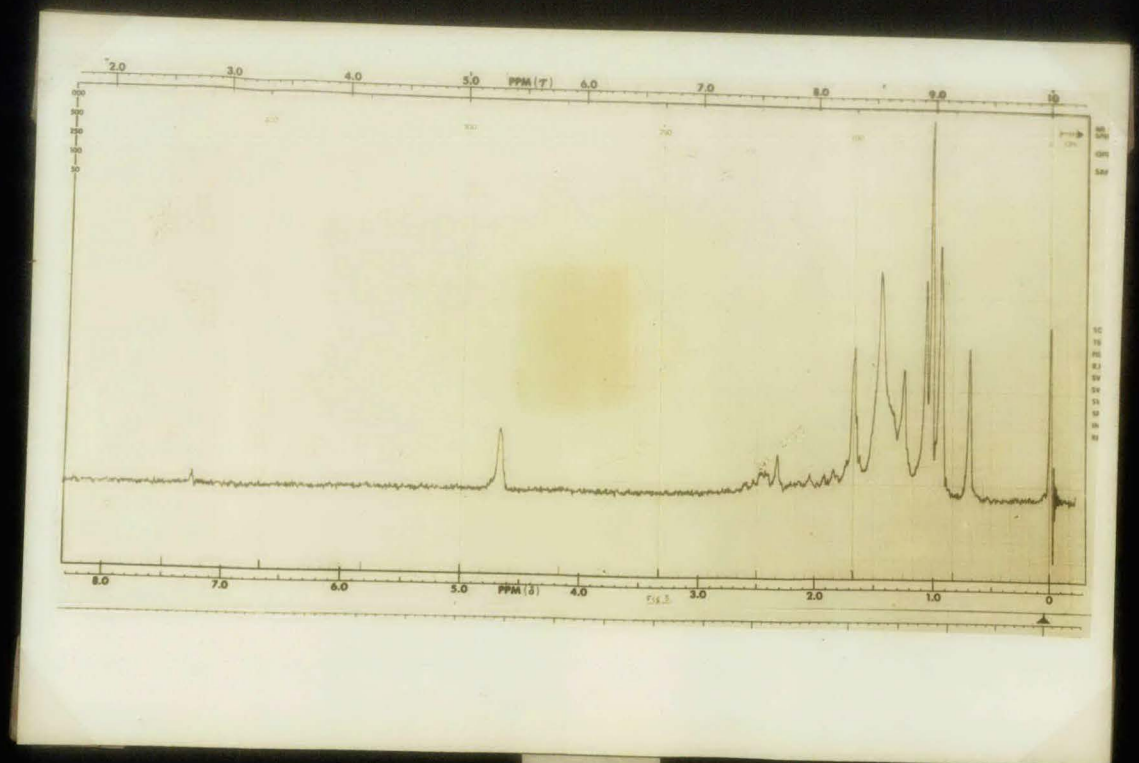

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9.0
10
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0

Fig. 5

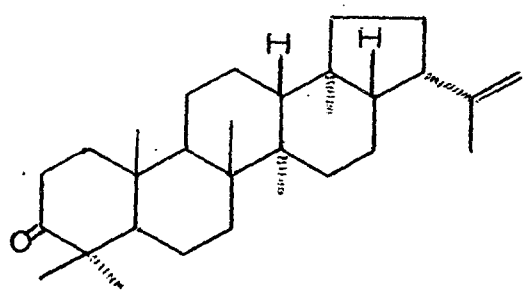
IV. Application of Nuclear Magnetic Resonance Spectra

As shown above NMR was applied as a tool for the elucidation of the structure of the ketone 108. The appearance of signals (Fig.5) at 0.70, 0.94, 0.95, 1.02 (6-H), and 1.08 ppm determined that six methyl groups were present on saturated carbons, the signal at 1.68 ppm showed the presence of additional methyl group on a double bond. The presence of a terminal methylene group appeared at 4.68 ppm. The peak at 2.33 and 2.43 ppm indicated the presence of active hydrogen atoms α to the keto group which was absent in the spectra of the deuterated ketone 108, the other signals remaining fixed. This observation is in conformity with the location of the keto group at C-3 position. The hydrocarbon 108a also showed peaks due to six methyl groups on saturated carbons and one methyl on a double bond and also the peak at 4.68 ppm assigned to exocyclic methylene protons. In the alcohol 108b the signals appeared at 0.69, 0.77, 0.83, 0.94, 0.99 (6-H) for six methyls on saturated carbons, and at 1.67 ppm for one methyl on a double bond. A signal at 3.22 ppm due to the proton at C-3 with one large and one small splitting gave evidence of the hydroxyl group at C-3 being equatorial, which is consistent with the retropinacoline rearrangement results. The signal of the terminal methylene group appeared at 4.68 ppm. The acetate 108c showed methyl signals at 0.68, 0.86 (9-H), 0.95, 0.98 ppm for the six methyl groups on saturated carbons and a methyl group on a double bond at 1.68 ppm. The acetate methyl group appeared at 2.05 ppm and the terminal methylene group at 4.73 ppm. In all the NMR spectras the presence of an isopropenyl group is shown to be present. The NMR studies also show that the ketone is a pentacyclic triterpene with an isopropenyl side chain and a keto group at 3-position.

Hence from a study of physical and chemical data of the ketone 108 and its derivatives, the structure 108 was proposed for the ketone. When we had

settled the structure of the ketone as 108 the Australian group of workers[11] reported the isolation of the alcohol Moretenol from Ficus macrophylla Desf., and assigned the structure 108 for the ketone, moretenone derived from their moretenol which had similar constants as our ketone. Subsequently, we identified our ketone as moretenone by mixed melting point and infrared comparison with an authentic specimen of moretenone kindly supplied by Prof. Ritchie.

In view of the recent work of Yosioka and coworkers[12,13] in the field of hopane series of triterpenes the structure of isoadiantone has been changed to 64 and accordingly we have changed the structure of moretenone 108 to 114 in which the C-21 isopropenyl side chain is in the stable α-orientation[12,13].



114

Examination of Fraction 2. Isolation of a new triterpene alcohol 3-epi moretenol, $C_{30}H_{50}O$, m.p. 223-4°, $[\alpha]_D$ -2.5° and elucidation of its structure.

The fraction 2 of the chromatogram, m.p. 200-215°, on chromatography and crystallisation from methanol afforded a solid 115, m.p. 223-4°, $[\alpha]_D$ -2.53°. Elemental analysis and mass spectrometric determination established the molecular formula to be $C_{30}H_{50}O$ (M^+ 426). The NMR spectrum (Fig. 6) showed

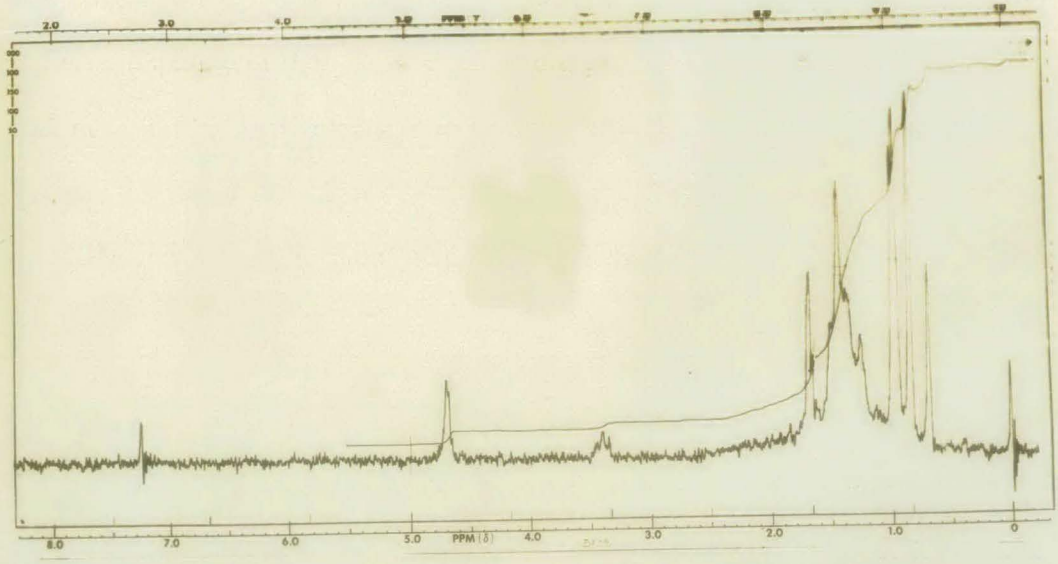


Fig. 6

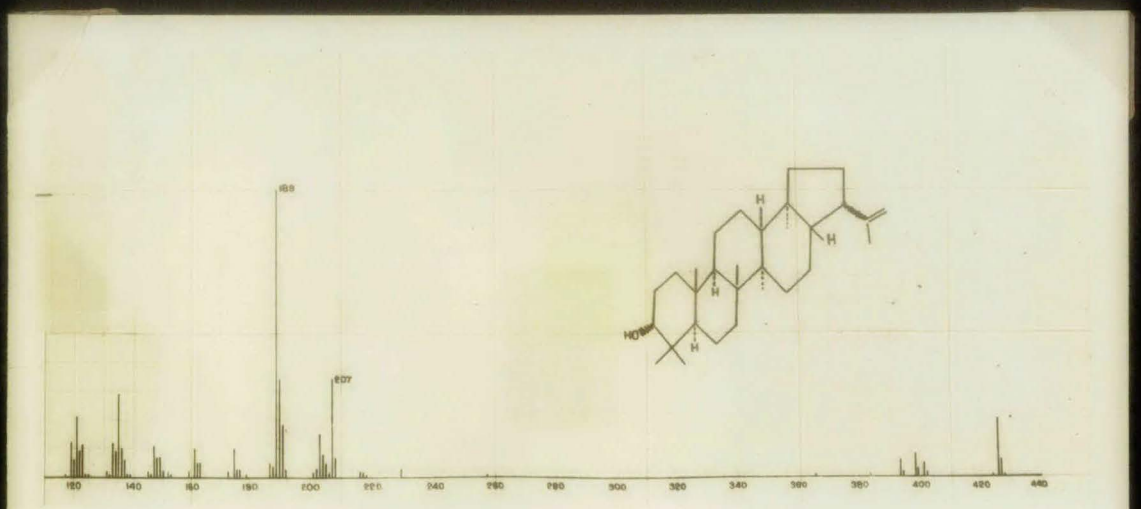
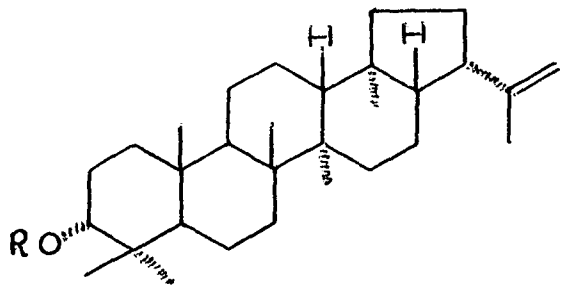


Fig. 7

peaks at 0.68, 0.83 (6-H), 0.95-0.98 (9-H) and 1.68 ppm corresponding to six methyl on saturated carbons and one methyl on a doubly bonded carbon respectively. A signal at 3.40 ppm (width at half height of 7 Hz) showed the proton at C-3 to be equatorial. The terminal methylene group appeared at 4.68 ppm. This compound afforded an acetate 115a, $C_{32}H_{52}O_2$, m.p. 233-4°, $[\alpha]_D$ -19.4° (m/e 189, 202, 408, 468) having NMR signals at 0.70, 0.84-0.88 (9-H), 0.98(6-H) ppm for six methyl groups on saturated carbons and 1.68 ppm for a methyl group on a doubly bonded carbon. The signal for the methyl of the acetate group appeared at 2.07 ppm. The signal at 4.68 ppm for the terminal methylene group partially overlapped that of the C-3 proton which was located at 4.64 ppm. The similarity of the mass spectral fragmentation pattern (Fig.7) and the NMR spectra of the alcohol and its acetate 115a to that of moretenol and its acetate suggested that the alcohol 115 might be 3-epimer of meretenol.

Chromium trioxide - pyridine oxidation on the alcohol gave a ketone, $C_{30}H_{48}O$, m.p. 200-201°, $[\alpha]_D$ +50° which was found to be identical with moretenone 108 by m.m.p. and comparison of infrared spectra with an authentic sample. Since the 3-β alcohol 108b was prepared by LAH reduction of moretenone 108 and was found to be different in every respect from the alcohol 115, the hydroxyl group at C-3 must be α-oriented. Thus the new triterpene was established as 3-epi-moretenol 115.



115 (R=H)
115a (R=-$COCH_3$)

The structure 115 was further confirmed by its partial synthesis from moretenone 114 by Meerwein-Pondorff reduction according to the method of Paton and coworkers[74] when 3-epimoretenol and moretenol were produced and separated by chromatography.

Acid Isomerisation of 3-epimoretenyl Acetate

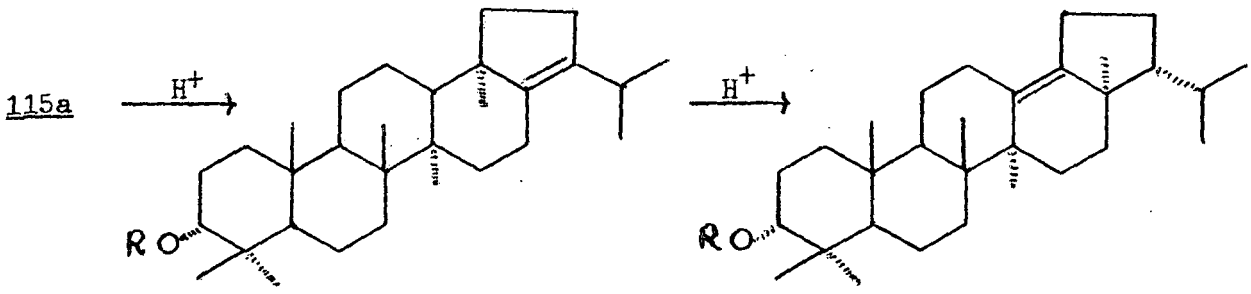
(i) Preparation of 3-epi-hop-17(21)-enyl Acetate 116

3-epi-moretenyl acetate 115a on acid isomerisation under mild conditions of Fazakerley and coworkers[42] furnished an acetate $C_{32}H_{52}O_2$, m.p. 222-3°, $[\alpha]_D$ +11.11°. The acetate on hydrolysis with methanolic potassium hydroxide gave an alcohol 116a, m.p. $C_{30}H_{50}O$, m.p. 185-6°, $[\alpha]_D$ +47° which on oxidation with CrO_3-Py afforded a ketone $C_{30}H_{48}O$, m.p. 197-8°, $[\alpha]_D$ +90°, identical with hopenone-I (m.m.p. and IR comparison). Hence the acetate 116 should be assigned 3-epi-hop-17(21)-enyl acetate 116 and the alcohol 3-epi-hop-17(21)-enol 116a. Our alcohol had similar m.p. and rotations as 3-epi-hop-17(21)-enol prepared by Arthur et al.[51]. They however have not described the acetate. Although we could not procure their sample we believe our compound is identical to Arthur's compound.

(ii) Preparation of 3-epi-hop-13(18)-enyl Acetate 117

The acetate 116 on further isomerisation under strong acid conditions of Fazakerley and coworkers[42] yielded an acetate 117, $C_{32}H_{52}O_2$, m.p. 179-81°, $[\alpha]_D$ -32.14°. The acetate 117, on hydrolysis with methanolic potassium hydroxide furnished an alcohol 117a, $C_{30}H_{50}O$, m.p. 219-21°, $[\alpha]_D$ -14.81°. Oxidation of the alcohol 117a with CrO_3-Py produced a ketone $C_{30}H_{48}O$, m.p. 148-50°, $[\alpha]_D$ +49°, which was subsequently identified as hopenone-II (m.m.p.). Hence the acetate can be assigned structure 117, and the alcohol 3-epi-hop-13(18)-en-ol 117a. The compound has not yet been isolated from

nature nor has it been prepared by synthesis. This appears to be the first report of these compounds.



116 (R=-$COCH_3$)
116a (R=H)

117 (R=-$COCH_3$)
117a (R=H)

Examination of fraction 3: and isolation of a paraffin alcohol $C_{28}H_{58}O$, m.p. 86-9°, $[\alpha]_D$ -13.8°

The fraction 3 of the chromatogram on rechromatography and crystallisation from chloroform and methanol gave an alcohol $C_{28}H_{58}O$, m.p. 86-89°, $[\alpha]_D$ -13.2°. This alcohol on acetylation furnished an acetate $C_{30}H_{60}O_2$, m.p. 85-6°, $[\alpha]_D$ -20.4°. It did not show any UV absorption maxima or any end absorption. From the above facts, it was evident that it was a branched chain saturated paraffin alcohol.

Examination of fraction 4 : Isolation and identification of Moretenol

Fraction 4 of the chromatogram on rechromatography and crystallisation from chloroform and methanol afforded an alcohol, m.p. 228-30°, $[\alpha]_D$ +25°. On acetylation with acetic anhydride and pyridine mixture it afforded an acetate m.p. 280-81°, $[\alpha]_D$ +24°. Elemental analysis of the alcohol and the acetate was found to correspond to the formula $C_{30}H_{50}O$ and $C_{32}H_{52}O_2$ respectively. On

oxidation with chromium trioxide and pyridine complex the alcohol afforded a ketone, $C_{30}H_{48}O$, m.p. 200-201°, $[\alpha]_D$ +52°. The ketone was identified as moretenone 114 by direct comparison with an authentic specimen of moretenone. The acetate was identical with moretenyl acetate by mixed melting point and comparison of IR spectra with an authentic sample of moretenyl acetate. Hence the alcohol m.p. 228-30°, $[\alpha]_D$ +25° was identified as Moretenol.

Examination of fraction 5 : Isolation and identification of β-sitosterol

Fraction 5 on crystallisation from chloroform and methanol mixture had m.p. 136-7°, $[\alpha]_D$ -32°. Elemental analysis showed the molecular formula as $C_{29}H_{50}O$. On treatment with acetic anhydride and pyridine solution it afforded an acetate $C_{31}H_{52}O_2$, m.p. 127-9°, $[\alpha]_D$ -40°. The acetate was identified as β-sitosterol acetate by direct comparison with an authentic specimen of β-sitosterol acetate. Hence the parent alcohol was identified as β-sitosterol.

Examination of fraction 6 : Isolation of a new nor-triterpene $C_{29}H_{46}O_4$, m.p. 228-9°, $[\alpha]_D$ -9.09° and investigation of its structure

Fraction 6 on rechromatography and crystallisation from methanol furnished fine needle shaped crystals having m.p. 228-9°(d), $[\alpha]_D$ -9.09°. Elemental analysis and mass spectrometric determination showed that the molecular formula was $C_{29}H_{46}O_4$ (M^+ 458). The compound had IR peaks (KBr disc) at 3360 (OH, broad), 2970 ($-CH_2-$, broad); 1467, 1453 (CH=CH- doublet); 1389, 1369 (gem dimethyl, sharp); 890, 875 (CH=CH-) cm^{-1}. The compound did not show any UV absorption in the region 220-300 mμ.

On acetylation with pyridine and acetic anhydride, the compound yielded a diacetate having m.p. 213-15°(d), $[\alpha]_D$ +47.5°. Elemental analysis and high

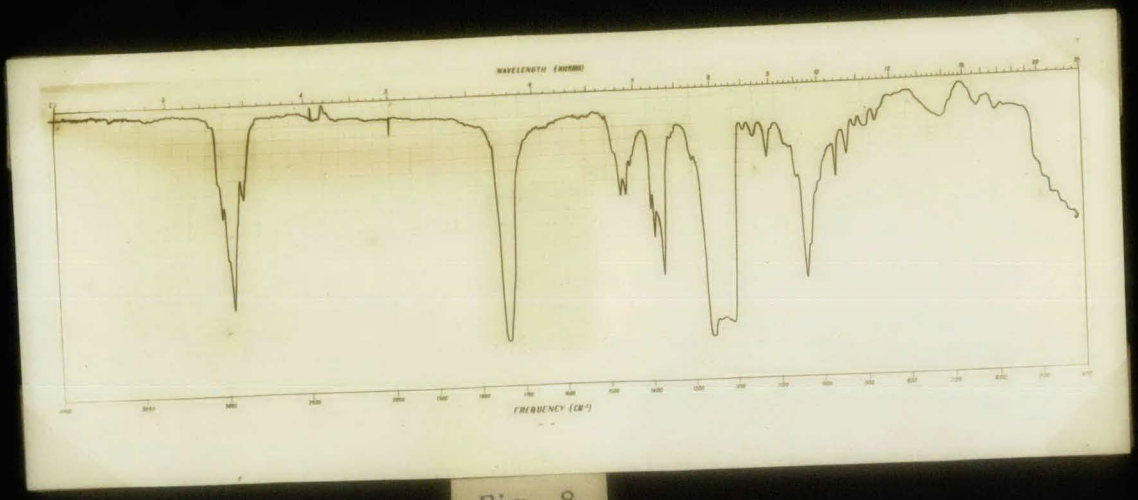


Fig. 8

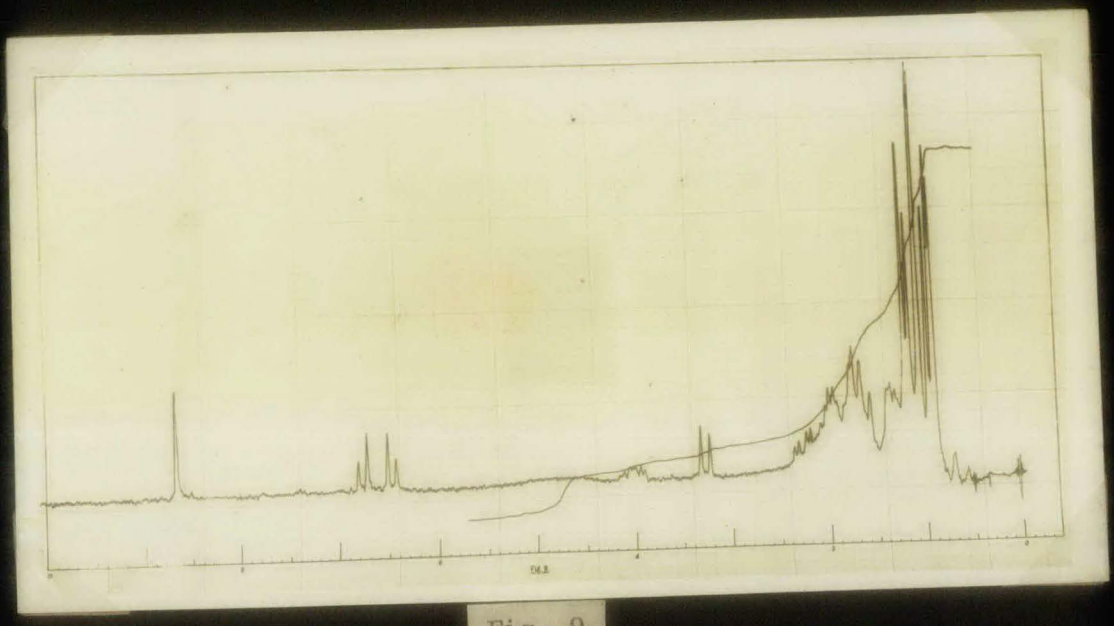


Fig. 9

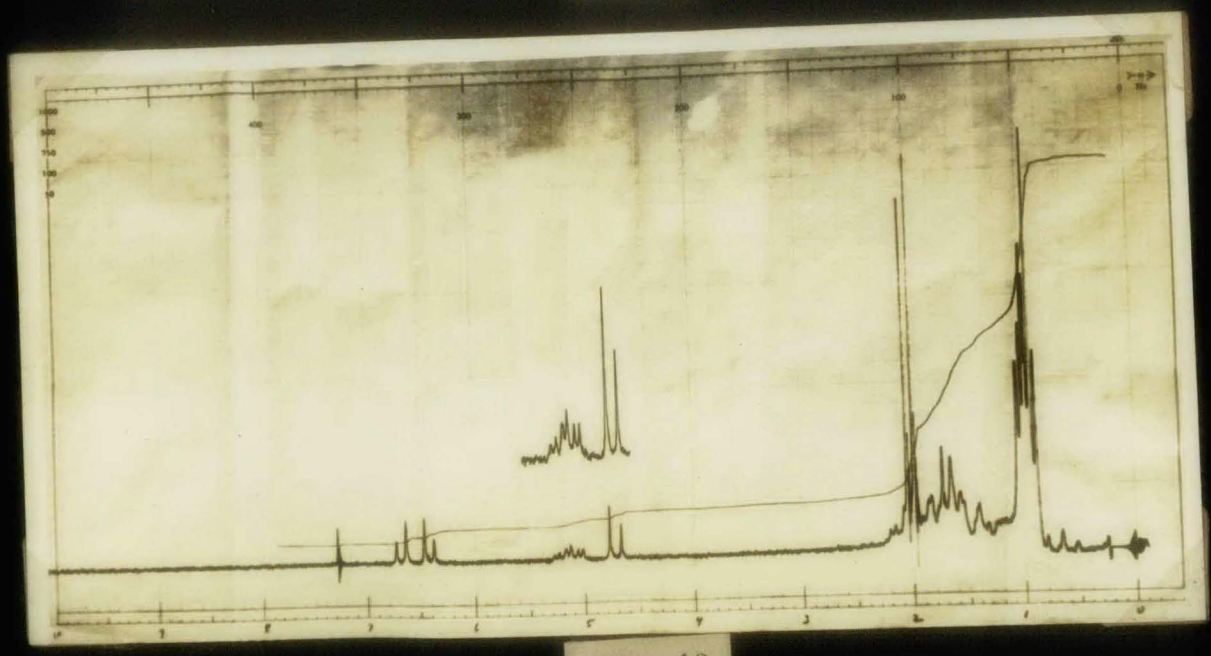


Fig. 10

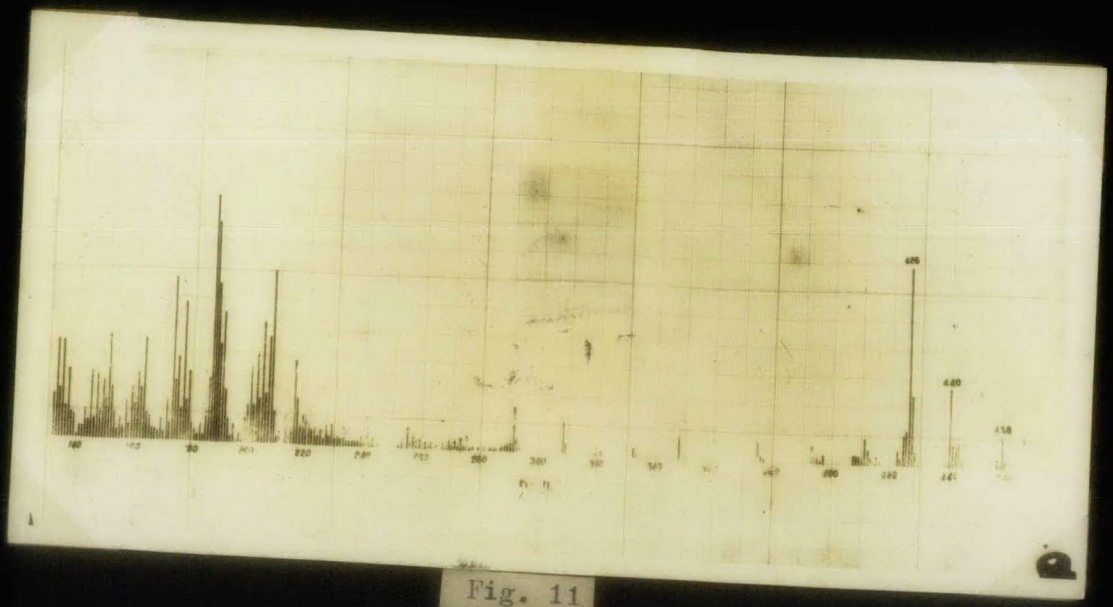


Fig. 11

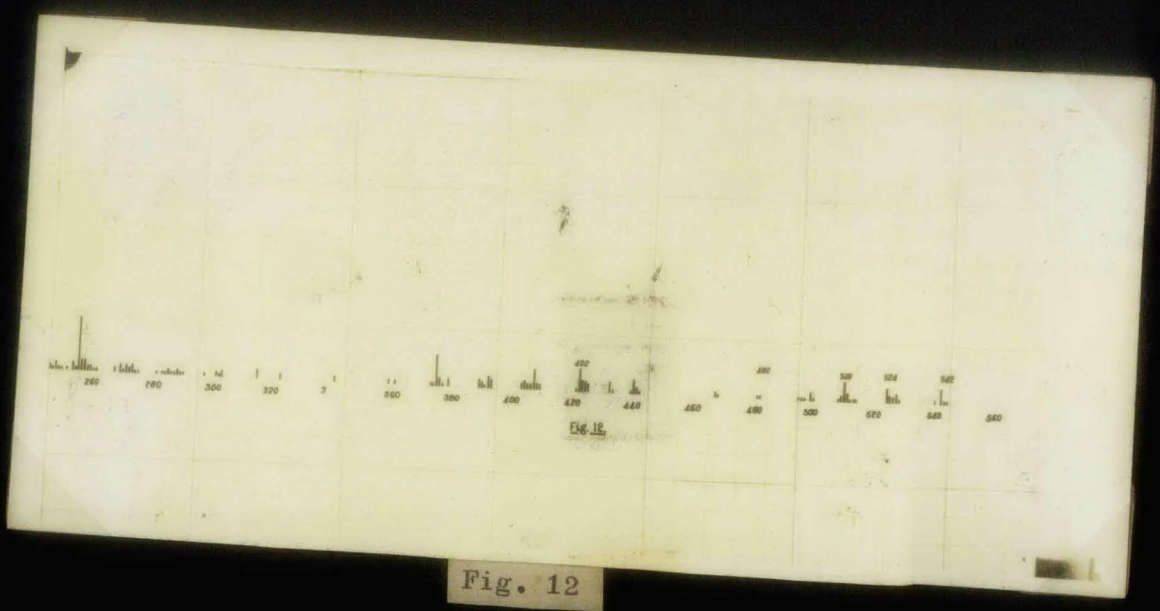


Fig. 12

resolution mass spectrometric determination suggested the molecular formula as $C_{33}H_{50}O_6$ (M^+ 542). The acetate had IR peaks ($CHCl_3$) (Fig.8) at 1737 ($-OCOCH_3$), 1467, 1453 (CH=CH, doublet); 1389, 1369 (gem dimethyl, sharp), 1245-50 ($-OCOCH_3$), 895-872 (CH=CH) cm^{-1} but no hydroxyl peak in the range 3100-3650 cm^{-1}.

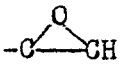
NMR spectra of the diol (Fig.9) showed peaks at 0.88, 0.91, 0.95, 1.04, 1.06, 1.14 and 1.18 ppm for seven tertiary methyl groups, two doublets at 2.16 and 2.20 ppm and 2.28 and 2.32 (two-O<u>H</u>, groups), 3.22 and 3.30 ppm (2H, <u>H</u>-C-OH) and a quartet of doublets at 4.00 ($-C\underline{H}_2-$) and another quartet at 6.42, 6.52, 6.72 and 6.80 ppm (AB quartet, CH=CH) .

The diacetate had NMR (100 Mc) signals (Fig.10) at 0.885, 0.930 (6-H), 0.960, 0.980, 1.01, 1.025 ppm for seven methyl groups on saturated carbons; 1.99 and 2.055 (6H, 2-$OCOC\underline{H}_3$), 4.700 and 4.800 ppm (2H, 2 <u>H</u>-C-$OCOCH_3$), 6.400, 6.490, 6.675, 6.750 ppm (AB quartet, CH=CH).

The mass spectra of the diol showed significant peaks at m/e 426, 440, 458 (M^+) (Fig.11). The mass spectra of the diacetate showed the significant peaks at m/e 422, 482, 510, 524, 542 (M^+) (Fig.12).

<u>Discussion</u>:

It has been found from the elemental analysis and mass spectral data that the molecular formula of the original alcohol and the diacetate is $C_{29}H_{46}O_4$ and $C_{33}H_{50}O_6$ respectively. Hence two of the oxygen atoms are present as two secondary hydroxyl groups which are acetylable. The UV spectra of the alcohol and the acetate showed no absorption in the region 220-300 mμ. Hence from the IR and UV spectra the absence of the carbonyl function in the molecule was

deduced. The fragment 524 (M^+ -18) in the mass spectra of the acetate indicated that a molecule of water might have been eliminated, which might be caused by the presence of a nonacetylable hydroxyl group in the acetate. But the absence of hydroxyl function was indicated by IR spectra which showed no peak around 3000-3600 cm^{-1}. This was confirmed by chemical reactions: (a) Chromic anhydride-Pyridine oxidation and (b) Phosphorous oxychloride-Pyridine dehydration on the acetate. Both the reactions gave back the original acetate, m.p.213-15°. Hence the presence of (OH) group was ruled out by these evidences. The two remaining oxygen functions might be present as ether linkage or oxide linkage. The presence of mass peaks at 426 and 510 of the alcohol and the acetate respectively might have resulted by the loss of one molecule of methanol and the presence of a group like $-CH_2-O-$ might be inferred. This was ruled out as there was no signal in the NMR of the alcohol and the acetate due to $-CH_2O-$ grouping. The absence of any peak due to $-OCH_3$ group precludes the possibility of the presence of a $-OCH_3$ group in the compound. The presence of oxide linkage of the type  was also eliminated by the absence of signals due to a proton attached to carbon of the oxide linkage.

The diacetate has been found to liberate one atom of iodine for one atom of oxygen when treated with potassium iodide in glacial acetic acid solution as compared to a similar blank solution. This experimental result seems to be very significant and the presence of a peroxide linkage such as -C-O-O-C- may be envisaged as in the case of ergosterol peroxide[75,76]. The presence of fragments due to loss of mass unit 32 in the spectra of the alcohol and the acetate may be accounted due to loss of two oxygen atoms.

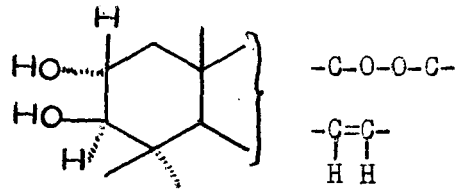
Perbenzoic acid titration on the acetate and the alcohol did not show any consumption of perbenzoic acid, although from the NMR spectra it was evident

that a double bond as -CH=CH- is present in the molecule which might consume one mole equivalent of perbenzoic acid. This anomalous observation might be explained if we assume that one mole equivalent of perbenzoic acid is consumed by the compound and an equivalent amount of iodine is liberated during the titration due to peroxide function thus offsetting the difference in the titration value.

The above interpretation is supported by the fact that on working up of the reaction product of perbenzoic acid titration, a different compound having melting point above 360° was isolated. On the basis of the above observations most probably -C-O-O-C- function is present in the compound. However, further work is necessary to corroborate this argument.

From the study of mass fragmentation pattern and NMR it is evident that there is no isopropenyl side chain in the alcohol, which is observed to be present in other compounds isolated from the same plant.

The diequatorial 2α, 3β configuration of the hydroxyl groups in the alcohol is unequivocally confirmed by examination of NMR spectra of its acetate. In the acetate, the proton at C-3 gives rise to a signal near 4.800 ppm which is split into an unsymmetrical doublet (J, 10 Hz) by the proton at C-2. The 10 Hz coupling between these implies a <u>trans</u> diaxial arrangement of the C-2 and C-3 protons[77]. The C-2 proton is further coupled to the methylene at C-1, and the signal for it is discerned as a quartet of doublets at 5 ppm (X part of ABXY). A similar pattern is observed in methyl alphitolate diacetate[78] and methyl maslinate diacetate and was recorded for other triterpenes with identical ring A[79]. Hence we can suggest a partial formula for the nor triterpene alcohol as in <u>118</u>.



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Hydrogenation of the acetate in presence of palladium-on-charcoal catalyst at ordinary pressure and room temperature afforded a compound having melting point 262-3°, acetylation of which gave an acetate, melting point 170°. Lithium aluminium hydride reduction of the alcohol in dioxan afforded a compound having melting point 301-3°, acetylation of which yielded an acetate having melting point 300°. Periodic acid titration on the original compound showed anomalous results and will be reported later. Further work is in progress.