## CHAPTER-IV

## Isolation of a New Triterpene, 29-Ethoxyhopane, $C_{32}H_{56}O$ and Investigations on its Structure.

### Section A: Establishment of the structure of the new triterpene:

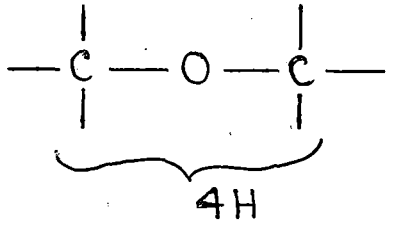
Fraction No. 1 (Chapter-III, Section B, Table-1) on careful chromatography followed by fractional crystallisation from a mixture of chloroform and methanol (3:1) furnished a crystalline solid, m.p. 179-80°, $(\alpha)_D^{27}.16^\circ$ (Chapter-III, Section C). The solid gave a positive Libermann-Burchard test but did not give any colour with tetranitromethane indicating that it was a saturated triterpene. The solid did not show any UV absorption in the region 200-300 nm. Elemental analysis and mass spectrometric determination ($M^+$456, mass spectra, Fig. 1) corresponded to the molecular formula $C_{32}H_{56}O$.

### Nature of the Oxygen function

The IR spectrum of the compound (Fig. 2) showed the absence of peaks in the hydroxyl and carbonyl regions. The appearance of a strong peak at 1105 $cm^{-1}$ indicated that the oxygen function was probably present as an ether linkage. The absence of prominent peaks in the regions 1250 $cm^{-1}$, 950-810 $cm^{-1}$, and 840-750 $cm^{-1}$ indicated that an epoxy linkage was probably

absent in the compound. Furthermore, the band at 1105 $cm^{-1}$ was a singlet indicating that the carbon atoms adjacent to the oxygen were probably unbranched[18]. These conclusions were further confirmed from the NMR and mass spectra of the triterpene.

The NMR spectrum (80 MHz) (Fig. 3) of the triterpene, $C_{32}H_{56}O$ showed signals between $\delta$ 0.7 to 0.95 for seven methyl groups. The NMR spectrum showed the absence of any signal due to olefinic protons. Moreover the presence of the oxygen function was also revealed by the appearance of a broad multiplet in the region $\delta$ 2.8 to 3.6 for four protons. The NMR band in this region indicated the presence of an ether or epoxy linkage[19] in the triterpene. Consequently, the two carbon atoms attached to the oxygen in the triterpene $C_{32}H_{56}O$ must hold four protons. Ethylene oxide ($CH_2\overset{O}{\diagup\diagdown}CH_2$) is the only epoxide in which this situation is met. In all other epoxides the number of protons attached to the two carbon atoms of the oxirane ring should be less than four. Since in the triterpene $C_{32}H_{56}O$, there were four such protons; evidently an epoxide linkage was ruled out. The triterpene, therefore contained the grouping (24).



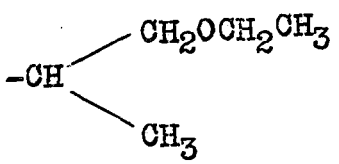

(24)

Nature of the skeleton of the Triterpene.

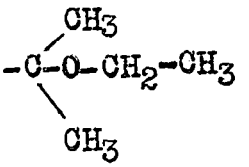
The number of rings in the triterpene was established as follows. The triterpene, $C_{32}H_{56}O$ did not contain any unsaturated linkage and the oxygen atom was present as an ether linkage. Hence writing $CH_2$ for O, the molecular formula of the parent saturated hydrocarbon came out to be $C_{33}H_{58}$. This corresponded to the general formula $C_nH_{2n-8}$. Hence the new triterpene was pentacyclic.

Further insight into the structure came out from a study of the fragmentation pattern in the mass spectra (Fig. 1) of the triterpene. The mass spectrum showed molecular ion peak ($M^+$) at m/e 456. The peaks at m/e 441 and 411 were due to the fragments formed from the molecular ion by the loss of 15 and 45 mass units respectively; attributable to the loss of a methyl and an ethoxy units respectively from the molecular ion. The peak at m/e 396 ($M^+$-60) was attributed to an ion formed due to the loss of a methyl and an ethoxy units from the molecular ion ($CH_3$+$OCH_2CH_3$, mass 60). An alternative explanation would be the loss of a $CH_2OCH_2CH_3$ unit plus one H (mass 60) from the molecular ion, but this explanation appeared less probable. The peak at m/e 369 ($M^+$-87) was clearly due to the loss of a $C_5H_{11}O$ (mass 87) unit from the molecular ion. This loss of $C_5H_{11}O$ unit was best explained by assuming the loss of an isopropyl group containing the ethoxy function (25a). It has already been proved that the triterpene contained the grouping (24) and hence the presence of

a grouping like (25b) was excluded.



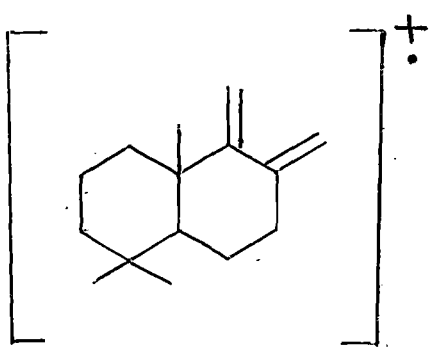
(25a)



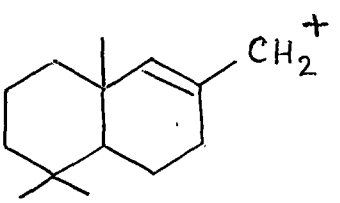
(25b)

Furthermore, since there was no significant loss of 59 mass units ($CH_2$-O-$CH_2$-$CH_3$) the possibility of the attachment of the ethoxy group to any of the tertiary methyls was excluded.

The peaks at m/e 204 and at m/e 191 were characteristic of a hopane or lupane type triterpene[20] and may be attributed to the species (26) and (27) respectively, arising from the molecular ion by the cleavage of ring C.



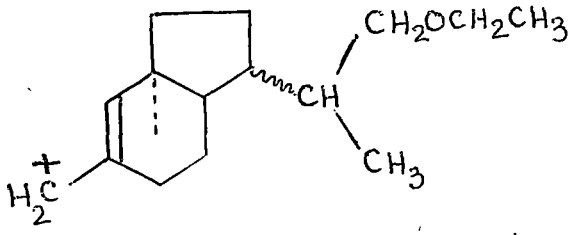
(26)



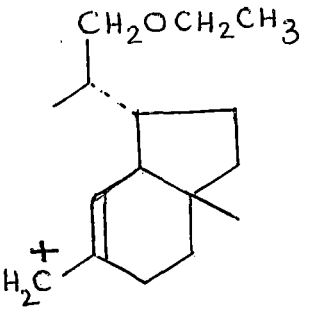
(27)

The appearance of these peaks also showed that the ethoxy function was not present in rings A or B.

The peak at m/e 235 clearly arose from a fragment consisting of ring D and E of the triterpene. The structure (28) or (29) might be attributed to this fragment depending on whether a hopane or lupane type of skeleton was present.



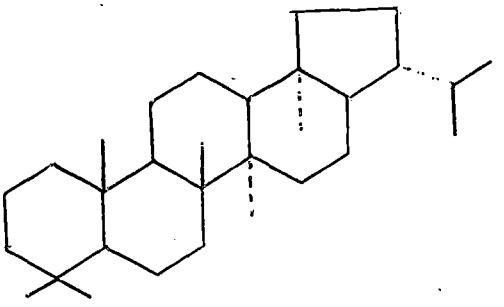

(28)



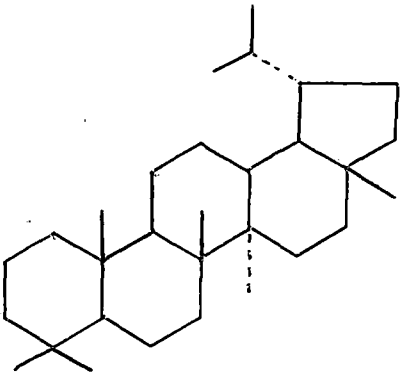

(29)

Further loss of a methyl and an ethoxy units ($CH_3 + OCH_2CH_3$, mass 60) gave the peak at m/e 175 and the loss of the entire isopropyl side chain with one additional hydrogen ($-CH(CH_2-OC_2H_5)(CH_3)$ + H, mass 88) afforded the peak at m/e 147.

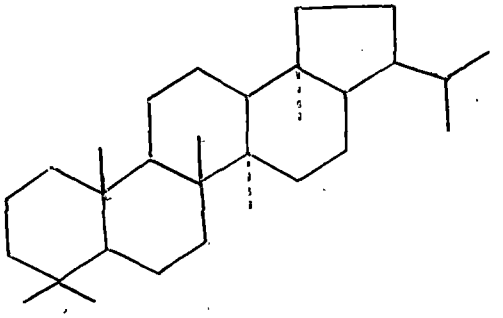
The mass spectrum therefore indicated the presence of the ethoxy function in the isopropyl side chain (the grouping like 25a) in a hopane or lupane type of skeleton. Though the mass spectrum closely corresponded to the presence of a hopane type nucleus (4), the presence of a lupane (30) or isohopane (31) type of nucleus could not be expected to give easily distinguishable fragmentation patterns[21] and hence could not be rejected



(4)



(30)

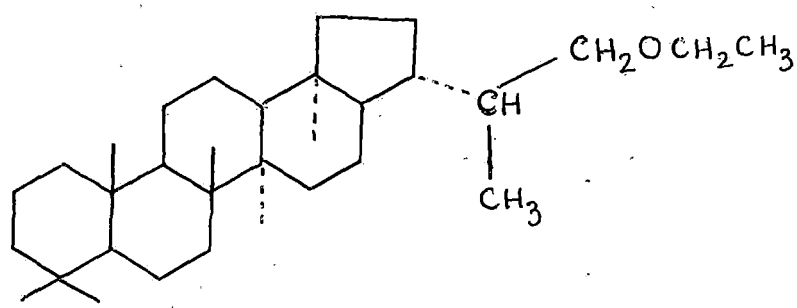


(31)

only on the basis of mass spectral evidence.

It was already pointed out that the triterpene $C_{32}H_{56}O$ occurred in the plant Oleandra nerifolia along with nerifoliol (17a) which contained the hopane type of nucleus (4). Therefore, from biogenetic consideration, it appeared reasonable that the same hopane type of nucleus (4) might be involved in the formation of the triterpene $C_{32}H_{56}O$, in the plant.

On the basis of the above considerations, the structure (32) for this new triterpene was proposed.

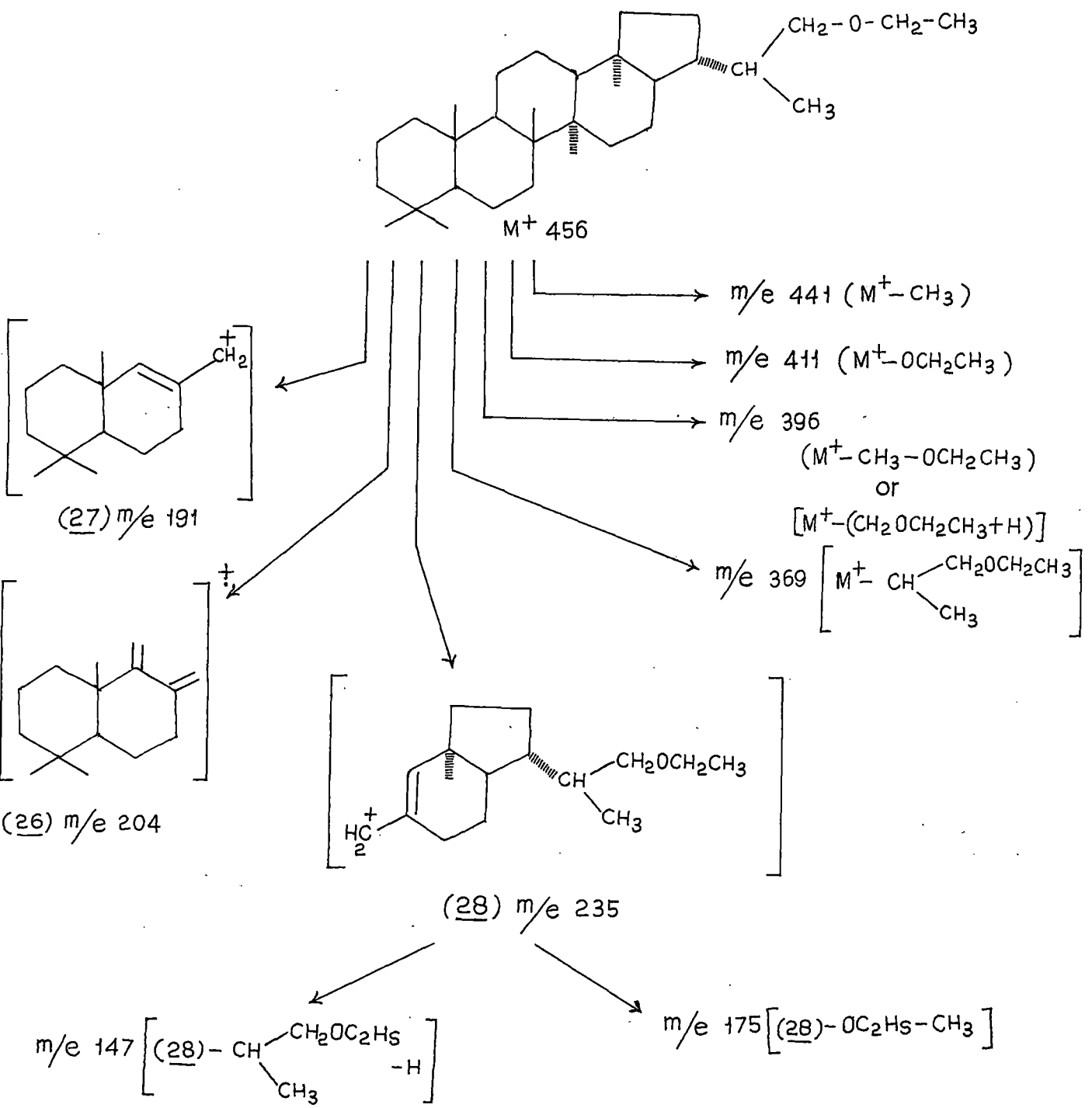



(32)

This structure (32) of the triterpene explained all the physical and chemical data. The mass fragmentation is schematically represented in Chart-II. The structure of the side chain was further substantiated by a detailed study of the NMR spectra of the triterpene.

## Chart-II

## Mass Fragmentation of the triterpene (32)

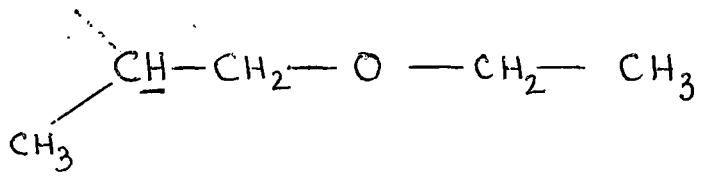


Detailed study of the NMR spectra (Fig. 3)

The $^1H$ NMR spectrum (80 MHz) of the triterpene was shown in Fig. 3. It could be anticipated that the chemical shifts of the two $CH_2$ groups adjacent to oxygen would not be very different and they might overlap. In the spectrum (Fig. 1) it was seen that this indeed happened.

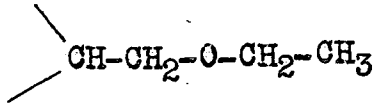
A better analysis could be possible on the basis of the plot expansion (Fig. 4). The $-CH_2-$ of the ethyl group ($-O-C\underline{H}_2-CH_3$) showing signal at $\delta$ 3.4 was a quartet as would be expected. The two protons of the other $CH_2$ group ($\overset{|}{C}H-C\underline{H}_2-O-C_2H_5$) were non equivalent, due to the adjacent asymmetric carbon atom (C-22). One of them showed signals near $\delta$ 3.0 and the other was superimposed in the quartet. They were coupled together with approximately a 10 $H_z$ coupling constant (geminal protons) and were also coupled to the adjacent CH group. The triplet at $\delta$ 1.175 was attributed to the protons of the methyl group ($-O-CH_2-C\underline{H}_3$). This methyl triplet at $\delta$ 1.175 was irradiated and decoupled from the adjacent $CH_2$ group, which collapsed to a single line at $\delta$ 3.425, and partially revealed the other proton of the non-equivalent pair of the $CH_2$ protons. The result of this irradiation at $\delta$ 1.175 (corresponding to $\nu = 4200$ $H_z$) was shown in the upper part of Fig. 4.

Fig. 5 shows the result of irradiation at $\nu$ = 4247 $H_z$ corresponding to $\delta$ 1.75 which appeared to be the chemical shift of the protons of the CH i.e.,



The coupling to this proton collapsed and the 10 $H_z$ coupling between the non-equivalent $CH_2$ protons (geminal protons) was now seen as the only splitting in the pattern. The quartet of the other $CH_2$ ($-O-\underline{CH_2}-CH_3$) was partially collapsed under these conditions.

These NMR data specifically confirm the presence of the grouping (33) in the triterpene and was completely consistent with



(33)

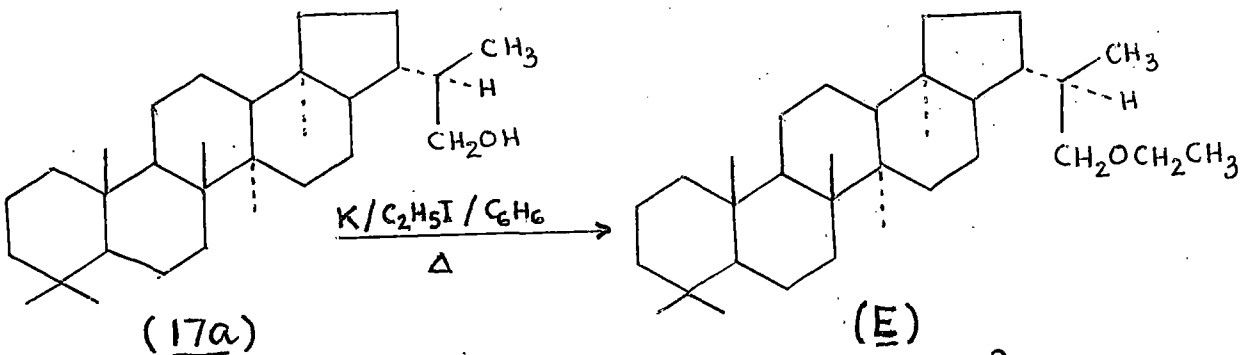
the structure (32) for the triterpene.

## Section B: Confirmation of the Structure (32) for the New Triterpene by Chemical Evidence and Establishment of the Stereochemistry at C-22 and the Complete Stereostructure (35)

The structure (32) for the new triterpene was further confirmed and the stereochemistry at each asymmetric centre, especially at C-22, was established by its correlation with a suitable member of the hopane series. As discussed earlier the presence of a hopane type of nucleus was suggested from mass fragmentation pattern of the triterpene and from biogenetic consideration. However, the above discussions could not throw any light on the stereochemistry at C-22. The chemical correlation studies were of two kinds, (A) the partial synthesis of the new triterpene from a known triterpene and (B) conversion of this new triterpene into a known triterpene.

### (A) Partial Synthesis of the New Triterpene (32) from Nerifoliol (17a)

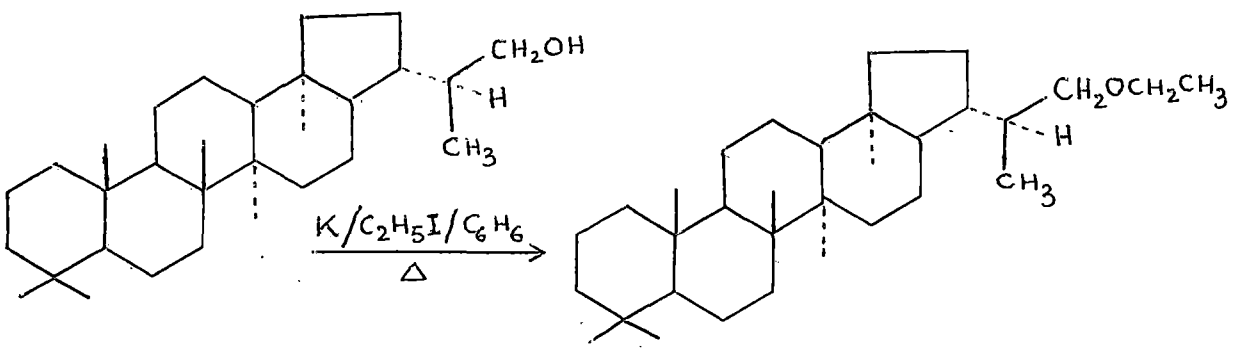
Nerifoliol (17a) was converted into its ethyl ether (E) by refluxing it with potassium metal and ethyl iodide in benzene[22,23].




(17a) (E)

The reaction product (E) m.p. 179-80°, $(\alpha)_D^{27}$.16° has been found to be identical (mmp, IR and TLC) with the triterpene (32) isolated from the fern Oleandra nerifolia.

Dryocrassol[12] (12a), epimeric with nerifoliol at C-22, isolated from the fern Polypodium juglandifolium Don[24] and Polypodium Wallichium[25] was similarly converted into its ethyl ether (34). The product (34), m.p. 148-50° showed depression of m.p. on admixture with the triterpene (32) and its IR spectrum

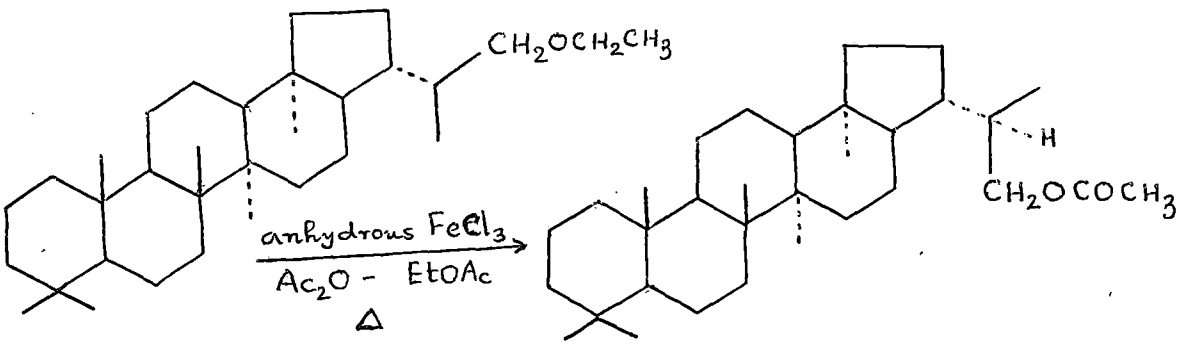



(12a) (34)

was distinctly different from that of the triterpene (32).

(B) Conversion of the Triterpene (32) into Nerifoliol acetate (17b)

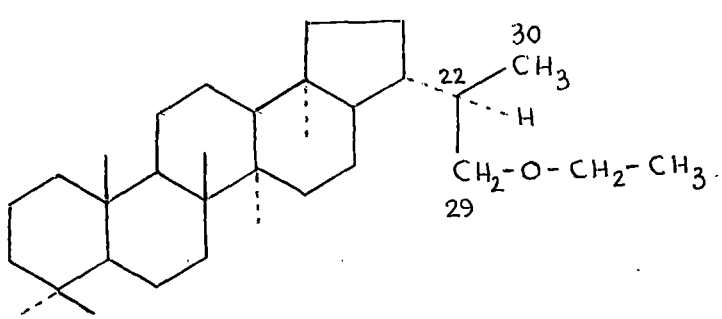
The methylene group adjacent to the oxygen atom in the triterpene (32) was converted into a carbonyl group by its reaction with anhydrous ferric chloride in acetic anhydride-ethyl acetate mixture[26].




(32) (17b)

The reaction product (17b), m.p. 195-96° has been found to be identical (m.m.p, IR and Co-TLC) with authentic nerifoliol acetate (17b).

The above evidences firmly established the structure (32) for the new triterpene. These observations also proved that the stereochemistry at each asymmetric centre of this new triterpene (32) was the same as that of nerifoliol (17a). Nerifoliol was shown by Ageta et al[12] to have the 22-R configuration and was named as hopan-29-ol. Consequently the new triterpene (32) also possessed the 22-R configuration. Thus the complete stereostructure of the new triterpene (32) was represented by (35) and the new triterpene was named 29-ethoxyhopane.




(35)

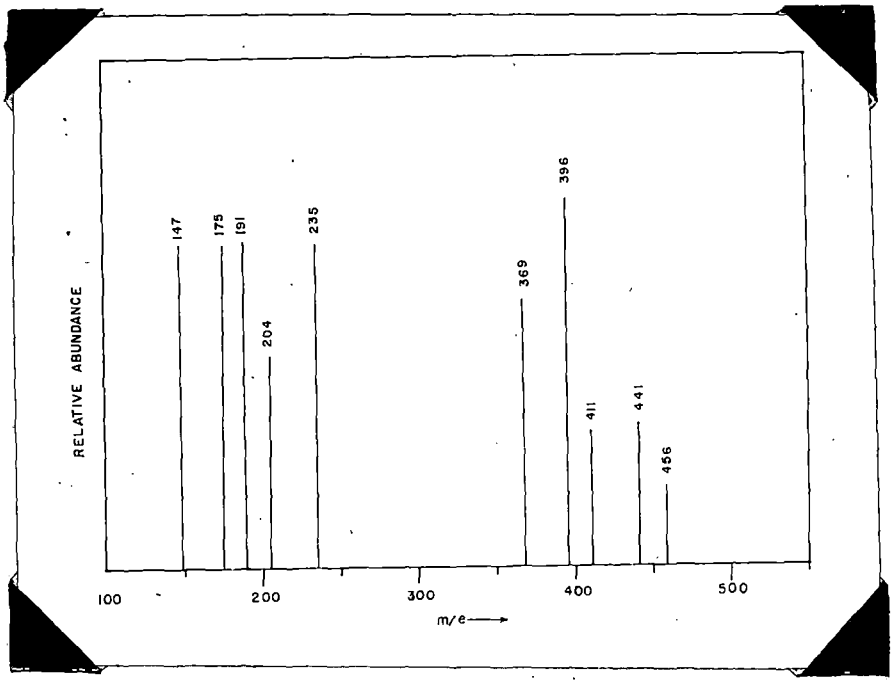



Fig. 1: Mass Spectrum of 29-Ethoxyhopane (35)

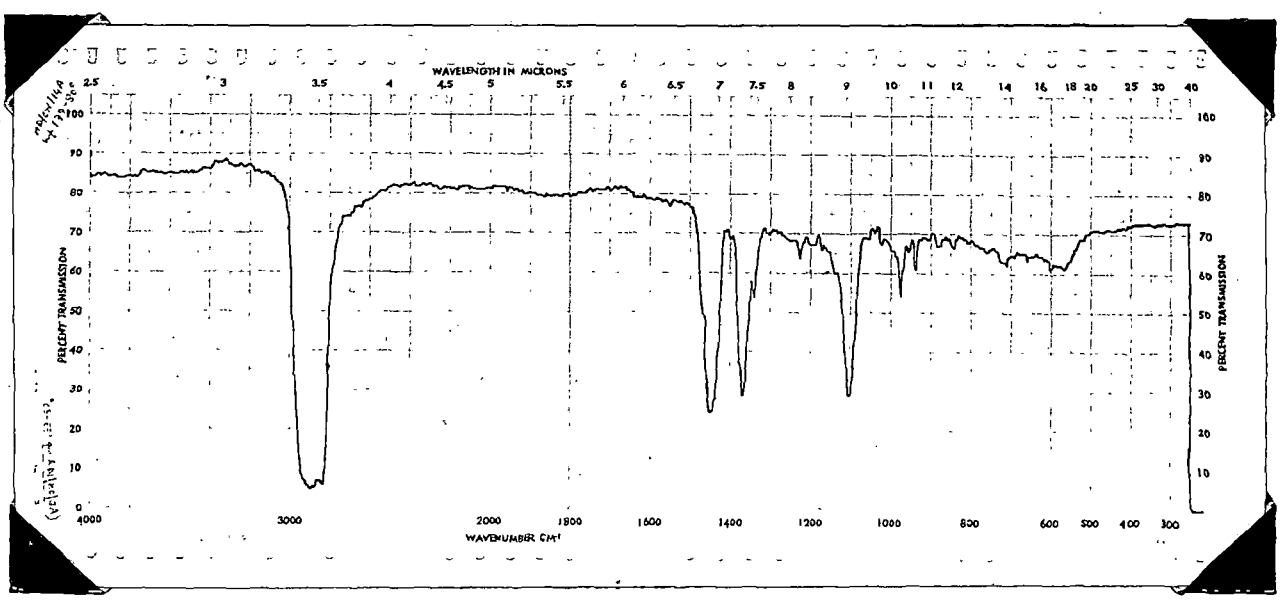



Fig. 2: IR spectrum of 29-Ethoxyhopane (35)

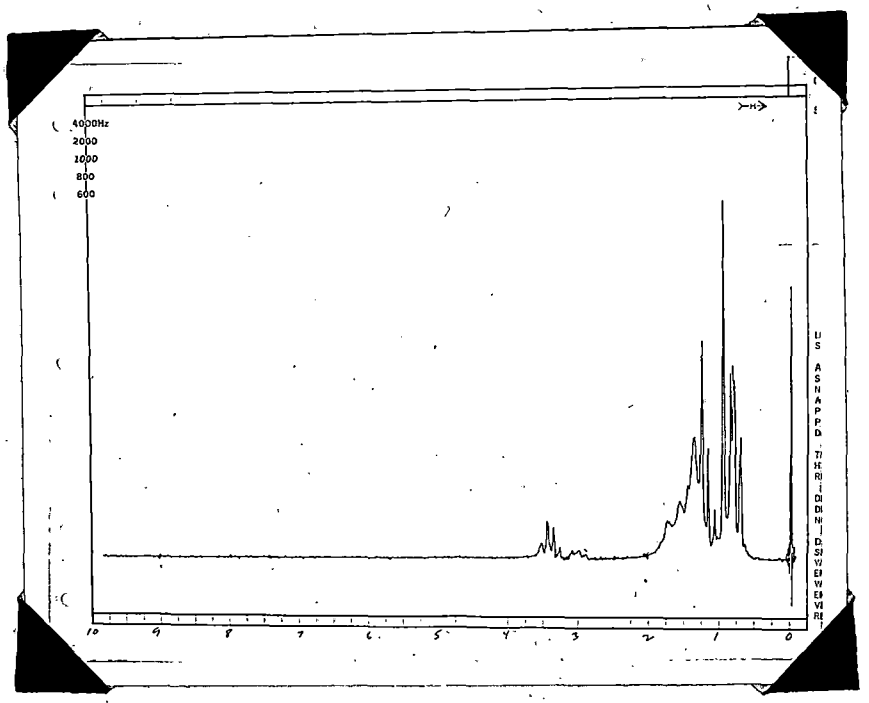


Fig. 3: NMR Spectrum of 29-Ethoxyhopane (35)

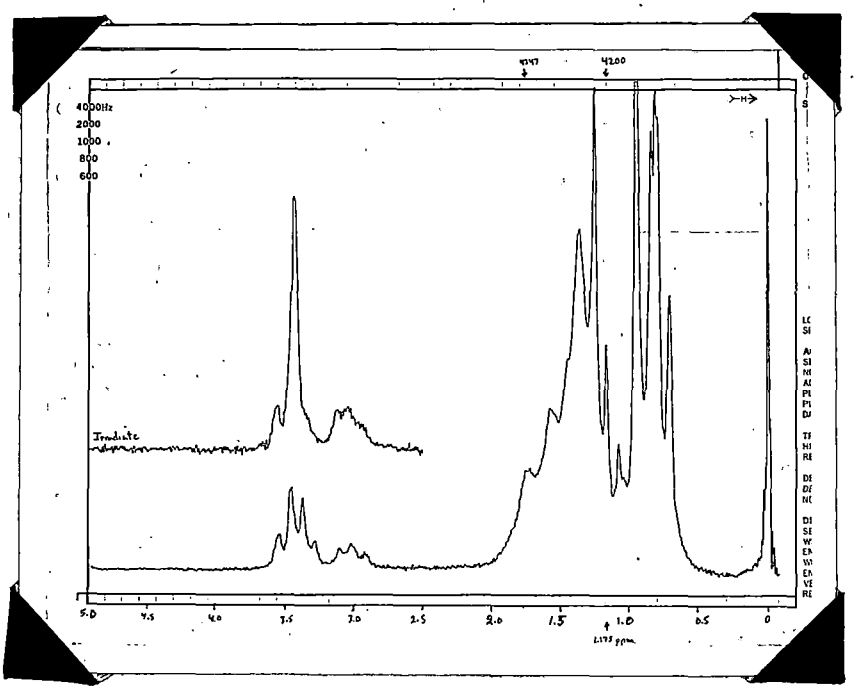



Fig.4: NMR Spectrum of 29-Ethoxyhopane (35), Plot Expansion. The result of irradiation at $\delta$ 1.175 (corresponding to $\nu$ = 4200 $H_Z$) was shown in the upper part of the figure.

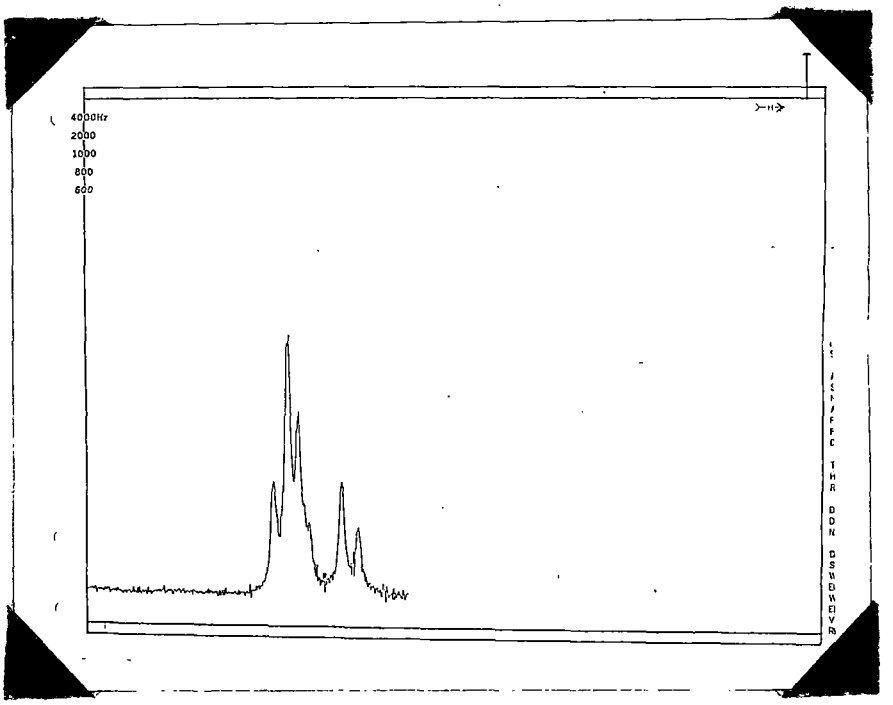


Fig. 5: NMR spectrum of 29-Ethoxyhopane (35). The result of irradiation at δ 1.75 (corresponding to $\nu$ = 4247 $H_z$)

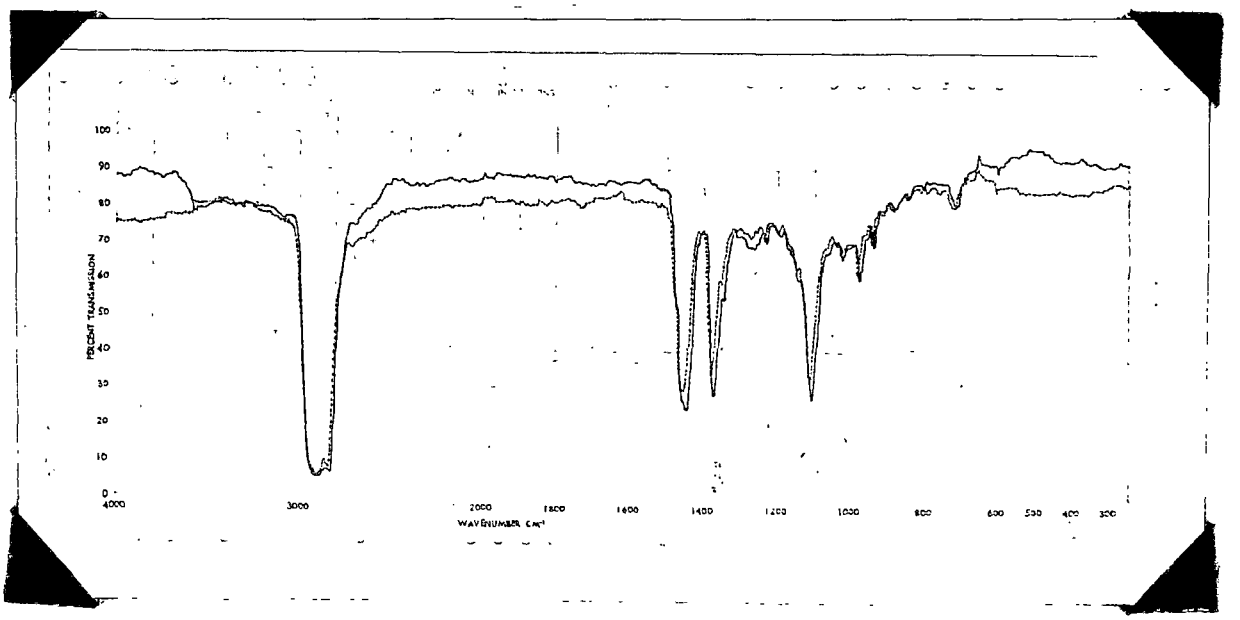


Fig. 6: IR comparison of synthetic 29-Ethoxyhopane (35) (dotted line) prepared from nerifoliol (17a), with an authentic specimen (Solid line) isolated from O. nerifolia.

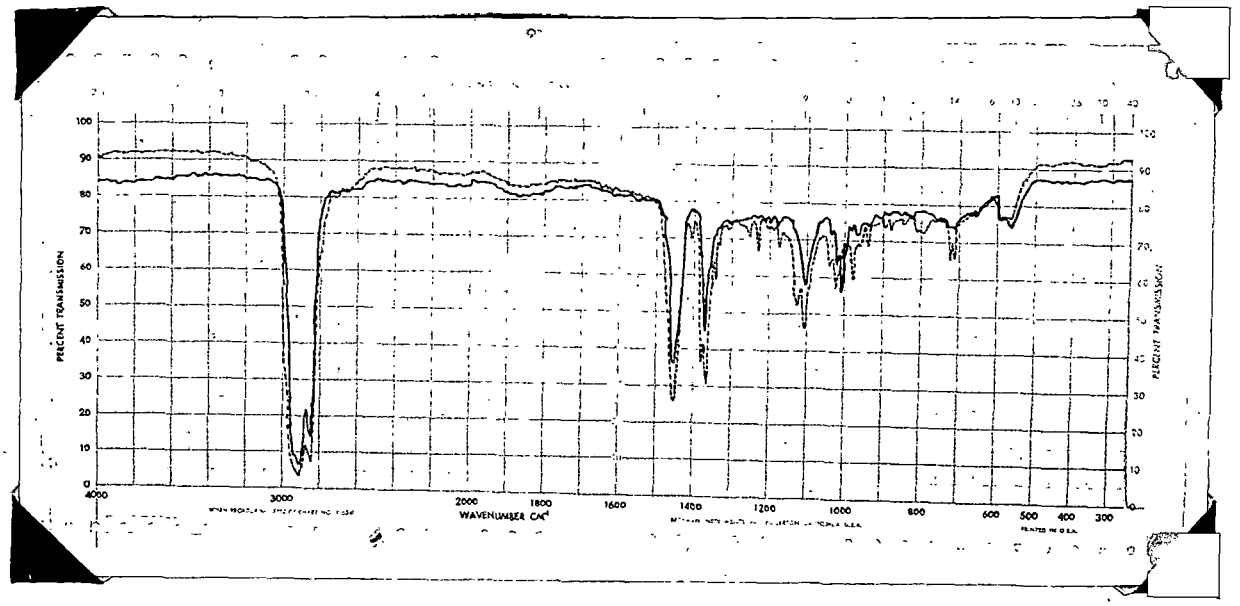



Fig. 7: IR comparison of synthetic Dryocrassol ether (30-Ethoxyhopane) (34) (dotted line) with an authentic specimen of 29-Ethoxyhopane (35) (Solid line) isolated from O.nerifolia.

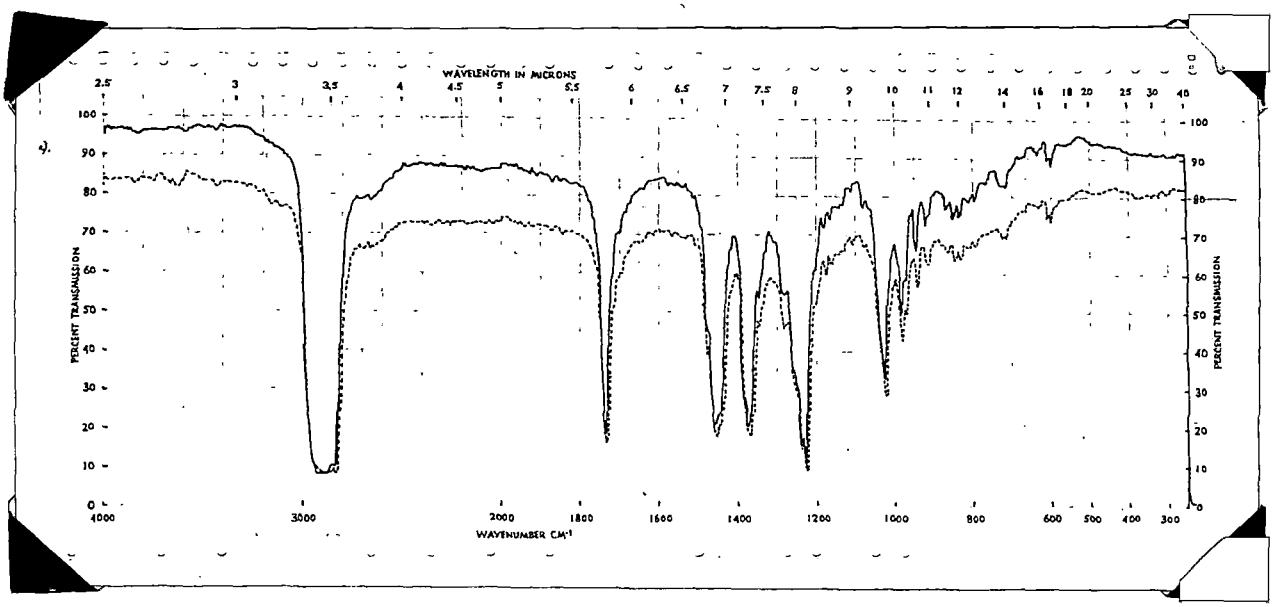



Fig. 8: IR comparison of synthetic Nerifoliol acetate (17b) (Solid line) prepared from 29-Ethoxyhopane (35), with an authentic specimen of Nerifoliol acetate (dotted line).