INVESTIGATION ON NATURALLY OCCURRING CARBOCYCLIC COMPOUNDS

ISOLATION, STRUCTURE ELUCIDATION AND PARTIAL SYNTHESIS OF TRITERPENES AND RELATED COMPOUNDS

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (SCIENCE)

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By

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ST - VERF

A C K N O W L E D G E M E N T S

The present thesis embodies the results of research carried out by the author at the Department of Chemistry, University of North Bengal, Raja Rammohunpur, Dist. Darjeeling.

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S U M M A R Y

The work embodied in the present thesis has been divided into three Parts.

PART-I

REINVESTIGATION ON THE FERN OLEANDRA NERIFOLIA CAV. HOOK: ISOLATION AND STRUCTURE ELUCIDATION OF A NEW TRITERPENE, 29-ETHOXYHOPANE.

Chapter-I:

In this chapter the morphological features of the fern Oleandra nerifolia Cav. Hook is described.

Chapter-II:

Section A:

This section gives a short review on the chemical constituents of ferns of Oleandraceae family.

Section B:

This section gives a short review on the structure of dryocrassol isolated from the Aspidiaceous fern.

Chapter-III:

This chapter deals with the present investigations on the neutral part of the benzene extract of the fern Oleandra nerifolia

and the isolation of a new triterpene, 29-ethoxyhopane along with filicene, nerifoliol and β -sitosterol.

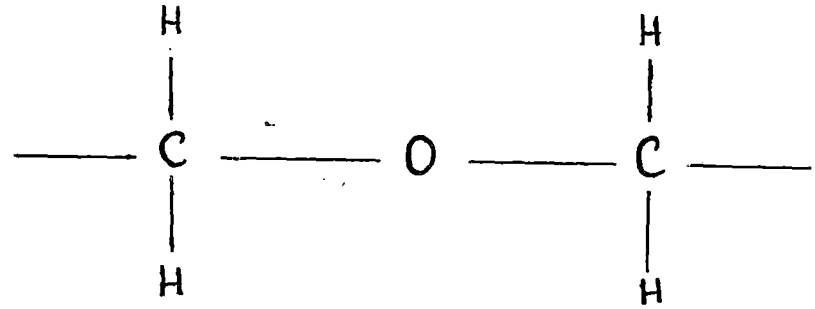
Chapter-IV:

In this chapter the structure elucidation of the new triterpene, 29-ethoxyhopane, isolated from the neutral part of the benzene extract of the fern Oleandra nerifolia is described in detail.

Section A:

This section deals with the establishment of the structure of the new triterpene.

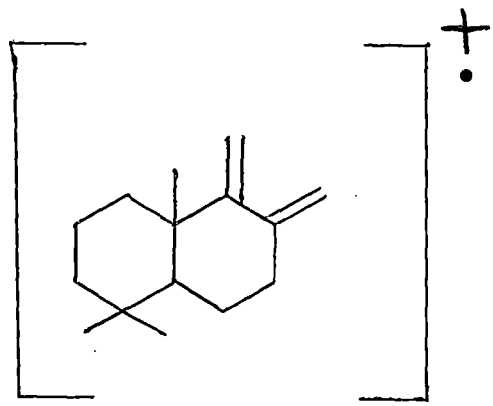
The new triterpene, 29-ethoxyhopane, $C_{32}H_{56}O$, m.p. 179-80°, $(\alpha)_D$ 27.16° gave positive Libbermann-Burchard test but did not give any colour with tetra-nitromethane indicating thereby that it was a saturated triterpene. The IR spectrum of the new triterpene $C_{32}H_{56}O$, showed bands at 1105 cm^{-1} indicating that the oxygen function was probably present as an ether linkage. The NMR spectrum showed signals between δ 0.7 to 0.95 (seven methyl groups) and a broad multiplet in the region δ 2.8 to 3.6 (four protons). The NMR band in the region δ 2.8 to 3.6 indicated the presence of an ether linkage. The new triterpene, thus, contains an ether linkage and the grouping like (I)



(I)

From an analysis of the molecular formula, the new triterpene was shown to be pentacyclic in nature.

The mass spectrum of the triterpene showed peaks at m/e 456 (M^+), 441 ($M^+ - CH_3$), 411 ($M^+ - OCH_2CH_3$), 396 ($M^+ - CH_3 - OCH_2CH_3$), 369 ($M^+ - CH(CH_3)(CH_2OC_2H_5)$), 235, 204, 191, 175 and 147. The peaks at m/e 235, 204 and 191 were characteristic of hopane or lupane type triterpene. The peaks at m/e 204 and 191 were explained as arising due to the formation of species (II) and (III) respectively.



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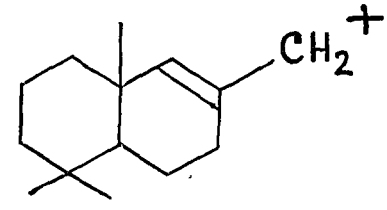
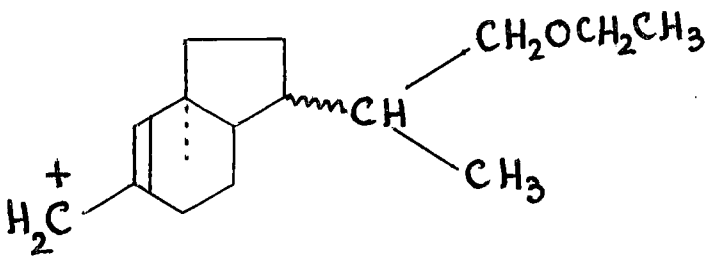


(III)

The peak at m/e 235 was explained as arising due to the species (IV) or (V) depending on whether a hopane or lupane type of nucleus was present.



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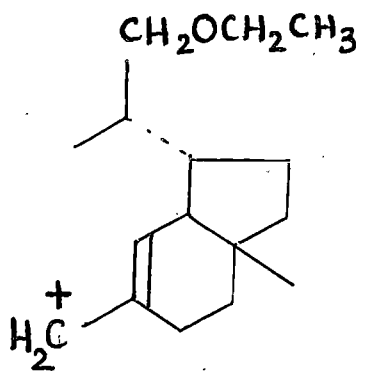
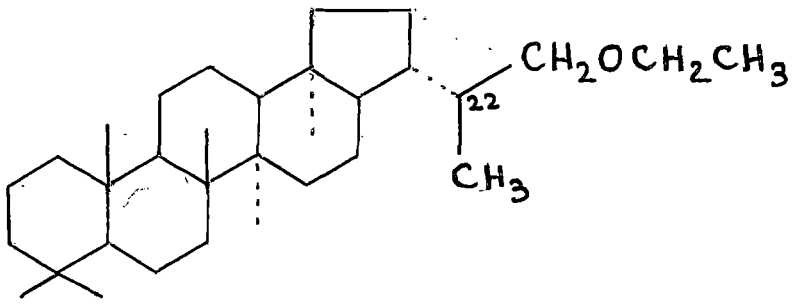


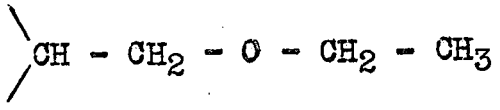
(V)

Further loss of (CH_3+OCH_2CH_3) and (CH<$^{CH_2OCH_2CH_3}_{CH_3}$ + H) units from (IV) or (V) gave peaks at m/e 175 and 147, respectively. Thus the mass spectra gave a detailed insight into the structure of the new triterpene. However, it did not prove whether a hopane, lupane, or isohopane type of nucleus was present in the triterpene. Since the new triterpene was found to occur with nerifoliol, containing a hopane type of nucleus, it appeared reasonable from biogenetic considerations that the same hopane type of nucleus was involved in the formation of the new triterpene in the fern. On the basis of these considerations, structure (VI) was proposed for the new triterpene.



(VI)

A detailed study of the NMR spectra (80 MH_z) of the triterpene confirmed the presence of the grouping (VII) in the triterpene.



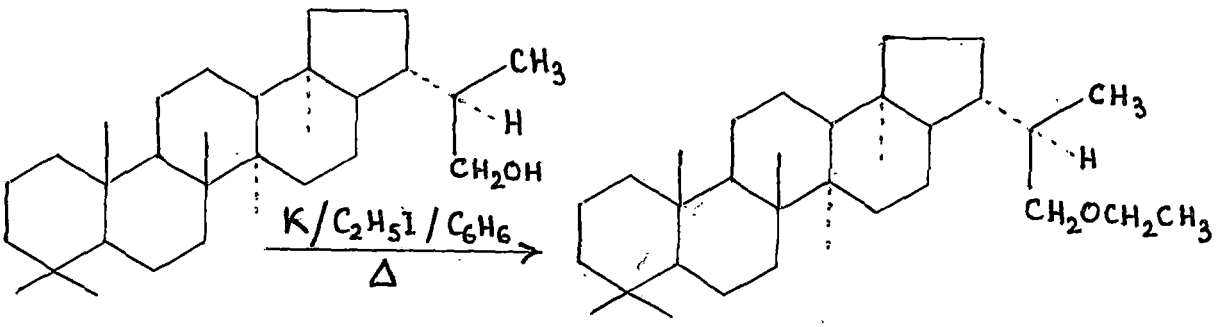
VII

Section B:

This section deals with the confirmation of the proposed structure (VI) of the triterpene by chemical evidences and settlement of the stereochemistry at C-22 and the complete stereo-structure of the triterpene. The chemical correlation studies described in this section were of two kinds (I) Partial

synthesis of the new triterpene from a known triterpene, and (II) Conversion of the new triterpene into a known triterpene.

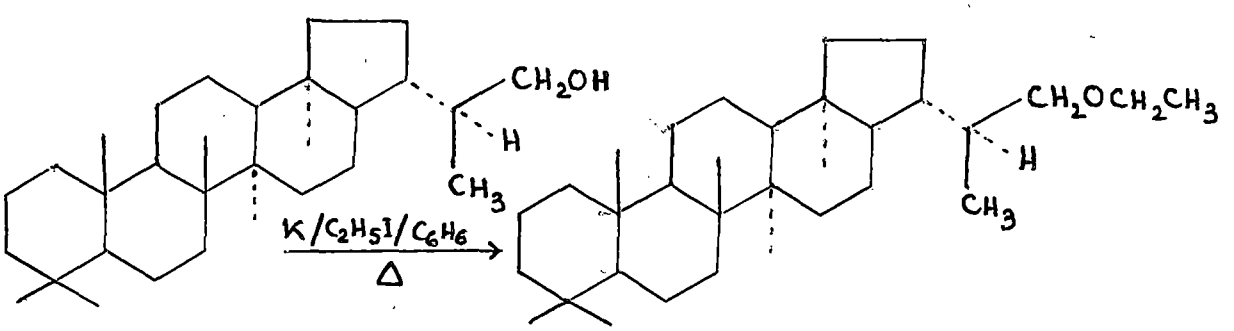
Nerifoliol (hopan-29-ol), (VIII) was converted into its ethyl ether (IX) by refluxing with potassium metal and ethyl iodide in benzene.



(VIII) (IX)

The reaction product was found to be identical in all respects with the new triterpene isolated from Oleandra nerifolia.

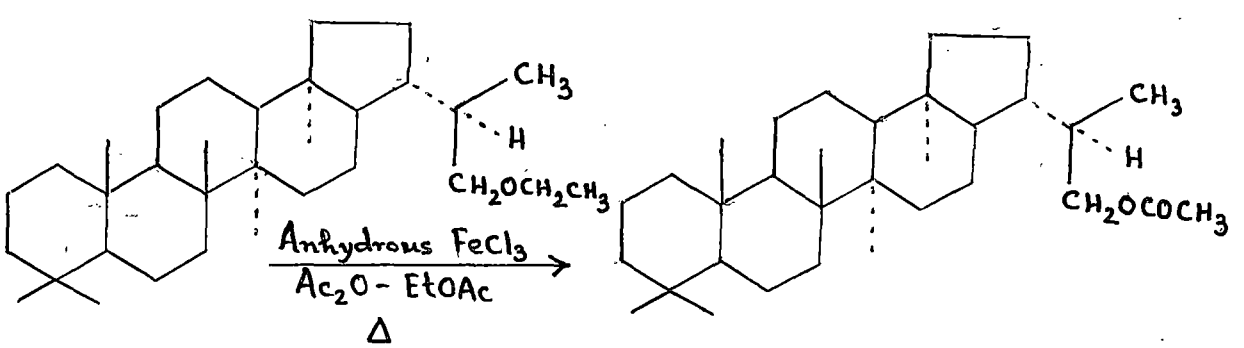
In this connection it may be mentioned that dryocrassol (hopan-30-ol), (X) was also converted into its ethyl ether (XI) by the same procedure.



(X) (XI)

The reaction product, however, was found to be distinctly different from the new triterpene isolated from Oleandra nerifolia.

The new triterpene was also converted into nerifoliol acetate (XII) by ~~oxidation~~ reaction with anhydrous ferric chloride in acetic anhydride-ethyl acetate mixture.



(IX) (XII)

The above observation confirmed the proposed structure (VI) for the new triterpene. Furthermore, nerifoliol possessed the 22-R configuration. It was, therefore, evident that the new triterpene did also possess the 22-R configuration. Thus the new triterpene was shown to be 29-ethoxyhopane (IX).

Chapter-V:

Experimental portion has been described in this chapter.

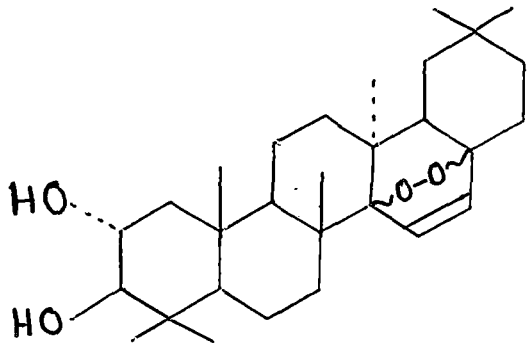
PART-II

PARTIAL SYNTHESIS OF 2α, 3β-DIACETOXY-28-NOR OLEANA-12, 17-DIENE: CONFIRMATION OF THE STRUCTURE OF BACCATIN:

Chapter-I:

This chapter gives a short review on the structure elucidation of baccatin.

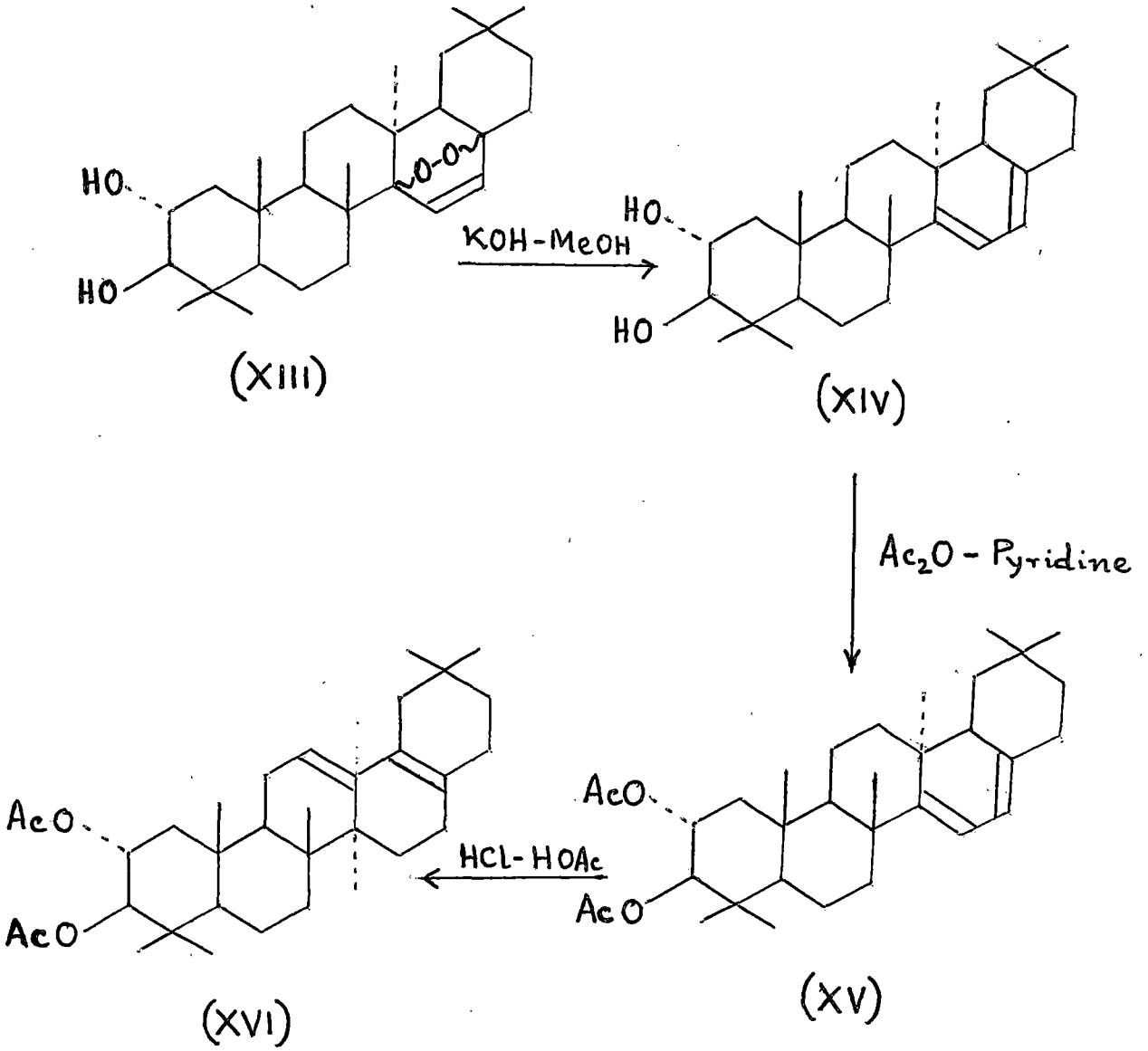
The benzene extract of the bark of Sapium baccatum Roxb. afforded the new nor-triterpene baccatin, along with taraxerone, taraxerol, 1-hexacosanol, β-sitosterol, 3, 3'-di-O-methyl ellagic acid and 3-acetoxy aleuritolic acid. From the physical and chemical data of baccatin and its various degradation products, the structure (XIII) was proposed for baccatin.



(XIII)

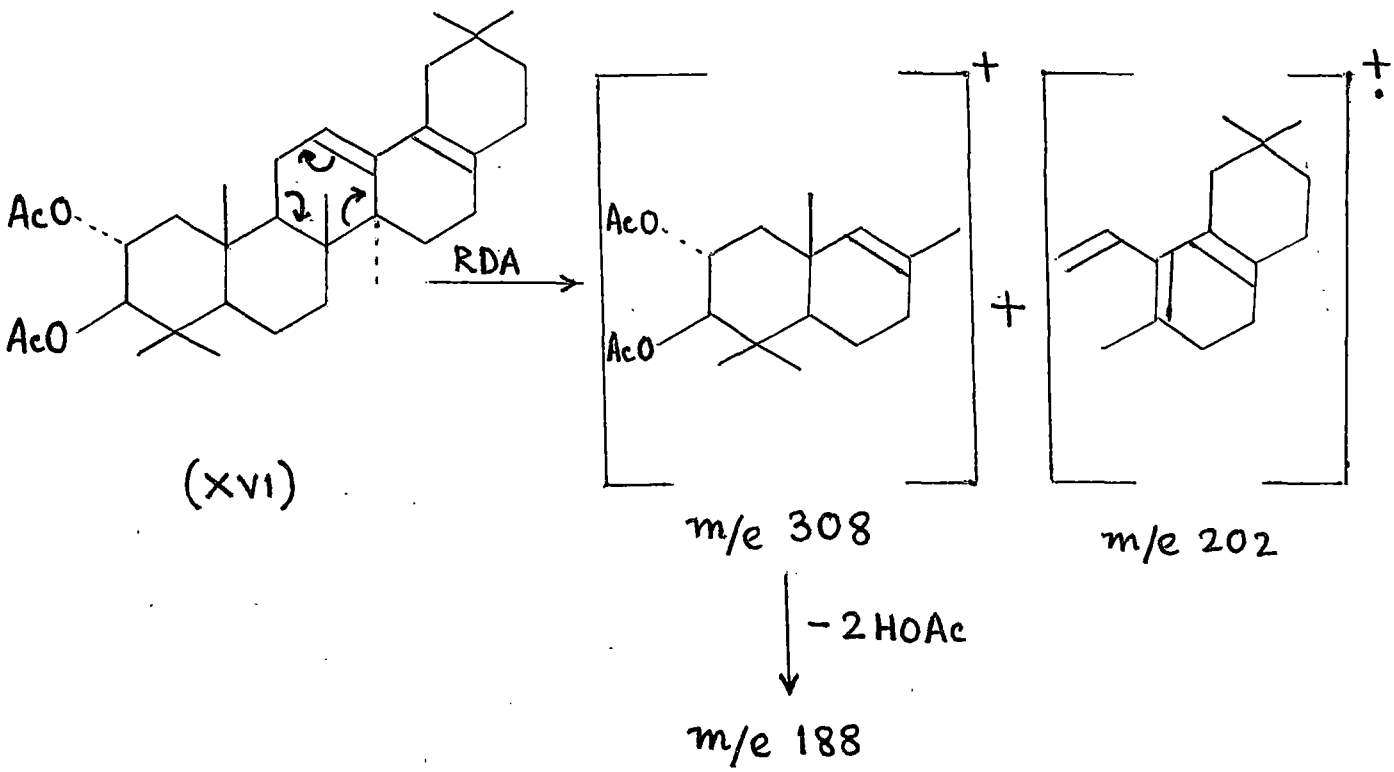
The presence of a taraxerene nucleus in baccatin was suggested mainly from biogenetic considerations; because of its occurrence with other triterpenes containing taraxerene nucleus.

Methanolic alkali treatment of baccatin afforded a homoannular diene-diol. The corresponding diacetate, on acid treatment, isomerised to a heteroannular diene-diacetate. The structure 2α, 3β-diacetoxy-28-nor-oleana-12, 17-diene (XVI) was proposed for this heteroannular diene-diacetate from physical data especially from its mass fragmentation pattern. The reactions were schematised as follows:



(XIII) (XIV) (XV) (XVI)

The mass peak at m/e 308, 202 and 188 of the heteroannular diene-diacetate was explained in the light of the following fragmentation.



From this mass fragmentation pattern, the structure (XVI) for the heteroannular diene-diacetate was established. This, in turn, confirmed the structure (XIII) for baccatin.

Chapter-II:

This chapter describes a successful partial synthesis of 2α, 3β-diacetoxy-28-nor oleana-12, 17-diene. The synthetic

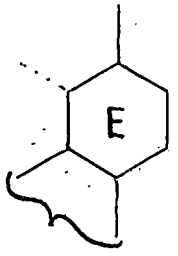
compound was shown to be identical with the heteroannular diene-diacetate previously obtained from the degradation of baccatin. This, in turn, confirmed the structure (XIII) for baccatin. This chapter was subdivided into two sections.

Section A:

This section describes the aims and objective of the present work.

A critical analysis showed that a few conclusions regarding the structure elucidation of baccatin required further confirmation.

The presence of a taraxerene nucleus in baccatin was suggested mainly from biogenetic consideration and this was supported by the structure (XVI) for the heteroannular diene diacetate obtained from it. However, all the physical and chemical data of the heteroannular diene diacetate could be explained with a fair degree of accuracy by assuming the presence of an ursane-type E ring (XVII) also in it and consequently in baccatin.



(XVII)

Furthermore, the presence of a 2α, 3β-diol system, suggested from NMR spectra, in baccatin required chemical proof.

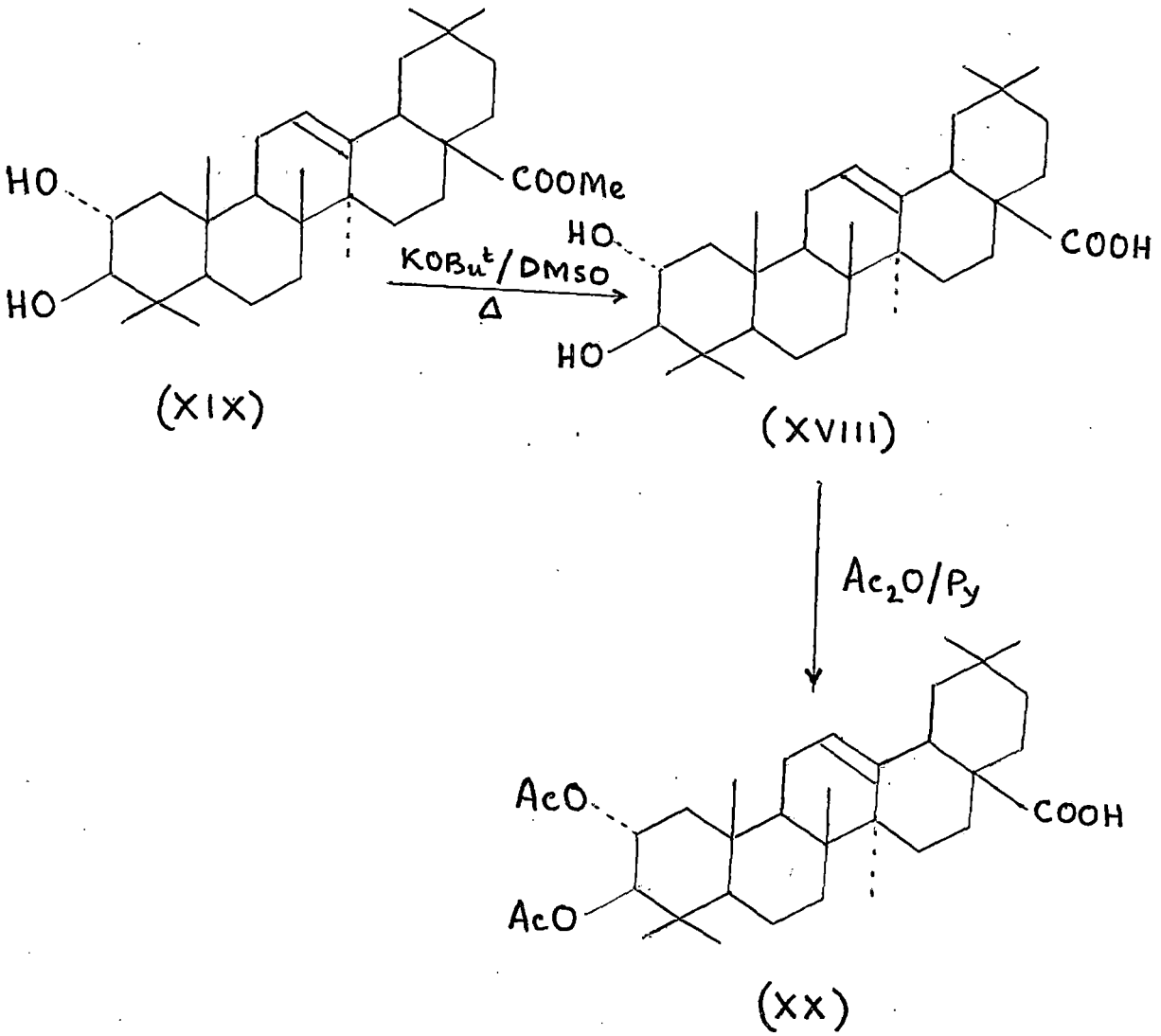
From these considerations it was thought that a partial synthesis of 2α, 3β-diacetoxy-28-nor oleana 12, 17-diene from a known triterpene, namely, crategolic acid, and subsequent demonstration of its identity with the heteroannular diene diacetate obtained from baccatin would remove the ambiguity and, in turn, would confirm the proposed structure (XIII) of baccatin.

Section B:

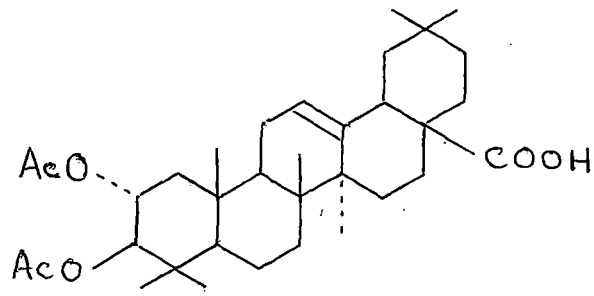
This section describes a successful partial synthesis of 2α, 3β-diacetoxy-28-mor oleana-12, 17-diene.

Benzene extract of the flowers of Eugenia Jambolana Lam was separated into acidic and neutral portions. Chromatography of the crude acid mixture gave Crategolic acid (XVIII) m.p. 266-69^{o}. The yield of pure crategolic acid by this method was very poor. The crude acid mixture on esterification followed by chromatography afforded methyl crategolate (XIX), m.p. 224-27^{o}, $(\alpha)_D$ 36^{o}. Hydrolysis of methyl crategolate with potassium tertiary butoxide in dimethyl sulfoxide gave crategolic acid identical with the acid obtained earlier. On acetylation,

crategolic acid gave crategolic acid diacetate (XX), m.p. 234-37°, $(\alpha)_D^{31°}$.

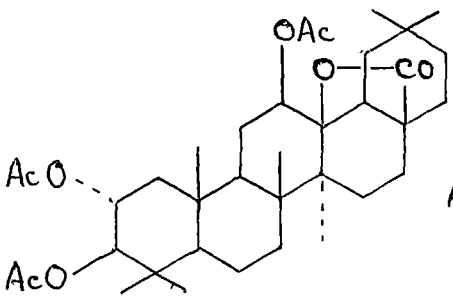


Oxidative decarboxylation of crategolic acid diacetate gave a mixture of products.

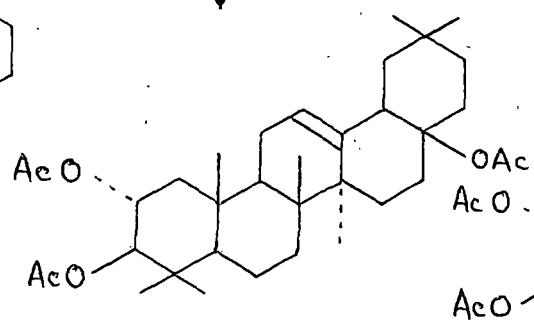


(XX)

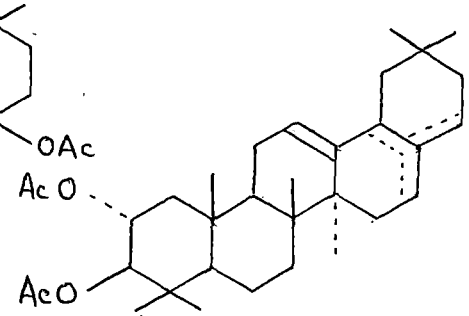
$Pb(OAc)_4 / C_6H_6 - Py / \Delta / N_2$ Atmos.



(XXI)



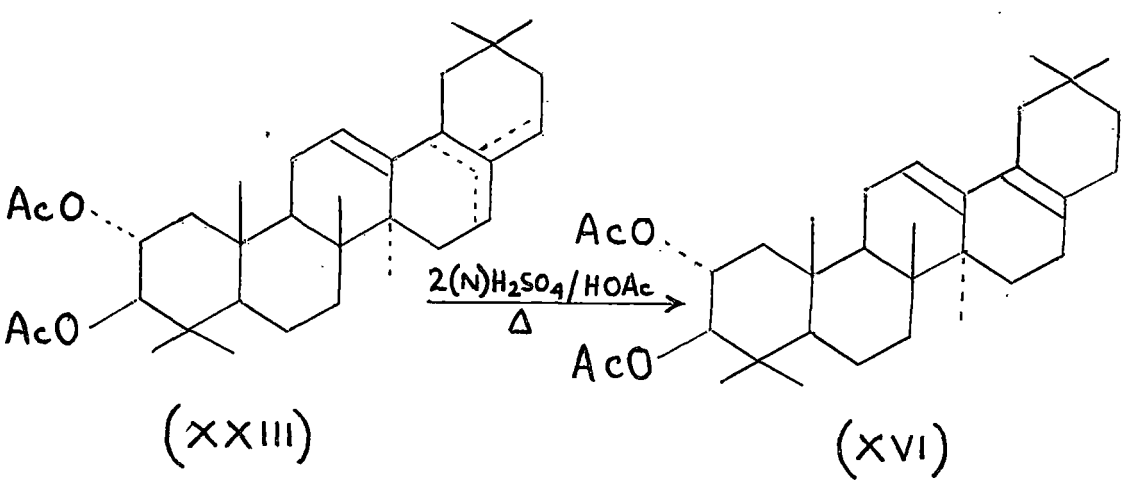
(XXII)



(XXIII)

Chromatography of the reaction product led to the successful separation of the mixture of diene-diacetates, $C_{33}H_{50}O_4$, represented by (XXIII), m.p. 114-170°, (TLC- three spots), IR ν_{max}^{nujol} 1740, 1240 cm^{-1}, UV λ_{max}^{MeOH} 237 (ε 9510), 244 (ε 10,050), 252 nm (ε 7590). The UV absorption spectra indicated that some amount of heteroannular conjugated diene was also present in the diene diacetate mixture.

On treatment with $2(N)H_2SO_4$ in acetic acid the mixture of the diene diacetates was isomerised completely into the conjugated system, 2α, 3β-diacetoxy-28-nor oleana-12, 17-diene, (XVI), m.p. 189-90°, IR ν_{max}^{nujol} 1745, 1650(W), 1255, 1220 cm^{-1}, UV λ_{max}^{MeOH} 237 (ε 27,000), 244 (ε 28,300), 252 nm (ε 20,200).



(XXIII) (XVI)

The synthetic 2α, 3β-diacetoxy-28-nor-oleana-12, 17-diene (XVI) was found to be identical in all respects with the hetero-annular diene-diacetate (XVI) previously prepared from the degradation of baccatin (XIII).

This unambiguous synthesis thus confirmed the proposed structure (XVI) of heteroannular diene diacetate obtained from baccatin and showed that it indeed contain an oleanane type E ring and a 2α, 3β-diacetoxy system. These confirmed the proposed structure (XIII) of baccatin.

Chapter-III:

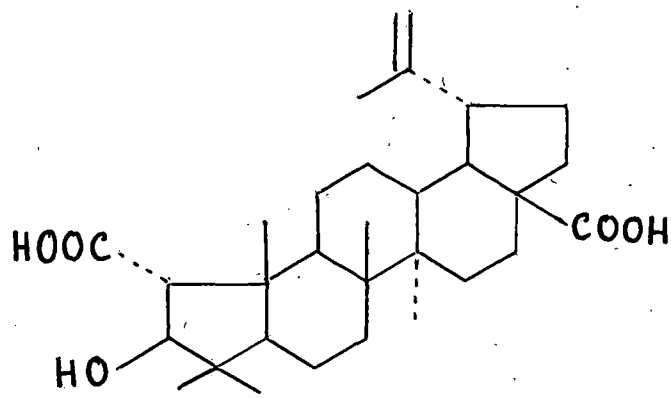
Experimental portion has been described in this chapter.

PART-III

PARTIAL SYNTHESIS OF ALL THE FOUR STEREOISOMERS OF DIMETHYL DIHYDROCEANOTHATE STARTING FROM BETULINIC ACID.

Chapter-I:

This chapter gives a short review on the isolation, structure elucidation and stereochemistry of ceanothic acid (XXIV).



(XXIV)

This chapter is divided into three sections.

Section A:

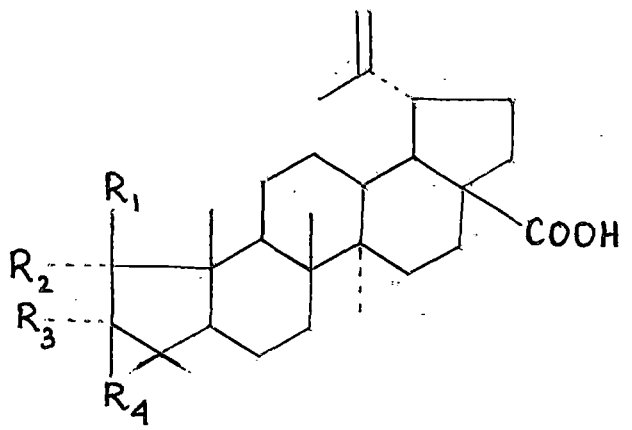
This section describes the isolation of ceanothic acid.

Section B:

This section deals with the structure elucidation of ceanothic acid.

Section C:

This section describes the establishment of the stereochemistry of ceanothic acid (XXIV) and its other three stereoisomers (XXV), (XXVI) and (XXVII).



(XXIV) $R_1 = R_3 = H$, $R_2 = COOH$, $R_4 = OH$

(XXV) $R_1 = R_4 = H$, $R_2 = COOH$, $R_3 = OH$

(XXVI) $R_2 = R_3 = H$, $R_1 = COOH$, $R_4 = OH$

(XXVII) $R_2 = R_4 = H$, $R_1 = COOH$, $R_3 = OH$

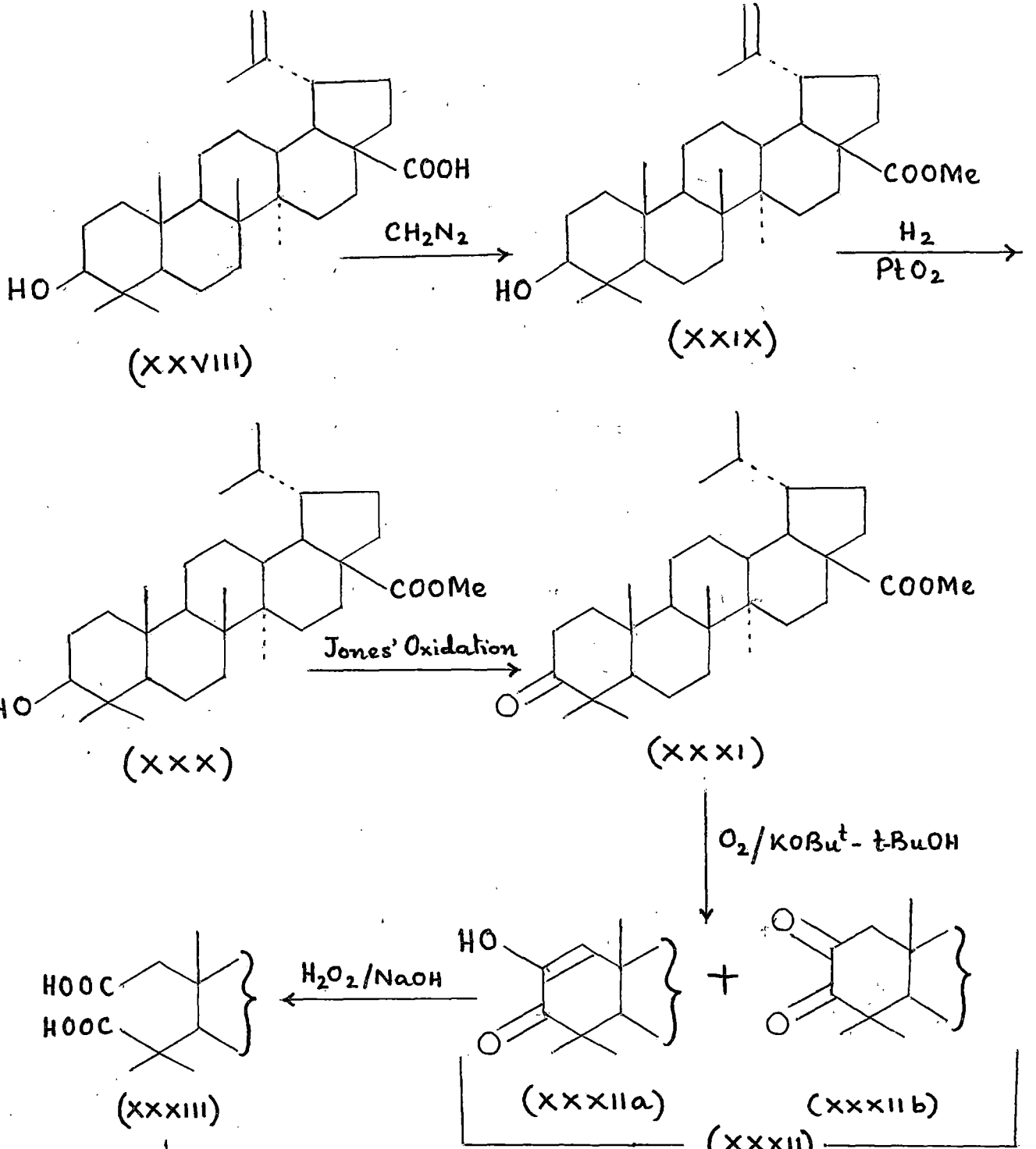
Chapter-II:

This chapter gives a short review on the previous attempts towards the partial synthesis of ceanothic acid and its stereo-isomers.

Chapter-III:

This chapter describes a successful partial synthesis of all the four stereoisomers of dimethyl dihydroceanothate starting from betulinic acid.

Betulinic acid (XXVIII) extracted from the acid part of the benzene extract of the bark of Bischofia Javonica Blume on esterification gave methyl betulinate (XXIX), m.p. 223-24°, $(\alpha)_D 5^\circ$. Hydrogenation of methyl betulinate (XXIX) afforded methyl dihydrobetulinate (XXX), m.p. 236-38°. Jones' oxidation of methyl dihydrobetulinate (XXX) afforded methyl dihydrobetulonate (XXXI), m.p. 191-93°, $(\alpha)_D$ 8°. Autoxidation of the latter by dry oxygen in presence of potassium tertiary butoxide in tertiary butanol afforded a solid, m.p. 131-33°, $(\alpha)_D$-1.96°, IR ν_{max}^{nujol} 3460, 1730, 1670, 1650, 860 cm^{-1}, UV λ_{max}^{MeOH} 269 nm (ϵ, 7532). In this solid the diosphenol (XXXIIa) was in equilibrium with the α-diketone (XXXIIb). Alkaline hydrogen peroxide oxidation of this equilibrium mixture (XXXII) afforded the A-seco acid (XXXIII), m.p. 175-77°, IR ν_{max}^{nujol} 1710 and 1680 cm^{-1}. Esterification of the A-seco acid (XXXIII) yielded the trimethyl ester (XXXIV), m.p. 146-47°, IR ν_{max}^{nujol} 1745 and 1725 cm^{-1}.



(XXVIII)

(XXIX)

(XXX)

(XXXI)

(XXXIIa)

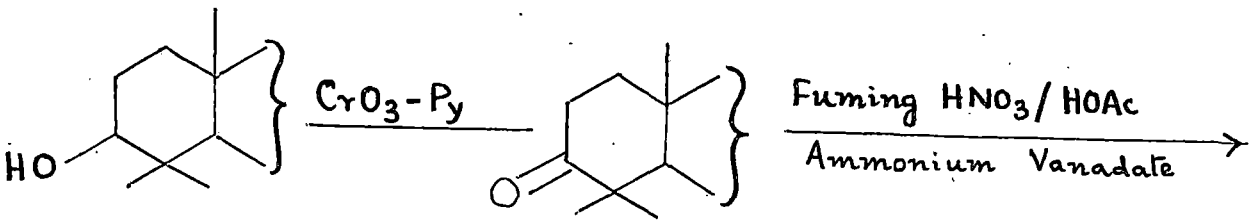
(XXXIIb)

(XXXII)

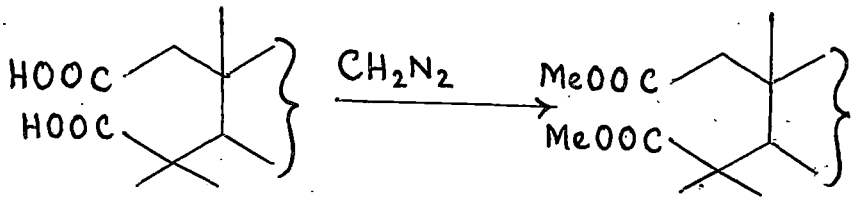
(XXXIII)

(XXXIV)

The overall yield of the trimethyl ester (XXXIV) by the above method was low. Finally a better method was also developed. Oxidation of methyl dihydrobetulinate (XXX) with anhydrous Chromium trioxide-Pyridine complex afforded methyl dihydrobetulonate (XXXI) in very good yield. Methyl dihydrobetulonate (XXXI) was directly converted into A-seco acid (XXXIII) by oxidation with fuming nitric acid in acetic acid in presence of ammonium vanadate as catalyst. Subsequent esterification of the A-seco-acid then afforded the trimethyl ester (XXXIV) in fairly good overall yield.

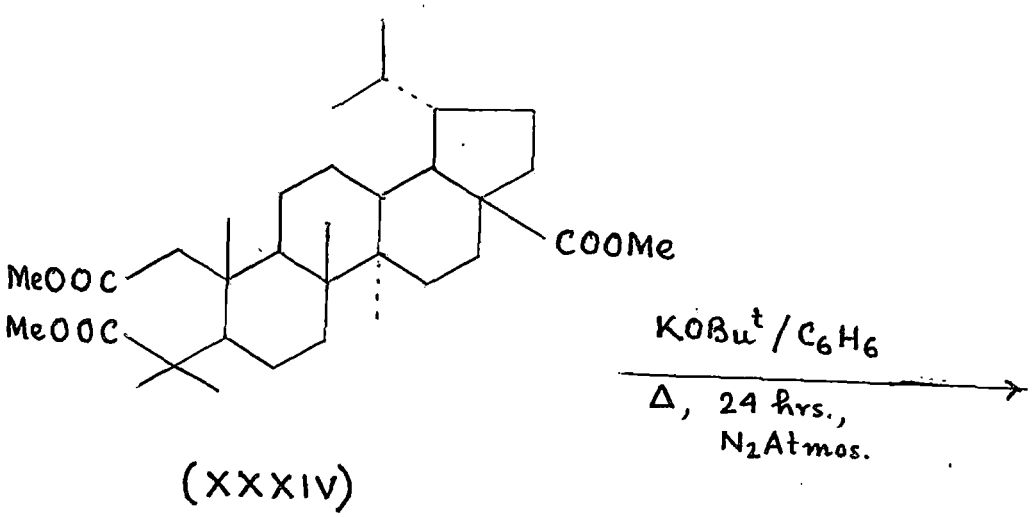


(XXX) (XXXI)

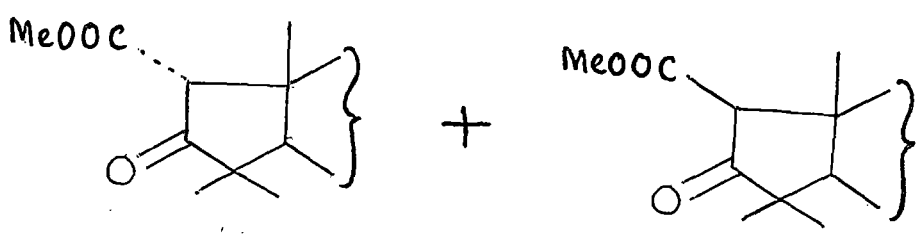


(XXXIII) (XXXIV)

Dieckmann condensation of the trimethyl ester (XXXIV) with potassium tertiary butoxide in benzene under nitrogen atmosphere followed by chromatographic separation afforded methyl-2α-methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate (XXXV), m.p. 191-93°, $(\alpha)_D$ 89°, IR ν_{max}^{nujol} 1755, 1725 cm^{-1} and its epimer methyl-2β-methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate (XXXVI), m.p. 175-77°, $(\alpha)_D$ 42°, IR ν_{max}^{nujol} 1750, 1720 cm^{-1}.



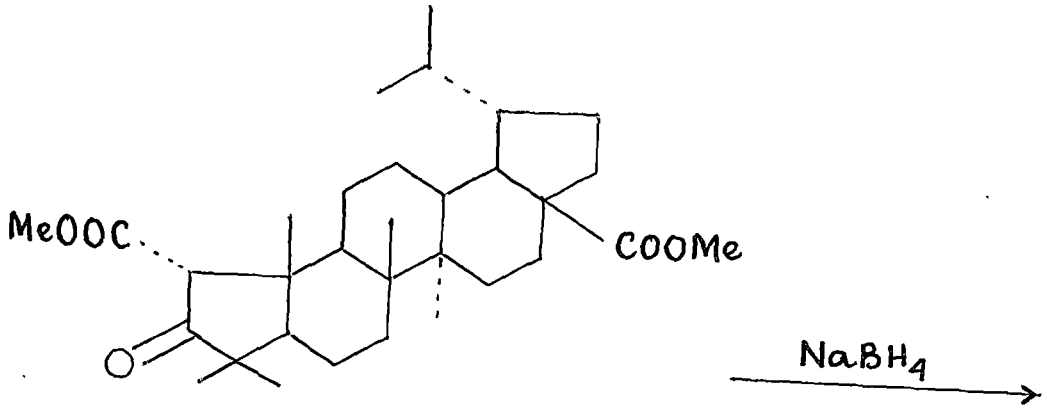
(XXXIV)



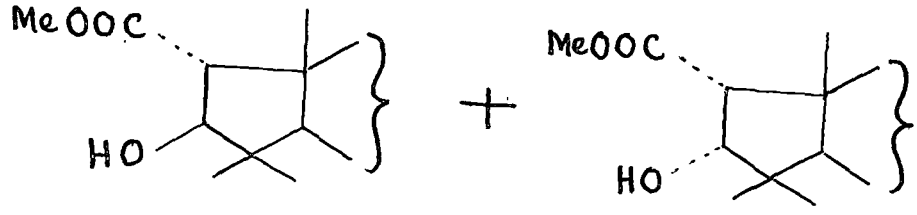
(XXXV) (XXXVI)

Sodium borohydride reduction of the β-ketoester (XXXV) in methanol-dioxan solution gave a mixture of two compounds. The

reaction product, on chromatography, first eluted a solid, m.p. 261-63°, $(\alpha)_D$ 22°, IR ν_{max}^{nujol} 3540, 1730, 1710 cm^{-1} which was found to be identical in all respects with an authentic specimen of dimethyl dihydroceanothate [methyl-3β-hydroxy-2α-methoxycarbonyl-A(1)-norlupan-28-oate] (XXXVII). Further elution with the same solvent afforded its C-3 epimer, methyl-3α-hydroxy-2α-methoxycarbonyl-A(1)-norlupan-28-oate (XXXVIII), m.p. 140-42°, IR ν_{max}^{nujol} 3560, 1745, 1705 cm^{-1}. Confirmation of this structure by NMR is in progress.



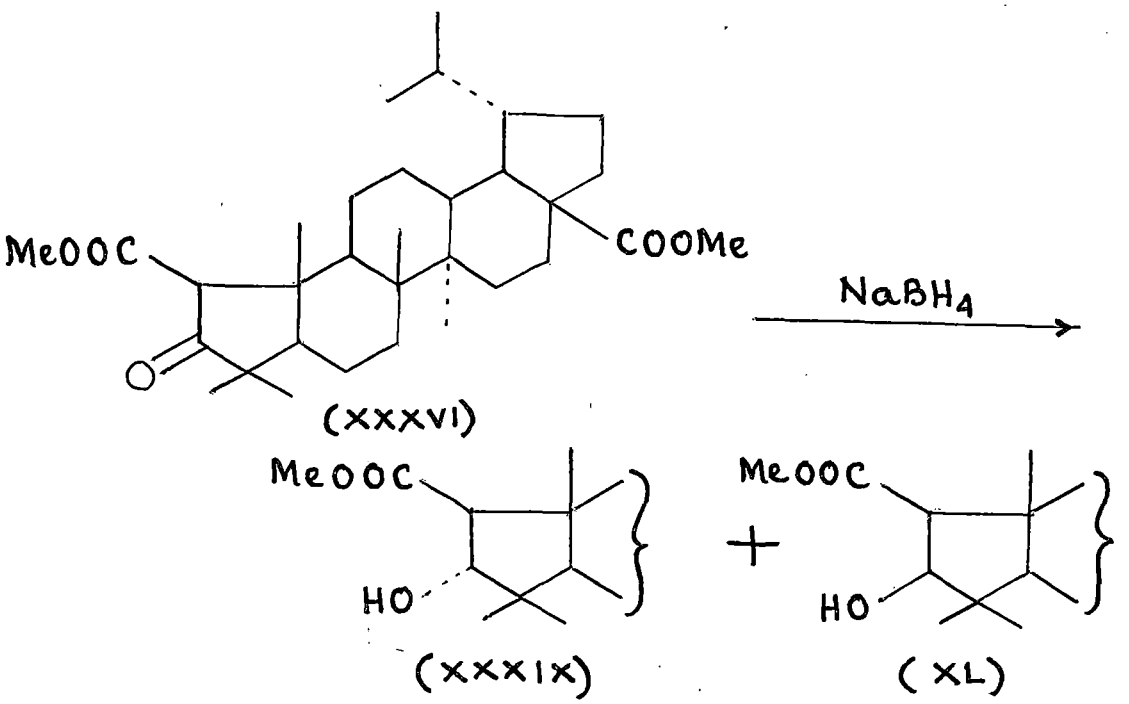
(XXXV)



(XXXVII) (XXXVIII)

Sodium borohydride reduction of the other β-ketoester (XXXVI) also gave a mixture of two compounds. Chromatography on

neutral alumina first eluted a solid A $C_{32}H_{52}O_5$, m.p. 202-203°, IR ν_{max}^{nujol} 3490, 1730, 1695 cm^{-1}, ^{1}H-NMR (80 MH_z) δ 0.7 to 1.1 (seven methyl groups), 2.1 (1H, OH), 3.06 (1H doublet, J = 7.0 H_z, CH-COOMe), 3.65 (3H singlet, $COOCH_3$), 3.7 (3H singlet, $COOCH_3$) and 4.18 (1H doublet, J = 7.0 H_z, CHOH). Further elution with more polar solvent afforded another solid B, $C_{32}H_{52}O_5$, m.p. 174-76°, IR ν_{max}^{nujol} 3540, 1740, 1690 cm^{-1}, ^{1}H-NMR (80 MH_z) δ 0.7 to 1.1 (seven methyl groups), 2.35 (1H doublet, J = 7.2 H_z, $CH-CO_2Me$), 2.8 (1H doublet, J = 4.5 H_z, OH), 3.65 (3H singlet, $COOCH_3$), 3.7 (3H singlet, $COOCH_3$), 4.02 (1H multiplet, CHOH). The above physical data indicated that the solids A and B were C-3 epimeric alcohols which fact was also evident from their methods of preparation. The assignment of conformations were done from the fact that the proton on C-2 in A was shifted downfield to δ 3.06 indicating thereby that the proton at C-2 and the hydroxyl group at C-3 in this compund A was on the same side of the ring, i.e., A was methyl-3α-hydroxy-2β-methoxy-carbonyl-A(1)-norlupan-28-oate (XXXIX). Consequently B was methyl-3β-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (XL).



Additional support for the above interpretation was found in the fact that, although the solution used for ^{1}H-NMR of (XL) was comparatively dilute, the signal for OH group was found further downfield than that in (XXXIX). This indicated the presence of an intramolecular hydrogen bond in (XL), which could only occur when the OH and COOMe groups were on the same side of the ring as in (XL). The ^{13}C-NMR spectra also supported the above structural assignments.

Chapter-IV:

Experimental portion has been described in this chapter.

C O N T E N T S

PART-II

PARTIAL SYNTHESIS OF 2α, 3β-DIACETOXY-28-NOR OLEANA-12, 17-DIENE: CONFIRMATION OF THE STRUCTURE OF BACCATIN.

PART-III

PARTIAL SYNTHESIS OF ALL THE FOUR STEREOISOMERS OF DIMETHYL DIHYDROCEANOTHATE STARTING FROM BETULINIC ACID.

PART-I

REINVESTIGATION ON THE FERN OLEANDRA NERIFOLIA CAV. HOOK: ISOLATION AND STRUCTURE ELUCIDATION OF A NEW TRITERPENE , 29- ETHOXYHOPANE.

PART-I

CHAPTER-I

Morphological Features of the Fern Oleandra nerifolia Cav. Hook.

The ferns of the genus Oleandraceae grow in the marshy hill slopes of the Eastern Himalayas, from Nepal eastwards, as well as in the Khasia range at an altitude of 2000-5000 ft[1a,1b]. This is also distributed in South India, Ceylon, Malaya, Polynesia[1a] and Central America[1a,1b]. Its sori round, inserted in a row near the base or below the centre of the compact free veinlets; involucre reniform; fronds entire lanceolate-elliptical, stems jointed, rhizome wide-creeping[1b]. This is a tropical genus of some ten species, with creeping and climbing shoots. It is usually placed in relation to Nephrolepis, and this may be accepted provisionally. It also has a kidney-shaped indusium covering sori superficially resembling those of Nephrodium[1c].

Oleandra nerifolia Cav. Hook (Syn. Oleandra neriformis Cav. Hook, Oleandra pistillaris (Sw) C. Chr[1d]):

Shoots woody, wide-creeping but often suberect, clothed with short adpressed scales which are often deciduous; stipes short, seldom 1 inch long, with the joint below the middle; fronds 4-8 inches long, ½ - 1½ inch broad, in opposite pairs or

often in terminal whorls, or more rarely scattered, from narrow-linear to oblong-acuminate; texture subcoriaceous, both sides glabrous or hairy underneath, sori in two rather irregular rows near the midrib[1b]. A scandent fern, when well developed with pendent main stem, the lateral stipe-bearing spurs grow upwards[1a].

CHAPTER-II

Section A: A Short Review on the Chemical Constituents of Ferns of Oleandraceae Family.

In recent years extensive works have been done in the field of Phytochemistry mainly due to two factors: (a) the development of modern techniques for structural investigations and (b) the potential usefulness of plants as a source of new therapeutic agents. But only a very limited studies have so far been done on the class of ferns. Of the fourteen families, with the exception of some genera of Polypodiaceae, very few individuals have been investigated by the Phytochemist. Some families, such as, Dipteridaceae and Salviniaceae are completely unexplored from the chemical point of view. However, recent discoveries of many interesting class of compounds such as new acylphlorglucinol derivatives and triterpenoids, indicate that the phytochemical investigation of ferns could be of great value both to the taxonomist, as an aid of classification, and to the natural product chemist in his search for new class of compounds with novel structures.

Only two species of the family Oleandraceae have so far been investigated. In this section is described the previous work done on the Oleandraceae family. It presents an up-to-date description of all organic compounds isolated from them . The work

on Oleandraceae family was mainly initiated by Pandey and Mitra.

Oleandra Wallichi (Hook) Pr.

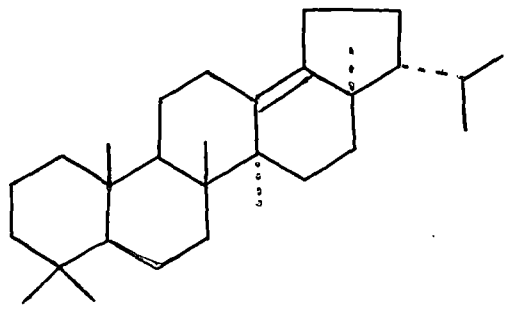
In 1967, Pandey and Mitra[2] reported the isolation of two triterpenic hydrocarbons from Oleandra Wallichi.

The residues obtained from the benzene extract of the rhizomes of O. Wallichi were partitioned between dilute alcohol (85%) and n-hexane. The n-hexane soluble fraction on chromatography gave two triterpene hydrocarbons, Wallichiene and Wallichienene.

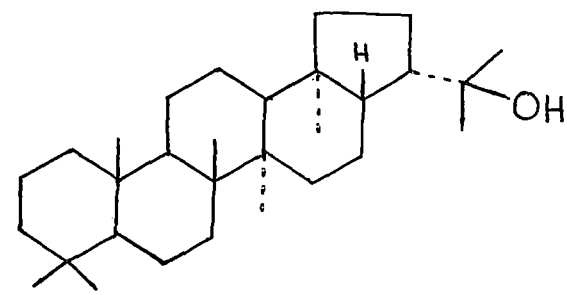
The hydrocarbon Wallichiene, $C_{30}H_{50}$, m.p. 196-97°, $(\alpha)_D 0°$, gave positive Libermann-Burchard, Noller's and tetranitromethane tests. The IR spectra showed bands at 1381 and 1370 (gem-dimethyl group) 1210 and 1195 (quaternary gem-dimethyl group) and 1175 and 1149 cm^{-1} (isopropyl group). The NMR spectra showed signals for eight quaternary methyl groups at τ 9.2, 9.15, 9.11, 9.00 and 8.90 but no signal for olefinic protons. The IR spectra also indicated the absence of a trisubstituted double bond in Wallichiene. Wallichiene was subsequently shown to be identical with hopene-II, (1) prepared previously[3] from hydroxy hopane (2) by dehydration and isomerisation. This structure (1) of Wallichiene was confirmed by physical and chemical evidences.

The hydrocarbon Wallichienene, $C_{30}H_{48}$, m.p. 210-12°, $(\alpha)_D$ 42° gave positive Libermann-Burchard, Noller's and tetranitromethane tests. The IR spectra showed bands at 795 and 780 cm^{-1}

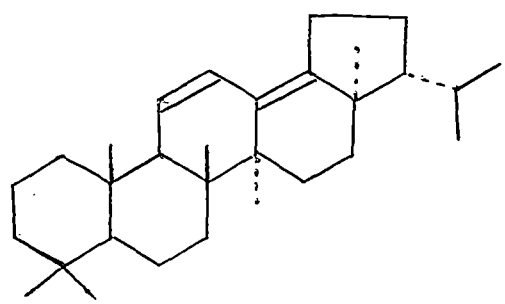
(di- or tri- substituted double bond). The characteristic UV absorption at 246 (log ε 4.37), 256 (log ε 4.43) and 265 nm (log ε 4.33) indicated the presence of a conjugated heteroannular diene system. Wallichienene, on hydrogenation, gave a dihydro compound $C_{30}H_{50}$, m.p. 196-97°, $(\alpha)_D$ 0° which was shown to be identical with Wallichiene or hopene-II, (1). Wallichienene was finally identified as neo-hopa-11,13(18)-diene[3,4], (3)



(1)



(2)



(3)

Though Wallichiene (hopene-II), (1), and Wallichienene [neo-hopa-11,13(18)-diene] (2), were prepared previously in the course of studies in the chemistry of related triterpenoids[3,4], Pandey and Mitra[2] reported their isolation for the first time from nature. The occurrence of pentacyclic triterpenoids with heteroannular conjugated diene system in nature is comparatively rare; however, a few such dienes have recently been reported[5]. Wallichienene was the first triterpene diene being isolated from a fern.

Oleandra nerifolia

Pandey and Mitra, in 1967, reported the isolation and structure elucidation of a new triterpene alcohol, nerifoliol, from the rhizomes of another fern oleandra nerifolia[6] [syn. oleandra pistillaris (Sw)C.Chr.]. Their works are described below.

The rhizomes of the fern O.nerifolia were extracted with benzene. The residue obtained from the neutral part was chromatographed over alumina. The n-hexane-benzene eluent yielded nerifoliol, $C_{30}H_{52}O$, m.p. 242-44°, $(\alpha)_D^{35°}$ (M^+428) which gave positive Libermann-Burchard test but negative test with tetranitromethane thus demonstrating that it might be a completely saturated triterpene. In addition, it was transparent in the

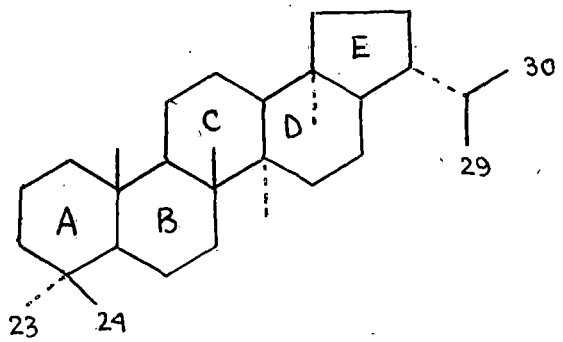
region 200 to 300 nm. The IR spectra showed bands at 3330 cm^{-1} with a supporting band at 1050 cm^{-1} indicating the presence of a primary hydroxyl function. The combination of peaks at 1390 and 1370 cm^{-1} indicated the presence of gem-dimethyl groups. The NMR spectra of the alcohol showed methyl signals at 44(3H), 49.50 (6H), 51.5(6H) and 58 (6H) cps, all corresponding to methyl groups.

On treatment with acetic anhydride and pyridine nerifoliol gave a monoacetate, $C_{32}H_{54}O_2$, m.p. 195-96°, $(\alpha)_D$ 20°, IR ν_{max}^{KBr} 1730, 1235 cm^{-1} (acetate). On oxidation with anhydrous chromium trioxide and pyridine nerifoliol furnished an aldehyde, nerifolial, $C_{30}H_{50}O$, m.p. 76°, IR ν_{max}^{KBr} 1730 cm^{-1}. Both nerifoliol and nerifolial, on oxidation with CrO_3 in acetic acid and benzene yielded nerifolic acid, $C_{30}H_{50}O_2$, m.p. 270-74°, $(\alpha)_D$ 16°, IR $_{max}^{KBr}$ 1730 cm^{-1} with an indefinite shoulder in the region 3571 to 3077 cm^{-1}. The corresponding methyl ester, methyl nerifoliate, $C_{31}H_{52}O_2$, m.p. 242-44°, $(\alpha)_D$ 8°, IR $_{max}^{KBr}$ 1730 cm^{-1} on reduction with lithium aluminium hydride in tetrahydrofuran gave back the original alcohol, nerifoliol.

From the above findings along with the biogenetic and chemotaxonomic considerations[7] Pandey and Mitra suggested[6] that nerifoliol was a pentacyclic triterpene alcohol having a hopane or modified hopane skeleton with a primary hydroxyl group. The presence of the primary hydroxyl group was also evidenced by the

NMR spectra of nerifoliol. The broad signal at τ 6.4 corresponding to two protons was attributed to the two protons of the $-CH_2OH$ group. Furthermore, this signal was resolved in two AB quartets (J = 11 cps) indicating that the carbon atom to which the group CH_2OH is bonded is asymmetric.

Nerifoliol when subjected to Huang-Minlon modification of W.K. reduction gave a hydrocarbon, $C_{30}H_{52}$, m.p. 190-92°, $(\alpha)_D$ 36°, which was shown[6] to be identical in all respects with hopane[3] (4). Therefore, Pandey and Mitra[6] concluded that the carbon skeleton of nerifoliol was the same as that of hopane. The nature of the hydroxyl group (primary) suggested that it was attached either to the gem-dimethyl or angular methyl groups or side chain.

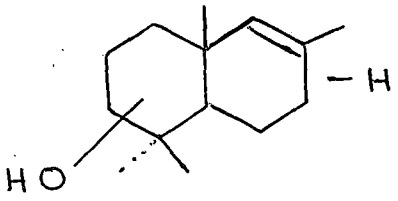


(4)

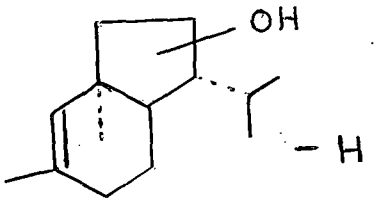
The relative ease with which methyl nerifoliate was hydrolysed (8% alcoholic Potassium hydroxide for 6 hours) excluded any of the angular positions as the possible site for the carboxyl group in nerifolic acid as there was no activating group present in the molecule. Evidently, Pandey and Mitra[6] suggested that C-23, 29 or 30 could be the possible positions for the carboxyl group as in case of medicagenic and desoxoglycyrrhetic acids[8,9]. The position C-24 (axial) was also excluded as the methyl ester would then be hydrolysed with great difficulty (cf. β-boswellic acid). They[6], therefore, concluded that the primary hydroxyl group in nerifoliol was attached to C-23, 29 or 30.

From a study of the mass fragmentation pattern of nerifoliol, Pandey and Mitra[6] concluded that the primary hydroxyl group was situated in the isopropyl side chain. The mass spectrum gave molecular ion peak, M^+ at m/e 428. The peaks at m/e 413 and 369 were due to the fragments formed from the molecular ion by the loss of 15 and 59 mass units. These were attributed to the ions formed by the loss of a methyl group (CH_3) and the isopropyl side chain carrying the hydroxyl function (C_3H_7O) respectively. Since there was no loss of 31 mass units (CH_2OH) to start with the mass spectra firmly excluded the possibility of the hydroxyl group being attached to C-23 or C-24 or any angular methyls and confirmed its presence at C-29 or C-30. The splitting of ring C gave rise to the fragments having m/e 207 and 191. The peak at

m/e 207 might belong to either to the left or right side of the molecule having oxygen in ring A (5) or ring E (6) or in the side



(5)



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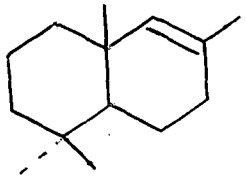

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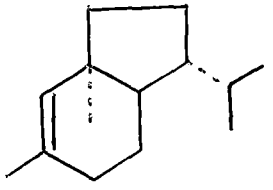
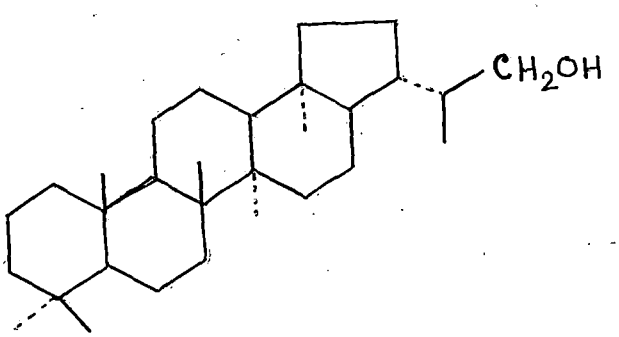


(8)

chain as shown. Similarly the peak at m/e 191 might belong to the left or right side of the molecule (7) or (8). That the succeeding lower mass peak at m/e 149 arose from the fragment

m/e 207 with the loss of 58 mass units was confirmed by the appearance of a metastable peak at m/e 107.2 (Calculated value 107.3) which was attributed to the loss of C_3H_6O unit, that is, the isopropyl side chain having the hydroxyl function, under transfer of one hydrogen. Thus the mass spectra of nerifoliol strongly favoured the position C-29 (or C-30) for the primary hydroxyl function in a hopane nucleus. From the above physical and chemical evidences Pandey and Mitra[6] assigned the structure (9) for nerifoliol.

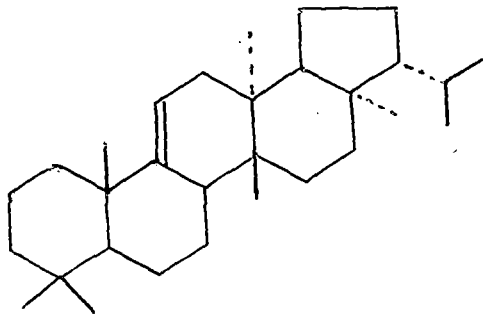


(9)

Incidentally, nerifoliol was the first triterpene primary alcohol isolated from a fern.

Section B: A Short Review on the Structure of Dryocrassol Isolated from the Aspidiaceous Fern.

In 1963, Ageta et al[10,11] reported the isolation of two triterpenoid hydrocarbons, fernene (10) and diploptene (hopene-b) (11) from the leaflets of the fern Dryopteris Crassirhizoma NAKAI (Aspidiaceae).



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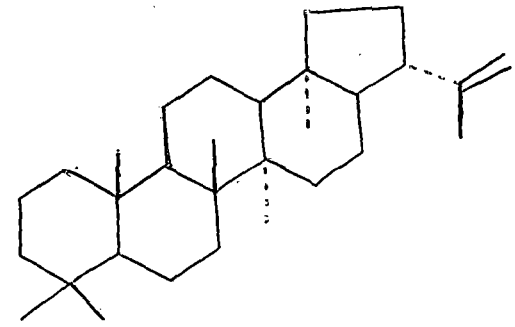
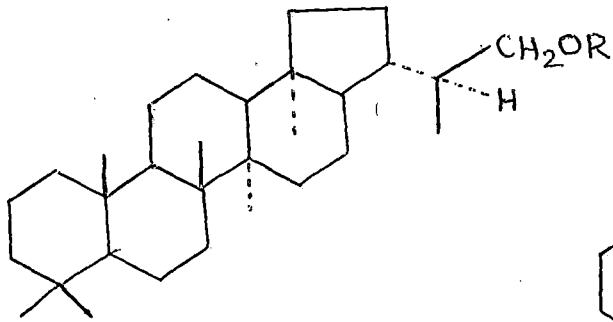


(11)

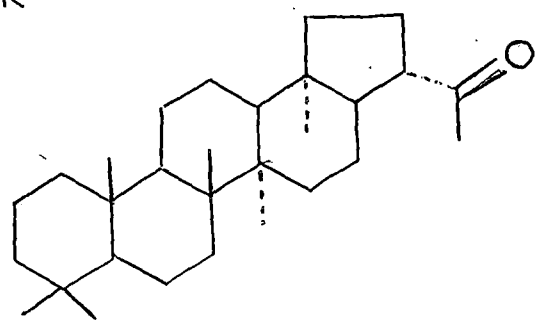
Further investigations on the triterpenoids from the same source were carried out by Ageta et al. They reported[12] the isolation of a new alcohol and its acetate, dryocrassol (12a)

and dryocrassol acetate (12b) along with 22-hydroxyhopane[13] (2), adiantone[14] (13), ferna-7, 9(11)-diene (14), fern-9(11)-ene-12 one[10,11] (15) and a sterol mixture. Dryocrassol acetate (12b) was also isolated from the leaves of Arachniodes standisii OHWI and Polystichum polyblepharum PR as the main triterpenoid constituent.

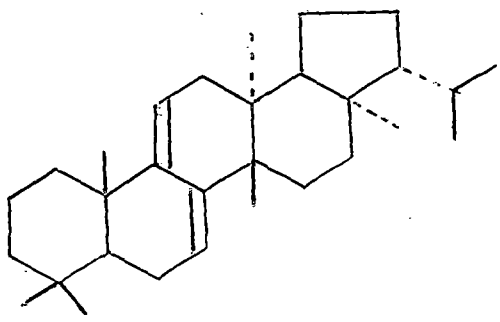


(12a) R = H

(12b) R = $COCH_3$



(13)



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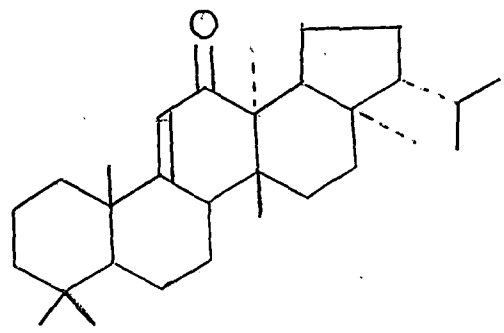
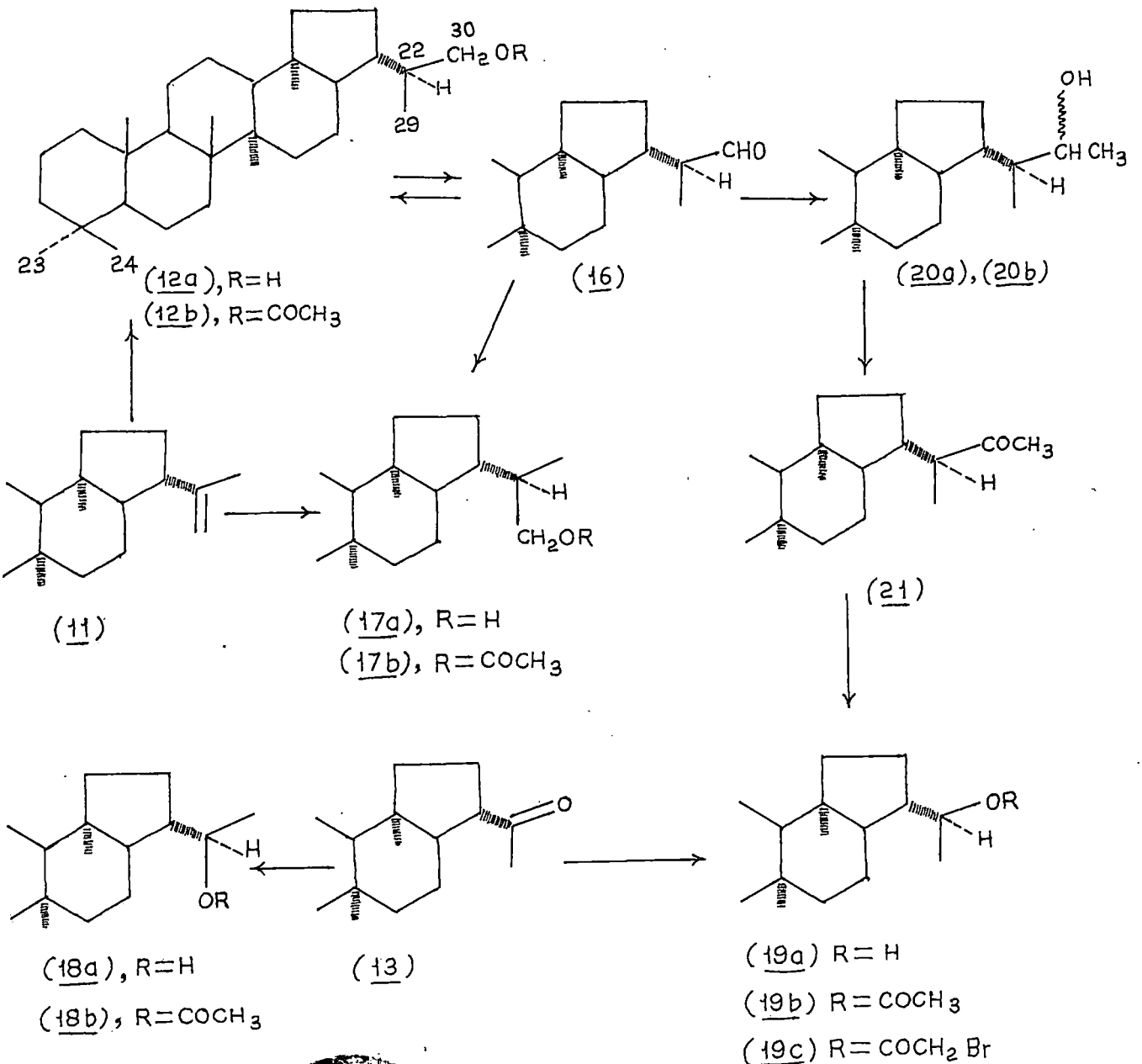


(15)

Dryocrassol (12a), $C_{30}H_{52}O$, m.p. 245-47°, $(\alpha)_D^{68°}$, IR ν_{max}^{KBr} 3330, 1026 cm^{-1} gave the acetate (12b), $C_{32}H_{54}O_2$, m.p. 196-98°, $(\alpha)_D^{58°}$, IR ν_{max}^{KBr} 1729, 1226 cm^{-1}. The NMR spectrum of dryocrassol showed signals each corresponding to three hydrogens at τ 9.15 (C-23), 9.21 (C-24), 9.19 (C-25), 9.04 (C-26), 9.04 (C-27), 9.27 (C-28), 8.97 (doublet, J = 6.5 Hz, C-29) and a multiplet corresponding to two hydrogens at τ 6.5. The mass spectrum of dryocrassol showed peaks at m/e 428 (M^+, 5%), 413 (2%), 369 (7%), 207 (100%) and 191 (64%). These NMR and mass spectra and also the same of dryocrassol acetate led Ageta et al[12] to suggest that dryocrassol was a triterpenoid of the hopane skeleton having a primary alcohol group in the side chain.

Chromic acid oxidation of dryocrassol in pyridine gave an aldehyde (16), m.p. 184-87°, $(\alpha)_D^{60°}$, IR ν_{max}^{KBr} 2700, 1725 cm^{-1} which was reduced into only dryocrassol (12a) with lithium aluminium hydride and into hopane[15] (4) by Wolff-Kishner method. Boiling of the aldehyde (16) with 5% methanolic potassium hydroxide afforded[12] unexpectedly a mixture of two alcohols, (12a) and (17a). The latter (17a), m.p. 242-44°, $(\alpha)_D^{35°}$ gave an acetate (17b), m.p. 214-16°. The alcohol (17a) was shown to be identical in all respects with nerifoliol[6]. Hydroboration of hop-22(29)-ene (11) gave also a 1:1 mixture of nerifoliol (17a) and dryocrassol (12a). Consequently, Ageta et al[12] concluded that either nerifoliol (17a) or dryocrassol (12a) should be hopane-29 (or 30)-ol having an epimeric centre at C-22.

Chart-1



Reduction of adiantone (13) with lithium aluminium hydride gave two isomeric alcohols adiantol A (less polar) (18a) m.p. 211-13°, $(\alpha)_D 40°$ [acetate (18b), m.p. 205-7°, $(\alpha)_D 35°$] and adiantol B (more polar) (19a), m.p. 252-56°, $(\alpha)_D 76°$ [acetate (19b), m.p. 222-24°, $(\alpha)_D 55°$]. The absolute configuration at C-22 of the latter alcohol (19a) was proved to be 22S by X-ray analysis of the corresponding bromoacetate[16] (19c). Grignard reaction of (16) with methyl magnesium iodide gave a mixture (1:1) of two alcohols (20a), m.p. 250-54°, IR ν_{max}^{KBr} 3430, 1127 cm^{-1} and (20b), m.p. 255-58°, IR ν_{max}^{KBr} 3500, 1090 cm^{-1} epimeric at C-30. Chromic acid oxidation of (20a) or (20b) in pyridine afforded the same methyl ketone (21), m.p. 239-42°, $(\alpha)_D 43°$, IR ν_{max}^{KBr} 1713 cm^{-1}. Baeyer-Villiger oxidation of the ketone (21) with perbenzoic acid yielded the acetate of an alcohol. This acetate was proved[12] to be identical with adiantol B acetate (19b). Ageta et al[12], therefore, concluded that the configuration at C-22 of (21), (20a), (20b), (16), (12a) and (12b) was 22S and that of (17a) and (17b) was 22R as shown in Chart-I. These workers also proposed the numbering of the side chain on the hopane skeleton. Thus nerifoliol (17a) was hopan-29-ol and dryocrassol (12a) was hopan-30-ol.

CHAPTER-III

Reinvestigation on the Neutral Part of the Benzene Extract of the Fern Oleandra nerifolia: Isolation of a New Triterpene 29-Ethoxyhopane, $C_{32}H_{56}O$, along with Filicene, Nerifoliol and β-Sitosterol.

Section A: Extraction:

Dried and powdered rhizomes of the fern Oleandra nerifolia (syn. Oleandra pistillaris) was extracted with benzene in a soxhlet apparatus for 20 hours. The gummy solid residue obtained after the evaporation of benzene was taken up in ether. The ether solution was washed with 10% aqueous sodium hydroxide solution and then with water till neutral and dried over anhydrous sodium Sulphate. Removal of ether gave a gummy residue which was chromatographed as diseussed in Section B.

Section B: Chromatography of the neutral part.

Table-1

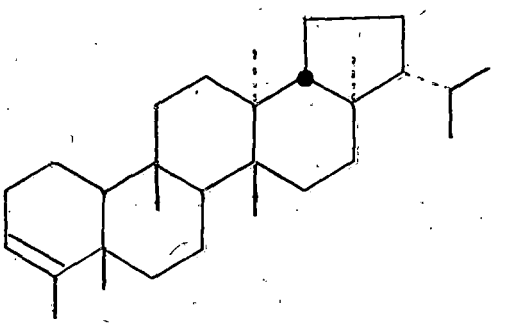
Fraction No.	Eluent	Eluate	Melting point of the residue in °C
1.	Petrol	Solid with oil	-
2.	Petrol: Benzene (3:2)	Solid (0.5 gm)	236-40°
3.	Petrol: Benzene (2:3)	Solid (1.0 gm)	130-34°

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

Section C: Examination of Fractions 1-3:

Fraction No. 1: Isolation of a New Triterpene, 29-Ethoxyhopane, $C_{32}H_{56}O$ and Filicene:

The fraction No. 1 (Table-1) on careful rechromatography over activated alumina afforded a waxy solid. The waxy solid on crystallisation from a mixture of chloroform and acetone furnished a solid $C_{30}H_{50}$, m.p. 226-28°, $(\alpha)_D 50°$, which was found to be identical (mmp, IR and TLC) with an authentic specimen of filicene (22).



(22)

The mother liquor from the crystallisation of filicene was found by TLC on 12% silver nitrate impregnated silica gel plate to be a mixture of three components. Fractional crystallisation of the residual solid from the mother liquor from a mixture

of chloroform and methanol (3:1) furnished a solid, m.p. 179-80°, $(\alpha)_D 27.16^\circ$. This compound was found to be a novel triterpene, $C_{32}H_{56}O$, namely, 29-ethoxyhopane. The chemistry and structure elucidation of this compound has been described in Chapter-IV.

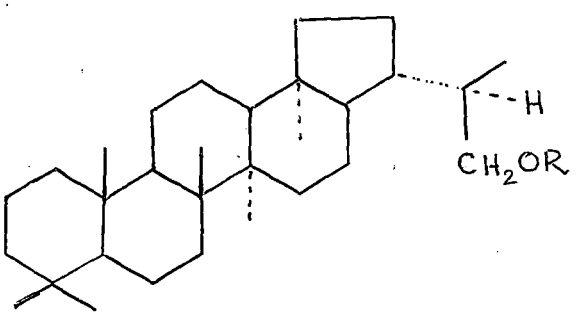
The residue from the final mother liquor after the separation of the above novel triterpene was rechromatographed over alumina impregnated with 20% silver nitrate. Elution of the column with petrol first gave a solid, which on crystallisation from a mixture of chloroform and methanol afforded a solid, m.p. 182-83°. The elemental analysis of this solid corresponded to the molecular formula $C_{30}H_{50}O$. Further elution of the column with petrol afforded another solid, which on crystallisation from a mixture of chloroform and methanol gave another solid, m.p. 163-64°. Elemental analysis suggested the molecular formula as $C_{30}H_{50}$. The structure elucidations of these two compounds were not possible because of their very poor yield. Further work is in progress to isolate them in quantity to enable us to investigate their structures.

Fraction No. 2: Isolation and Identification of Nerifoliol:

Rechromatography of the fraction No. 2 (Table-1) over a column of active alumina and elution with a mixture of petrol and benzene (2:3) gave an alcohol, $C_{30}H_{52}O$, m.p. 242-44°, $(\alpha)_D 35^\circ$, (M^+428), IR ν_{max}^{nujol} 3320 cm^{-1}.

On acetylation, the alcohol furnished an acetate, $C_{32}H_{54}O_2$, m.p. 195-96°, $(\alpha)_D^{20°}$, IR ν_{max}^{nujol} 1730, 1225 cm^{-1}.

The physical and chemical data of the alcohol and its acetate showed that they were identical with nerifoliol (hopan-29-ol) (17a), isolated by Pandey and Mitra[6] from the same plant Oleandra nerifolia, and its acetate (17b) respectively.



(17a) R = H

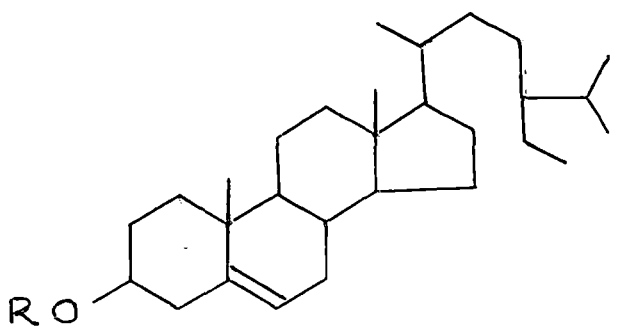
(17b) R = $COCH_3$

Fraction No. 3: Isolation and Identification of β-Sitosterol:

Fraction No. 3 (Table-1) on rechromatography over a column of active alumina and elution with a mixture of petrol and benzene (1:4) gave a solid, which on crystallisation from a mixture of chloroform and methanol furnished fine needle shaped crystals of an alcohol, $C_{29}H_{50}O$, m.p. 136-37°, $(\alpha)_D$-32°.

On acetylation it gave an acetate, $C_{31}H_{52}O_2$, m.p. 127-29°, $(\alpha)_D$-40°.

The alcohol and its acetate were identified as β-sitosterol (23a) and β-sitosteryl acetate (23b) respectively by direct comparison (m.m.p., IR and Co-TLC) with their respective authentic specimens.



(23a) R = H

(23b) R = $COCH_3$.

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:

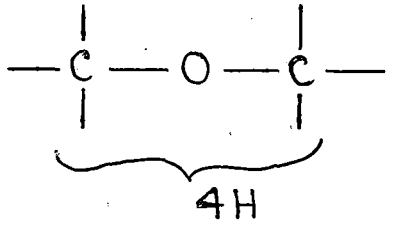
Fraction No. 1 (Chapter-III, Section B, Table-1) on careful chromatography followed by fractional crystallisation from a mixture of chloroform and methanol (3:1) furnished a crystalline solid, m.p. 179-80°, $(\alpha)_D^{27}.16^\circ$ (Chapter-III, Section C). The solid gave a positive Libermann-Burchard 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 δ 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 δ 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).



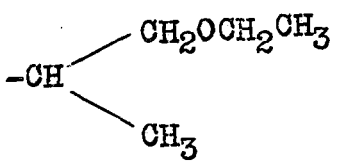
(24)

Nature of the skeleton of the Triterpene.

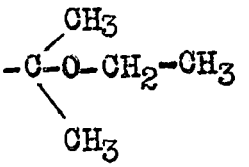
The number of rings in the triterpene was established as follows. The triterpene, $C_{32}H_{56}O$ did not contain any unsaturated linkage and the oxygen atom was present as an ether linkage. Hence writing CH_2 for O, the molecular formula of the parent saturated hydrocarbon came out to be $C_{33}H_{58}$.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



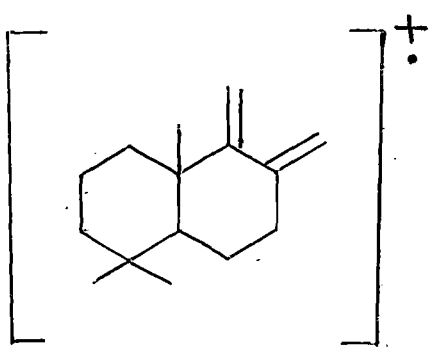
(25a)



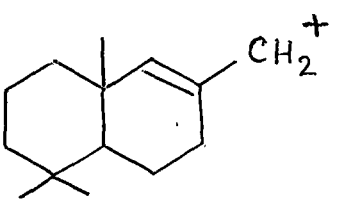
(25b)

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.



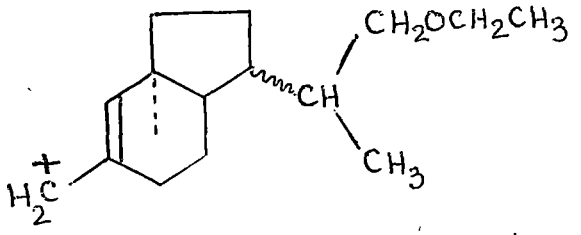
(26)



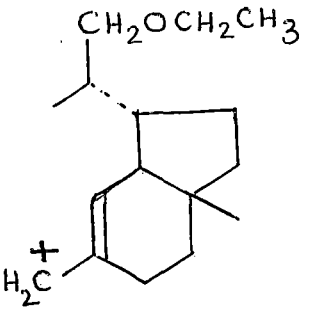
(27)

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.



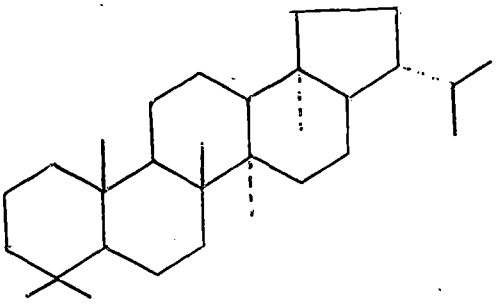
(28)



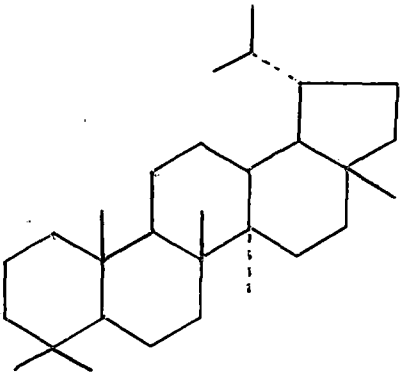
(29)

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.

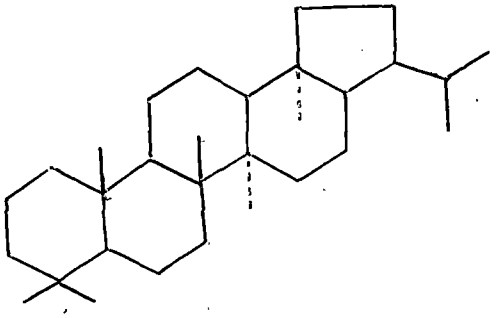
The mass spectrum therefore indicated the presence of the ethoxy function in the isopropyl side chain (the grouping like 25a) in a hopane or lupane type of skeleton. Though the mass spectrum closely corresponded to the presence of a hopane type nucleus (4), the presence of a lupane (30) or isohopane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



(4)



(30)

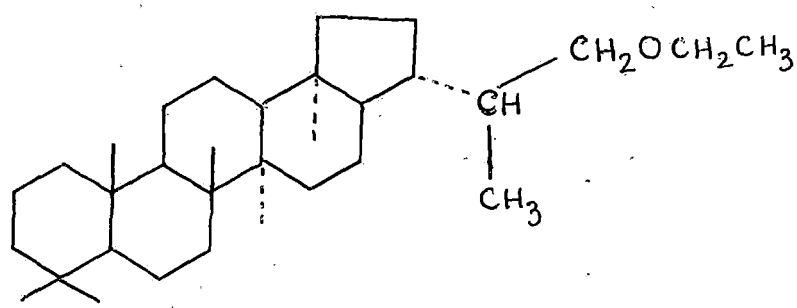


(31)

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.

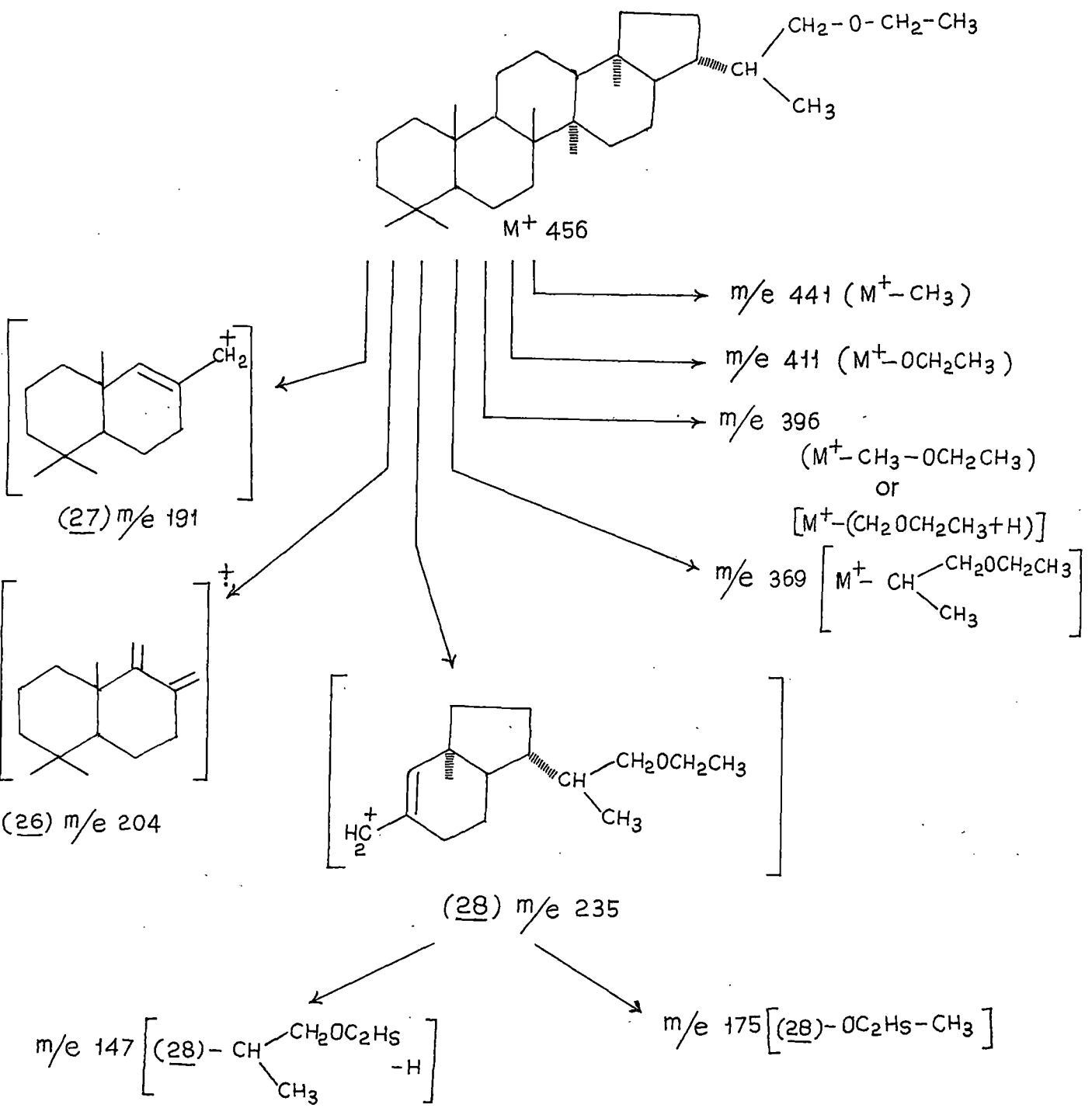


(32)

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)

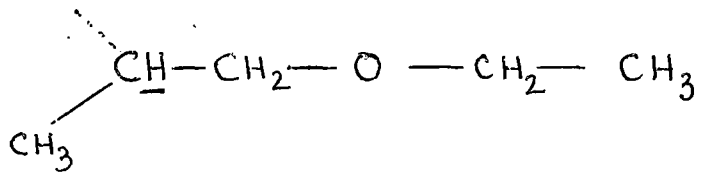


Detailed study of the NMR spectra (Fig. 3)

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.

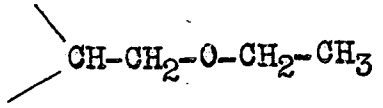
A better analysis could be possible on the basis of the plot expansion (Fig. 4). The $-CH_2-$ of the ethyl group ($-O-C\underline{H}_2-CH_3$) showing signal at δ 3.4 was a quartet as would be expected. The two protons of the other CH_2 group ($\overset{|}{C}H-C\underline{H}_2-O-C_2H_5$) were non equivalent, due to the adjacent asymmetric carbon atom (C-22). One of them showed signals near δ 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 δ 1.175 was attributed to the protons of the methyl group ($-O-CH_2-C\underline{H}_3$). This methyl triplet at δ 1.175 was irradiated and decoupled from the adjacent CH_2 group, which collapsed to a single line at δ 3.425, and partially revealed the other proton of the non-equivalent pair of the CH_2 protons. The result of this irradiation at δ 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 ν = 4247 H_z corresponding to δ 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)

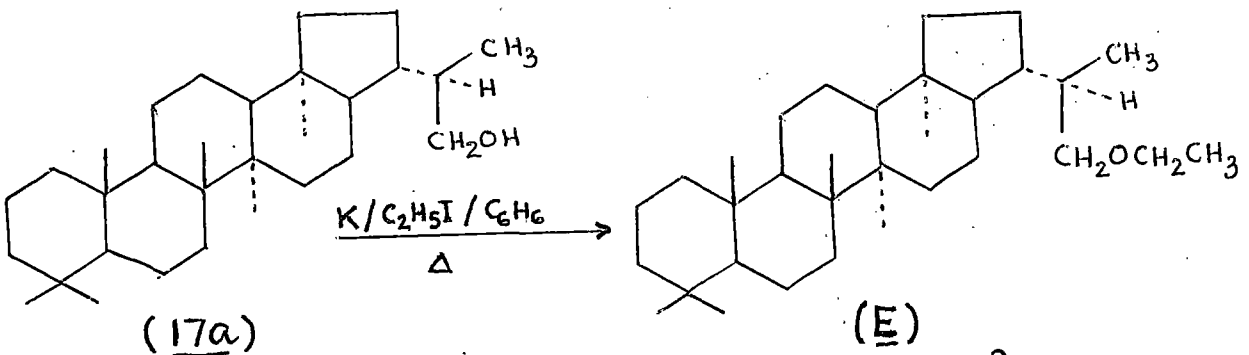
the structure (32) for the triterpene.

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)

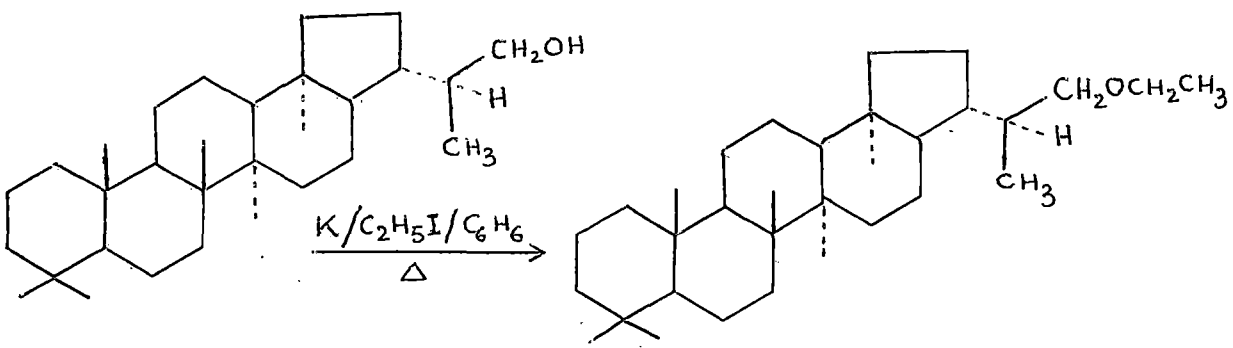
Nerifoliol (17a) was converted into its ethyl ether (E) by refluxing it with potassium metal and ethyl iodide in benzene[22,23].



(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

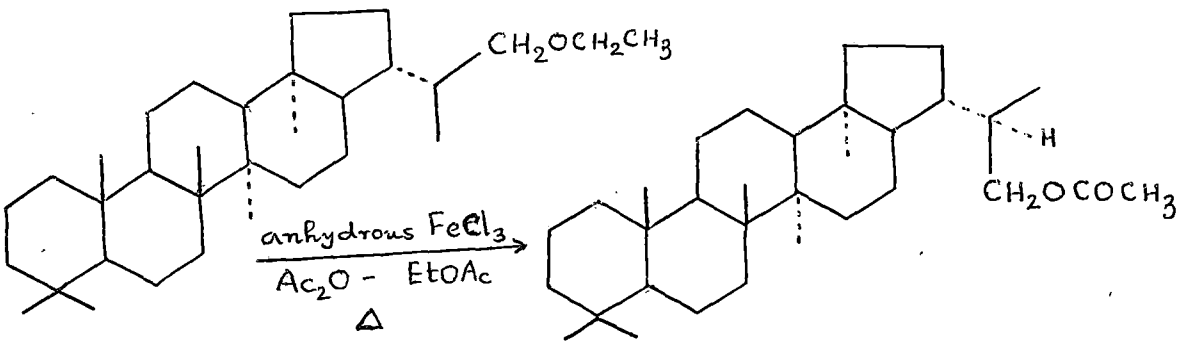


(12a) (34)

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

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

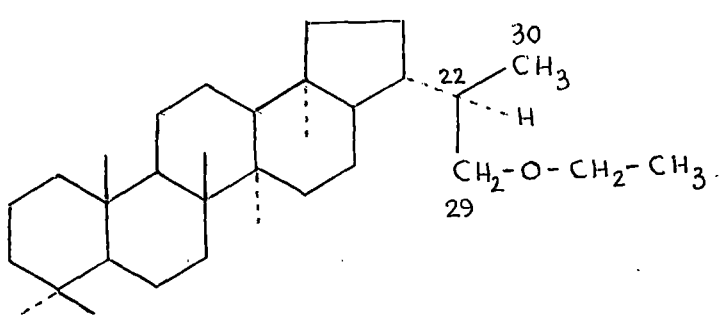
The methylene group adjacent to the oxygen atom in the triterpene (32) was converted into a carbonyl group by its reaction with anhydrous ferric chloride in acetic anhydride-ethyl acetate mixture[26].



(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)

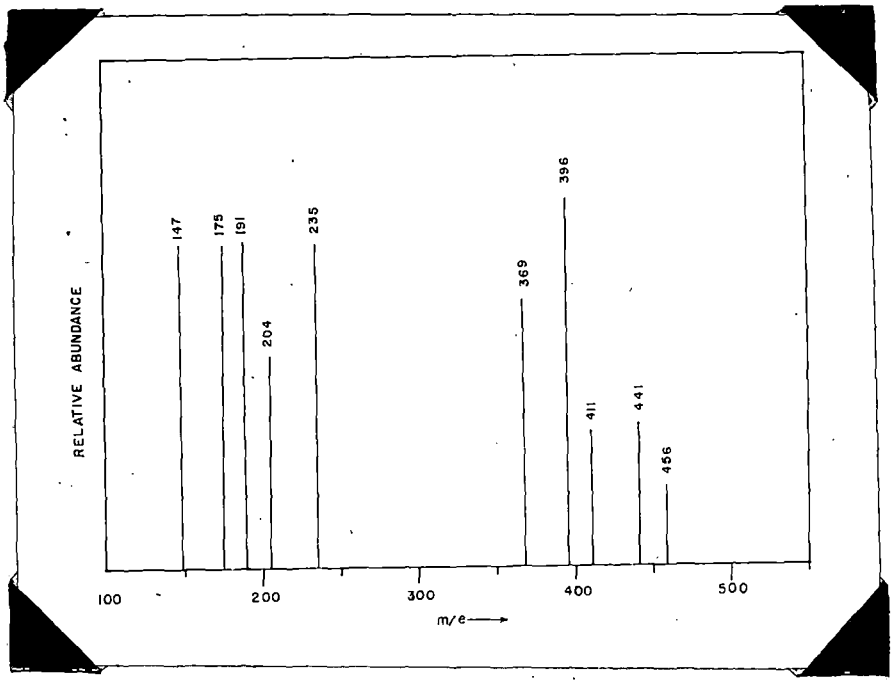


Fig. 1: Mass Spectrum of 29-Ethoxyhopane (35)

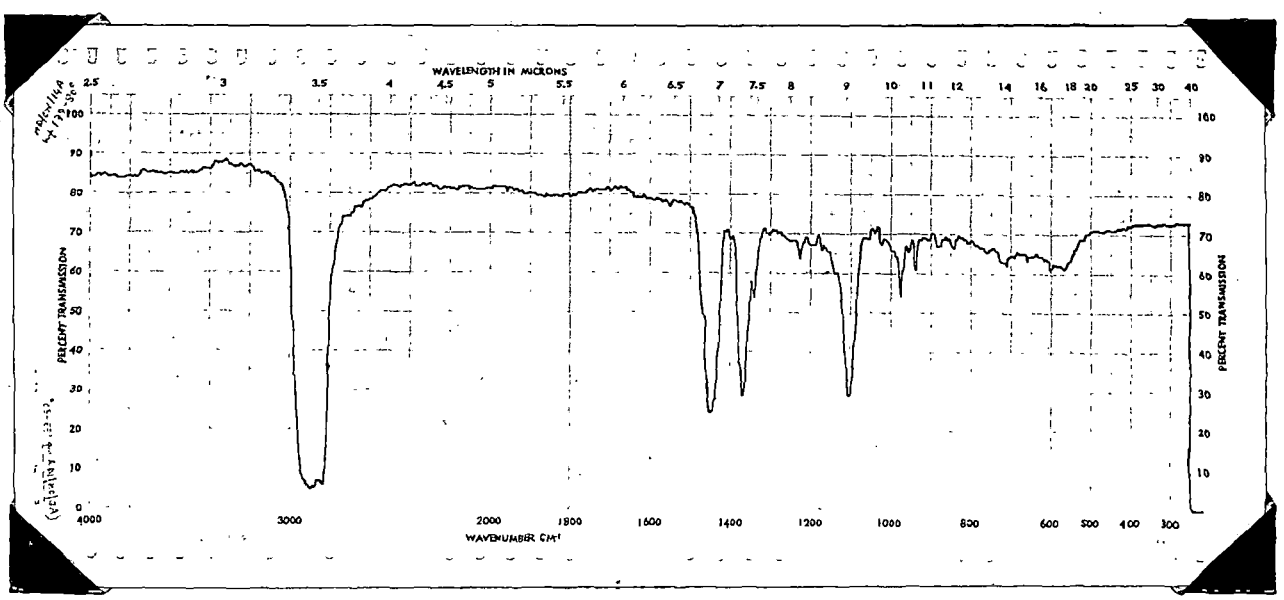


Fig. 2: IR spectrum of 29-Ethoxyhopane (35)

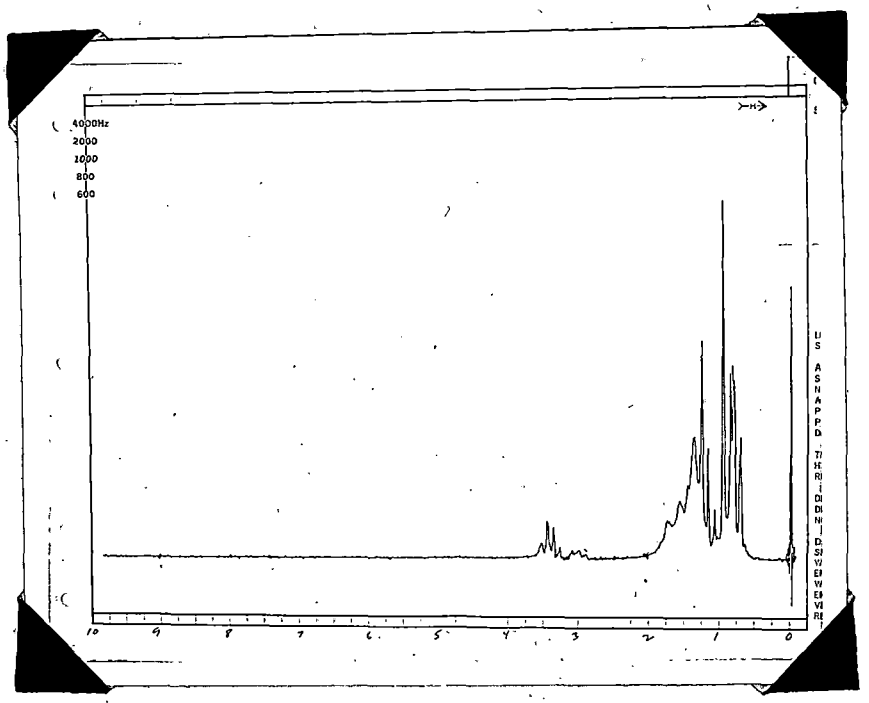


Fig. 3: NMR Spectrum of 29-Ethoxyhopane (35)

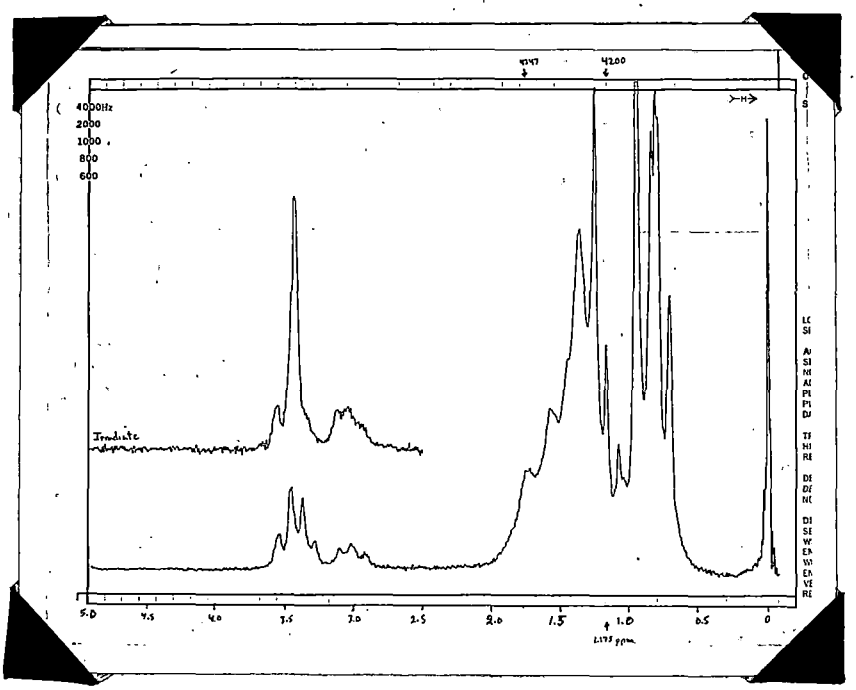


Fig.4: NMR Spectrum of 29-Ethoxyhopane (35), Plot Expansion. The result of irradiation at δ 1.175 (corresponding to ν = 4200 H_Z) was shown in the upper part of the figure.

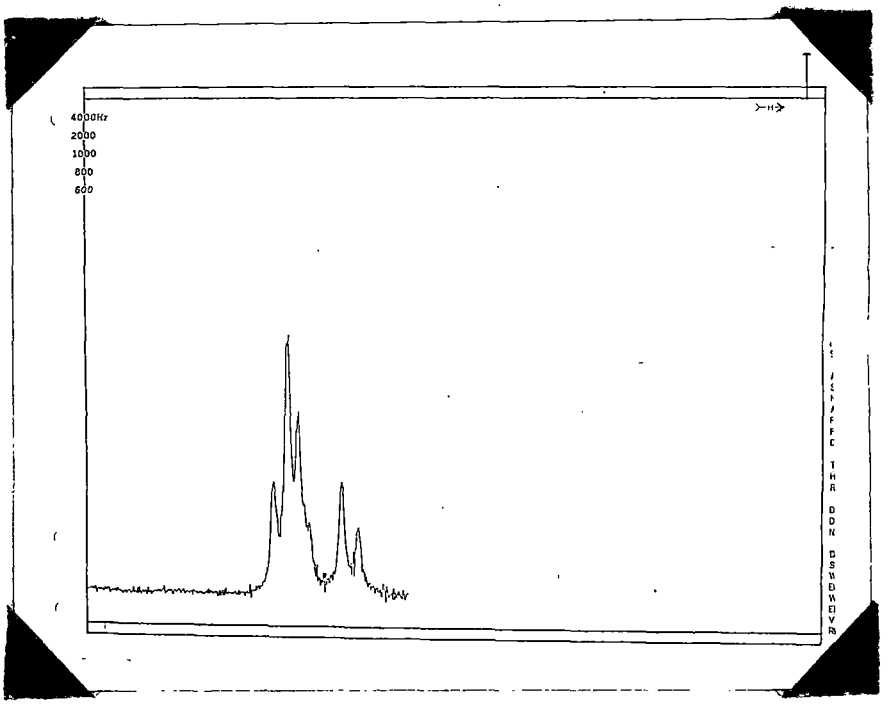


Fig. 5: NMR spectrum of 29-Ethoxyhopane (35). The result of irradiation at δ 1.75 (corresponding to ν = 4247 H_z)

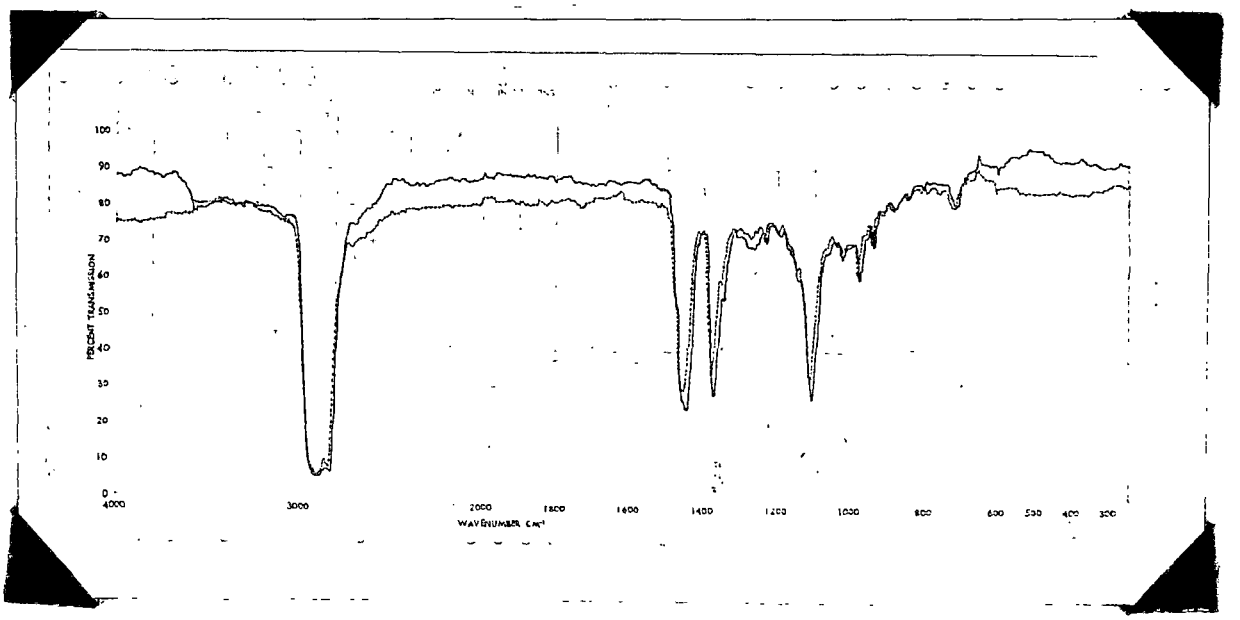


Fig. 6: IR comparison of synthetic 29-Ethoxyhopane (35) (dotted line) prepared from nerifoliol (17a), with an authentic specimen (Solid line) isolated from O. nerifolia.

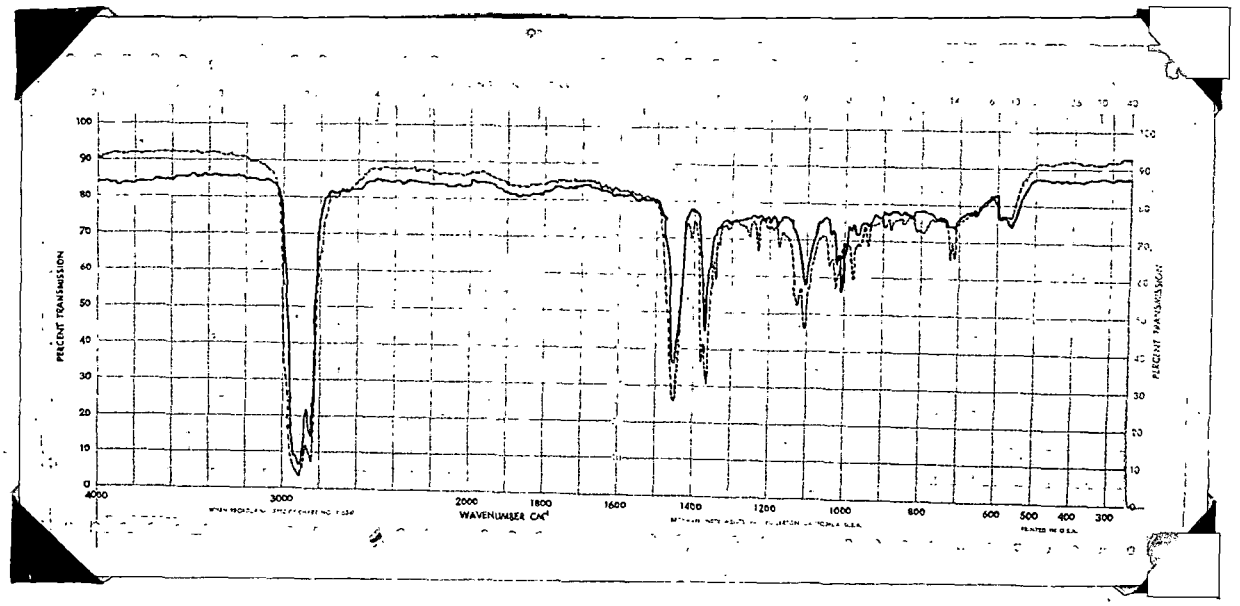


Fig. 7: IR comparison of synthetic Dryocrassol ether (30-Ethoxyhopane) (34) (dotted line) with an authentic specimen of 29-Ethoxyhopane (35) (Solid line) isolated from O.nerifolia.

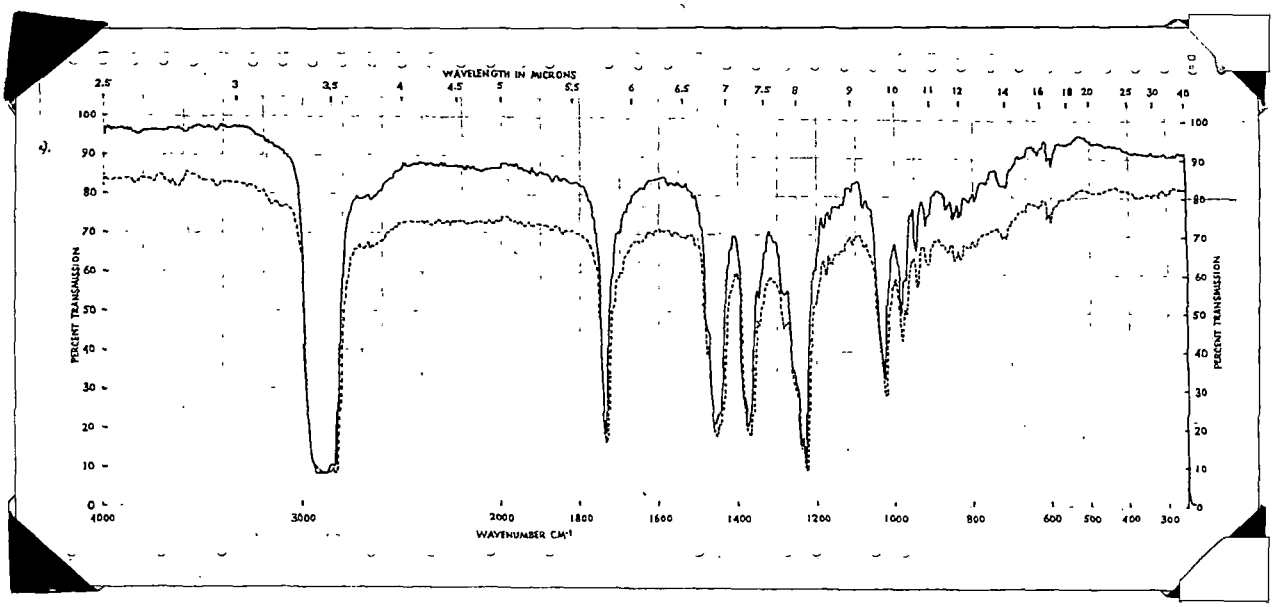


Fig. 8: IR comparison of synthetic Nerifoliol acetate (17b) (Solid line) prepared from 29-Ethoxyhopane (35), with an authentic specimen of Nerifoliol acetate (dotted line).

CHAPTER-V

EXPERIMENTAL

Melting points are uncorrected. The petrol used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution. NMR spectra were determined on a Varian HA-80 spectrophotometer using chloroform -d solution containing tetramethylsilane as internal reference. The IR spectra were recorded in a Beckmann IR-20 spectrophotometer. U.V. absorption spectra were taken in a Beckmann DU-2 spectrophotometer using hexane solution. The mass spectra were determined with an MS-50 mass spectrophotometer, using direct sample introduction into the ion source. Silica gel G for column chromatography was of 60-120 mesh and was activated at 120°. Silver nitrate impregnated Silica Gel was made by the method of Gupta and Sukh Dev[27] and activated at 110-20° (12 hours). TLC examinations were carried out on 12% Silver nitrate impregnated silica gel plate. The plates were activated at 110-20° (30 minutes) and then stored in a dessicator. Acetic anhydride-sulphuric acid (9:1) spray followed by heating (120°, 15 minutes) was used for visualisation of TLC spots.

Extraction:

Dried and powdered rhizomes of the fern *oleandra nerifolia* (2 kg) was extracted with benzene in a soxhlet

apparatus for 20 hours. Benzene was distilled off and the residual gummy material (6 g) was taken up in ether. The ether solution was washed with 10% aqueous sodium hydroxide solution and then with water till neutral and dried (Na_2SO_4). Removal of ether gave a gummy residue (4 g).

Chromatography of the above Gummy Residue:

The above gummy residue (4 g) was dissolved in benzene (12 ml) and was placed on a column of alumina (250 g; deactivated with 10 ml of 10% aqueous acetic acid). The chromatogram was developed with petrol and eluted with the following solvents (Table-II).

Table-II

Eluent	Fractions 100 ml each	Residue on Evaporation	Melting point in ^{o}C
Petrol	1-10	Solid with oil (0.8 g)	-
Petrol: Benzene (4:1)	11-15	Trace oil (0.1 g)	-
Petrol: Benzene (3:2)	16-22	Solid (0.5 g)	236-40^{o}
Petrol: Benzene (2:3)	23-32	Solid (1.0 g)	130-34^{o}

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

Rechromatography of Fractions 1-10 (Table-II, Chapter-V):

The oily solid (0.8 g) obtained from fractions 1-10 (Table-II, Chapter-V) was dissolved in benzene (3 ml) and placed on a column of active alumina (50 g). The chromatogram was developed with petrol and eluted with the following solvents (Table-III).

Table-III

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-4	Oil
	5-10	Waxy solid (0.5 g)

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

Examination of Fractions 5-10 (Table-III, Chapter-V):

Isolation of Filicene (22):

Fractions 5-10 (Table-III, Chapter-V) were combined (0.5 g) to furnish a waxy solid which on careful crystallisation from a mixture of chloroform and acetone yielded a solid (0.3 g),

m.p. 226-28°, $(\alpha)_D$ 50°, (TLC-single spot). It was found to be identical (m.m.p, IR and Co-TLC) with an authentic specimen of filicene[17] (22).

Found :	C, 87.86;	H, 12.12.
Calc. for $C_{30}H_{50}$:	C, 87.73;	H, 12.27%

Isolation of the New Triterpene, 29-Ethoxyhopane, $C_{32}H_{56}O$ (35):

The mother liquor from the crystallisation of filicene (22) on evaporation gave a crude mass (0.1 g), which was found by TLC to be a mixture of three components. Careful fractional crystallisation of this crude mass (0.1 g) from a mixture of chloroform and methanol (3:1) afforded crystals of (35) (10 mg), m.p. 179-80°, $(\alpha)_D$ 27.16° (TLC-single spot).

Found:	C, 84.16;	H, 12.22.
Calc. for $C_{32}H_{56}O$:	C, 84.14;	H, 12.36%

Mass spectrum: M^+ 456 Fig-1

IR : ν_{max}^{nujol} 1105 cm^{-1} Fig-2

UV : No absorption in the region 200-300 nm.

NMR Spectra (80 MH_z) : δ 0.7-0.95 (7 methyl groups)

δ 2.8-3.6 (multiplet, 4 protons)

Fig-3, Fig-4, Fig-5.

Isolation of Two other unidentified Compounds:

The mother liquor from the crystallisation of 29-ethoxyhopane (35) on evaporation gave a crude mass (50 mg), which was dissolved in benzene (1 ml) and was placed on a column of alumina (5 g) impregnated with 20% silver nitrate. The chromatogram was developed with petrol and eluted with the following solvents (Table-IV).

Table-IV

Eluent	Fractions 25 ml each	Residue on evaporation
Petrol	1-3	Oil
	4-5	Solid (m.p. 180-82°)
	6-7	Solid (m.p. 160-63°)

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

Fractions 4-5 (Table-IV) were combined and on crystallisation from a mixture of chloroform and methanol gave crystals (6 mg), m.p. 182-83°, (TLC-single spot).

Found:	C, 84.16;	H, 11.64
Calc. for $C_{30}H_{50}O$:	C, 84.44;	H, 11.81%

Fractions 6-7 (Table-IV) were combined to yield a solid which on crystallisation from a mixture of chloroform and methanol afforded yet another solid (4 mg), m.p. 163-64°, (TLC-single spot).

Found:	C, 87.61;	H, 12.16
Calc. for $C_{30}H_{50}$:	C, 87.73;	H, 12.27%.

The structure elucidations of these two compounds were not possible because of their very poor yield. Further work is in progress to isolate them in quantity to enable us to investigate their structures.

Examination of Fractions 16-22 (Table-II, Chapter-V): Isolation of Nerifoliol (17a):

The solid (0.5 g) obtained from fractions 16-22 (Table-II, Chapter-V) was dissolved in benzene (5 ml) and placed on a column of active alumina (30 g). The chromatogram was developed

with petrol and eluted with the following solvents (Table-V).

Table-V

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-4	Oil
Petrol: Benzene (4:1)	5-6	Nil
Petrol: Benzene (3:2)	7-8	Nil
Petrol: Benzene (2:3)	9-14	Solid, m.p. 241-43° (0.4 g)

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

The solid (0.4 g) from fractions 9-14 (Table-V) on crystallisation from a mixture of chloroform and methanol afforded fine crystals of (17a), m.p. 242-44°, $(\alpha)_D$ 35° (TLC-single spot).

Found: C, 83.91; H, 12.34

Calc. for $C_{30}H_{52}O$: C, 84.04; H, 12.22%

IR ν_{max}^{nujol} 3320 cm^{-1} (OH)

Mass spectrum: M^{+} 428.

The physical and chemical data of the compound (17a) showed that this was identical with nerifoliol (17a) isolated by Pandey and Mitra[6] from the same plant O. nerifolia.

Acetylation of Nerifoliol (17a): Preparation of Nerifoliol Acetate(17b):

A solution of the compound (17a; 200 mg) in pyridine (2 ml) and acetic anhydride (2 ml) was heated on a water bath for 4 hours. After working up in the usual way it gave a solid residue (200 mg), which was dissolved in benzene (4 ml) and placed over a column of active alumina (10 g). The chromatogram was developed with petrol and eluted with the following solvents (Table-VI).

Table-VI

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-5	Solid (180 mg), m.p. 194-96°

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

The solid (180 mg) from fractions 1-5 (Table-VI) on crystallisation from a mixture of chloroform and methanol afforded crystals of (17b), m.p. 195-96°, $(\alpha)_D^{20°}$.

Found: C, 81.48; H, 11.48.

Calc. for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56%

IR ν_{max}^{nujol} : 1730, 1225 cm^{-1} ($OCOCH_3$)

Mass spectrum: M^+ 470.

The physical and chemical data of the compound showed that it was identical with nerifoliol acetate (17b) prepared by Pandey and Mitra[6].

Examination of Fractions 23-32 (Table-II, Chapter-V): Isolation and Identification of β-Sitosterol:

The solid (1.0 g) obtained from the fractions 23-32 (Table-II, Chapter-V) was rechromatographed over active alumina (60 g). Elution with a mixture of petrol and benzene (1:4) gave a solid which on crystallisation from a mixture of chloroform and methanol furnished fine needle shaped crystals of an alcohol, m.p. 136-37°, $(\alpha)_D$-32°.

Found:	C, 83.56;	H, 11.76
Calc. for $C_{29}H_{50}O$:	C, 83.98;	H, 12.15%.

The alcohol (200 mg) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The product on crystallisation from a mixture of chloroform and methanol furnished crystals of the acetate, m.p. 127-29°, $(\alpha)_D$-40°.

Found:	C, 81.23;	H, 11.32.
Calc. for $C_{31}H_{52}O_2$:	C, 81.52;	H, 11.48%.

The alcohol and the acetate were identified as β-sitosterol and β-sitosteryl acetate respectively by direct comparison (m.m.p, IR and Co-TLC) with their respective authentic specimens.

Potassium Metal-Ethyl Iodide Treatment[22,23] of Nerifoliol (17a): Partial Synthesis of the New Triterpene, 29-Ethoxy hopane (35):

To a solution of nerifoliol (17a) (300 mg) in dry benzene (25 ml) was added potassium metal (400 mg) and the mixture was refluxed under anhydrous condition for 3 hours. The reaction mixture was then allowed to cool to room temperature. Freshly prepared dry ethyl iodide (25 ml) was added dropwise to the reaction mixture. The reaction mixture was again refluxed for 3 hours and then allowed to cool to room temperature. Excess potassium metal was decomposed by cautious addition of ethanol. The reaction mixture was diluted with water and the organic layer extracted with petrol. The petrol benzene layer was washed with saturated sodium chloride solution till neutral and dried (Na_2SO_4). Removal of solvent gave a gummy residue (300 mg), which was dissolved in benzene (5 ml) and placed on a column of active alumina (30 g). The chromatogram was developed in petrol and eluted with the following solvents (Table-VII).

Table-VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-2	Oil
	3-8	Solid (100 mg), m.p. 176-78°

Contd....

Table-VII (Contd)

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol: Benzene (4:1)	9-11	Nil
Petrol: Benzene (3:2)	12-14	Nil
Petrol: Benzene (2:3)	15-22	Solid (160 mg), m.p. 240-42°

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

The solid (100 mg) from fractions 3-8 (Table-VII) on crystallisation from a mixture of chloroform and methanol furnished a crystalline solid, m.p. 179-80°, $(\alpha)_D$ 27.16° which was found to identical (m.m.p, Co-TLC and Co-IR, Fig. 6) with 29-ethoxyhopane (35) isolated from the plant Oleandra nerifolia as described above.

Found: C, 84.36; H, 12.53.

Calc. for $C_{32}H_{56}O$: C, 84.14; H, 12.36%.

IR ν_{max}^{nujol} 1105 cm^{-1}

Co IR Fig. 6

The solid (160 mg) from fractions 15-22 (Table-VII) on crystallisation from a mixture of chloroform and methanol afforeded crystals, m.p. 242-44° which were found to be identical (m.m.p, Co-TLC and IR) with the starting material nerifoliol (17a).

Potassium Metal-Ethyl Iodide Treatment of Dryocrassol (12a): Preparation of Dryocrassol Ethyl Ether (30-Ethoxyhopane) (34):

Dryocrassol[12,24,25] (12a; 100 mg) dissolved in dry benzene (10 ml) was similarly treated with potassium metal (180 mg) and ethyl iodide (10 ml). The gummy residue (100 mg) obtained after working up the reaction mixture in the same manner was dissolved in benzene (2 ml) and was placed over a column of active alumina (15 g). The chromatogram was developed with petrol and eluted with the following solvents (Table-VIII).

Table-VIII

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-3	Oil
	4-5	Solid (30 mg), m.p. 146-49°
Petrol: Benzene (4:1)	6-7	Nil
Petrol: Benzene (3:2)	8-9	Nil

Contd...

Table-VIII (Contd.)

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol: Benzene (2:3)	10-12	Solid (50 mg), m.p. 243-46°

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

The solid (30 mg) from fractions 4-5 (Table-VIII) on crystallisation from a mixture of chloroform and methanol afforded crystals of (34), m.p. 148-50°. This compound (34) showed depression of melting point on admixture with the natural product (35) and its IR spectra was distinctly different from that of 29-ethoxyhopane (35) isolated from the natural source [Co IR of Synthetic dryocrassol Ether (34) and natural 29-ethoxyhopane (35) -Fig.7].

Found: C, 84.28; H, 12.47.

Calc. for $C_{32}H_{56}O$: C, 84.14; H, 12.36%.

IR ν_{max}^{nujol} 1110 cm^{-1}

Co IR of Synthetic Dryocrassol Ether and 29-ethoxyhopane (natural)- Fig. 7

Fractions 10-12 (Table-VIII) were combined (50 mg) and on crystallisation from a mixture of chloroform and methanol afforded a solid, m.p. 244-47° which was found to be identical (m.m.p., IR and Co-TLC) with the starting material dryocrassol (12a).

Anhydrous Ferric Chloride-Acetic Anhydride-Ethyl acetate Treatment of 29-Ethoxyhopane (35): Preparation of Nerifoliol Acetate (17b):

The triterpene 29-ethoxyhopane (35; 5 mg) was dissolved in a mixture of dry acetic anhydride and ethyl acetate mixture (1:1; 5 ml) with gentle heating. After cooling to room temperature, anhydrous ferric chloride (1.25 mg) was added and the reaction mixture kept at 80° for 24 hours. The reaction mixture was cooled, poured into water and extracted with hexane. The organic layer was washed with water, 5% aqueous $NaHCO_3$ solution and again with water till neutral and then dried (Na_2SO_4). Removal of the solvent gave a residue (3 mg), which on crystallisation from a mixture of chloroform and methanol afforded nerifoliol acetate (17b; 2 mg), m.p. 195-96° which was found to be identical (no m.m.p. depression, Co-TLC and superimposable IR spectra - Fig. 8) with an authentic specimen.

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PART-II

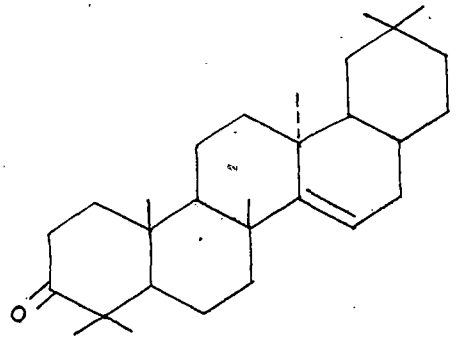
PARTIAL SYNTHESIS OF 2α, 3β - DIACETOXY - 28 - NOR OLEANA - 12, 17 - DIENE : CONFIRMATION OF THE STRUCTURE OF BACCATIN.

PART-II

CHAPTER-I

A Short Review on the Structure Elucidation of Baccatin.

Investigations on the benzene extract of the bark of *Sapium baccatum* Roxb in this laboratory led to the isolation of taraxerone (1), taraxerol (2), 1-hexacosanol (3), β-Sitosterol (4), 3,3'-di-O-methyl ellagic acid (5) and 3-acetoxy aleuritolic acid (6)[1-6].



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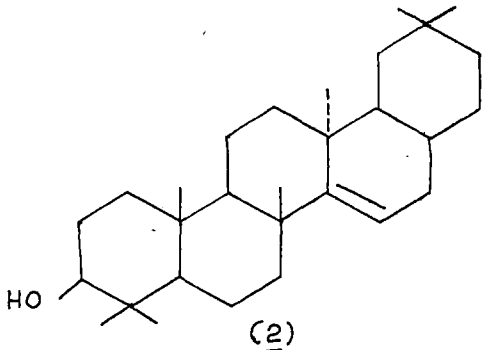
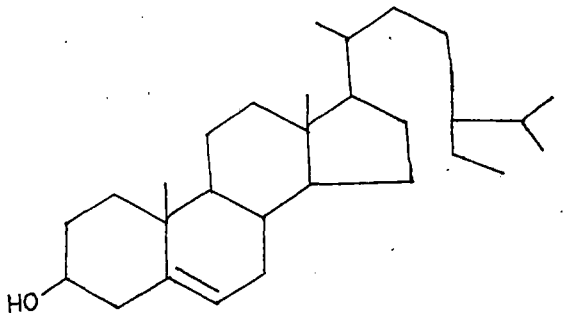


(2)

$CH_3-(CH_2)_{24}-CH_2OH$

(3)



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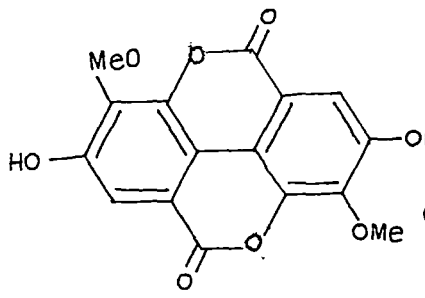

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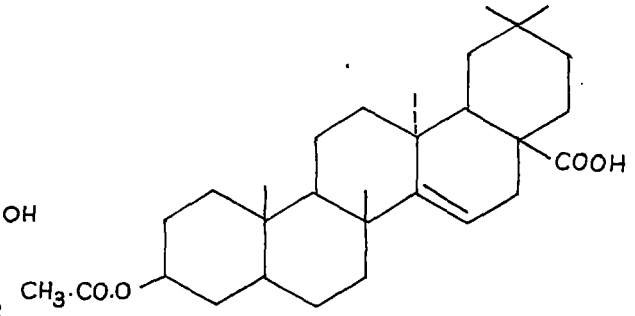
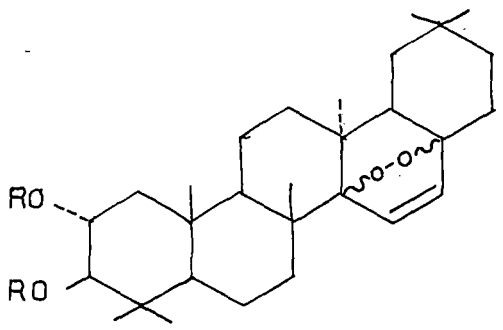


(6)

Further investigations on the more polar fractions of the neutral portion of the same extract afforded a new nor-triterpene peroxide, $C_{29}H_{46}O_4$ which was named baccatin (7a)[7,8].



(7a) R = H

(7b) R = $COCH_3$

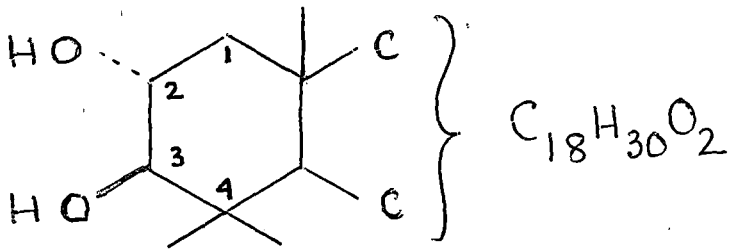
A short review on the structure elucidation[7,8] of baccatin (7a) is given below:

Baccatin (7a), $C_{29}H_{46}O_4$ (M^+458), m.p. 228-29° (d), $(\alpha)_D$ -9.09° showed IR absorption at ν_{max}^{KBr} 3360 (OH), 2970, 1453, 1389, 1369 (gem dimethyl) and 890, 875 cm^{-1} (CH = CH) and did not show any U.V. absorption in the region 220-300 nm. It readily formed an acetate (7b), $C_{33}H_{50}O_6$ (M^+542), m.p. 213-15°, $(\alpha)_D$ 47.5°, IR $\nu_{max}^{CHCl_3}$ 1737 ($OCOCH_3$), 1467, 1453, 1389, 1369 (gem dimethyl), 1245 ($OCOCH_3$) and 895-872 cm^{-1} (-CH = CH-) but no hydroxyl peak in the region 3650-3100 cm^{-1} and also did not show any U.V. absorption in the region 220-300 nm. These data indicated that baccatin (7a) contained two easily acetylable hydroxyl groups.

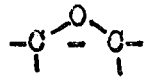
The position and stereochemistry of the two hydroxyl groups were established[7,8] from a study of NMR spectrum of baccatin (7a) and its diacetate (7b). The NMR spectrum (100 MH_Z) of baccatin (7a) showed signals at δ 0.88-1.18 (7 tert methyl groups), two doublets at δ 2.16 and 2.20 and at δ 2.28 and 2.32 (two - OH groups), an unsymmetrical doublet at δ 3.22 and 3.30 (H-C-OH), a quartet of doublets at δ 3.86, 3.95, 4.01 and 4.04 (H-C-OH) and another quartet at 6.42, 6.52, 6.71 and 6.81 (AB quartet, $-\overset{|}{C}-CH = CH-\overset{|}{C}-$). The NMR spectrum (100 MH_Z) of the diacetate (7b), showed peaks at δ 0.88-1.025 (7 tert-methyl groups), 1.99 and 2.055 (6H, 2-O-CO-CH_3), an unsymmetrical

doublet at δ 4.66 and 4.76 (H<u>C</u>-OCOCH$_3$, J = 10 H$_z$), a quartet of doublets at δ 5.01, 5.04, 5.13 and 5.16 (H<u>C</u>-OCOCH$_3$, J = 10 H$_z$ and 10.5 H$_z$) and another quartet at δ 6.40, 6.49, 6.675 and 6.75 (AB quartet, -<u>C</u>-CH = CH-<u>C</u>-). The NMR spectra of baccatin (<u>7a</u>) and its diacetate (<u>7b</u>) were explained[7,8] by assuming that the two hydroxyl groups were present in the diequatorial 2α, 3β-configuration as shown in the partial structure (<u>8</u>). It was argued that the downfield shift in the NMR spectra of the doublet at δ 3.22 and 3.30 and the quartet of doublets in the region δ 3.86-4.01 of baccatin (<u>7a</u>) to δ 4.66 and 4.76 for the doublet and in the region δ 5.01-5.16 for the quartet of doublets, respectively, in its diacetate (<u>7b</u>) was characteristic of protons attached to carbons bearing hydroxyl functions. The doublet at δ 3.22 and 3.30 in the spectrum of baccatin (<u>7a</u>) was thought of as arising from the splitting of the C$_3$-H signal by the proton on C$_2$. In the diacetate (<u>7b</u>) this doublet was shifted to δ 4.66 and 4.76 (J = 10 H$_z$). It was further interpreted that the signal due to C$_2$-H likewise splitted into a doublet by C$_3$-H and this doublet then splitted into a quartet of doublets by coupling with the two geminal protons on C$_1$ and appeared in the region δ 3.86-4.06 in the case of baccatin (<u>7a</u>) and in the region δ 5.01-5.16 in the case of the diacetate (<u>7b</u>). Citing examples from triterpenoid fields[9,10] it was further proposed that this part of the spectrum corresponded to the X-part of the ABXY -type of spectrum

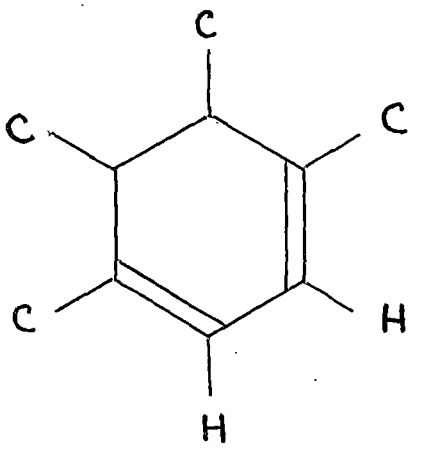
similar to those observed in analogous cases. Moreover, the 10 H_z coupling constant between the C_2 and C_3 protons implied a trans -diaxial arrangement for these two protons and hence a trans-di-equatorial, i.e., a 2α, 3β-configuration for the two hydroxyl groups. These observations established the partial formula (8) for baccatin (7a) corresponding to ring A of the compound with gem-dimethyl groups at C_4.



(8)

The diacetate (7b) liberated one atom of iodine for one atom of oxygen when titrated with potassium iodide in glacial acetic acid. This indicated the presence of two active oxygen atoms either in a peroxide linkage of the type -C-O-O-C- or two epoxide linkage of the type  . The primary argument against the presence of the epoxide linkage was that epoxides were stable

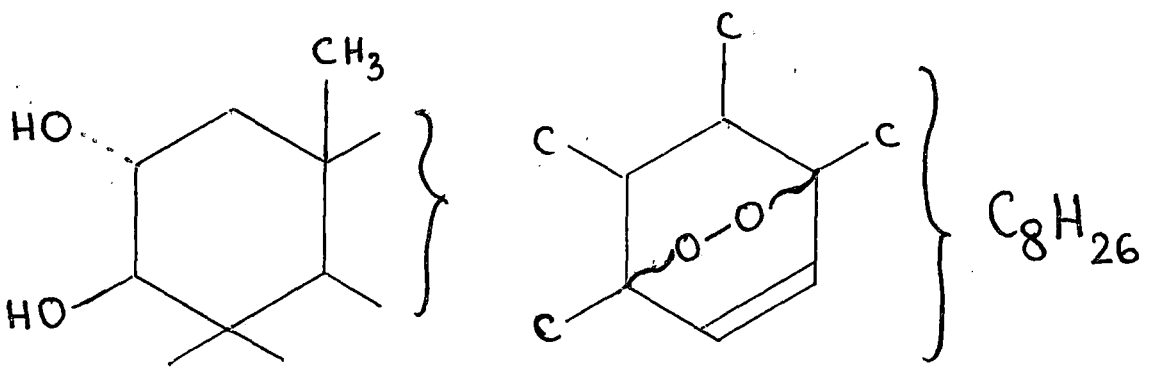
towards alkaline reagents[11,12] whereas baccatin (7a) was found to be very much sensitive to alkali. Thus when baccatin (7a) or its diacetate (7b) was treated with 10% methanolic potash, a new product, $C_{29}H_{46}O_2$ (M^+ 426), m.p. 237-40°, IR ν_{max}^{nujol} 3280 (OH), 1050 and 840 cm^{-1} was obtained. The new compound showed UV absorption at λ_{max}^{MeOH} 282 nm (ϵ, 8300) suggesting that it was a homo-annular conjugated diene as represented by the partial structure (9). The diene, $C_{29}H_{46}O_2$ on acetylation gave a



(9)

diacetate $C_{33}H_{50}O_6$ (M^+ 510), m.p. 226-27°, UV λ_{max}^{MeOH} 282 nm (ϵ, 8030). The NMR spectrum (100 MH_z) of this diacetate showed

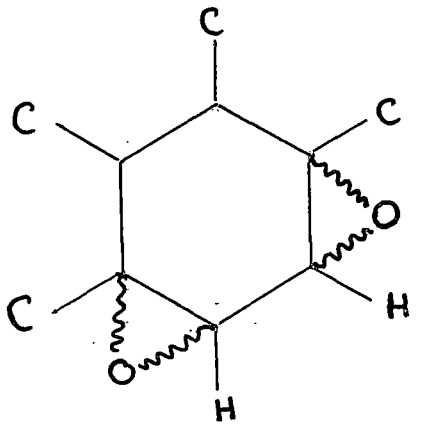
signals at δ 0.9-1.15 (7 tertiary methyl groups), 2.0 and 2.08 (6H, two -O-CO-CH_3 groups), a doublet at 4.7 and 4.8 (H on the acetoxy bearing C-3), a quartet of doublets centred at 5.1 (H on the acetoxy bearing C-2) and a multiplet at 5.58 attributable to two vinyl protons present in the diene system as shown in partial structure (9). From these observations Khastgir et al[7,8] suggested the presence of a perxoy linkage in baccatin (7a). This conclusion was further substantiated by the conversion of an alcoholic solution of the diene diol, $C_{29}H_{46}O_2$ by eosin sensitized photooxidation into a peroxide identical with baccatin (7a). Furthermore they proposed that the presence of abundant peaks at (M^+-32) in the mass spectrum of baccatin (7a) and its diacetate (7b) could also be accounted for by the presence of a peroxide linkage. The peak at (M^+-32) in the peroxide arose from the loss of an oxygen molecule from the parent peak and had been previously reported in case of several peroxides[13-15]. Having thus established the presence of a peroxide linkage in baccatin, they infered that the quartet between δ 6.42 and 6.81 in the NMR spectrum of both baccatin (7a) and its diacetate (7b) arose from a disubstituted double bond of the type $-\overset{|}{C}-CH = CH-\overset{|}{C}-$ present in the same ring as the peroxide moiety. Thus they extended the partial structure of baccatin (7a) to (10).



(10)

Further evidence for the presence of a peroxy linkage in baccatin (7a) was adduced from a study of its expected[12,16,17] rearrangement when a benzene solution of baccatin diacetate (7b) was adsorbed in a column of basic alumina for 48 hours. The rearranged new product, $C_{33}H_{50}O_6$ (M^+ 542), m.p. 263-64°, IR ν_{max}^{nujol} 1720, 1250 ($-OCOCH_3$), 1040 and 920 cm^{-1} showed NMR (100 MH_z) signals at δ 0.9-1.18 (7 tertiary methyl groups), 2.0 and 2.06 (two-$OCOC\underline{H}_3$), symmetrical doublet at 3.00 ($J = 2H_z$, $-C(O)-C\underline{H}-$), symmetrical doublet at 3.5 ($J = 2H_z$, $-C(O)-C\underline{H}-$) unsymmetrical doublet at 4.7, 4.8 (H on the acetoxy bearing C-3) and quartet of doublets at 5.0, 5.03, 5.06 and 5.09 (H on the acetoxy bearing C-2). The significant difference in the NMR spectrum of baccatin

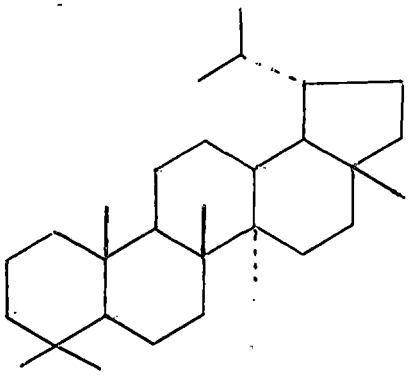
diacetate (7b) and the rearrangement product, $C_{33}H_{50}O_6$ was the absence of the AB type quartet (assigned to $-\overset{|}{C}-CH = CH-\overset{|}{C}-$ group in baccatin) between δ 6.4 - 6.75 in case of the rearranged product. Khastgir et al[7,8] proposed that the double bond and the perxoy linkage had probably been involved in the rearrangement. From a study of the physical data of the rearranged product, $C_{33}H_{50}O_6$ and of its hydrolysis product, $C_{29}H_{46}O_4$, m.p. 242-43°, IR ν_{max}^{nujol} 3340 (OH), 1040 and 920 cm^{-1}; they assigned the partial diepoxide structure (11) to the rearranged product, $C_{33}H_{50}O_6$.



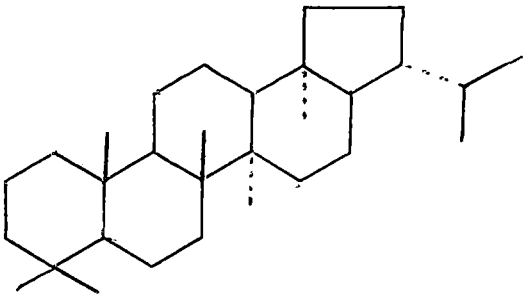
(11)

From the computation of the molecular formula of baccatin (7a) and the diene, $C_{29}H_{46}O_2$, Khastgir et al[7,8] concluded that baccatin (7a) was pentacyclic. The NMR spectrum of baccatin (7a) and its various degradation products showed the presence of seven tertiary methyl groups in each of these compounds. The mass spectrum of these compounds did not show any fragment which could be assigned to the loss of isopropyl or isopropenyl groups. From these observations they suggested that a lupane (12) or hopane (13) type

of nucleus was not possible for baccatin (7a).

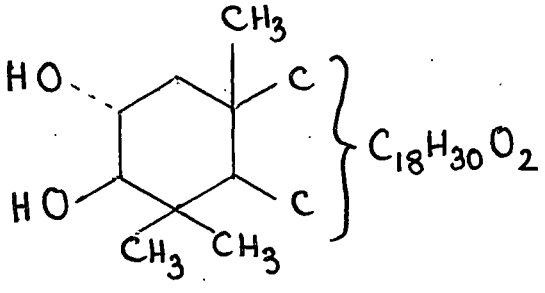


(12)

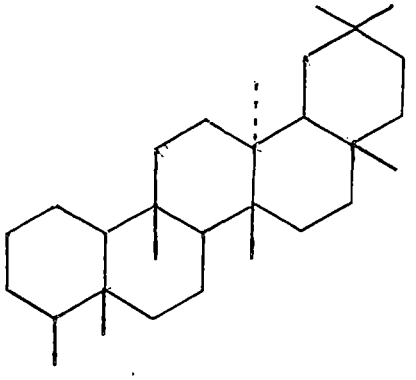


(13)

Ring A of baccatin (7a) was shown from the NMR spectrum to be represented by the partial structure (8). Hence the presence of a friedelane type skeleton (14) was ruled out as it could not explain the ABXY pattern observed in the NMR spectrum of baccatin (7a) and its diacetate (7b).

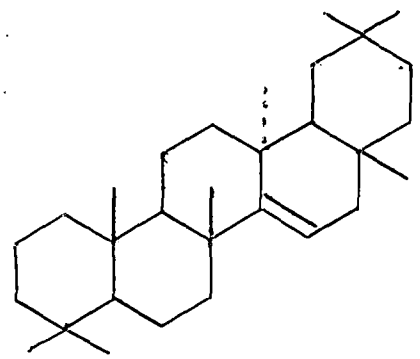


(8)



(14)

Baccatin (7a) was obtained from the benzene extract of the trunk bark and Stem of Sapium baccatum Roxb. along with taraxerone (1), taraxerol (2) and 3-acetoxy aleuritolic acid (6). These three compounds contain the Δ^{14} -taraxerene nucleus (15).



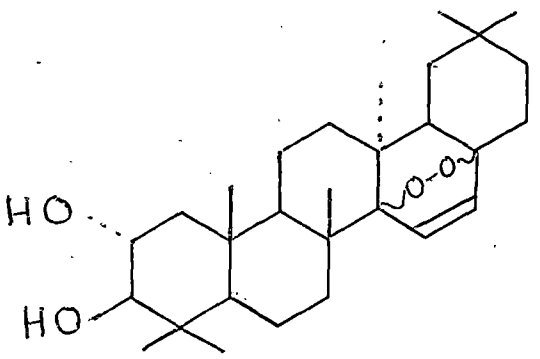
(15)

Therefore, from biogenetic considerations, Khastgir et al[7,8] proposed that the same Δ^{14} - taraxerene type of nucleus (15) might be involved in the formation of the nor-triterpene peroxide, baccatin (7a), in the plant.

From an analysis of the physical data and the partial structure (10) for baccatin (7a) and (9) for the diene, $C_{29}H_{46}O_2$, they concluded that no tertiary methyl groups were present at the peroxide bridge-head.

On the basis of the above considerations, Khastgir et al[7,8] proposed from biogenetic point of view the structure (7a) for

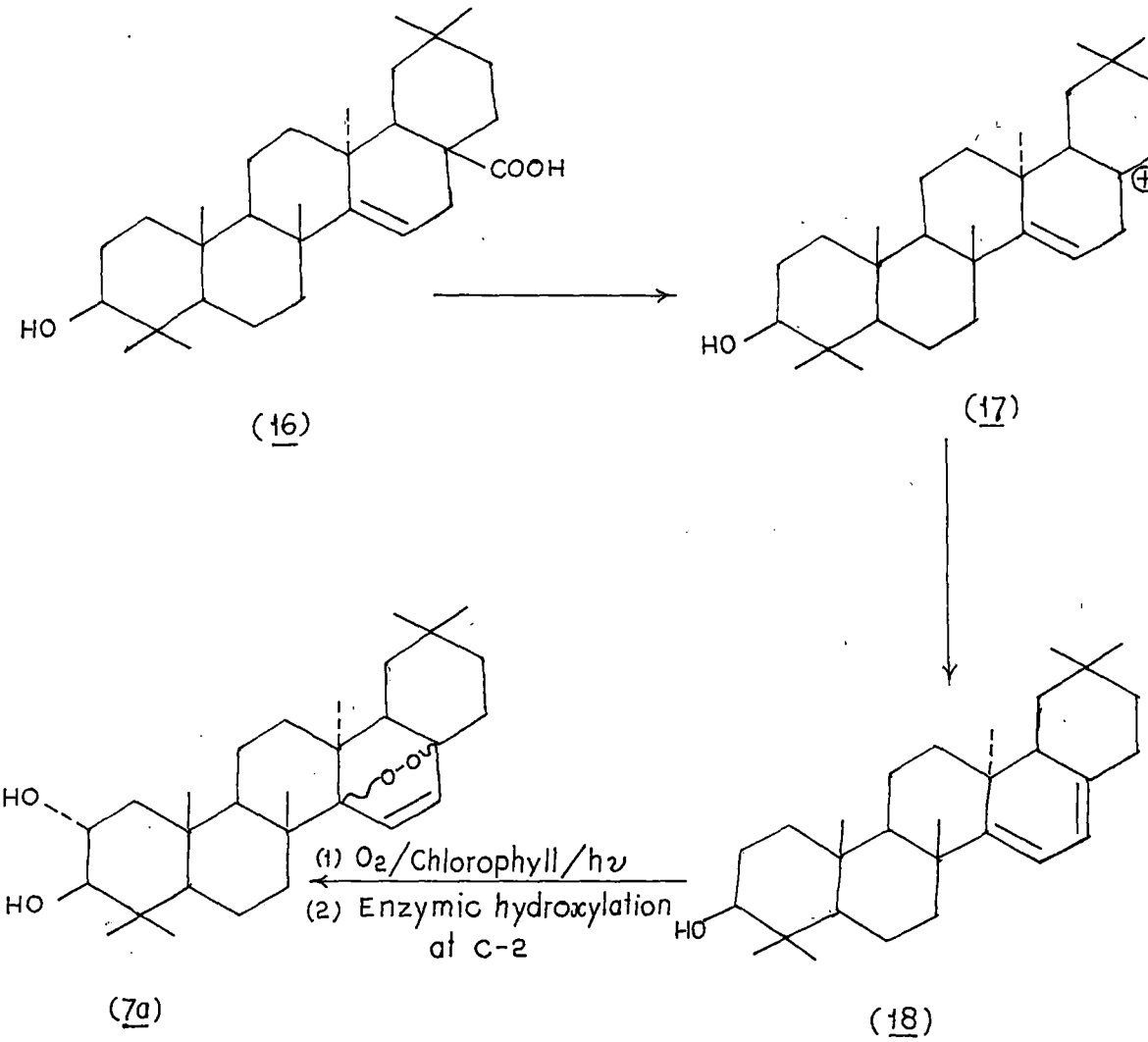
baccatin, which explained all the available chemical and physical evidences in a satisfactory manner.



(7a)

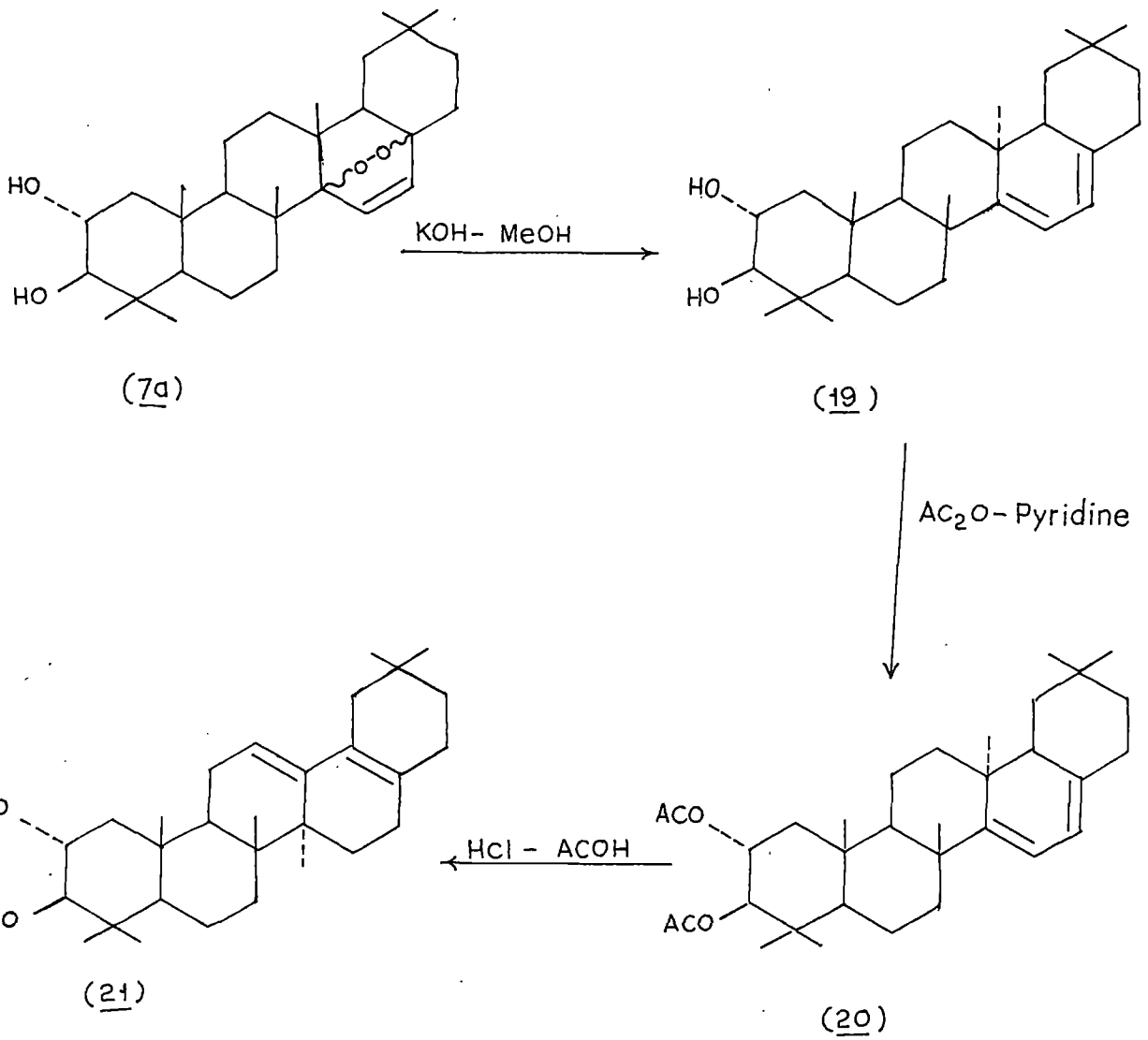
Khastgir et al[7,8] also suggested a biogenetic route for the formation of baccatin (7a) from aleuritolic acid (16) in the plant as shown in Chart-I. According to them decarboxylation of aleuritolic acid (16) in the plant took place generating the carbonium ion (17) which readily eliminated a proton to form the homoannular conjugated diene (18). Photooxidation and enzymic hydroxylation of (18) then gave rise to the nortriterpene peroxide, baccatin (7a).

Chart-I



(16) (17) (18) (7a)

Treatment of baccatin (7a) with methanolic alkali gave the homoannular conjugated diene, $C_{29}H_{46}O_2$, (19) and this on acetylation afforded the diene-diacetate, $C_{33}H_{50}O_4$ (20). Treatment of the latter with a mixture of hydrochloric acid and acetic acid gave a compound, $C_{33}H_{50}O_4$, m.p. 189-90°. The U.V. spectrum of this compound showed absorptions at λ_{max}^{MeOH} 237 (ϵ, 27,000), 244 (ϵ, 28,300) and 252 nm (ϵ, 20,200) thereby suggesting the presence of a heteroannular conjugated diene system in the rearranged product. The NMR spectrum (100 MH_z) of the rearranged product showed signals at δ 0.85 - 1.14 (7 tertiary methyl groups), 1.96, 2.0 (6H, 2-OCOCH_3) unsymmetrical doublet at 4.64, 4.75 (H on the acetoxy bearing C-3), quartet of doublets at 4.95, 5.03, 5.08, 5.2 (H on the acetoxy bearing (C-2) and 5.46 (1H, Vinyl proton). The mass spectrum of the rearranged product showed significant peaks at m/e 510 (M^+), 495 (M^+-15), 450 (M^+-60), 435 (M^+-60-15), 390 (M^+-60-60), 375 (M^+-60-60-15), 308, 202, 188. On the basis of these data Khastgir et al[7,8] assigned the structure 2α, 3β-diacetoxy-28-nor oleana-12,17-diene, (21) for the rearranged product.



(7a)

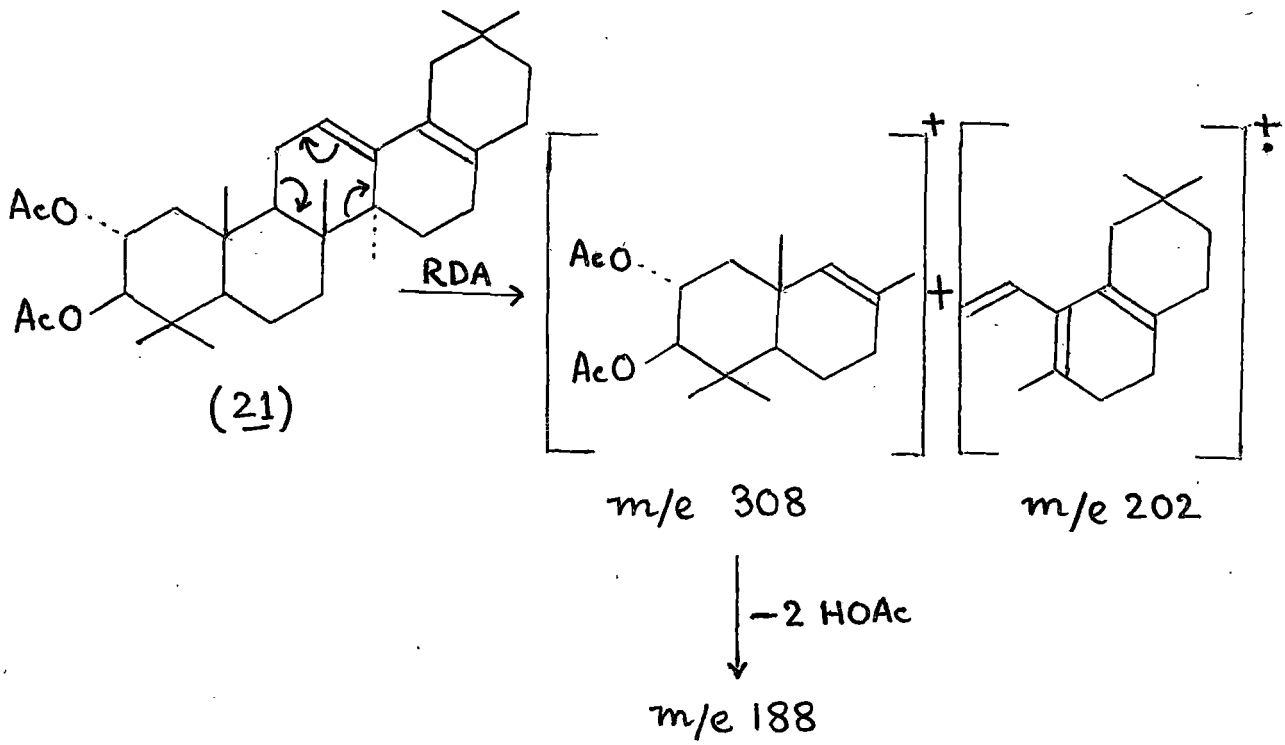
(19)

(21)

(20)

The mass fragmentation pattern of the rearranged product (21) was explained as shown in Chart-II.

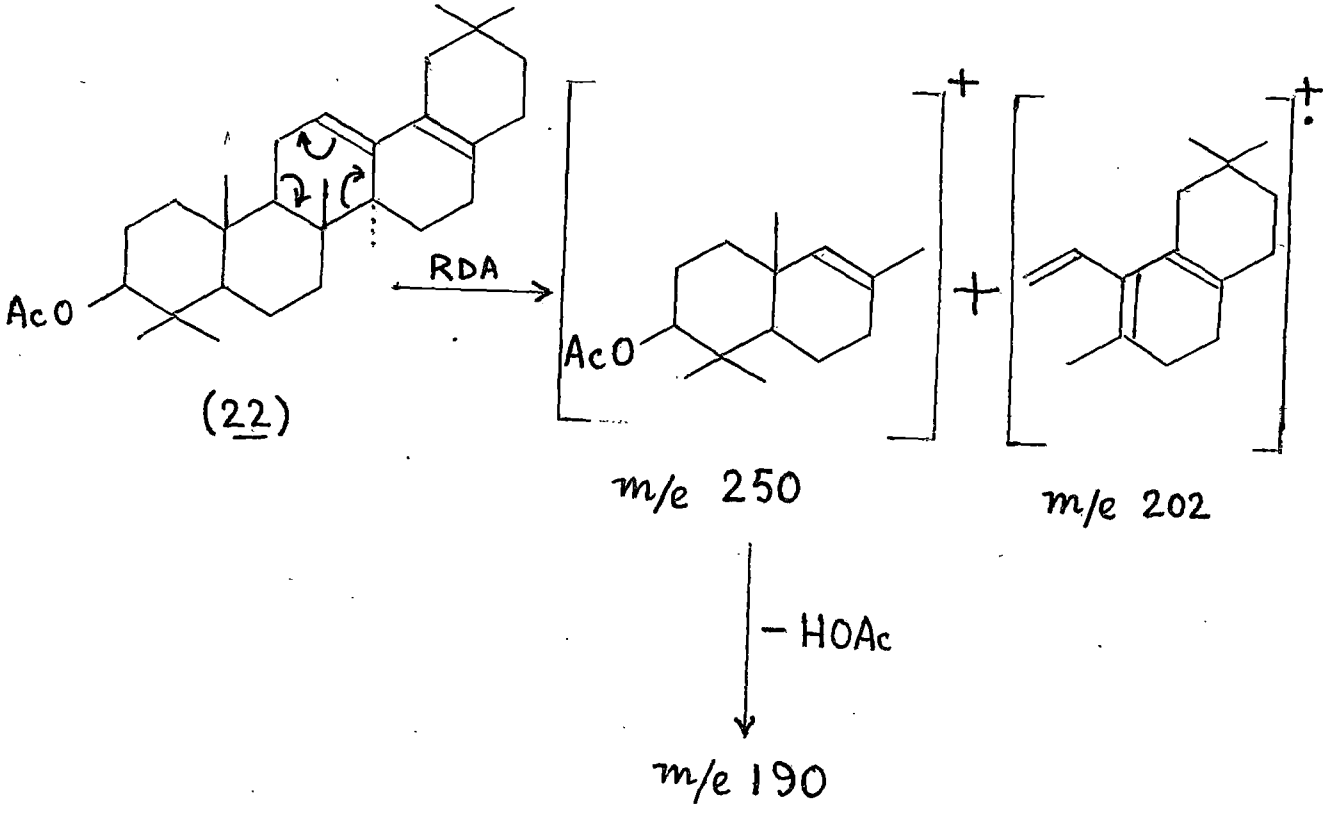
Chart-II



In accordance with the known mass fragmentation pattern of Δ^{12}-oleanenes[18] Khastgir et al[7,8] proposed that the fragments at m/e 308, 202 and 188 were diagnostic of the system shown in

(21). In order to correlate the above observation, they also prepared the compound (22) having a similar heteroannular dienic system starting from acetyl oleanolic acid. They argued that if the peaks at m/e 202 and 188 were indeed diagnostic of structure (21), then the compound (22) should also be expected to exhibit analogous peaks at m/e 202 and 190 corresponding to the fragmentation shown in Chart-III.

Chart-III

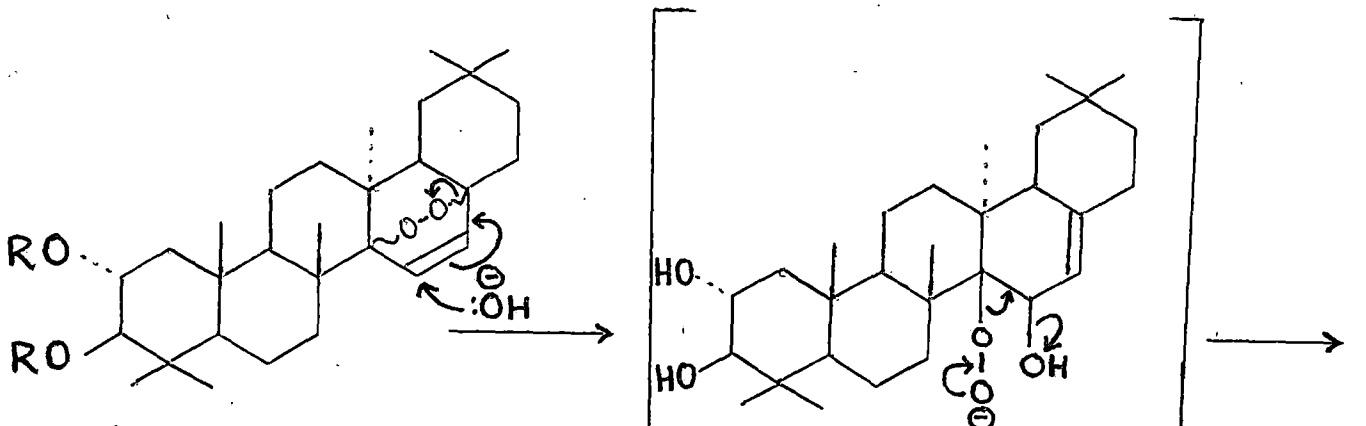


(22)

In accordance with their expectation, the mass spectrum of (22) showed prominent peaks at m/e 452 (M^+), 437 (M^+-15), 392 (M^+-60), 377 (M^+-60-15), 202 and 190. Thus by comparison of the mass spectra of compounds (21) and (22) Khastgir et al[7,8] confirmed the structure (21) for the rearranged diene diacetate. This in turn confirmed the structure (19) for the homoannular diene, $C_{29}H_{46}O_2$ obtained by the alkali treatment of baccatin (7a). Thus they concluded that baccatin must have the structure (7a) and its diacetate (7b).

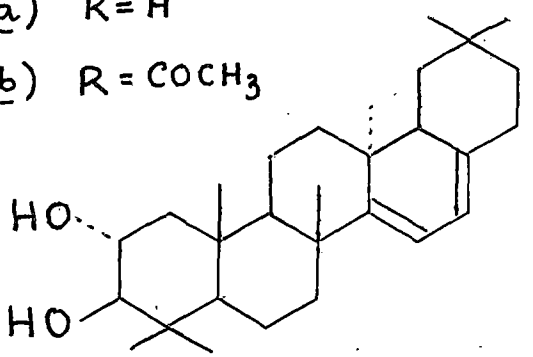
Khastgir et al[7,8] have also proposed a probable mechanism for the transformation of baccatin (7a) or its diacetate (7b) to the homoannular diene (19) by treatment with methanolic alkali as shown in Chart-IV.

Chart-IV



(7a) R = H

(7b) R = $COCH_3$



(19)

An examination of the Dreiding model of (7a) or (7b) with a β-peroxide linkage showed that both rings C and D assumed rigid boat conformation with severe interaction between the hydrogen atoms at C-12 and C-19. In the diene (19) ring C took up half-chair conformation and ring D became almost flat with complete disappearance of the above interactions. They suggested that most probably this relief of strain facilitated the transformation of the peroxide (7a) or (7b) to the homoannular diene (19).

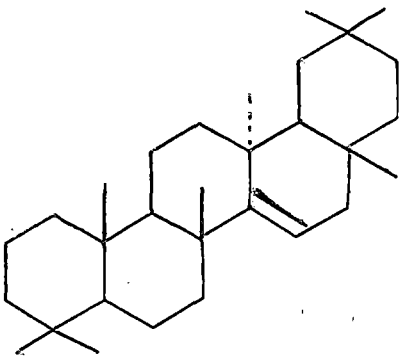
CHAPTER-II

Partial Synthesis of 2α, 3β-Diacetoxy 28-Nor Oleana-12,17-diene: Confirmation of the Structure of Baccatin:

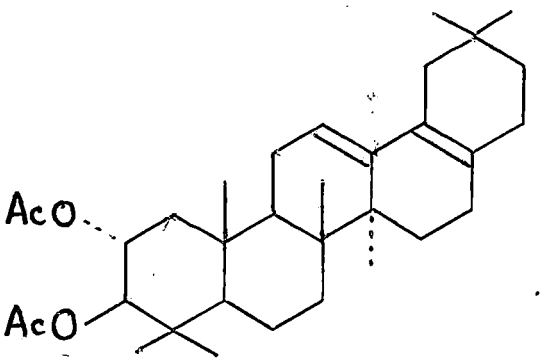
Section A: Aims and Objective of the Present Work.

The present investigation dealt with a detailed analysis of the physical and chemical data that led to the structure (7a) for baccatin[7,8]. Although this structure satisfactorily explained the available data, it was felt that a few conclusions derived in the elucidation of the structure required further confirmation.

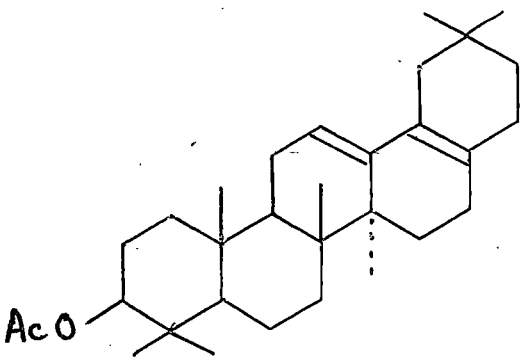
The presence of a taraxerene type of nucleus (15) in baccatin (7a) was suggested[7,8] mainly from biogenetic point of view. This conclusion was supported[7,8] by a similarity in the mass fragmentation pattern of 2α, 3β-diacetoxy-28-nor-Oleana-12, 17-diene (21), a degradation product of baccatin (7a), and that of 3β-acetoxy-28-nor-oleana-12, 17-diene (22) prepared from acetyl oleanolic acid. However, the presence of an ursane-type E ring such as shown in (23) in the heteroannular diene (21) (and consequently in baccatin) could also explain the mass fragmentation and other physical data to a fair degree of accuracy. It was, therefore, thought that it was necessary to prove that (21) (and



(15)



(21)



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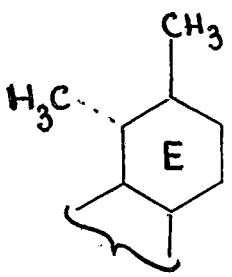

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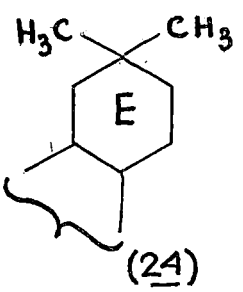


(24)

consequently baccatin) indeed contained an oleanane-type E ring as shown in (24).

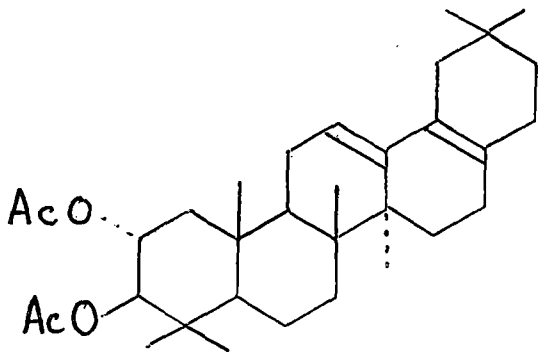
Furthermore, the presence of a 2α, 3β-diol system in baccatin was proposed from the analysis of the NMR spectra of

baccatin and its various degradation products. A chemical correlation of this conclusion was also warranted.

Considering these facts a partial synthesis of baccatin or any one of its degradation product was needed to confirm the proposed structure (7a) for baccatin. The present work (described in detail in Section B) constituted a successful partial synthesis of 2α, 3β-diacetoxy-28-nor Oleana-12, 17-diene (21), a degradation product of baccatin, from a known triterpene, namely, crategolic acid, by well established chemical methods.

Section B: Partial Synthesis of 2α, 3β-diacetoxy-28-Nor-Oleana-12, 17-diene (21):

The objective of the present study was to develop a partial synthesis of 2α, 3β-diacetoxy 28-nor oleana-12, 17 diene (21). It was thought that it would be better to start with a compound having the same type of ring A oxygenation pattern. Furthermore, in the desired synthetic pathway it would be necessary to convert a normal triterpene into a 28-nor derivative. This could be accomplished if the starting material contained some type of active function at C-28. With this view in mind, it was found that some derivatives of crategolic acid (25) which met all the above requirements, would be the best starting material. The



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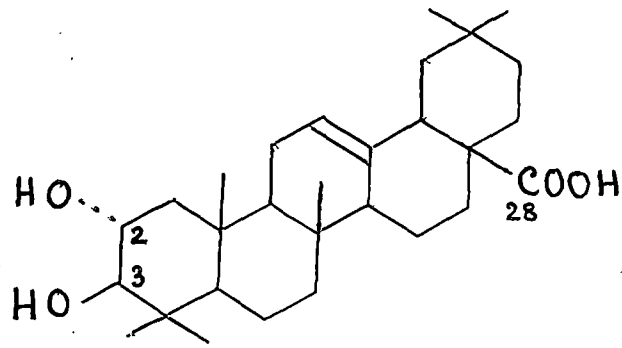
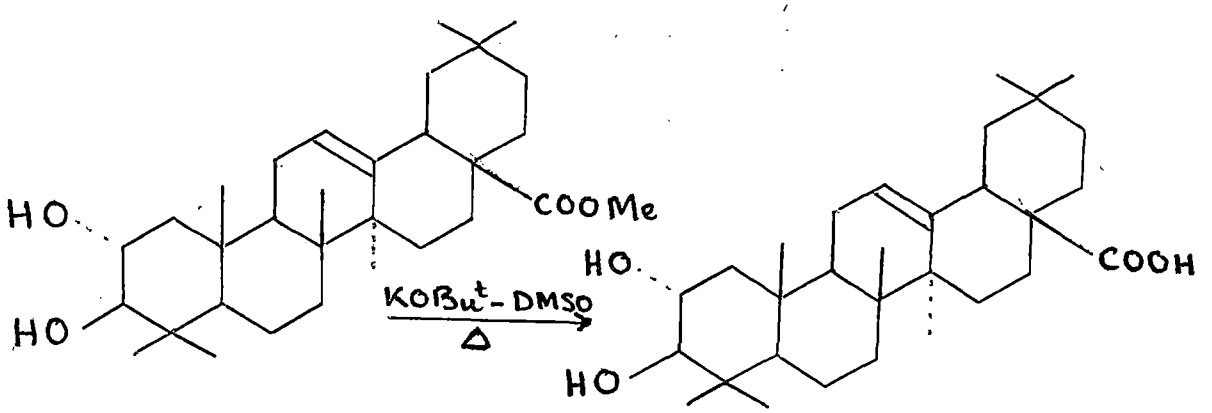


(25)

decarboxylation of the C-28 carboxyl group with concommitant oxidation by any well established method, such as that with lead tetraacetate[19-24], might be expected to afford the synthetic objective. Furthermore, the action of such a reagent on crategolic acid (25) itself (a compound containing a 2,3-diol system) would be expected to lead to a scission of the C_2-C_3 bond. Thus it was necessary to protect these two hydroxyl groups by acetylation prior to such oxidation.

The present work describes in detail the successful development of a method for the partial synthesis of 2α, 3β-diacetoxy 28-nor oleana-12, 17-diene, (21).

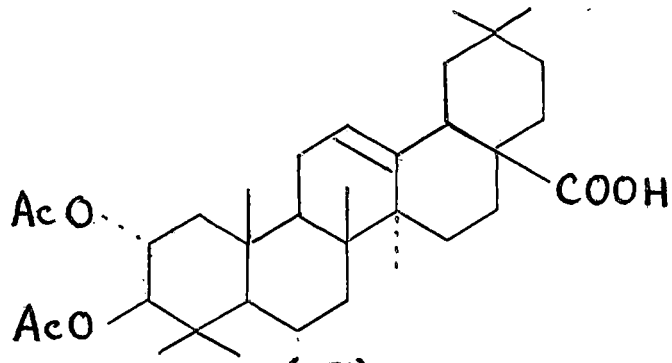
The flowers of Eugenia jambolana Lam[25,26] were used as the source of crategolic (syn. maslinic) acid (25). The benzene extract of the flowers of Eugenia jambolana Lam was separated into acidic and neutral portions. The acid part was directly chromatographed over silica gel[25]. The gummy material eluted by a mixture of benzene and ether (1:4) on rechromatography and crystallisation from aqueous methanol afforded crategolic acid (25), m.p. 266-69°, IR ν_{max}^{nujol} 3350, 1680 cm^{-1} identical with an authentic sample. However, the yield of pure crategolic acid (25) by this method was very poor. Most of the acid part remained as a gummy material from which the separation of pure crategolic acid (25) was not possible. Thus a somewhat different route was undertaken as already followed by Sengupta and Das[25,26] for the isolation of crategolic acid (25). The crude acid mixture was esterified with diazomethane and chromatographed on alumina. The solid eluted by a mixture of benzene and ether (1:4) on rechromatography followed by crystallisation from a mixture of benzene and petrol afforded satisfactory yield of methyl crategolate (26), m.p. 224-27°, $(\alpha)_D 36°$, IR ν_{max}^{nujol} 3200, 3100, 1710, 1230 cm^{-1} identical with an authentic sample of methyl crategolate. Methyl crategolate (26) was then hydrolysed with potassium tertiary butoxide and dimethyl sulfoxide following the method of Chang and Wood[27] to give crategolic acid (25), m.p. 266-69° identical with the acid obtained earlier.



(26) (25)

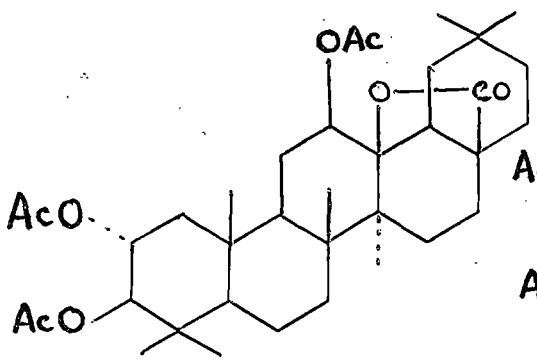
The above crategolic acid (25), on acetylation, afforded crategolic acid diacetate (27), m.p. 234-37°, IR ν_{max}^{nujol} 1740, 1680, 1250 cm^{-1}, $(\alpha)_D$ 31°.

Oxidative decarboxylation of crategolic acid diacetate (27) with lead tetraacetate following the method of Cambie et al[24] gave a mixture of products which could contain the lactone triacetate (28) the triacetate (29) and the isomeric mixture of diacetates (30).



(27)

$Pb(OAc)_4$ / C_6H_6-Py / Δ / N_2 Atmos.



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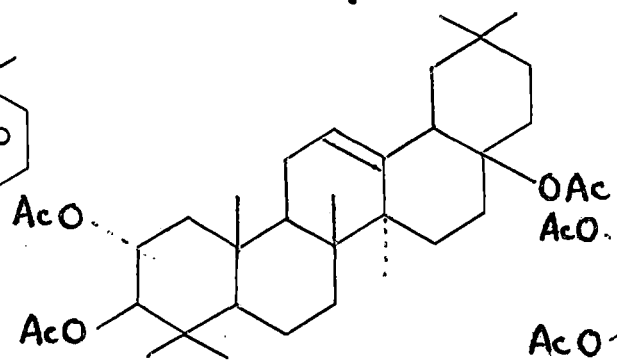

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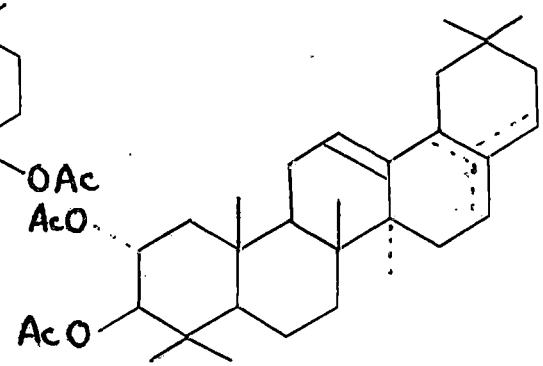
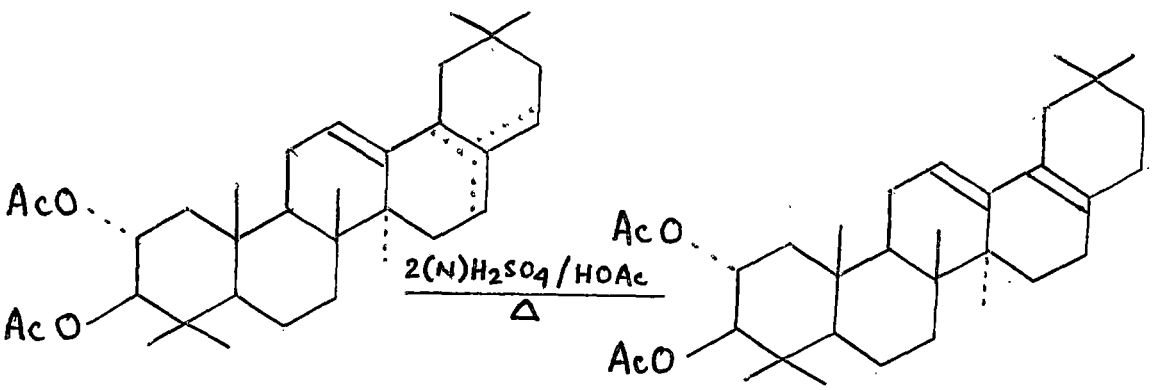


(30)

The reaction product was chromatographed over alumina. Elution with a mixture of petrol and benzene (4:1) afforded a solid mass which showed a melting point range from 114° to 170°. T.L.C. examination showed that it was indeed a mixture of three compounds. The IR spectrum showed bands at 1740, 1240 cm^{-1} (acetate). Elemental analysis closely corresponded to the molecular formula, $C_{33}H_{50}O_4$. This solid was, therefore, a mixture of the diacetates represented by the three diene diacetates (30). The UV spectrum of the mixture (30) showed peaks at λ_{max}^{MeOH} 237 (ϵ, 9510), 244 (ϵ, 10,050) and 252 nm (ϵ, 7590) thereby indicating that some amount of the heteroannular conjugated diene system was present in the mixture. The more polar fractions of the lead tetraacetate reaction product were not investigated further.

It could be expected that on acid treatment the mixture of dienes represented by (30) would rearrange completely to the single thermodynamically stable heteroannular conjugated diene (21). This was indeed found to be true. When the mixture (30) was heated with 2(N) sulfuric acid in acetic acid it rearranged to a product which on chromatography followed by crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals of 2α, 3β-diacetoxy-28-nor oleana-12, 17 diene (21), $C_{33}H_{50}O_4$, m.p. 189-90°, (TLC-single spot), IR ν_{max}^{nujol} 1745, 1650 (w), 1255, 1220 cm^{-1}, U.V λ_{max}^{MeOH} 237 (ϵ, 27,000), 244 (ϵ, 28,300) and 252 nm (ϵ, 20,200).



(30) (21)

This synthetic 2α, 3β-diacetoxy-28-nor oleana-12, 17-diene (21) was found to be identical (mmp, Co-TLC, UV, IR) with the heteroannular diene diacetate (21) previously obtained from the degradation of baccatin (7a).

The above unambiguous synthesis of 2α, 3β-diacetoxy-28-nor oleana-12, 17-diene (21) conclusively established the structure (21) proposed for the heteroannualr diene diacetate prepared from baccatin. The heteroannular diene diacetate thus contained a 2α, 3β-diacetoxy system and an oleanane type E ring and not an ursane type E ring. The presence of a

2α, 3β-diol system in baccatin was thus firmly established. The synthesis also provided a chemical proof for the presence of a traraxerene skeleton in baccatin and, in turn, confirmed beyond doubt the proposed structure (7a) for baccatin.

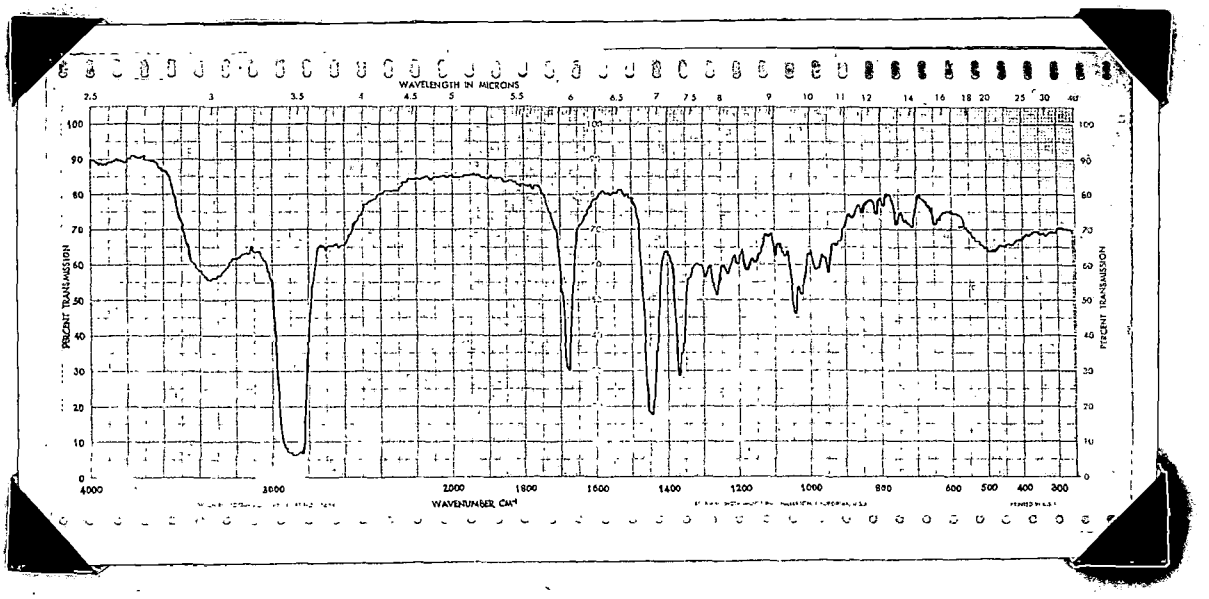


Fig. 1: IR spectrum of Crategolic Acid (25)

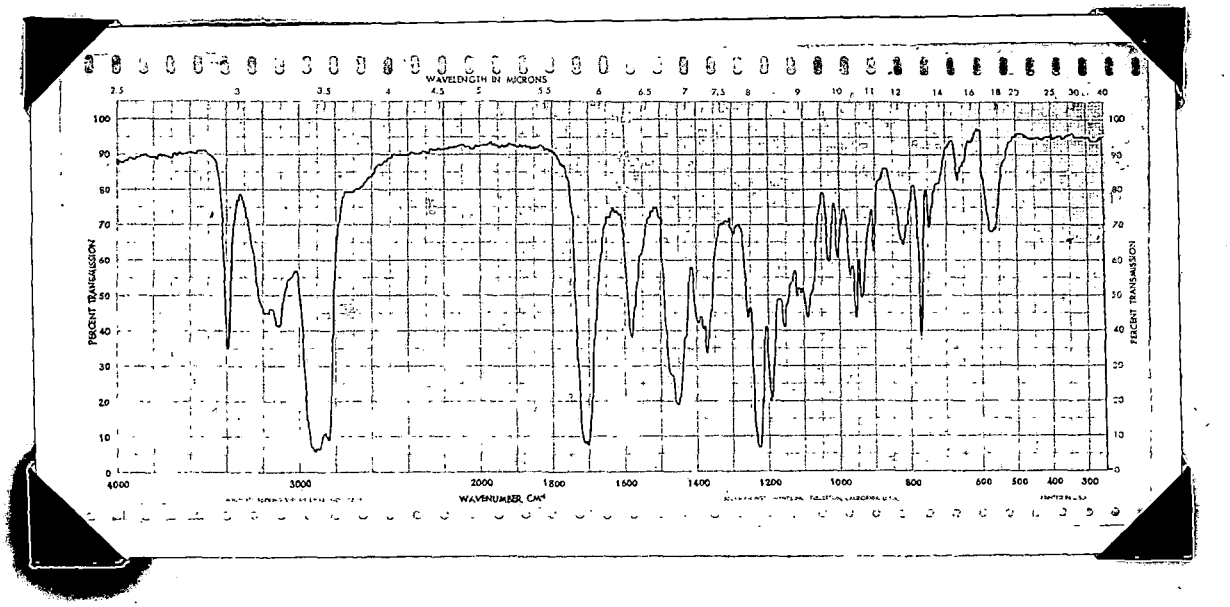


Fig. 2: IR spectrum of Methyl Crategolate (26)

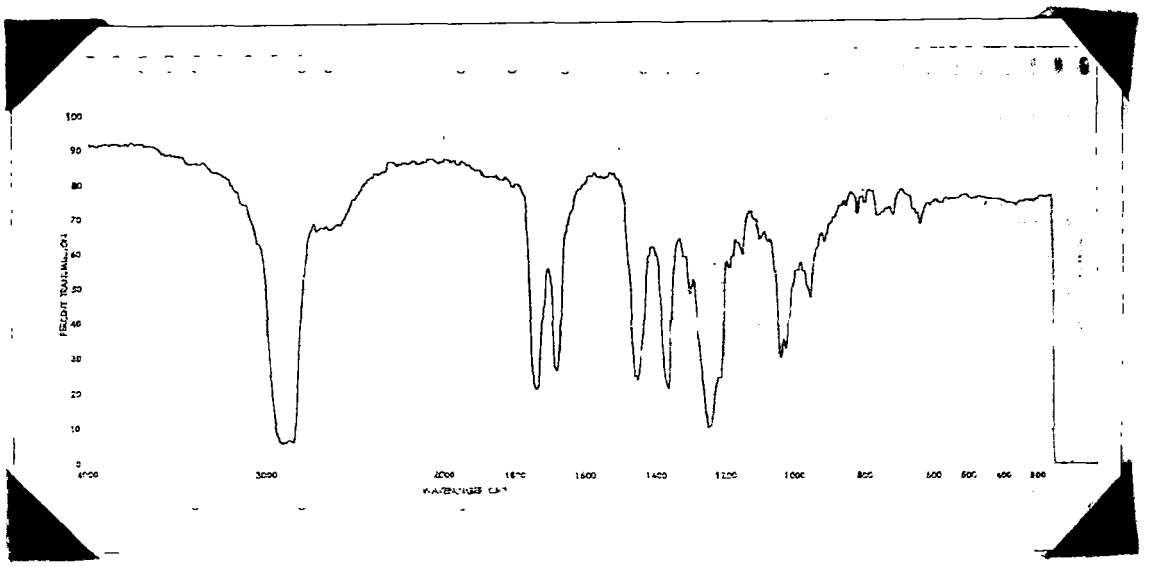


Fig. 3: IR spectrum of Crategolic Acid Diacetate (27)

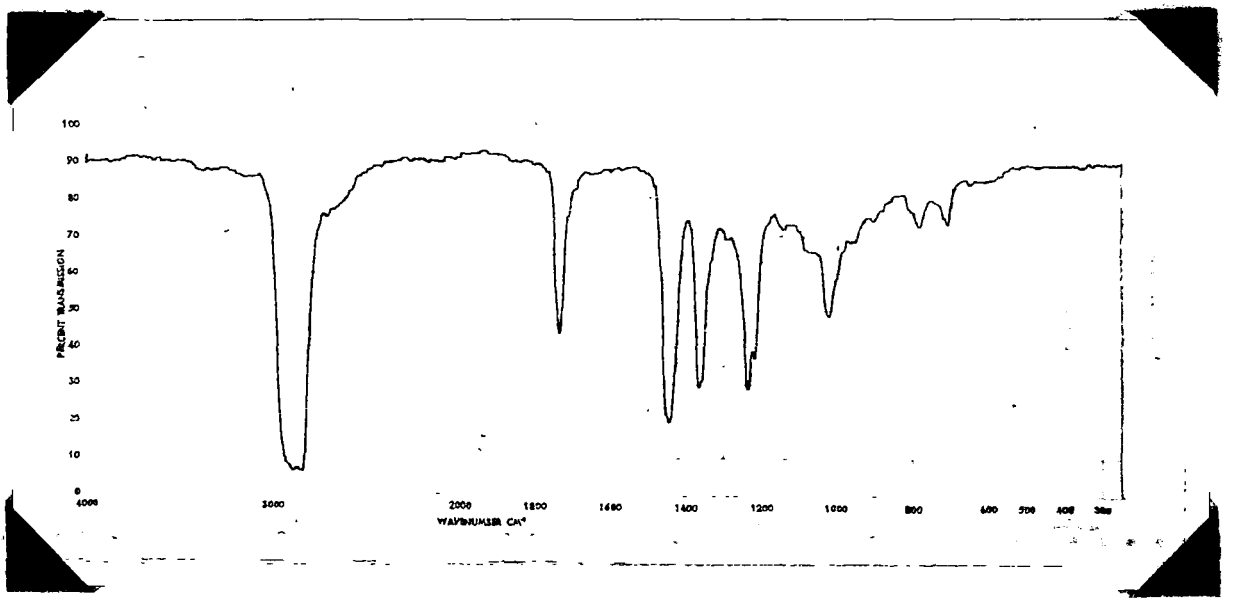


Fig. 4: IR spectrum of the Mixture of Diene Diacetates (30)

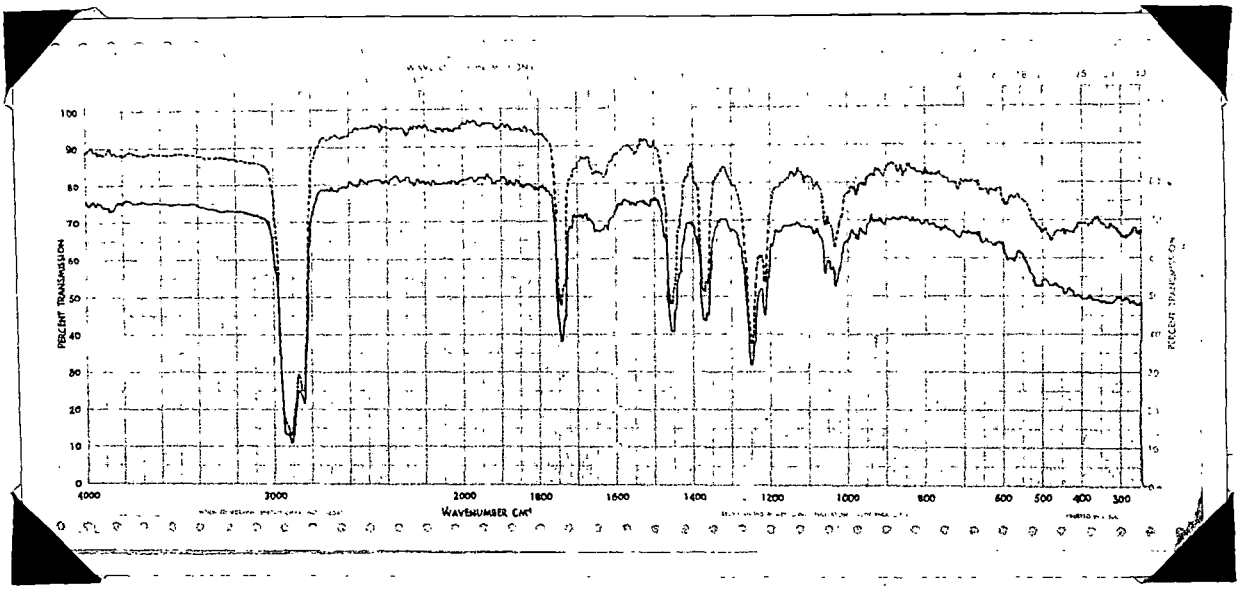


Fig. 5: IR comparison of synthetic 2α, 3β -Diacetoxy-28-Nor Oleana-12,17-diene (21) (Solid line) with an authentic specimen (dotted line) previously obtained from the degradation of baccatin (7a)

CHAPTER-III

EXPERIMENTAL

Melting points are uncorrected. The petrol used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution. The I.R. spectra were recorded in Beckmann IR-20 spectrophotometer. The U.V. absorption spectra were taken in Beckmann DU-2 spectrophotometer in methanol solution. TLC was carried out on 12% silver nitrate impregnated silica gel G (E.Merck) plate and the spots were developed with sulfuric acid-acetic anhydride (1:9) mixture. Silica gel used for column chromatography was of 60-120 mesh (B.D.H) and alumina used for column chromatography was of active, basic grade (B.D.H).

Extraction of the Flowers of *Eugenia Jambolana* Lam and Separation of the Acid Part:

The dried and powdered flowers of *Eugenia Jambolana* Lam (2 kg) were extracted with benzene in a soxhlet apparatus for 24 hours. Benzene was distilled off and the gummy residue (85 g) was digested with ether. The ether solution was separated from ether-insoluble material by filtration. The ether solution was extracted with 10% aqueous potassium hydroxide solution (4 x 200 ml). The aqueous alkaline layer was acidified with cold dilute hydrochloric acid and the precipitated dark brown coloured acidic material (30g.) was separated by filtration.

Chromatography of the Acid Part: Isolation of Crategolic Acid (25):

A solution of the above acidic material (10 g) in benzene (100 ml) was placed over a column of silica gel (300 g) and the column was eluted with the following solvents (Table-I).

Table-I

Eluent	Fractions 250 ml each	Residue on evaporation
Benzene	1-4	Brown gum
Benzene: Ether (4:1)	5-18	Brown gum
Benzene: Ether (3:2)	19-27	Gummy solid (0.8 g), m.p. 232-272°
Benzene: Ether (2:3)	28-34	Gummy solid (0.3 g), m.p. 221-37°
Benzene: Ether (1:4)	35-41	Gummy solid (0.5 g), m.p. 259-266°
Ether	42-46	Trace gummy solid
Chloroform	47-51	Nil
Methanol	52-58	Gummy solid, m.p. 217-232°

The gummy solid (0.5 g) obtained from the fractions 35-41 (Table-I) was rechromatographed over a column of silica gel (30 g). The brownish solid (0.3 g) eluted with ether was dissolved in hot methanol, decolorised with active charcoal and filtered. Concentration of the methanol solution and dilution with water afforded crystalline crategolic acid (25, 0.2 g), m.p. 266-69° identical with an authentic specimen.

Found: C, 76.09; H, 10.16.

Calc. for $C_{30}H_{48}O_4$: C, 76.23; H, 10.24%

IR ν_{max}^{nujol} 3350, 1680 cm^{-1}

Fig-1

Esterification of the Acidic Part: Isolation of Methyl Crategolate (26):

The ether solution of the total crude acid mixture (15 g) mentioned before was esterified with an ethereal solution of diazomethane prepared from nitrosomethylurea (15 g) in the usual way and the mixture kept overnight. The excess of diazomethane was decomposed with acetic acid (8 ml). The ether solution was washed with water, 10% aqueous potassium hydroxide solution and again with water till neutral and then dried (Na_2SO_4). Evaporation

of ether gave a gummy residue (14.5 g). The crude ester mixture (14.5 g) dissolved in benzene (20 ml) was placed over a column of alumina (900 g; deactivated with 54 ml of 10% aqueous acetic acid) and the column was eluted with the following solvents (Table-II).

Table-II

Eluent	Fractions 250 ml each	Residue on evaporation
Petrol	1-12	Oil (2.4 g)
Petrol: Benzene (1:1)	13-20	Oil (1.1 g)
Benzene	21-33	Oily Solid (5.3 g), m.p. 104-54°
Benzene: Ether (4:1)	34-39	Oil (0.4 g)
Benzene: Ether (3:2)	40-45	Oil (0.8 g)
Benzene: Ether (2:3)	46-51	Oil (0.6 g)
Benzene: Ether (1:4)	52-60	Oily Solid (1.2 g), m.p. 217-22°
Ether	61-66	Gum (0.1 g)
Chloroform	67-75	Gum (0.2 g)

The solid (1.2 g) obtained from fractions 52-60 (Table-II) was rechromatographed over a column of alumina (54 g; deactivated with 3.2 ml of 10% aqueous acetic acid). The solid (0.9 g) eluted with ether on crystallisation from a mixture of benzene and petrol afforded crystals (0.6 g) of methyl crategolate (26), m.p. 224-27°, $(\alpha)_D$ 36° identical with an authentic specimen.

Found: C, 76.32; H, 10.18.

Calc. for $C_{31}H_{50}O_4$: C, 76.54; H, 10.28%

IR ν_{max}^{nujol} 3200, 3100, 1710, 1230 cm^{-1}

Fig-2

Hydrolysis of Methyl Crategolate (26): Preparation of Crategolic Acid (25):

To a suspension of dry potassium tertiary butoxide (prepared from 2.8 g of metallic potassium and 35 ml of dry tertiary butanol) in distilled dimethyl sulfoxide (20 ml) was added a solution of methyl crategolate (25; 1 g) in distilled dimethyl sulfoxide (50 ml) and the reaction mixture was heated on an oil bath at 120° for 8 hours. The mixture was allowed to cool to room temperature and allowed to stand overnight. The reaction mixture was diluted with water (500 ml) and acidified

with cold dilute hydrochloric acid. The precipitated material was extracted with chloroform. The chloroform solution was washed with water till neutral and dried (Na_2SO_4). Removal of the solvent gave a brown coloured gummy material (1.2 g) dissolved in benzene (12 ml) and was placed over a column of silica gel (75 g) which was eluted with the following solvents (Table-III).

Table-III

Eluent	Fractions 100 ml each	Residue on evaporation
Benzene	1-6	Oil
Benzene: Ether (4:1)	7-11	Oil
Benzene : Ether (3:2)	12-15	Trace oil
Benzene: Ether (2:3)	16-19	Trace oil
Benzene: Ether (1:4)	20-24	Trace oil
Ether	25-33	Partially crystalline solid (0.9 g), m.p. 262-67°

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

Fractions 25-33 (Table-III) were combined (0.9 g) dissolved in hot methanol, decolorised with active charcoal and filtered. Concentration of the methanol solution and dilution with water afforded crategolic acid (25, 0.6 g), m.p. 266-69° identical with the crategolic acid obtained earlier.

Acetylation of Crategolic Acid (25): Preparation of Crategolic Acid Diacetate (27):

To a solution of crategolic acid (25; 0.6 g) in pyridine (20 ml) was added acetic anhydride (20 ml) and the mixture was heated on a water bath for 12 hours. The reaction mixture was allowed to stand overnight at room temperature, then diluted with water (500 ml) and the precipitated material was extracted with ether. The ether layer was washed with water till neutral and dried (Na_2SO_4). Removal of solvent gave a slightly coloured residue (0.6 g) which was dissolved in benzene (6 ml) and placed on a column of silica gel (60 g) which was eluted with the following solvents (Table-IV).

Table-IV

Eluent	Fractions 100 ml each	Residue on evaporation
Petrol	1-4	Oil
Petrol: Benzene (4:1)	5-8	Nil
Petrol: Benzene (3:2)	9-12	Nil
Petrol: Benzene (2:3)	13-16	Trace oil
Petrol: Benzene (1:4)	17-20	Trace oil
Benzene	21-25	Trace oil
Benzene: Ether (9:1)	26-36	Solid (0.5 g), m.p. 232-35^{0}
Benzene: Ether (4:1)	37-40	Trace oil

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

The solid (0.5 g) from fractions 26-36 (Table-IV) was dissolved in hot methanol, boiled with active charcoal and filtered. Removal of the solvent gave a white solid (450 mg). The solid (450 mg) upon crystallisation from a mixture of ether and petrol afforded

needle shaped crystals (350 mg) of pure crategolic acid diacetate, (27), m.p. 234-37°, $(\alpha)_D$ 31° .

Found:	C, 73.26;	H, 9.35.
Calc. for $C_{34}H_{52}O_6$:	C, 73.35;	H, 9.41%

IR ν_{max}^{nujol} 1740, 1680, 1250 cm^{-1}

Fig-3

Lead Tetraacetate Decarboxylation of Crategolic Acid Diacetate (27):

To a solution of crategolic acid diacetate (27; 300 mg) in dry benzene (25 ml) was added pyridine (0.3 ml) followed by lead tetraacetate (0.5 g). The reaction mixture which immediately turned dark brown was stirred under nitrogen atmosphere for one hour at room temperature and then refluxed for 4 hours. The cooled mixture was filtered and the filtrate was concentrated under vacuum to yield a yellowish residue (300 mg) which was dissolved in benzene (3 ml) and placed on a column of alumina (30 g; deactivated with 1.8 ml of 10% aqueous acetic acid), which was eluted with the following solvents (Table-V).

Table-V

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-12	Trace oil
Petrol: Benzene (4:1)	13-20	Foamy solid (63.3 mg), m.p. 114-170°
Petrol: Benzene (3:2)	21-28	Trace oil
Petrol: Benzene (2:3)	29-32	Trace oil
Petrol: Benzene (1:4)	33-37	Trace oil
Benzene	38-42	Trace oil

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

Examination of the Solid from Fractions 13-20 (Table-V):

Isolation of the Mixture of Diene Diacetates (30):

The foamy solid (63.3 mg) from fractions 13-20 (Table-V) showed a range of melting point from 114 to 170° and three spots on TLC.

Found: C, 77.73; H, 9.58.

Calc. for $C_{33}H_{50}O_4$: C, 77.95; H, 9.44%

IR ν_{max}^{nujol} 1740, 1240 cm^{-1}

Fig-4

UV λ_{max}^{MeOH} 237 (ϵ, 9510), 244 (ϵ, 10,050) and 252 nm (ϵ, 7590).

The above physical data indicated that it was a mixture of the diene diacetates represented by structure (30).

Acid Isomerisation of the Mixture of Diene Diacetates (30): Preparation of 2α, 3β-Diacetoxy-28-nor oleana-12,17-diene (21):

To a solution of the above mixture of diene diacetates (30; 60 mg) in glacial acetic acid (3 ml) was added a 2(N) sulfuric acid solution (0.3 ml). The mixture was heated on a water bath for 2.5 hours, cooled to room temperature and poured into ice-cold water (100 ml), whereby a yellowish solid separated out. The latter solid was extracted with ether, and the ether layer was washed with water till neutral and dried (Na_2SO_4). Removal of the solvent gave a yellow gum (66.3 mg) which was dissolved in benzene (3 ml) and placed on a column of alumina

(12 g; deactivated with 0.7 ml of 10% aqueous acetic acid). The column was eluted with the following solvents (Table-VI).

Table-VI

Eluent	Fractions 25 ml each	Residue on evaporation
Petrol	1-10	Trace oil
Petrol: Benzene (9:1)	11-17	Trace oil
Petrol: Benzene (4:1)	18-35	Solid (40 mg), m.p. 184-86°

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

The solid (40 mg) from fractions 18-35 (Table-VI) on crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals (25 mg) of 2α, 3β-diacetoxy-28-nor oleana-12, 17-diene (21), m.p. 189-90°.

TLC : Single spot Rf = 0.34, Solvent- Benzene.

Found: C, 77.83; H, 9.53.

Calc. for $C_{33}H_{50}O_4$: C, 77.95; H, 9.44%

U.V. λ_{max}^{MeOH} 237 (ϵ, 27,000), 244 (ϵ, 28,300) and 252 nm (ϵ, 20,200).

IR ν_{max}^{nujol} 1745, 1650 (w), 1255, 1220 cm^{-1}

The 2α, 3β-diacetoxy 28-nor oleana-12, 17-diene (21) synthesised in this manner was found to be identical (m.m.p, U.V. absorption, Co-TLC, superimposable IR spectra - Fig-5) with an authentic specimen of 2α, 3β-diacetoxy-28-nor oleana-12, 17-diene (21) previously obtained[7,8] from the degradation of baccatin (7a).

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PART-III

PARTIAL SYNTHESIS OF ALL THE FOUR STEREOISOMERS OF DIMETHYL DIHYDROCEANOTHATE STARTING FROM BETULINIC ACID.

PART-III

CHAPTER-I

A Short Review on the Isolation, Structure Elucidation and Stereochemistry of Ceanothic Acid.

Section A : Isolation

Ceanothic acid was first isolated by Julian, Pikl and Dawson[1] from the root bark of *Ceanothus americanus* and has subsequently been isolated from a number of Australian plants belonging to *Rhamnaceae* species[2,3]. Ceanothic acid was characterised by Julian *et al*[1] as a hydroxy dicarboxylic acid and attributed the molecular formula $C_{29}H_{44}O_5$. It was further characterised by the preparation of a dimethyl ester and of a dimethyl ester monoacetate. In 1958, Boyer *et al*[2] isolated emmolic acid from *Emmenospermum alphitonioides* F.Muell (*Rhamnaceae*) which was subsequently shown to be identical with Ceanothic acid by Birch and co-workers[4]. de Mayo and Starratt[5] in 1962 also isolated ceanothic acid from *Ceanothus americanus*. They, however, could not isolate the acid by adopting the procedure used by Julian *et al*[1] and consequently developed a somewhat modified procedure which led to the successful isolation of pure ceanothic acid. de Mayo and Starratt[5] remarked on the variability

of the plant and subsequent workers[6] have also made similar observations. By adopting the modified procedure, de Mayo and Starratt[5] were able to isolate different proportions of the various acid constituents. Their method leading to ceanothic acid has been described here. The ground root bark of Ceanothus americanus was extracted continuously with ether in a soxhlet apparatus for 33 hours. The residue obtained by removing ether was extracted with light petroleum under reflux for 3 hours. The process was repeated four times. The residue, in ethereal solution, was extracted exhaustively with 2% potassium hydroxide solution. During the extraction a solid was separated at the interface and this was removed by filtration. Acidification of the alkaline solution and isolation with ether gave the crude acid mixture which was further defatted by extraction with light petroleum. The benzene solution of the defatted residual mixture was added to a column of silica gel. Elution with a mixture of benzene and ether (20:1) gave an acid, m.p. 350-54° (decomp.), $(\alpha)_D$ 39°. Further elution with the same solvent mixture then gave betulinic acid, m.p. 270-85°. Elution with benzene-ether (10:1) then gave a material which after several crystallisations first from a mixture of ether and benzene and then of ether and methanol gave pure ceanothic acid, m.p. 356-57° (gas evolution) (lit.[1] m.p. 354°), $(\alpha)_D$ 38°, ν_{max}^{nujol} 3480, 1720, 1641 and 883 cm^{-1}. de Mayo and Starrat[5] also showed that the molecular

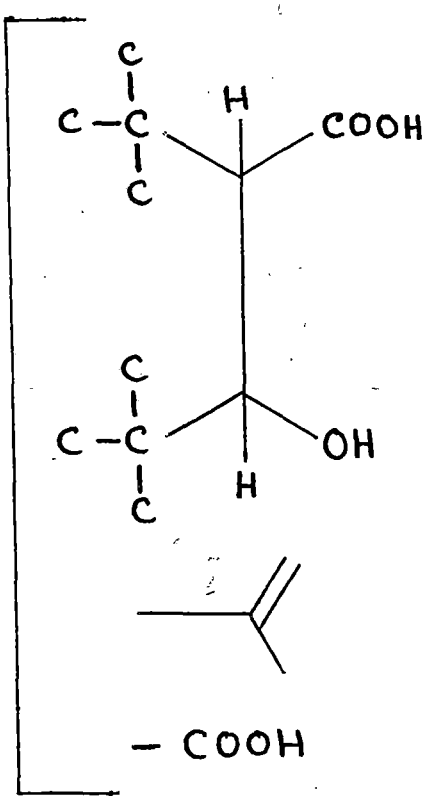
formula, $C_{29}H_{44}O_5$, suggested by Julian et al[1] for ceanothic acid was incorrect. The actual molecular formula was shown to be $C_{30}H_{46}O_5$.

Section B: Structure Elucidation of Ceanothic Acid.

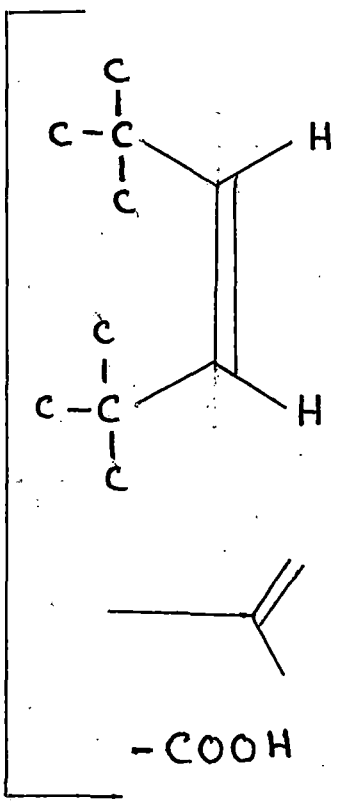
The systematic approach to the structure of ceanothic acid stems from the work of de Mayo and Starratt[5]. Their work is summarised below.

The infrared spectra of ceanothic acid and of its dimethyl ester showed bands at ν_{max}^{nujol} 833 and 889 cm^{-1} respectively, which disappeared on hydrogenation to their respective saturated dihydro compounds. This indicated the presence of an exo methylene group. The NMR spectrum of the ester showed bands at τ 5.37 (2H, d, $J \sim 7.7$ c.p.s) attributed to the methylene group and at τ 8.36 (3H, S) attributed to a vinylic methyl group[7]. These observations, in the absence of any other double bond, indicated the presence of an isopropenyl group. The NMR spectrum further showed singlets (1H each) at τ 5.98 and τ 7.51 suggesting that both the hydroxyl groups and one of the carboxyl groups were attached to carbon atoms bearing only one hydrogen atom, since these signals were in appropriate positions for the respective methine hydrogens[7]. The proximity of the hydroxyl group and the secondary carboxyl group was established from a study of the "lactone",

previously obtained by Julian et al[1], by heating ceanothic acid to its melting point. This substance was shown to be an unsaturated acid by its conversion, with diazomethane, to the corresponding ester. In addition to the signals at τ 5.40 and τ 8.46 in the NMR spectrum, indicative of the continuing presence of the isopropenyl group, signals for two hydrogens producing an AB pattern (doublets at τ 4.16 and τ 4.66; $J_{AB} \sim 5.4$ c.p.s) now appeared. The formation of this pattern was suggested as due to the transformation indicated in the conversion of (1) to (2), that is, the dehydration-decarboxylation of a β-hydroxy acid.



(1)



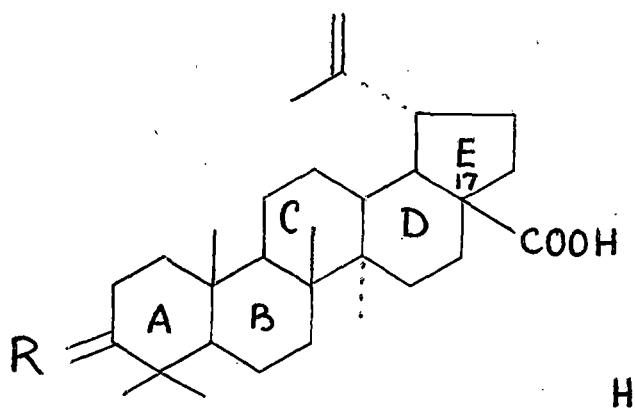
(2)

The above view was confirmed from a study of the pyrolysis of the benzoate of dimethyl ceanothate, whereby a molecule of benzoic acid was eliminated and an anhydroacid was obtained. The latter showed the ultraviolet end absorption expected for an isolated isopropenyl group and an $\alpha\beta$-unsaturated ester. Furthermore, a signal at τ 3.9 (1H, S) in the N.M.R. spectrum confirmed the presence of the expected olefinic hydrogen in addition to those of the isopropenyl group[7]. Since dihydroceanothic acid and its dimethyl ester showed no ultraviolet absorption in the region from 200 to 300 nm, they were, presumably, saturated and consequently ceanothic acid was pentacarbocyclic. Because of the presence of an isopropenyl group and its occurrence along with betulinic acid, de Mayo and Starratt[5] assumed that ceanothic acid was probably related to the lupeol-betulin-betulinic acid (3, R = H, OH) series. One of the characteristic transformations of this group of substances was the ready acid catalysed expansion of the terminal, E, ring to give derivatives of the β-amyrin series[8]. In those substances having a carboxyl function at C_{17} concomitant lactonisation occurred. Betulinic acid (3, R = H, OH) for example, was converted into (4). Ceanothic acid was similarly converted, by refluxing with formic acid for 3 hours, into a γ-lactone, ν_{max} 1696 (carboxyl) and 1762 cm^{-1} (γ-lactone) with the simultaneous disappearance of the isopropenyl

group. The resulting monocarboxylic acid lactone was further characterised as the acetate, indicating the non-participation of the hydroxyl group in the lactonisation process. One of the carboxylic acid groups remained as evidenced by the formation of a mono-methyl ester lactone. To accommodate the presence of the functions indicated in (1) in ceanothic acid, de Mayo and Starratt[5] suggested that some modification of ring A of betulinic acid was necessary. The grouping in (1) was suggestive of the occurrence of a 'biogenetic' pinacolic rearrangement at some stage in the genesis of ceanothic acid, such as may take place in the formation of gibberellic acid[9] and of the aldehyde in magnamycin[10]. Oxidation of methyl dihydroceanothate with sodium dichromate gave the corresponding Ketone diester. Alkaline hydrolysis resulted in the elimination of carbon dioxide expected of a β-ketoester and the formation of a ketonic monoester. This substance showed an unresolved band in the infrared spectrum at ν_{max} 1738 cm^{-1} for the cyclopentanone and ester, while its precursor showed bands at $\nu_{max}^{CCl_4}$ 1750 (cyclopentanone) and 1727 cm^{-1} (ester).

The above experiments led de Mayo *et al* to propose two structures (5) and (6) for the ketone, but its properties suggested that it was (6), a substance previously prepared by Ruzicka *et al*[11,12] from betulonic acid (3, R = O) by hydrogenation, nitric acid oxidation to (7), followed by pyrolysis and

esterification. Direct preparation of this substance from methyldihydrobetulonate and comparison of it, and its various derivatives showed them to be identical in every respect. Therefore, they proposed four possible structures(8), (9), (10) and (11) for ceanothic acid.



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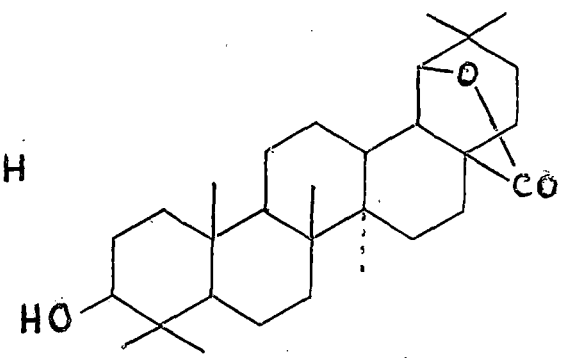

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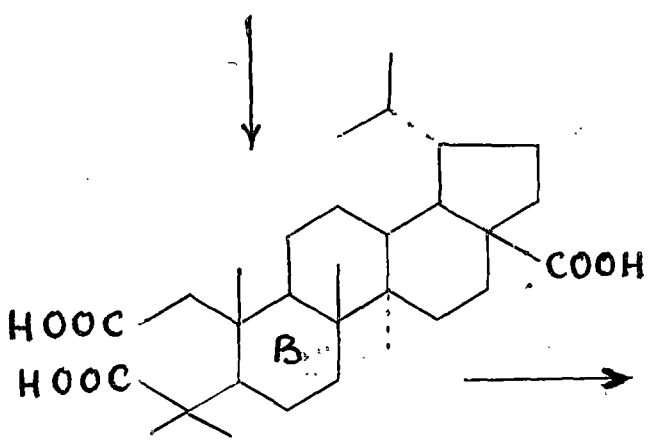

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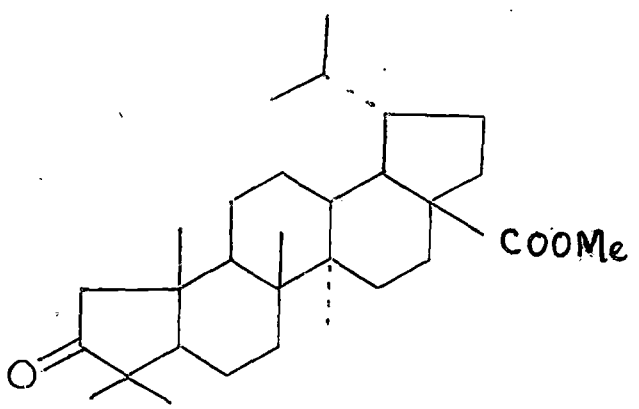

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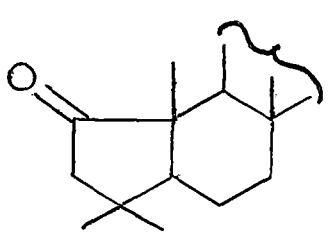

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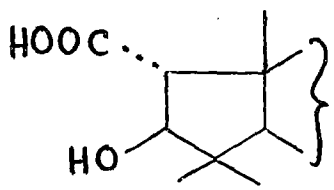

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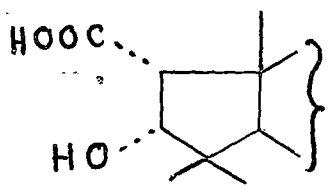

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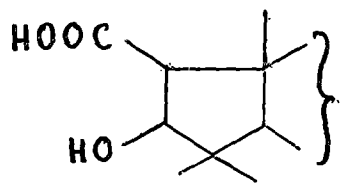

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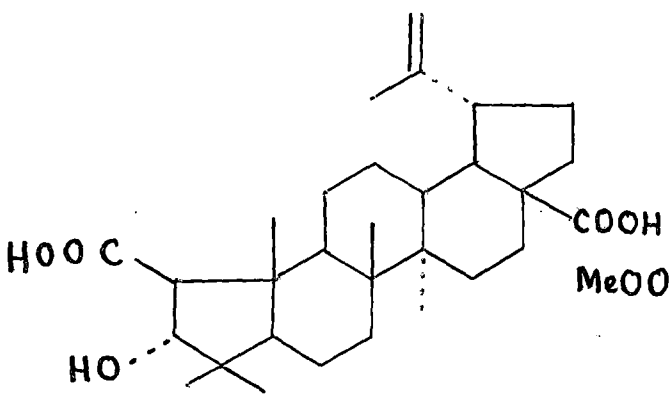

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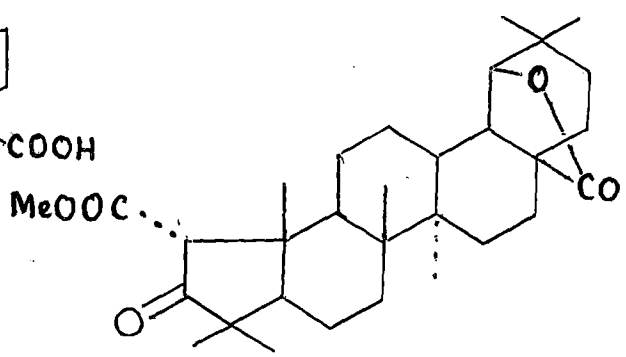


(12)

The N.M.R. spectrum of dimethyl ceanothate showed that the methine hydrogens adjacent to the carbomethoxyl and hydroxyl groups were singlets; that is, although the hydrogens were on adjacent carbon atoms, the coupling constant was close to zero. In contrast, the ketone (12), derived from methyl ceanothate lactone, on reduction with sodium borohydride gave an epimeric alcoholic lactone, methyl isoceanothate lactone. The N.M.R. spectrum of the latter showed a doublet at τ 7.0, the methine hydrogens being coupled, and a quartet at $\tau 5.9^{7}$, the hydrogen on the carbon bearing oxygen being split by the adjacent methine hydrogen and by the hydroxyl hydrogen. This suggested that in

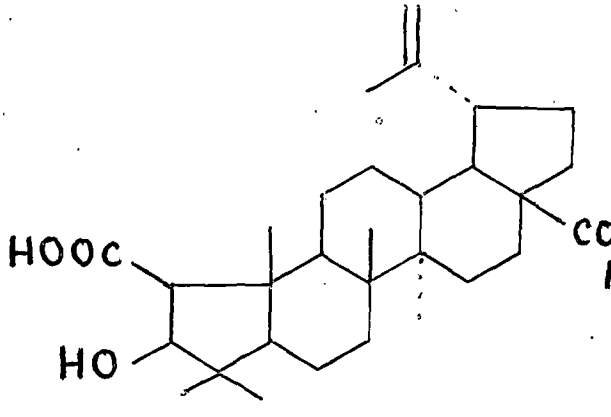
dimethyl ceanothate the hydrogen atoms were at an angle of about 90°[13,14] and, therefore, in the trans relationship.

Further evidence supporting this view was obtained from a study of infrared spectra. Treatment of methyl dehydroceanothate lactone (12), with sodium methoxide resulted in a rapid epimerisation of the carbomethoxyl group-which must, therefore, be in the unstable configuration - and the formation of the isomeric methyl dehydroepiceanothate lactone. Reduction of this with sodium borohydride then gave methyl epiceanothate lactone. Having prepared three of the four possible epimeric hydroxy esters, de Mayo and Starratt[5] carefully examined the carbonyl and hydroxyl regions of the infrared spectra of these substances in solution. It was found that methyl dihydroceanothate showed a normal unbonded ester and, in agreement, the hydroxyl group showed intermolecular hydrogen bonding only in the most concentrated solution. In contrast, both the epimeric methyl isoceanothate lactone and methyl epiceanothate lactone showed a bonded ester group and a bonded hydroxyl band even in dilute solution. These results supported the trans and cis configurations, respectively, allocated to dimethyl ceanothate and methyl isoceanothate lactone. They suggested that methyl epiceanothate lactone was, also, probably cis.

Considering all the above observations, de Mayo and Starratt[5] suggested that only two structures (8) and (11)

remained for ceanothic acid. From an inspection of models they suggested that the β-carbomethoxyl group was under more severe non-bonded interaction than the α-epimer. In view of the observed ready epimerisation of methyl dehydroceanothate lactone, they preferred the stereostructure (11) for ceanothic acid.

In 1961, just prior to the publication of the work of de Mayo and Starratt[5], Mechoulam[15] published a paper in which he assigned the β-configuration of the carboxyl group in ring A and a cis relationship between this carboxyl and the adjacent hydroxyl groups (13). His arguments were as follows. None of the secondary and the tertiary carbomethoxyl groups of dimethyl ceanothate underwent hydrolysis with 10% KOH solution



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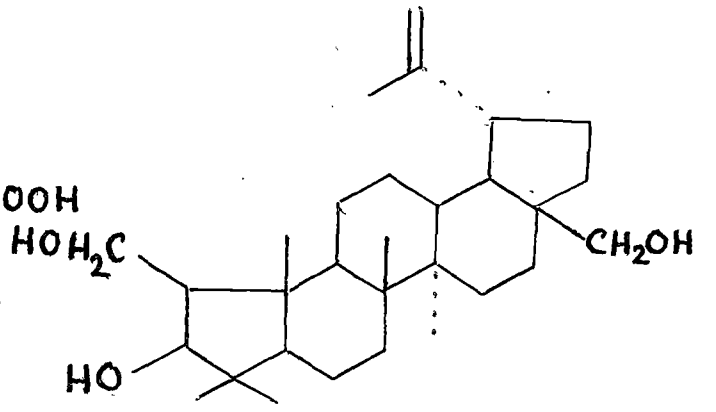


(14)

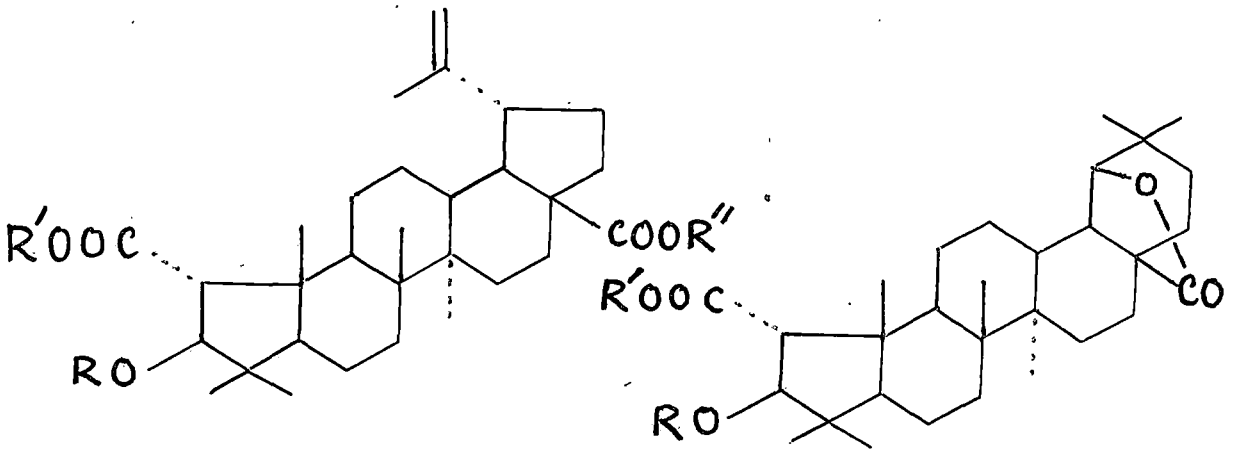
and consequently both of them were hindered. Inspection of the model showed that a 2β-, but not 2α-, carboxyl group was considerably hindered; therefore, they ascribed a β-carboxyl configuration to the natural product. Furthermore, they showed that both dimethyl ceanothate and dimethyl - 3-oxo-ceanothate on lithium aluminium hydride reduction, gave the same triol[2] (14), m.p. 226-28°, $(\alpha)_D$ 46° (EtOH). They argued that since for steric reasons the latter would be expected to give the 3β-ol by this type of reduction, the natural product thus contained β-hydroxyl grouping. The cis relationship, thus obtained, was supported by the presence of an intramolecular hydrogen bond as evidenced from the I.R. spectrum of a very dilute solution of the triol (14). Later Eade, Kornis and Simes[16,17] and Mechaulam[18] himself negated the idea of cis relationship of the carboxyl and hydroxyl group and gave further evidence in support of the structure proposed by de Mayo and Starratt[5].

Eade et al[16] using lithium fluoride optics, examined the I.R. spectrum of dimethyl ceanothate at various concentrations. From the results, they concluded that intramolecular hydrogen bonding was absent. Inspection of the model showed that intramolecular hydrogen bonding would be expected to occur only when the adjacent methoxycarbonyl and hydroxyl groups were cis[19]. Thus in dimethyl ceanothate these groups were, presumably, trans.

In contrast the work of Mechaulam[15], Eade et al[16] reported that lithium aluminium hydride reduction of dimethyl dihydroceanothate and the corresponding ketone, dimethyl 3-oxo- dihydroceanothate gave different triols. They explained this observation on the basis of two possible factors: (I) the reducing agent could chelate with the hindering group and this might affect the stereochemical course of the reduction of the ketone, and (II) epimerisation of the methoxycarbonyl group might occur during reduction.

In 1958, Boyer et al[2] reported that dimethyl ceanothate was stable to boiling 10% ethanolic potassium hydroxide for 2 hours. But Eade et al[16] found that when refluxed for 48 hours with 20% methanolic potassium hydroxide, dimethyl ceanothate was converted into an equilibrium mixture from which the major product, mono-methyl ester (15A) could be isolated in 50% yield. Methylation of (15A) gave a dimethyl ester (15B) which was isomeric with dimethyl ceanothate. The corresponding acid (15C), isomeric with ceanothic acid, was named isoceanothic acid. Lactonisation (H_2SO_4/Acetic acid/Benzene) of compound (15D) [obtained by acetylation of (15A)] gave the lactone (16B) indicating that it was the methoxycarbonyl group which had been hydrolysed by alkali. Both lactone (17A) (normal series, prepared by lactonisation of dimethyl ceanothate) and lactone (16C) (iso-series) were converted into the corresponding oxo-esters (18A)

and (18B) respectively having different physical constants; both (18A) and (18B), however, yielded the same nor-ketone (18C).



(15A) $R = R' = H$, $R'' = CH_3$

(15B) $R = H$, $R' = R'' = CH_3$

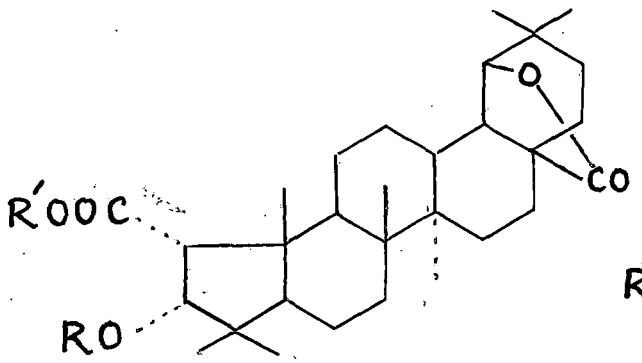
(15C) $R = R' = R'' = H$

(15D) $R = COMe$, $R' = H$, $R'' = CH_3$

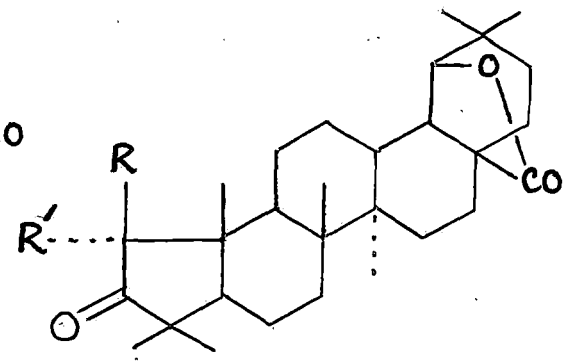
(16A) $R = R' = H$

(16B) $R = COMe$, $R' = H$

(16C) $R = H$, $R' = Me$



(17A) R = H, R′ = CH_3



(18A) R = COOMe, R′ = H

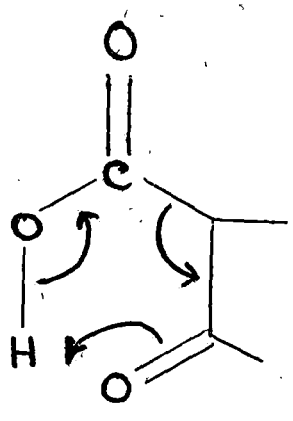
(18B) R = H, R′ = COOMe

(18C) R = R′ = H

Thus the methoxycarbonyl group in ceanothic acid and that in isoceanothic acid were epimeric. From an examination of the model, Eade et al[16] suggested that if the methoxycarbonyl group in ring A had the β-configuration it would be under considerable non-bonded interaction. Such interaction would be virtually absent if it possessed the α-configuration. Thus they concluded that the carboxyl group in ring A of ceanothic acid probably possessed the more strained β-configuration leading to the α-assignment for the hydroxyl group. The ring A substituents of isoceanothic acid were also assigned a trans relationship since the infrared spectra of the dimethyl ester (15B) showed the

absence of any intramolecular hydrogen bonding. Consequently formation of isoceanothic acid from ceanothic acid required an opening of ring A in a reverse aldol type reaction to an A-seco derivative followed by subsequent ring closure.

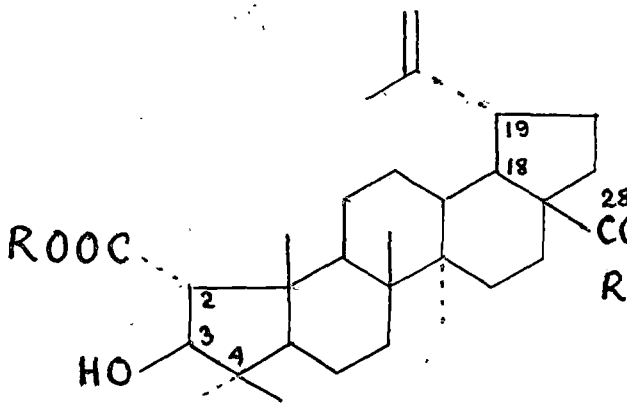
Crowley[20], in 1962, reported the natural occurrence of a 2,3 - seco triterpene and this supported the suggested biogenesis[3] of ceanothic acid by ring closure of a similar 2,3-seco derivative derived from 2-hydroxybetulinic acid; the latter has recently been isolated along with ceanothic acid from Alphitonia Whitei Braid[3]. Eade et al[16] found that the β-keto acid derived from the iso-series by oxidation of either (15A) or (16A) were quite stable and could be dried in vacuo at 100°. However, each acid underwent decarboxylation only at higher temperatures, for example, (16A) gave (18C) at 220°C. They attributed this stability as due to the difficulty in finding a cyclic planar transition state (A). In this connection, they cited the example of a stable β-keto acid, camphor-3-carboxylic acid, which, according to them was stereochemically similar.



(A)

Section C: Stereochemistry of Ceanothic Acid.

The stereochemistry of ceanothic acid was finally established by Eade et al[21,22]. These authors observed[17] that the ester group at C_2 in dimethyl ceanothate (19, R = Me) could be epimerised only with great difficulty, whereas in the corresponding ester, dimethyl ceanothate lactone (19A, R = Me), with a modified ring E area, it could be comparatively easily epimerised and was accompanied by simultaneous hydrolysis. They suggested[17] that this considerable difference in reactivity might be caused by a long range conformational transmission effect. They expected that a comparison of the coupling constants



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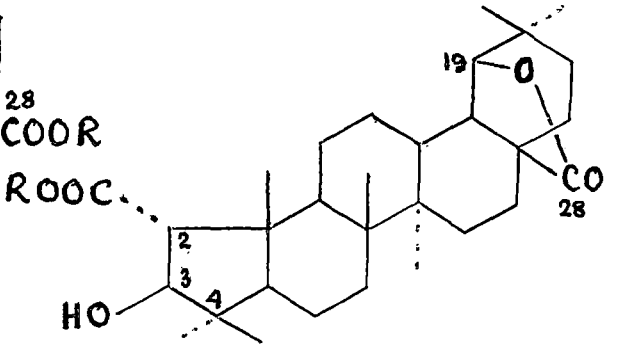


(19 A)

of the C_2 and C_3 protons of the four possible C_2- CO_2Me/C_3-OH isomers (and their acetates) in the dimethyl ceanothate series [carbon skeleton type (19)] with those of the corresponding compounds in the lactone series [carbon skeleton type (19A)] would reveal any significant differences in the shape of ring A between the relevant compounds. An explanation might also be found for the singlet for the C3 proton in the N.M.R. spectrum of dimethyl ceanothate[5] which was not in agreement with the structure then assigned to ceanothic acid even if ring A existed in the α-envelope conformation[23,24]. Further they expected that the preparation of the entire group of four isomers in each series would solve the controversy whether the "methyl epiceanothate" of de Mayo and Starratt had a cis or trans relationship of the ring A substituents[5,17].

Eade et al[21] finally proposed structure (19) for ceanothic acid in which the ring A substituents, while still having a trans relationship, possessed the configurations opposite to those originally put forward by de Mayo and Starratt[5]. Their works[22] are described below.

Preparation of the Dimethyl Ceanothate Series.

Of the four isomers in the dimethyl ceanothate series, two have been previously prepared. One was dimethyl ceanothate itself (19, R = Me) and the other was its C2 epimer (23) [methyl

-3β-hydroxy-2β-methoxycarbonyl-A(I)-Norlup-20(29)-en-28-oate] (Scheme 1) reported by Eade et al[17] who incorrectly formulated it as the trans isomer of dimethyl ceanothate epimerised at both C2 and C3 and was originally given the name dimethyl epiceanothate in accordance with the nomenclature used by de Mayo and Starratt[5]. This isomer (23) was first prepared[17] by epimerisation of the C2 methoxycarbonyl group by prolonged treatment of dimethyl ceanothate with concentrated methanolic sodium methoxide to give after separation of the mixture through the half ester of (23), followed by methylation with diazomethane to give (23). Eade et al[22] prepared this compound by the procedure[5] for the analogous compound in the lactone series. Oxidation of dimethyl ceanothate (19) with Jones' reagent gave the known methyl 2α-methoxycarbonyl-3-oxo-A(1)-Norlup-20(29)-en-28-oate (dimethyl dehydroceanothate) (20) which was rapidly epimerised by alkali to an equilibrium mixture containing 40% of the starting material and 60% of the isomer, epimeric at C2, methyl 2β-methoxycarbonyl-3-oxo-A(1)-Norlup-20(29)-en-28-oate (22) (dimethyl epidehydroceanothate). Reduction of (22) with sodium borohydride gave the cis isomer (23) as the sole product.

Reduction of (20) with sodium borohydrohydride gave a 1:1 mixture of dimethyl ceanothate (19, R = Me) and its C3 epimer (21) which were readily separated by column chromatography.

The fourth isomer, methyl 3α-hydroxy-2β-methoxycarbonyl-A(1)-Norlup-20(29)-en-28-oate (24) was prepared by long heating

of a solution of methyl 3α-hydroxy 2α-methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate (21) in methanolic sodium methoxide whereupon partial epimerisation of the C2 methoxycarbonyl group occurred. After re-methylation of the total crude product, the isomer (24) could be separated from the starting material by column chromatography.

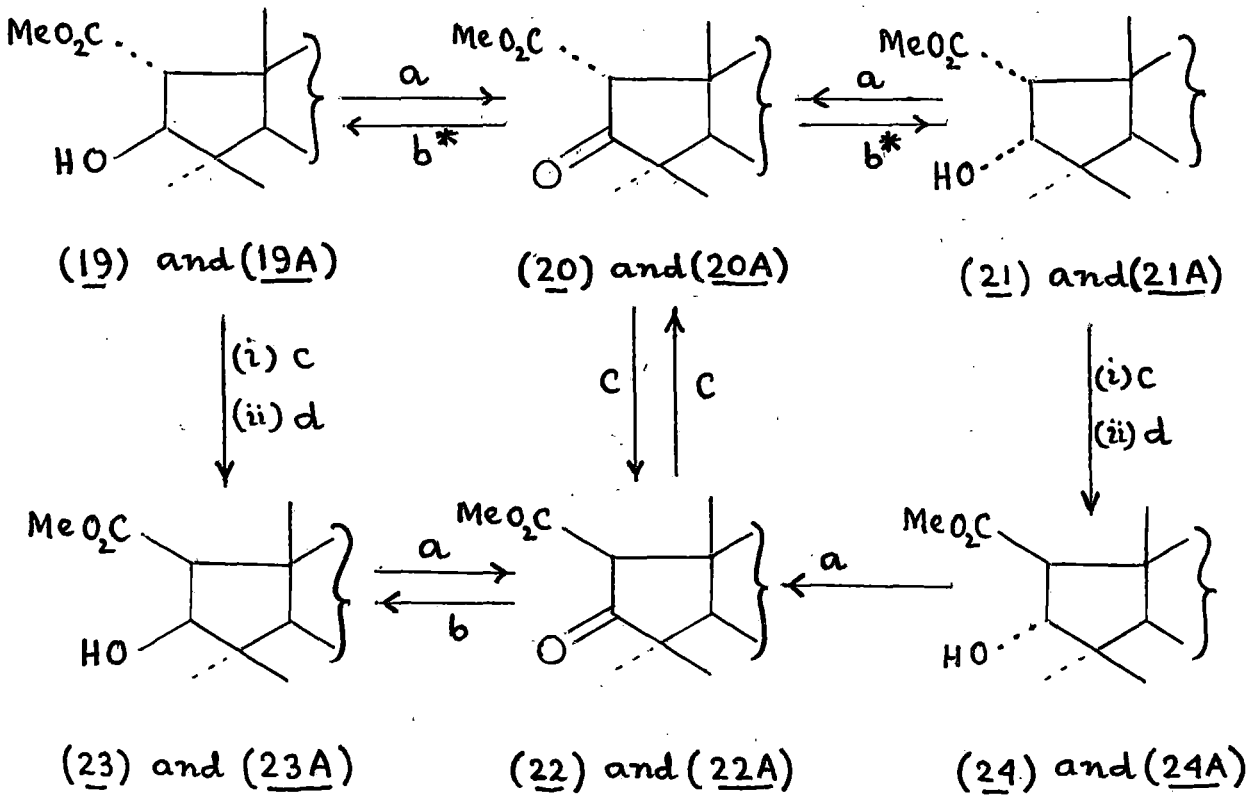
The identity of each of the above alcohols was established by oxidation (Jones' reagent) to the corresponding ketones. Eade et al[22] also converted each alcohol into its corresponding acetate.

Preparation of the Lactone Series.

Three of the four isomers in this series were previously reported[5,17]. Methyl ceanothate lactone (19A, R = Me) was prepared from ceanothic acid by formic acid catalysed lactonisation followed by hydrolysis and methylation[5] and also by lactonisation of dimethyl ceanothate in acetic acid and sulphuric acid followed by deacetylation[17]. The compounds (21A) and (23A) were prepared by borohydride reduction of the corresponding ketones (20A) and (22A). Eade et al[22] prepared the fourth and previously unknown isomer (24A) by isomerisation of (21A) with sodium methoxide in methanol, followed by remethylation with diazomethane. Each of the above alcohols was converted to the corresponding ketone and acetate.

A summary of the preparation of the four isomers by Eade et al[22] in the two series is shown in Scheme-1. The

Scheme-1 (Preparation of Isomers).



a. Jones' Reagent, b. Sodium borohydride

c. Sodium methoxide, d. Diazomethane.

* (20) $\xrightarrow{b}$ (19) + (21), but (20A) $\xrightarrow{b}$ (21A) Only.

epimerisation of both ketones in each series were reversible equilibria. Eade et al[22] also showed that the equilibrium mixture contained 60% of (22) or (22A) and 40% of (20) or (20A).

N.M.R. Results.

Eade et al[22] measured the coupling constants between the protons on C-2 and C-3 for each isomer and its acetate belonging to both the series. They also calculated the vicinal coupling constants ($J_{2,3}$) of the four isomers for the three possible ring A conformations from the dihedral angle measured from Drieding models. These were shown in Table-1. Of these three possible conformations, they argued that the α-envelope seemed least

Table-1

Coupling Constants of C-2 and C-3 Protons.

	Configuration of ring A substituents.			
	2α,3β;	2β,3β;	2α, 3α;	2β, 3α
Observed $J_{2,3}$ (H_z)				
Dimethyl Ceanothate Series:				
C3-OH	1.0	7.3	7.0	9.0
C3-OAc	0.2	7.6	7.6	9.5
Lactone Series:				
C3-OH	1.0	7.4	7.0	9.0
C3-OAc	0	7.6	7.7	9.5

Contd..

Table-1 (Contd.)

	Configuration of ring A substituents.			
	2α, 3β ;	2β, 3β ;	2α, 3α ;	2β, 3α
Calculated $J_{2,3}(H_z)$				
α-Envelope	1.8-2.9	8.2	8.2	1.8-2.9
β-Envelope	-0.3-0.0	6.2-6.9	5.7-6.7	5.9-7.1
Half Chair	0.3-0.8	7.2-8.0	7.5-7.9	3.5-5.0

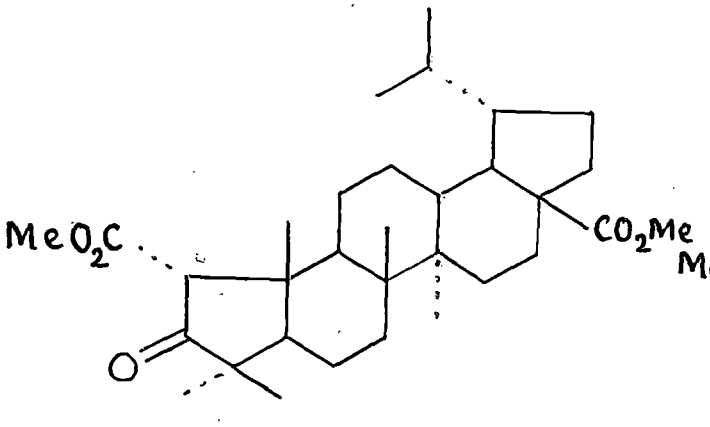
likely since examination of the models showed strong 10β-methyl/4β-methyl interaction which would be expected to destabilise this conformation. In addition, the investigation by Fishman[25] on analogous 16,17-disubstituted steroids lacking these methyl/methyl interactions suggested that the α-envelope need not be considered. The only significant difference between the three conformations were the two trans couplings in the α-envelopes[25] and hence these results, although excluding the α-envelope, did not distinguish between the other two conformations. Eade et al[22] pointed out that the observed couplings were clearly consistent with the proposed formula for ceanothic acid (19, R = Me) but

obviously did not conform with the original stereochemistry suggested by de Mayo and Starratt[5].

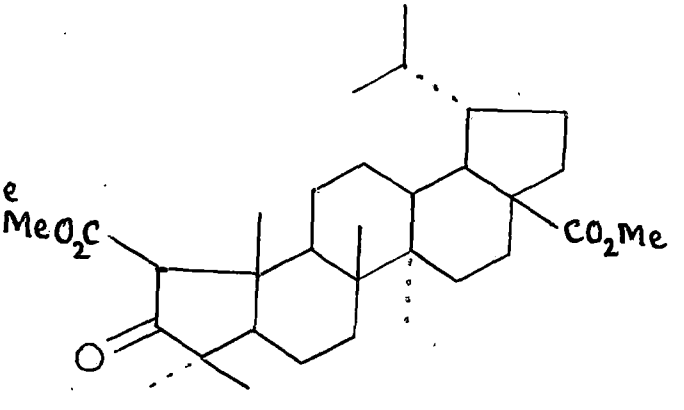
Table-1 shows that there are no significant differences between the coupling constants of the C-2 and C-3 protons among the members of the dimethyl ceanothate series compared to those among the analogous compounds in the lactone series and hence the investigation[22] did not yield any conclusive evidence which had been proposed[17] to explain the differences in reactivity in the ring A moiety between the two series. The coupling constants in Table-I, together with the interconversions summarised in Scheme-I, established unequivocally that in the epiceanothate series the ring A substituents were cis to one another, each having the β-configuration.

In addition to the evidence presented above in relation to the vicinal coupling between the protons on C-2 and C-3, the long range (4σ) coupling[26] between the 10 β-methyl group and the C-2 proton furnished further support for their assignment[22]. This coupling was studied using the following three pairs of isomers: (i) methyl dehydroceanothate lactone (20A) and its 2β-epimer (22A); (ii) dimethyl dehydroceanothate (20) and its 2β-epimer (22); (iii) dimethyl dihydrodehydroceanothate [methyl-2α-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-oate] (25) and its 2β-epimer (26). The last pair of isomers were prepared by Eade et al[22] from dimethyl dihydroceanothate by

methods identical with those used to prepare (20) and (22) from dimethyl ceanothate. In each of these six compounds the C-2 proton appeared as a singlet at approximately δ 3.0; however, this signal for each of the 2β-methoxycarbonyl (epi) derivatives was broad ($W_{\frac{1}{2}}$ 2.5 H_z) compared to that in each of the 2α-methoxycarbonyl compounds ($W_{\frac{1}{2}}$ 1.5 H_z). This broadening was confirmed by spin-spin decoupling. Decoupling of the C-2 proton signal (at δ 3.08) of dimethyl epidehydroceanothate (22) increased the intensity of the C-10 methyl signal (at δ 1.06) markedly, while irradiation of this methyl frequency resulted in a sharpening of the C-2 proton signal ($W_{\frac{1}{2}}$ became 1.6 H_z). Similar sharpening of the C-2 proton signal (from $W_{\frac{1}{2}}$ 2.5 H_z to $W_{\frac{1}{2}}$ 1.6 H_z) was also observed when the relevant methyl signals in both (22A) and (26) were irradiated. The observed couplings demonstrated the pseudo-axial character of the 2α-hydrogens in (22), (22A) and (26) and supported the half-chair or β-envelope conformation of ring A for these derivatives when the 2α-proton and 10β-methyl showed some degree of co-planarity.



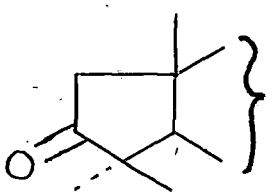
(25)



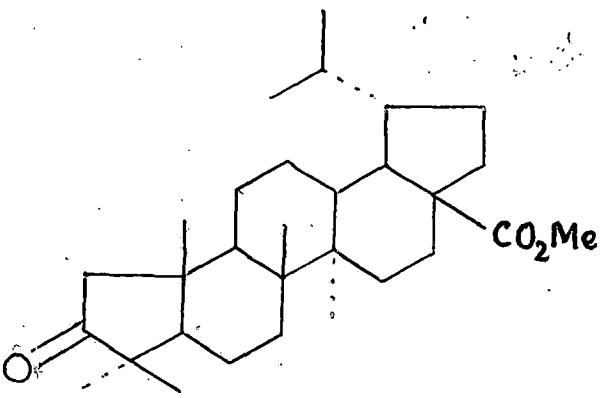
(26)

Circular Dichroism Results.

Eade et al[22] studied the circular dichroism of the two epimeric ketones (20) and (22) together with that of the corresponding nor ketone methyldecarboxydehydroceanothate [methyl-3-Oxo-A(1)-norlup-20(29)-en-28-oate] (27) and also of the corresponding sets of the three ketones in the lactone series (20A), (22A) and (27A) and in the dimethyl dihydroceanothate series (25), (26) and (28).



(27) and (27A)



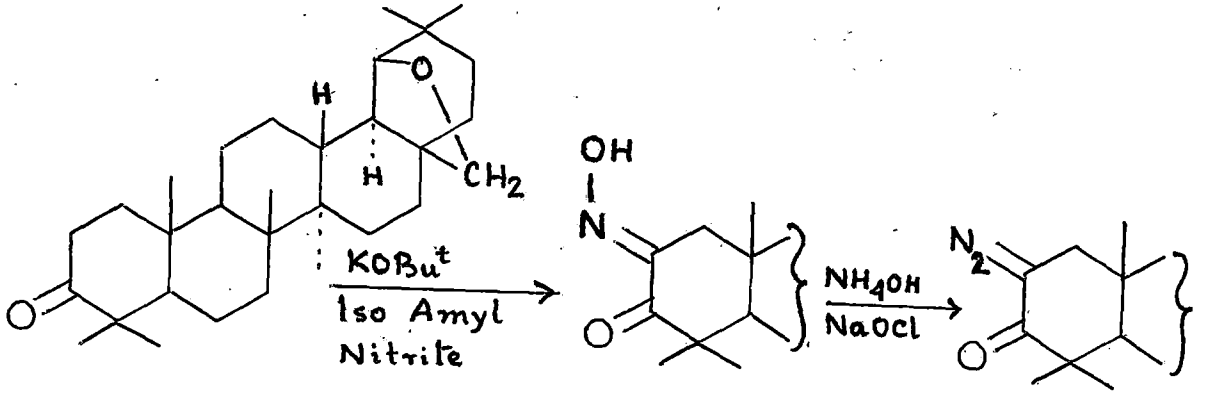
(28)

Unfortunately the changes in circular dichroism between members of each set did not yield any information about the configuration of the C-2 methoxycarbonyl group. The differences between the epimeric members of each set were quite small. Work on the circular dichroism of A-nor-steroid derivatives[27] showed that changes in configuration of substituent methyl groups in the cyclopentanone ring did not yield diagnostic changes in the circular dichroism of these compounds. The investigations of Eade et al[22] thus gave further support to this interpretation with respect to cyclopentanone structure.

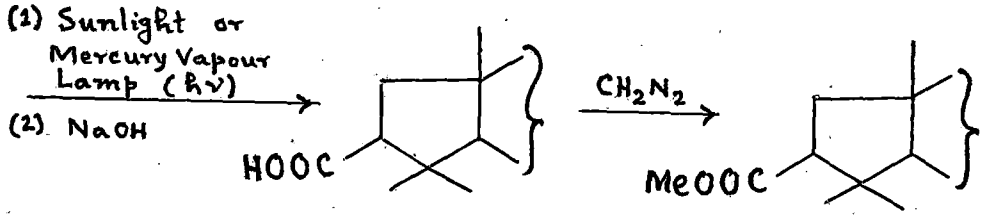
CHAPTER-II

Previous attempts towards the Partial Synthesis of Ceanothic Acid and its Stereoisomers.

In 1965 Huneck[28] with a view to devising a method for the partial synthesis of ceanothic acid developed a procedure for contraction of Ring A in normal triterpenoids to give suitable A-nor carboxylic acids. He utilised the method of Horner[29] and Sus et al[30] involving the Wolff rearrangement of cyclic diazo-ketones with concommitant ring contraction under photochemical conditions. The first publication of Huneck[28a] reported the conversion of 3-Oxo-allobetulan (29) to 19β, 28-epoxy-2β-methoxy-carbonyl-A-nor-18α H-oleanan (33) according to the following sequence.

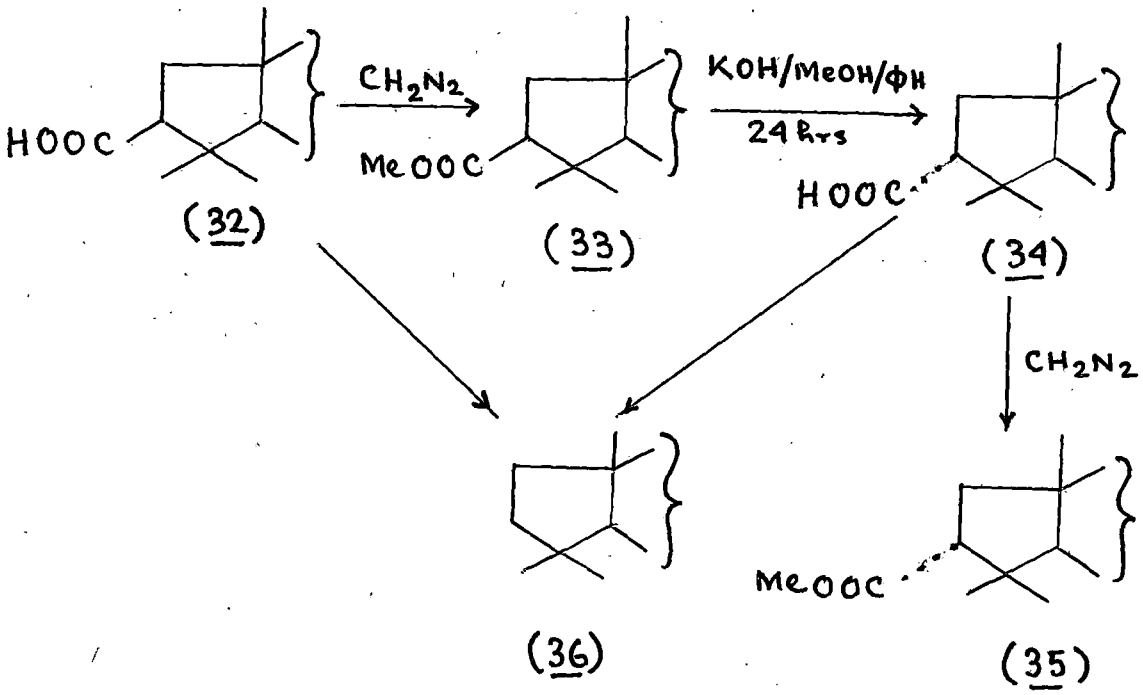


(29) (30) (31)



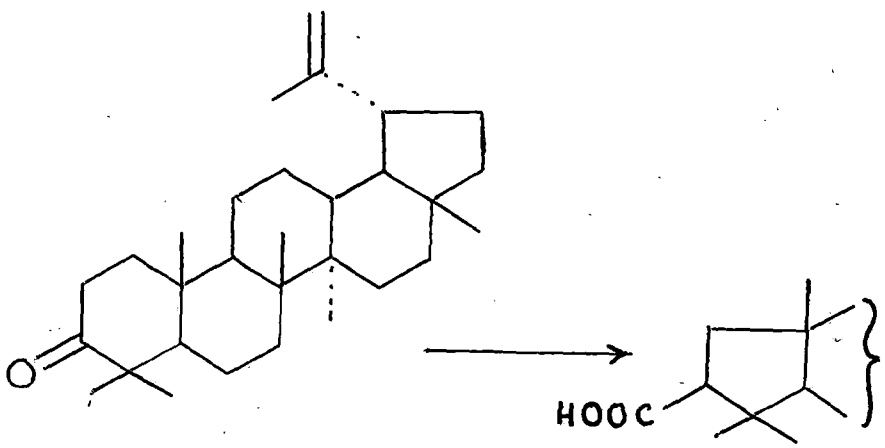
(32) (33)

The β-isomer (33) on treatment with methanolic potassium hydroxide for 24 hours gave the 2α-carboxy-derivative (34), which on methylation gave 2α-carbomethoxy compound (35). The structures of the acids (32) and (34) were established by their conversion to 19β, 28-epoxy-A-nor-18α H-oleanan (36).

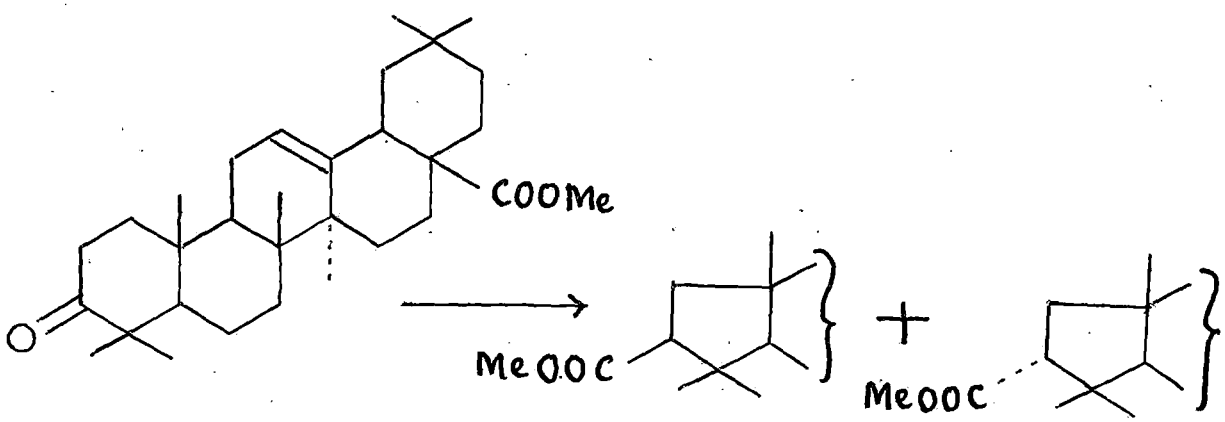


(32) (33) (34) (35) (36)

In another paper, Huneck[28b] reported the conversion of 3-oxolup-20(29)-ene (37) to 2β-carboxy-A-norlup-20(29)-ene (38) and methyl oleanonic ester (39) to a mixture of 2β-methoxycarbonyl-A-nor-Δ^{12}-oleanene-17-methylester (40) and its 2α-epimer (41) by similar reactions.

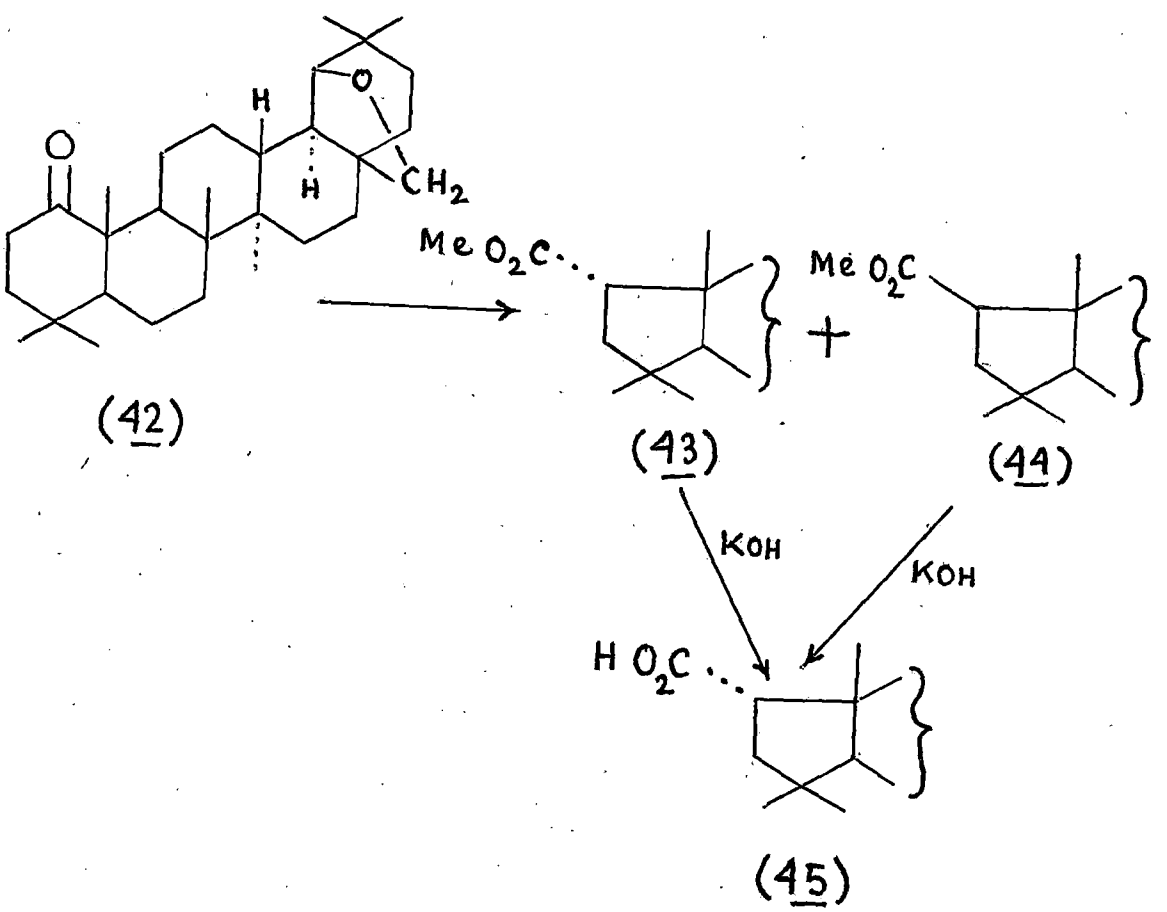


(37) (38)



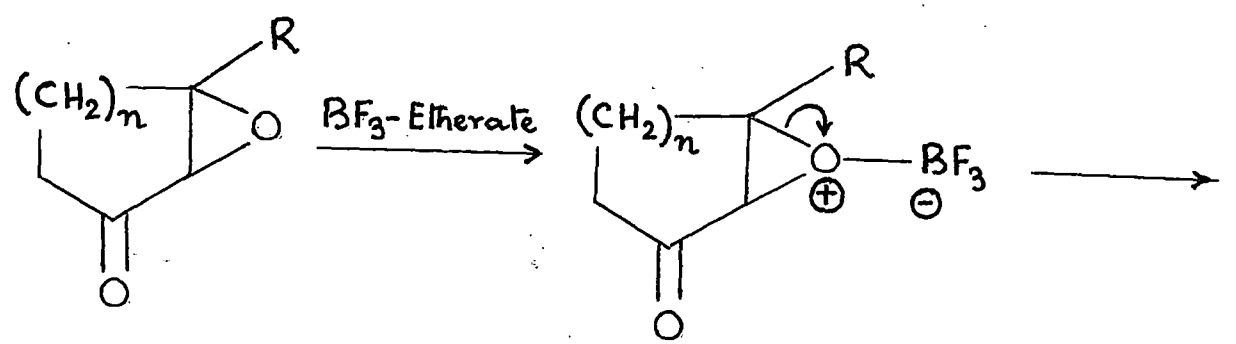
(39) (40) (41)

He also showed[28c] that the appropriate 1-oxotriterpenoids can be converted to the A-nor-1-carboxy compounds by application of the same procedure. Thus 19β, 28-epoxy-1-oxo-18α H-oleanan (42) gave a mixture of 1α-methoxycarbonyl-19β, 28-epoxy-A-nor-18α H-oleanane (43) and its 1β-epimer (44) by similar reactions. Both (43) and (44) on treatment with alkali gave the 1α-carboxy compound (45).

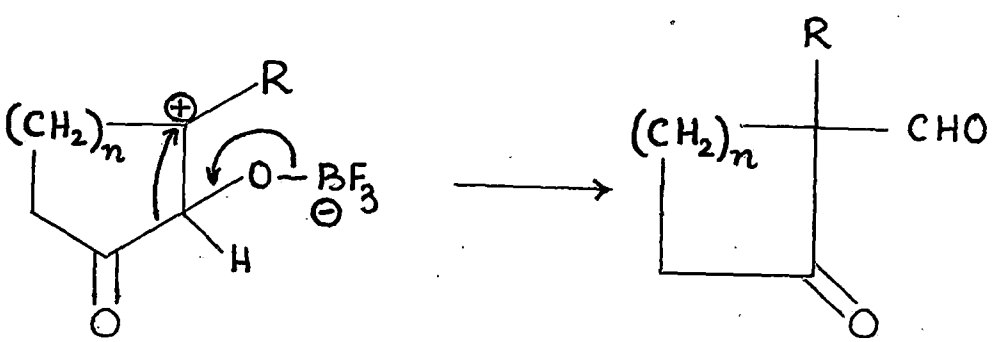


(42) (43) (44) (45)

House and Wasson[31] have shown that, epoxy ketones of the type (46; n>1) in the presence of BF_3-etherate undergo rearrangement with reduction in ring size of the cyclic ketone, and produced the keto-aldehyde (49) possibly via the ion (47) and/or (48).

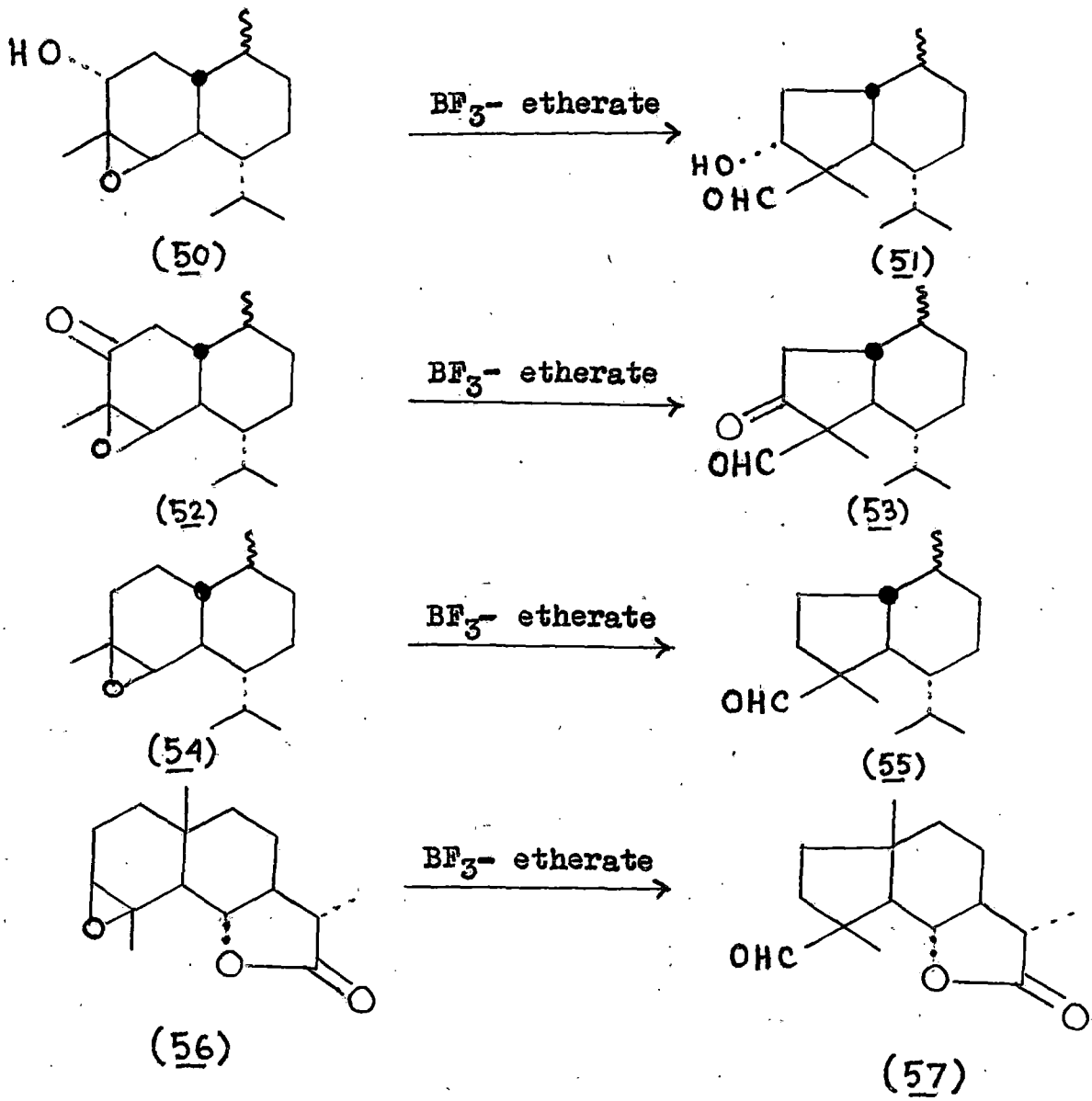


(46) (47)

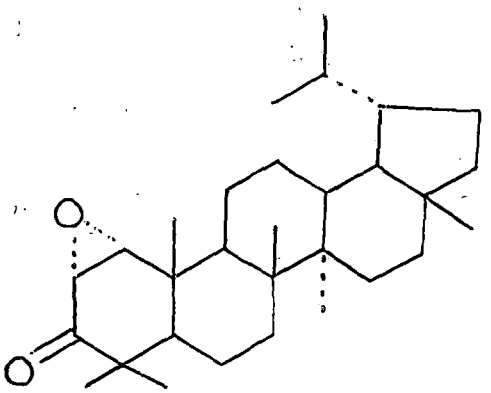


(48) (49)

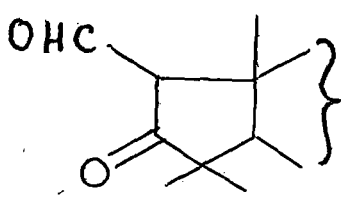
Kartha and Chakravarti[32] studied the action of BF_3-etherate on some sesquiterpene oxides and found that in each case an aldehyde was formed by contraction of a six membered ring to a five membered ring as shown below:



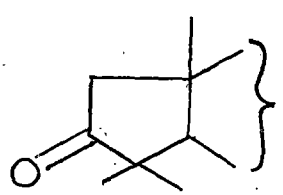
In 1968, Chatterjee and colleagues[33] attempted to prepare the keto aldehyde (59) by ring contraction of 1∝, 2∝-epoxylupan-3-one (58) by following the same procedure as described by House et al[31] and Kartha et al[32] . However, in this reaction, they could not isolate the keto aldehyde (59) but obtained only the ketone, A-norlupan-2-one (60) in good yield.



(58)



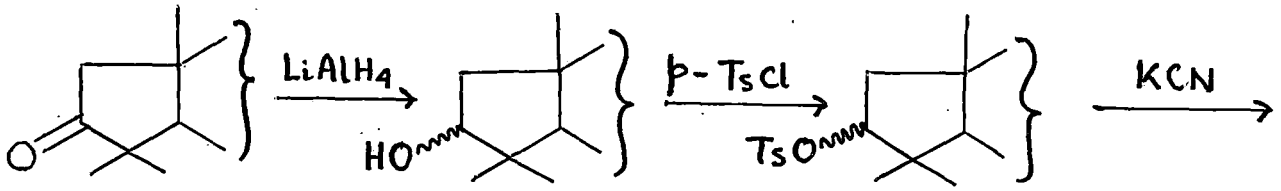
(59)



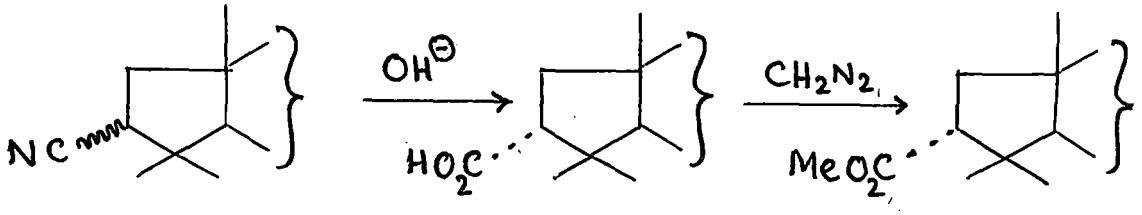
(60)

Most probably the intermediate keto aldehyde (59), that was formed, underwent easy loss of formyl group under the reaction condition. The object of their work was to develop a method for the partial synthesis of ceanothic acid starting from the keto

aldehyde (59). They, however, finally achieved the synthesis of 2α-methoxycarbonyl-A-norlupan (61) by following the sequence of reaction shown below.

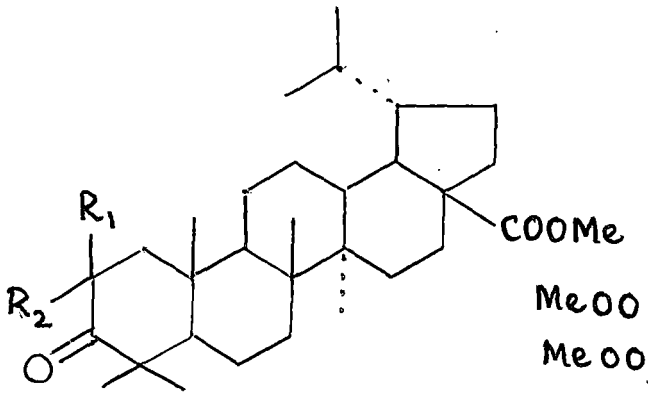


(60)



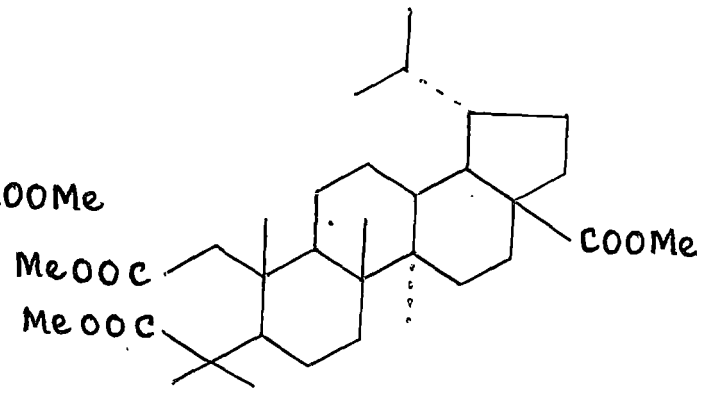
(61)

A partial synthesis of dimethyl dihydroceanothate was reported from this laboratory[34,35]. The diosphenol (62B) obtained by the autoxidation of methyl dihydrobetulonate (62A) on oxidation with hydrogen peroxide followed by esterification afforded the trimethyl ester (63). Dieckmann condensation of (63)

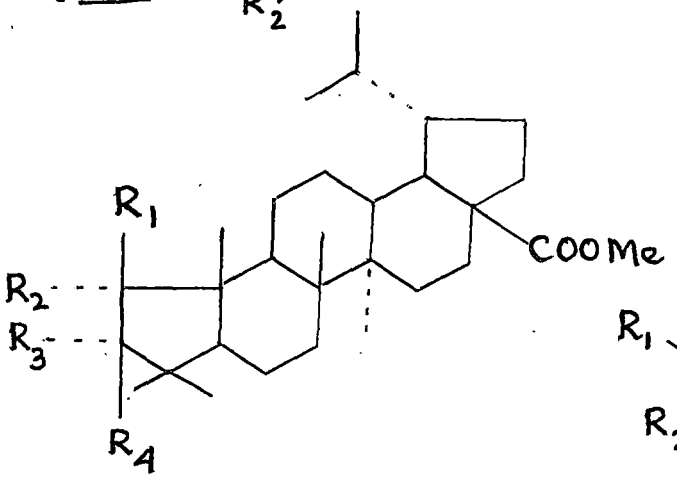


(62A) $R_1 = R_2 = H$

(62B) $R_1, R_2 >= O$

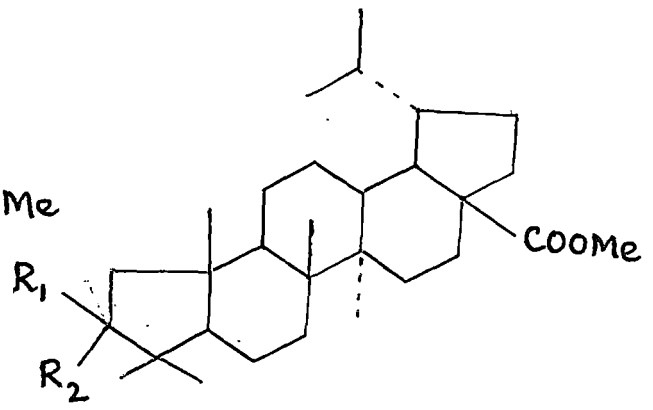


(63)



(64A) $R_1 = H$, $R_2 = COOMe$, $R_3, R_4 >= O$

(64B) $R_1 = R_3 = H$, $R_2 = COOMe$, $R_4 = OH$



(65A) $R_1 = OH$, $R_2 = COOH$

(65B) $R_1, R_2 >= O$

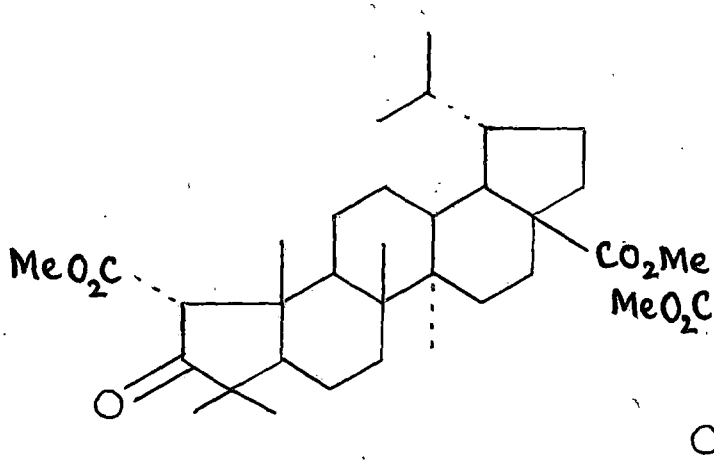
furnished methyl-2α-methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate (64A) which on $NaBH_4$ reduction gave the desired dimethyl dihydroceanothate (64B) identical with an authentic specimen. But then it was not possible to synthesize the other three stereoisomers arising from different spatial disposition of groups in ring A. Furthermore, the diosphenol (62B) under basic condition underwent benzilic acid rearrangement to the α-hydroxy acid (65A) which on treatment with lead dioxide in acetic acid gave the nor-ketone (65B). Attempted carbomethoxylation of this nor-ketone (65B) to give the β-keto ester of the type (64A) using dimethylcarbonate in the presence of various bases in different solvents was found to be unsuccessful.

CAHPTER-III

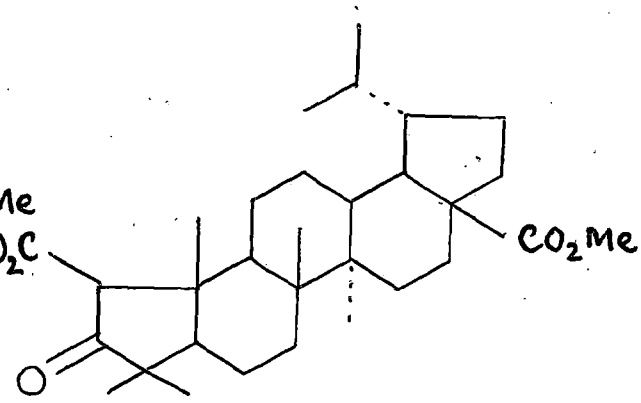
Partial Synthesis of All the Four Stereoisomers of Dimethyl Dihydroceanothate starting from Betulinic Acid.

The objective of the present study was to develop a partial synthesis of all the four stereoisomers of dimethyl dihydroceanothate starting from betulinic acid. In order to achieve this goal, it was necessary (I) to convert the six membered ring A present in the starting material into a five membered ring of the product without affecting the structural and stereochemical features present in the other parts of the starting material and (II) to introduce the substituents at the two asymmetric centres in ring A with proper stereochemistry.

The second problem could best be solved by introducing the stereochemical centres in a stepwise predetermined fashion on some synthetic intermediate. Since Eade *et al*[22] have shown that the C-3 hydroxyl group in dimethyl ceanothate series (except in one case) could be introduced by sodium borohydride reduction of the corresponding C-3 ketones, it was thought that the β-keto esters (66) and (67) would be the best intermediates for this purpose.



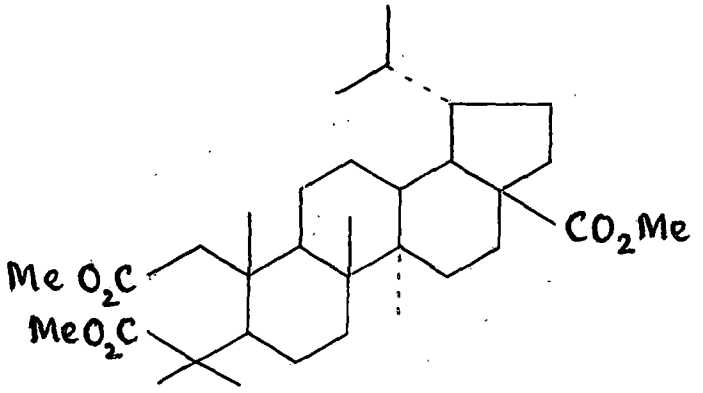
(66)



(67)

These compounds contained the C-2 methoxycarbonyl group in proper stereochemistry and once the synthesis of these could be attained, the introduction of C-3 hydroxyl group in proper stereochemistry would not be very difficult.

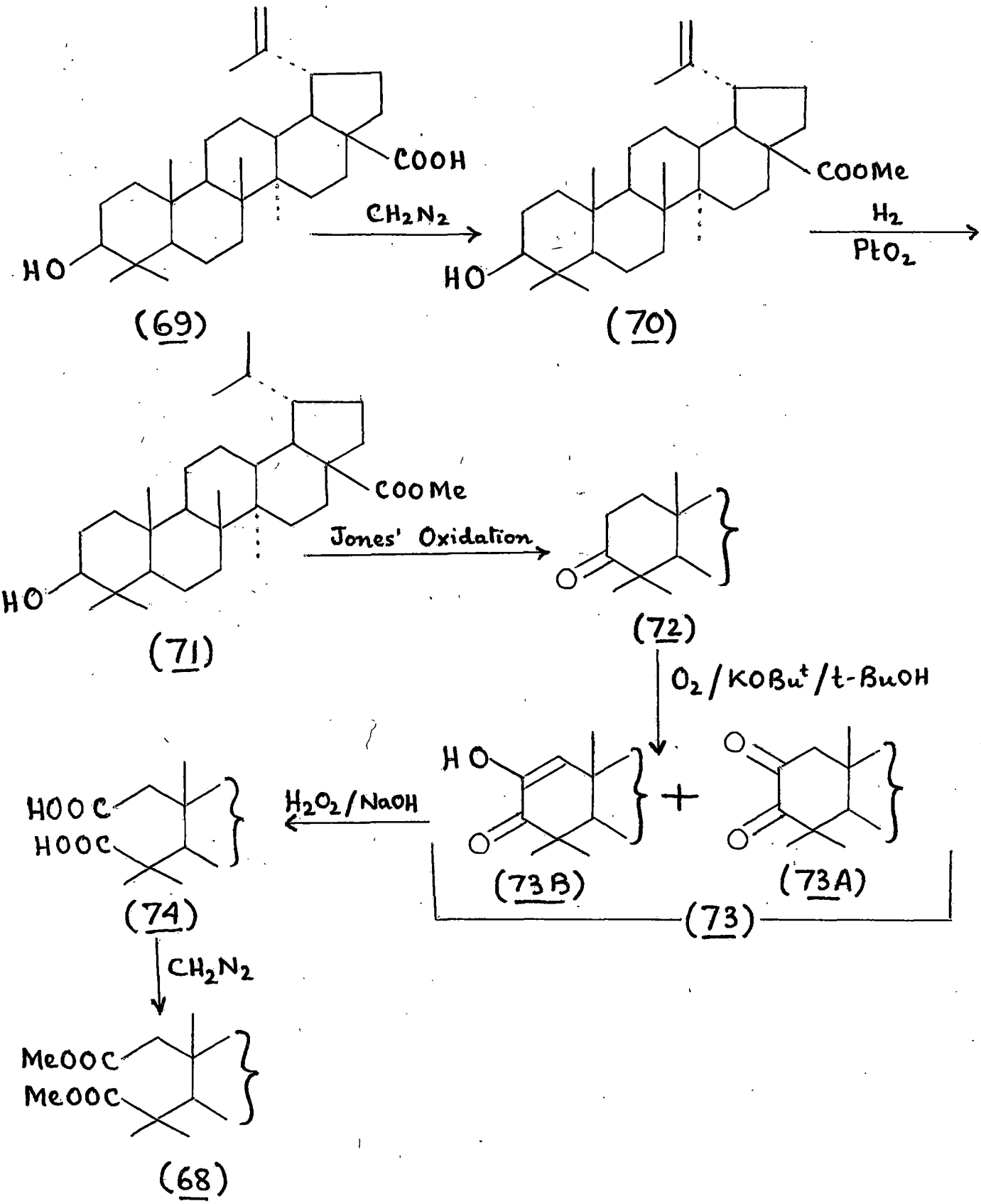
Regarding the first problem, that is, the construction of the five membered A ring skeleton present in (66) and (67), it had to be noted that both these were β-keto esters. Hence, theoretically, they could be prepared by Dieckmann condensation of the corresponding 2, 3-seco trimethyl ester (68). Thus the problem was reduced to the synthesis of the trimethyl ester (68). This trimethyl ester (68) could be obtained by oxidative opening of ring A of a betulinic acid derivative. But betulinic acid



(68)

itself contained an isopropenyl side chain, and the exocyclic double bond might be expected to undergo disruption under drastic oxidation condition required for ring opening. Thus, it was necessary to reduce the double bond of betulinic acid at an early stage.

Betulinic acid (69) extracted from the acid part of the benzene extract of the bark of Bischofia Javonica Blume on esterification with diazomethane gave methyl betulinate (70), m.p. 223-24°, $(\alpha)_D$ 5°, ν_{max}^{nujol} 3560 (OH), 1715 ($COOCH_3$), 1660 and 880 cm^{-1} (= CH_2). Hydrogenation of methyl betulinate (70) in presence of PtO_2-catalyst in a mixture of acetic acid and ethyl acetate gave methyl dihydrobetulinate (71), m.p. 236-38°, ν_{max}^{nujol} 3560 (OH), 1710 cm^{-1} ($COOCH_3$). Jones' oxidation of methyl

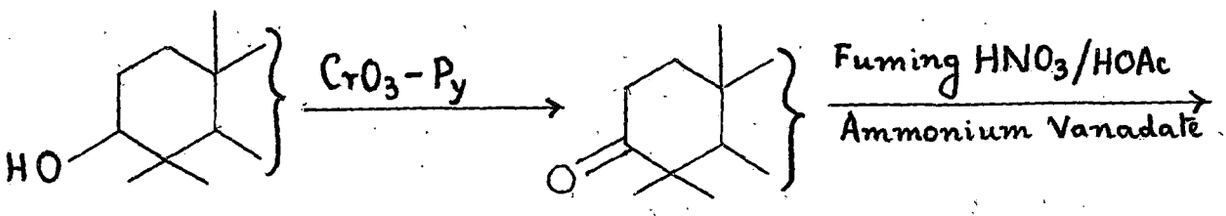


(69) (70) (71) (72) (73) (73B) (73A) (74) (68)

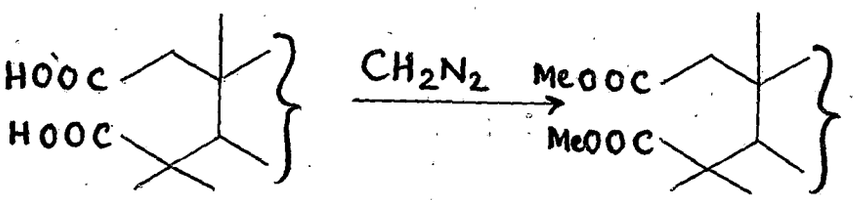
dihydrobetulinate (71) afforded methyl dihydrobetulonate (72), m.p. 191-93°, $(\alpha)_D$ 8°, ν_{max}^{nujol} 1730 ($COOCH_3$), 1705 cm^{-1} (CO). Autoxidation of (72) by passing a stream of oxygen in presence of potassium tertiary butoxide in tertiary butanol furnished a product, m.p. 131-33°, $(\alpha)_D$-1.96°, ν_{max}^{nujol} 3460 (OH), 1730 ($COOCH_3$), 1670, 1650, 860 cm^{-1}, λ_{max} 269 nm (ϵ, 7532). It gave a positive ferric chloride colouration for diosphenol. T.L.C. showed two spots indicating that it was a mixture of two tautomeric forms the α-diketone (73A) and the diosphenol (73B). Hydrogen peroxide oxidation[36] of the mixture of (73A) and (73B) in presence of sodium hydroxide gave after acidification the A-seco acid (74), m.p. 175-77°, ν_{max}^{nujol} 1710 and 1680 cm^{-1} (COOH) which on esterification with diazomethane gave the desired trimethyl ester (68), m.p. 146-47°, ν_{max}^{nujol} 1745 and 1725 cm^{-1} ($COOCH_3$) identical with the compound reported in literature[3,12].

The overall yield of the triester (68) from methyl dihydrobetulinate (71) by the above procedure was very poor. To increase the yield it was felt that (I) a shorter reaction path and (II) a better reaction condition were necessary. After several experimentation such a synthetic pathway could be arrived at. Oxidation of methyl dihydrobetulinate (71) with Chromium trioxide-pyridine complex[37] gave in very good yield methyl dihydrobetulonate (72) which on oxidation with fuming nitric acid in acetic acid in presence of ammonium vanadate[12,38] directly afforded the

seco-acid (74). The latter on esterification gave the triester (68) in fairly good overall yield.



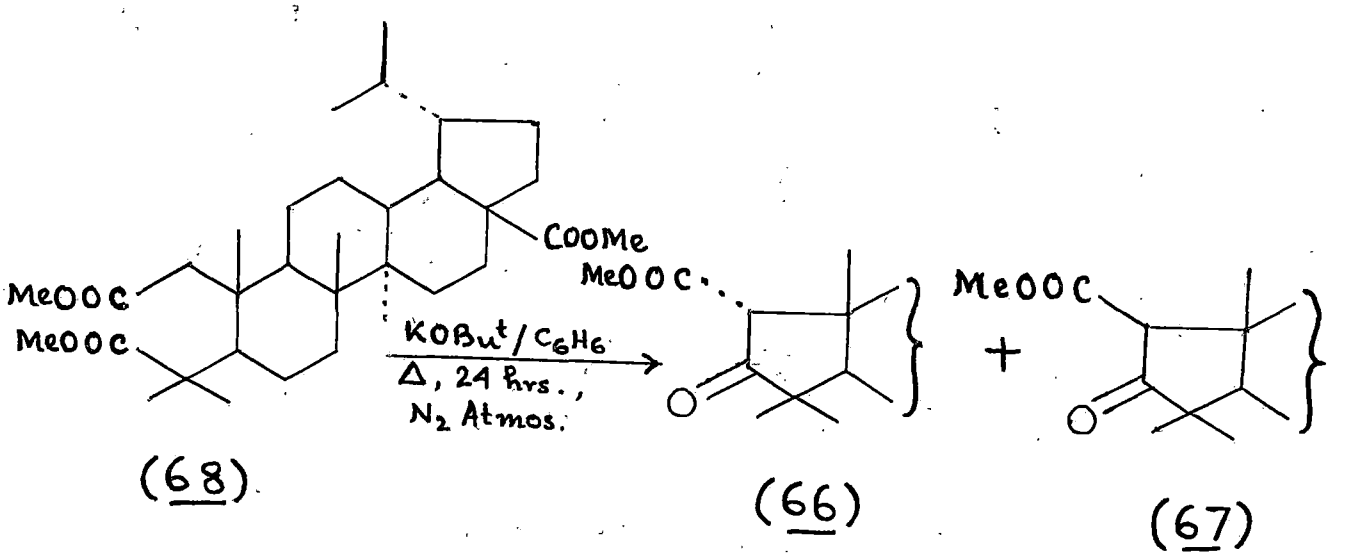
(71) (72)



(74) (68)

Dieckmann condensation was performed by refluxing the trimethyl ester (68) with potassium tertiary butoxide in benzene solution for 24 hours in an atmosphere of nitrogen[39,40]. During the period of reaction a portion of the solvent was removed with

a Dean-Stark trap. The gummy reaction product obtained after usual work up was chromatographed over deactivated alumina. A mixture of petrol and benzene (3:2) first eluted a solid A, m.p. 189-91°. Further elution with the same solvent mixture gave another solid B, m.p. 173-75°. The proportions of two solids varied with the reaction condition and the time of refluxing. Solid A on crystallisation from methanol afforded needle shaped crystals, m.p. 191-93° (TLC-homogenous), $(\alpha)_D$ 89°, ν_{max}^{nujol} 1755 (cyclopentanone), 1725 cm^{-1} (CO_2Me) having the same m.p, rotation and I.R. spectra reported in literature[22] for dimethyl dehydrodihydroceanothate, i.e., methyl-2α-methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate (66). Solid B on crystallisation from methanol afforded needle shaped crystals, m.p. 175-77° (TLC-homogenous), $(\alpha)_D$ 42°, ν_{max}^{nujol} 1750 (cyclopentanone), 1720 cm^{-1} (CO_2Me) having the same m.p., rotation and I.R. spectra reported in literature[22] for methyl -2β-methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate,(67).



(68) (66) (67)

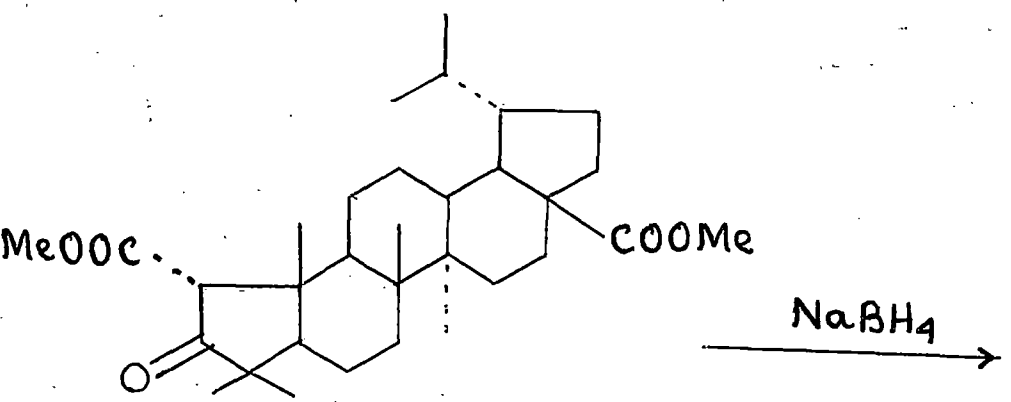
Thus the most important synthetic goal, i.e., the construction of a five membered A-ring without affecting the stereochemistry of various ring junctions was achieved. Furthermore, in the β-ketoesters (66) and (67) the stereochemistry at C-2 was also known.

Sodium borohydride reduction of the β-ketoester (66) in a mixture of methanol and dioxan gave an oily residue which was chromatographed over deactivated alumina. Elution with a mixture of petrol and benzene (2:3) first afforded a solid C, m.p. 257-59°. Further elution with the same solvent gave another solid D, m.p. 140-42°.

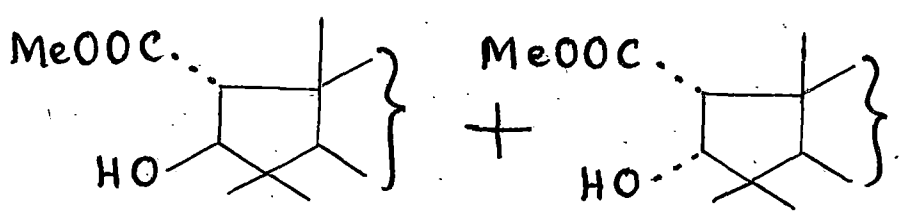
Solid C, m.p. 257-59° on crystallisation from methanol gave needle shaped crystals, m.p. 261-63°, $(\alpha)_D$ 22°, ν_{max}^{nujol} 3540 (OH), 1730 (CO_2Me), 1710 cm^{-1} (CO_2Me), and was found to be identical (m.m.p., Co-TLC and I.R. comparison) with an authentic sample of dimethyldihydroceanothate[5] [methyl-3β-hydroxy-2α-methoxycarbonyl-A(1)-norlupan-28-oate] (75) supplied by Professor P. de Mayo.

Solid D on crystallisation from methanol gave crystals, m.p. 140-42°, (TLC-homogenous), ν_{max}^{nujol} 3560 (OH), 1745 (CO_2Me) and 1705 cm^{-1} (CO_2Me). From the method of preparation and by analogy with the previous work[22] it was evident that this compound must be C-3 epimer of dimethyldihydroceanothate, i.e. methyl-3α-

hydroxy-2 ∝ -methoxycarbonyl-A(1)-norlupan-28-oate, (76). Further confirmation of this structure by NMR spectra is in progress.



(66)



(75) (76)

Attempts to reduce the β-keto ester (67) by sodium borohydride under conditions in which the β-ketoester (66) underwent smooth reduction failed. However, when the β-ketoester (67) was stirred with a large excess of sodium borohydride in a

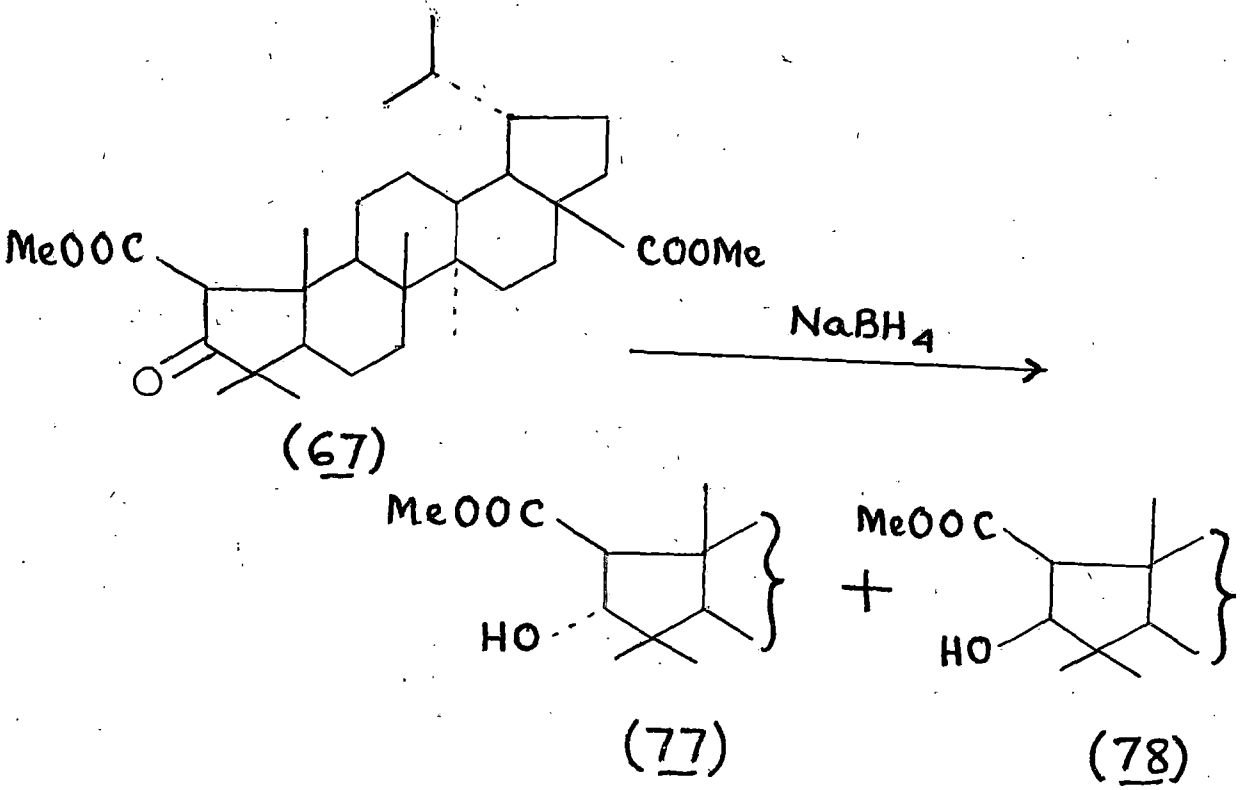
mixture of methanol and dioxan, for a sufficiently long time the desired reduction took place. The oily reaction product was shown by TLC studies to be a mixture of two compounds which could be resolved by chromatography over a column of deactivated alumina. Elution with a mixture of petrol and benzene (1:1) afforded a solid E, m.p. 197-201°. Further elution with a mixture of petrol and benzene (3:7) gave another solid F, m.p. 168-72°. Evidently E and F must be the C-3 epimers. This observation was at variance with the work of Eade et al[22] who showed that dimethyl epidehydroceanothate (22) on reduction (of course under somewhat milder condition) afforded only the 3β-hydroxy compound (23).

The solid E, m.p. 197-201°, on crystallisation from aqueous methanol afforded crystals, m.p. 202-203° (TLC-single spot), IR ν_{max}^{nujol} 3490 (OH), 1730 (CO_2Me), 1695 cm^{-1} (CO_2Me). The NMR spectrum (80 MH_z) of the compound showed signals at δ 0.7 to 1.1 (seven methyl groups), 2.1 (one proton, OH) 3.06 (1H, doublet, J = 7 H_z, CH-COOMe i.e., C-2 proton), 3.65 (3H, singlet, $-COOCH_3$), 3.7 (3H, singlet, $-COOCH_3$) and 4.18 (1H, doublet = 7 H_z; CHOH i.e., C-3 proton). The signal due to OH at δ 2.1 in the spectrum was a singlet slightly broadened line which moved upfield on heating.

The solid F, m.p. 168-72° on crystallisation from aqueous methanol afforded crystals, m.p. 174-76° (TLC-single spot), IR ν_{max}^{nujol} 3540 (OH), 1740 (CO_2Me), 1690 cm^{-1} (CO_2Me). The NMR

spectrum (80 MH_z) of the compound showed signals at δ 0.7 to 1.1 (seven methyl groups), 2.35 (1H, doublet, J = 7.2 H_z; C<u>H</u>-CO_2Me, i.e., the C-2 proton) a doublet centred at 2.8 (1H, doublet, J = 4.5 H_z; O<u>H</u>), 3.65 (3H, singlet, $COOCH_3$), 3.7 (3H, singlet, $COOCH_3$), 4.02 (1H, doublet of doublets, J = 7.2 H_z and 4.5 H_z; C<u>H</u>OH i.e. C-3 proton). The doublet due to OH moved upfield on heating to 60°C.

It was evident from the method of preparation that solid <u>E</u> and solid <u>F</u> were C-3 epimers, in one the hydroxy at C-3 was α and in the other it was β. However, at variance with the work of Eade <u>et al</u>[22] on analogous compounds, the coupling constant values were found to be nearly equal. Since this vicinal coupling constants were nearly equal there was no way to deduce at this stage in which compound was the 3-hydroxy was β and in which it was α. However, the correct conformations were established by other means. The fact that the proton on C-2 in <u>E</u> was shifted downfield to δ 3.06 indicated that the proton at C-2 and the hydroxy group at C-3 in this compound <u>E</u> was on the same side of the ring. Consequently, this compound <u>E</u> was the <u>trans</u> compound, i.e. methyl - 3α-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (<u>77</u>). Evidently <u>F</u> was the <u>cis</u>-compound, i.e., methyl-3β-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (<u>78</u>).



Additional support for the above interpretation was found in the fact that, although the solution used for NMR of (78) was more dilute, the signal for OH group was found further downfield than that in (77), which meant that (78) was more strongly hydrogen bonded compared to (77). This can only occur when they are on the same side of the ring as in (78). ^{13}C NMR spectra were also run on the compounds (77) and (78). Such a spectrum of the compound (77) showed 31 strong lines, one of which represented two carbons. The ^{13}C NMR spectra of (78) showed 30 strong lines (and 5 weak lines, probably due to noise level)

two of which represented two carbons. Thus both compounds had 32 carbons as expected. Both compounds had a peak near 82 ppm which evidently was the carbon bearing the hydroxyl group.

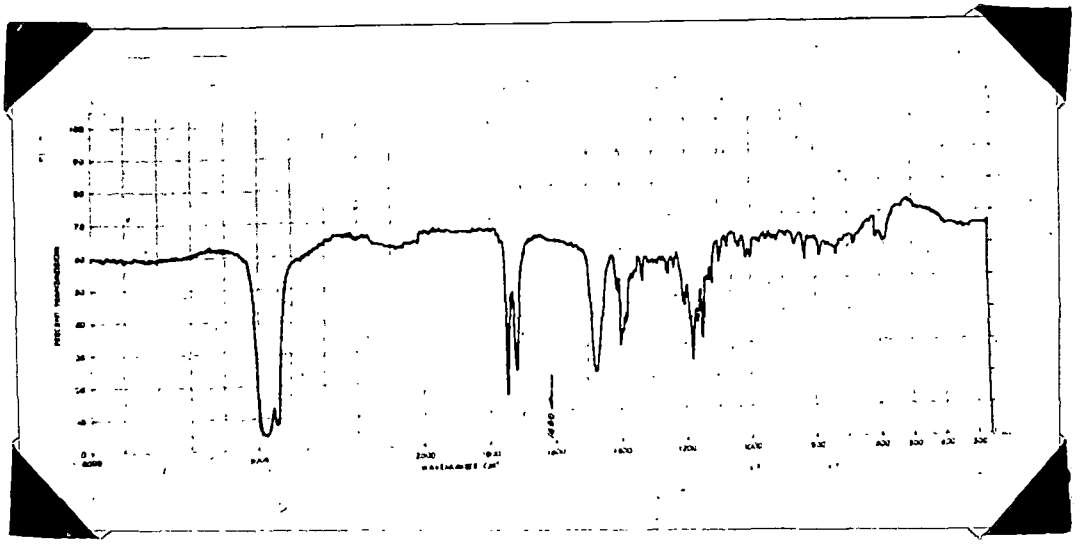


Fig. 1: IR Spectrum of Methyl Dihydrobetulonate (72)

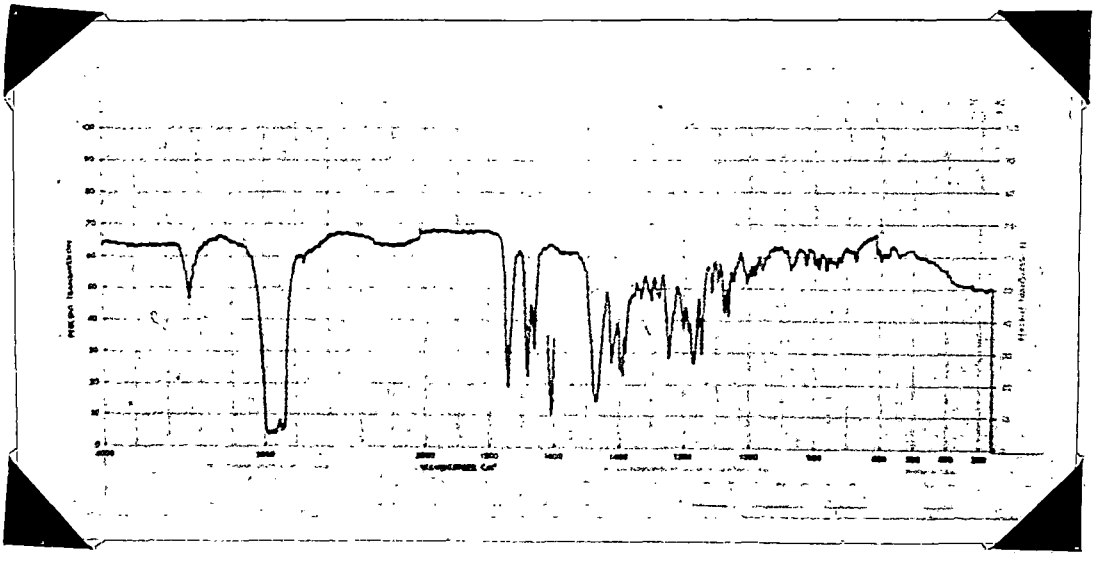


Fig. 2: IR Spectrum of Diosphenol (73)

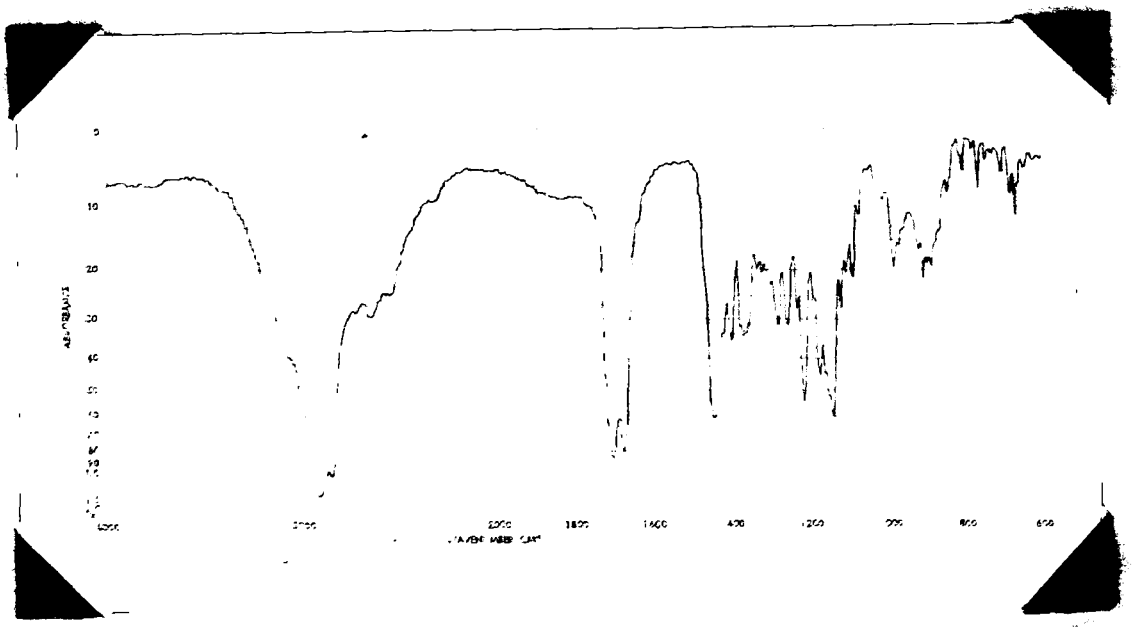


Fig. 3: IR Spectrum of Seco-A-acid (74)

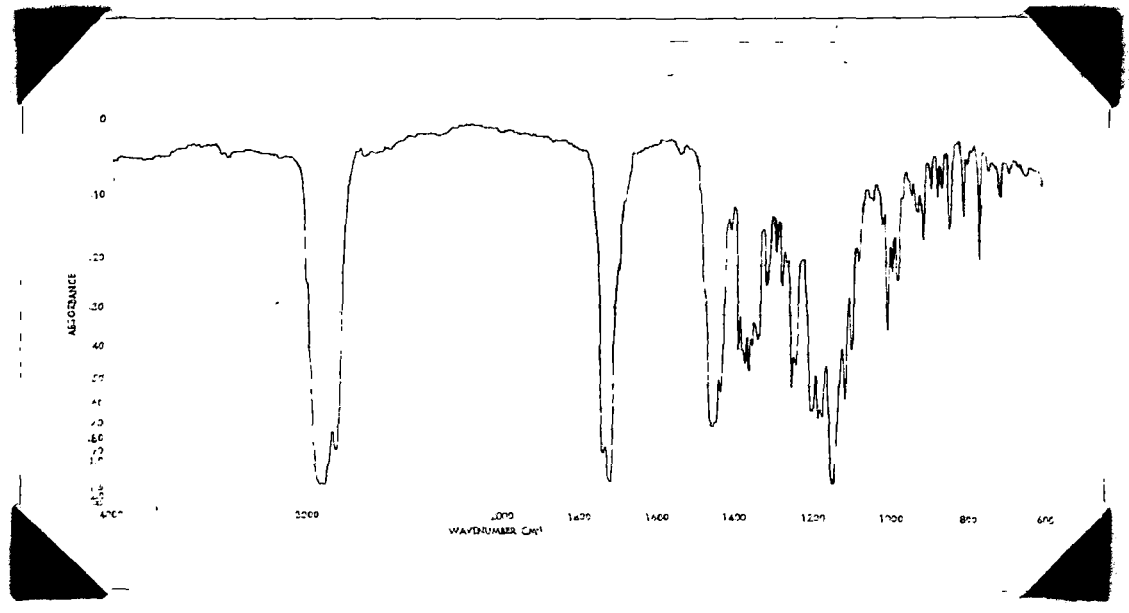


Fig. 4: IR Spectrum of Trimethyl Ester (68)

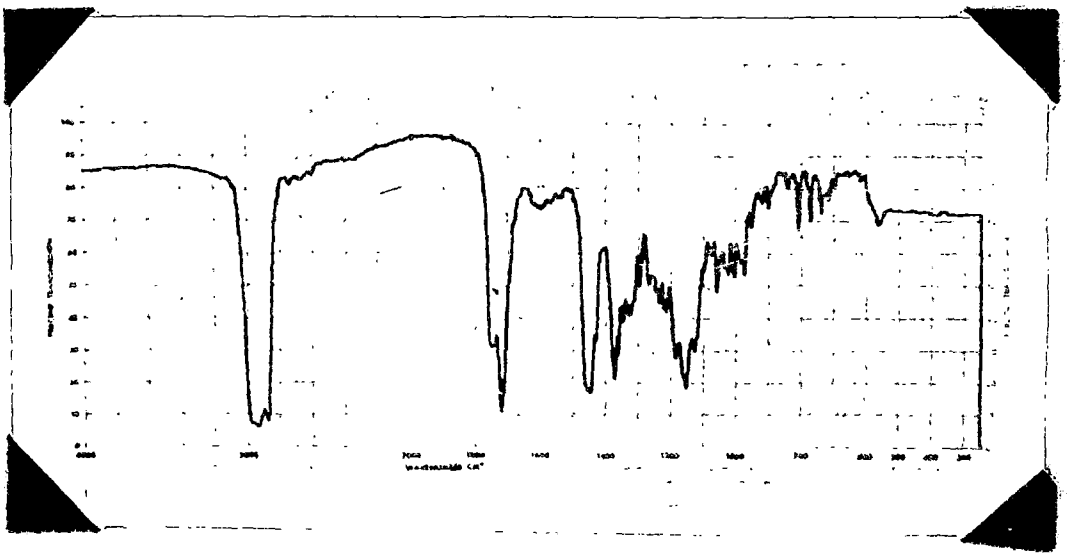


Fig. 5: IR Spectrum of Methyl-2α-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-oate (66)

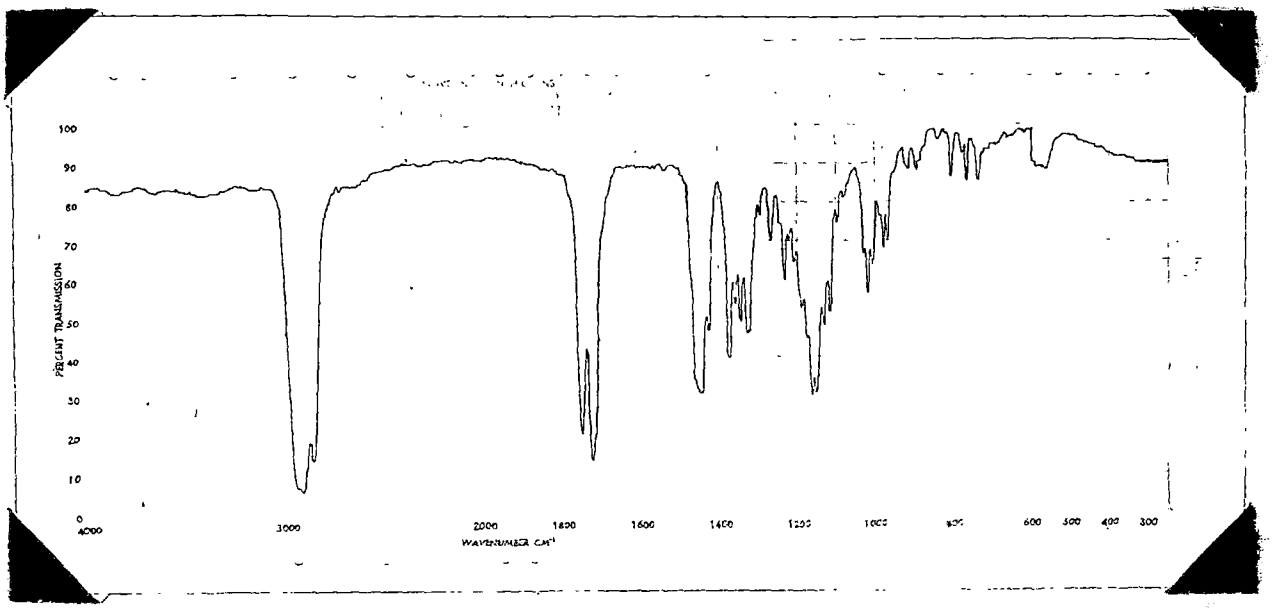


Fig. 6: IR Spectrum of Methyl-2β-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-oate (67)

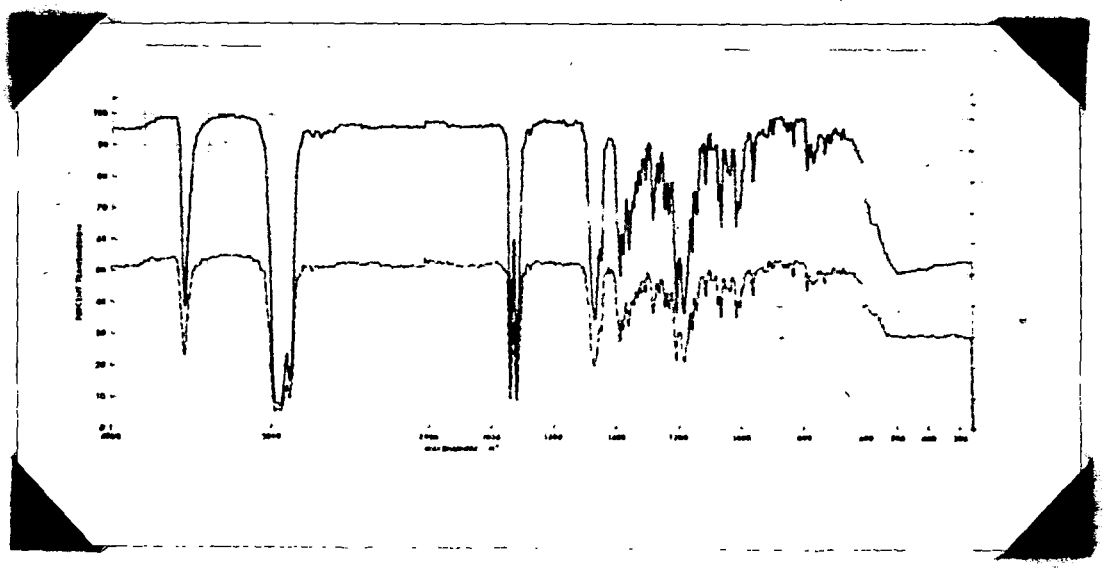


Fig.7: IR comparison of Dimethyl Dihydroceanothate (75) (solid line) with an authentic specimen (dotted line).

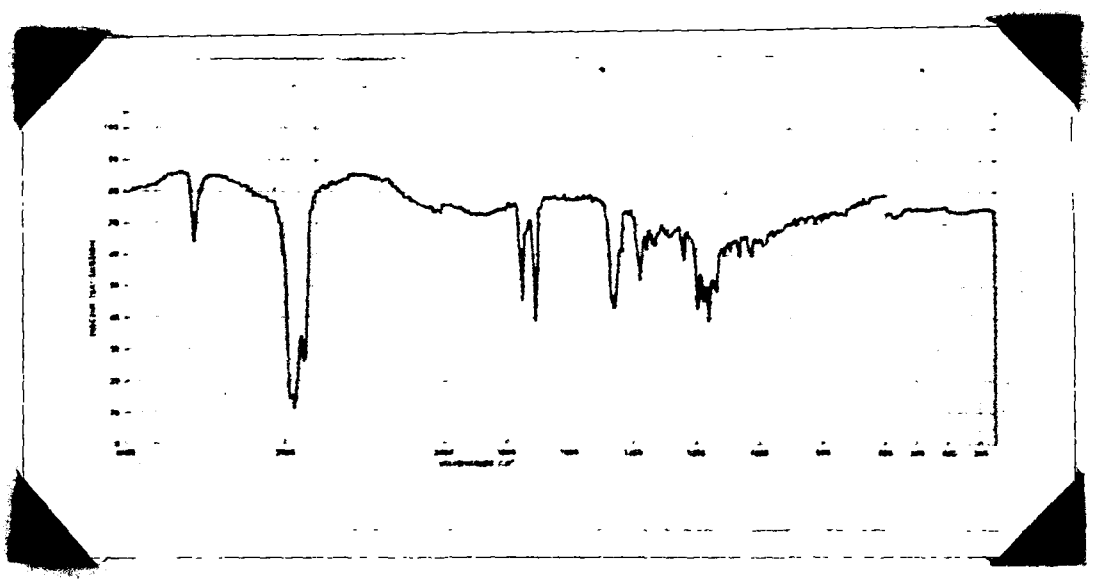


Fig. 8: IR Spectrum of Methyl-3α-hydroxy-2α-methoxy-carbonyl-A(1)-norlupan-28-oate (76)

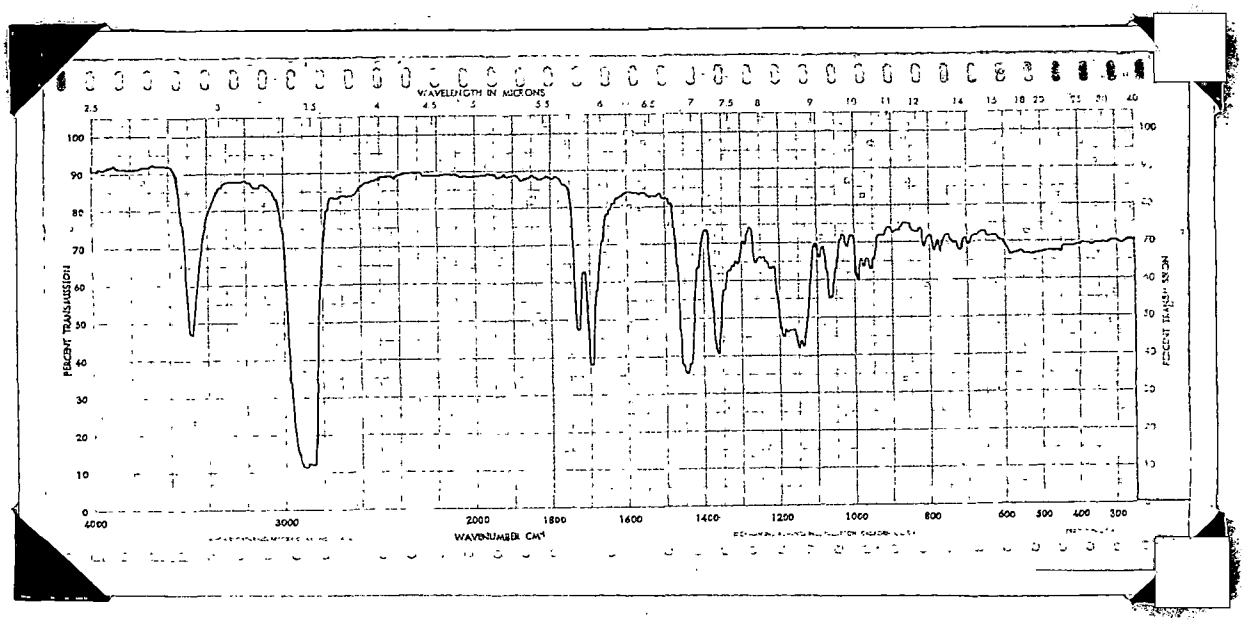


Fig.9: IR Spectrum of Methyl-3α-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (77)

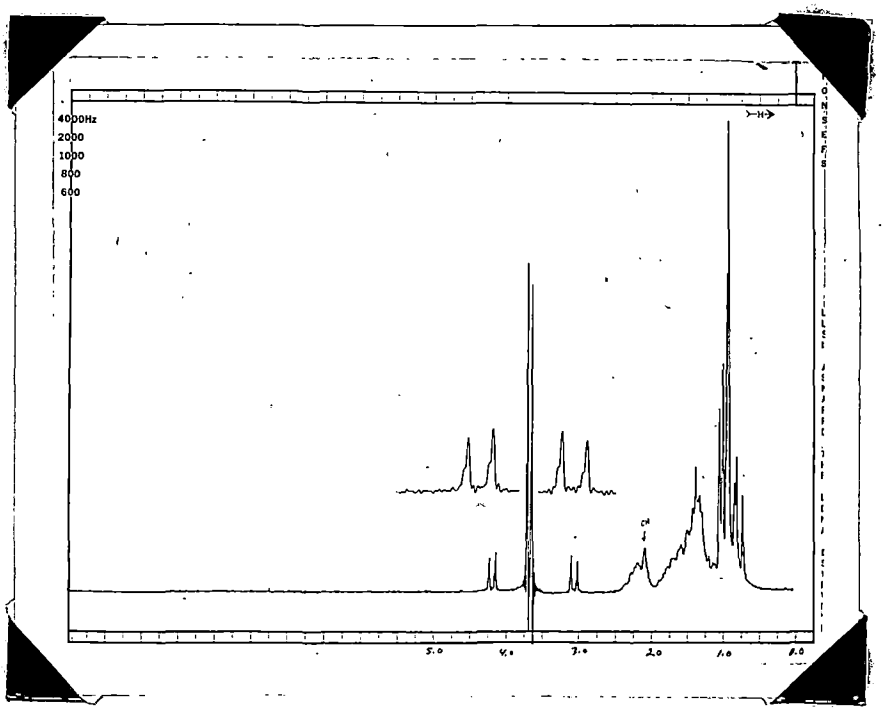


Fig.10: ^{1}H-NMR spectrum of Methyl-3α-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (77)

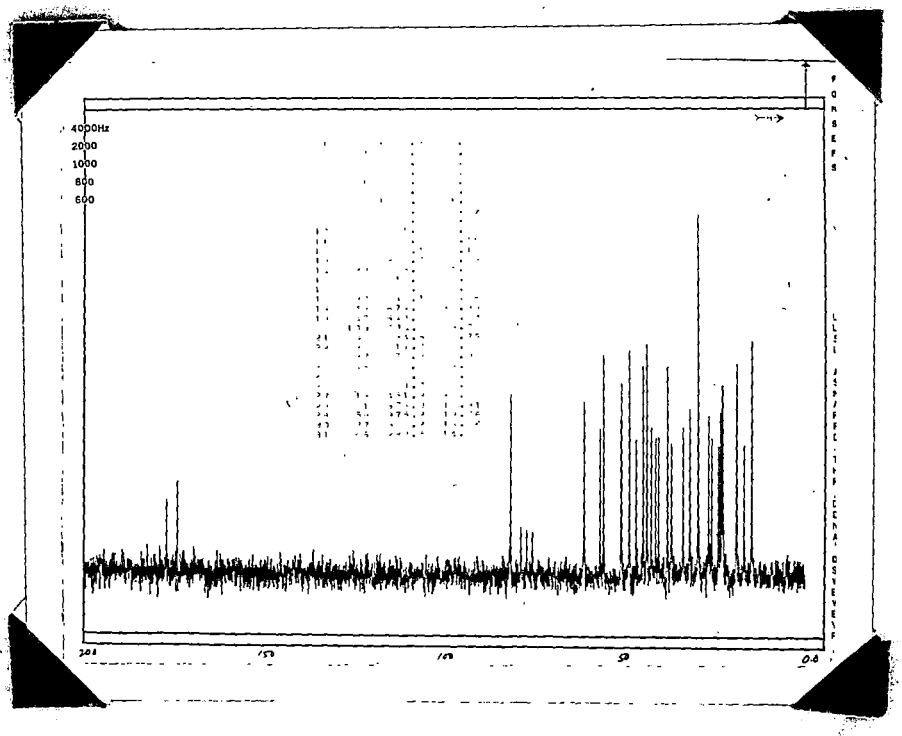


Fig. 11: ^{13}C-NMR spectrum of Methyl-3α-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (77)

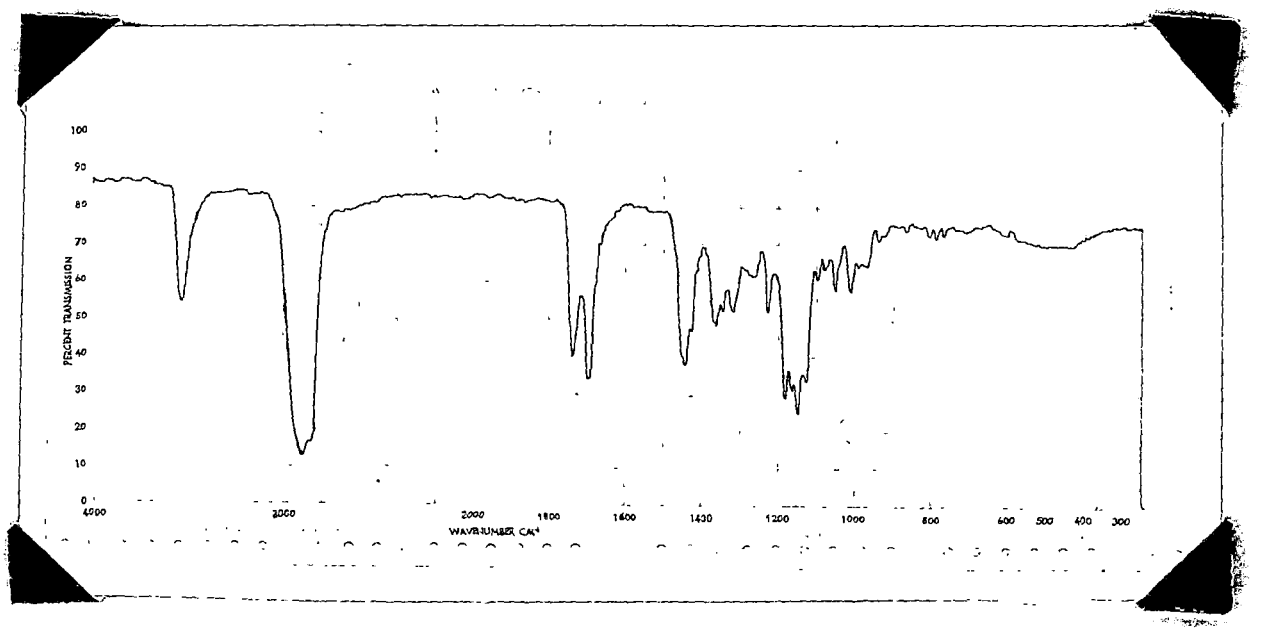


Fig. 12: IR spectrum of Methyl-3β-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (78)

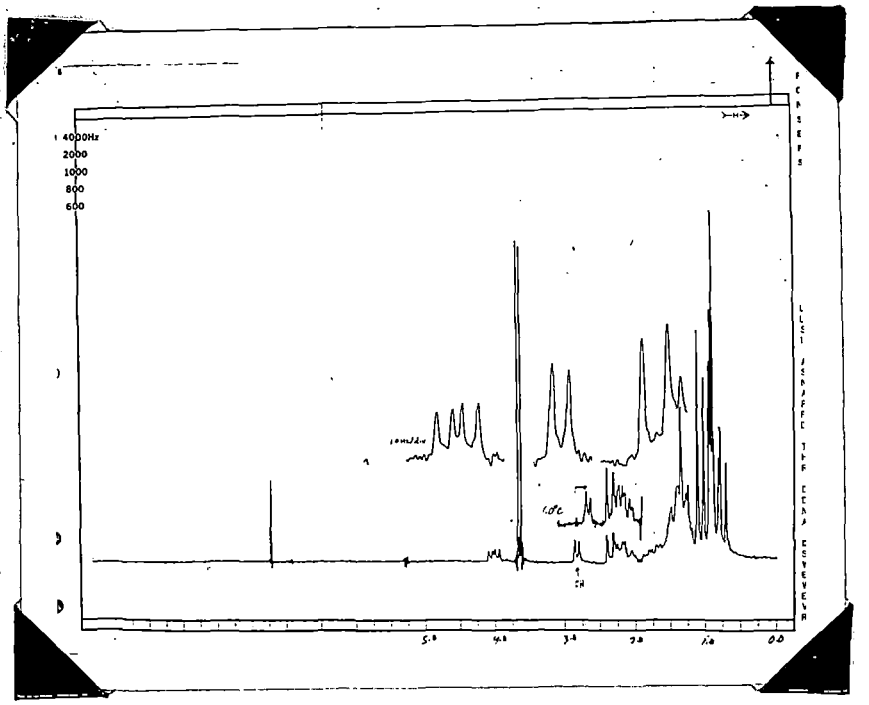


Fig. 13: ^{1}H-NMR spectrum of Methyl-3β-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (78)

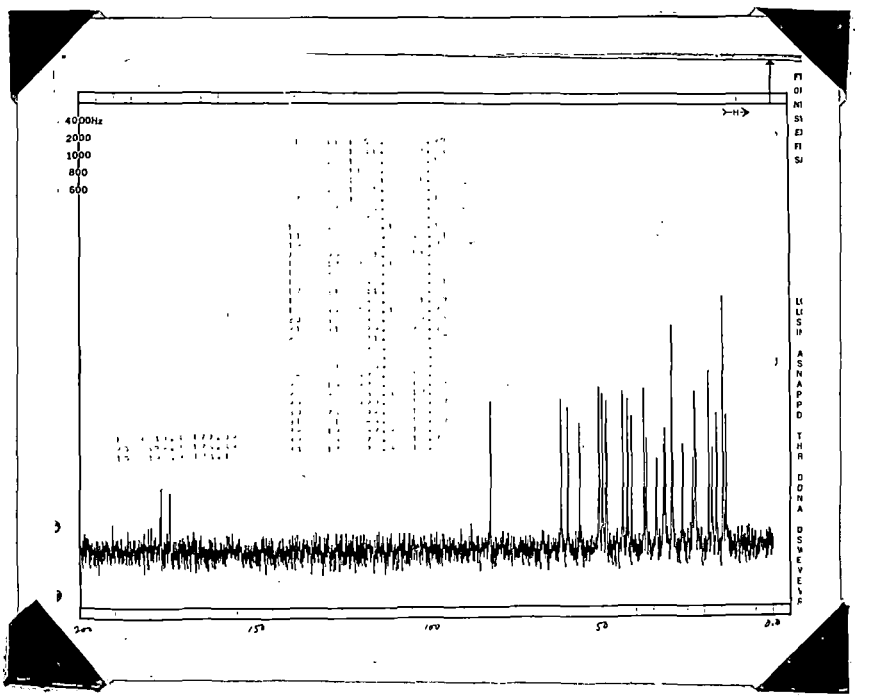


Fig. 14: ^{13}C-NMR spectrum of Methyl-3β-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (78).

CHAPTER-IV

EXPERIMENTAL

Melting points are uncorrected. The petrol used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution. I.R. spectra were recorded on a Beckmann IR-20 spectrophotometer. U.V. absorption spectra were taken in Beckmann DU-2 and Ziess VSU-1 spectrophotometers in methanol solution. TLC was carried out on 12% silver nitrate impregnated Silica Gel G (E.Merck) chromatographic plate and the spots were developed with H_2SO_4 - Ac_2O (1:9) mixture. ^1H - NMR spectra were determined in Varian HA-80 spectrophotometer and ^{13}C -NMR spectra were determined in Varian FT-80A spectrophotometer; in each case using $CDCl_3$ solution and tetramethylsilane as internal standard.

Extraction of Biscofia Javonica Blume: Isolation of Betulinic Acid (69):

Dried and powdered trunk bark of Biscofia javonica Blume (5 kg) was extracted with benzene in a soxhlet apparatus for 24 hours. Benzene was distilled off and the residual gummy mass (25 g) was taken up in ether (2.5 liter). The ether solution was washed with 10% aqueous sodium hydroxide solution (4 x 300 ml).

The alkaline solution was acidified with cold and dilute (10%) hydrochloric acid (1.5 liter) when some insoluble solids separated out, which were taken up in ether. The ether solution was washed with water till neutral and dried (Na_2SO_4). On removal of ether, a gummy residue of betulinic acid (69; 5 g) was obtained. This was not purified and used directly for the next step.

Esterification of Betulinic Acid (69): Preparation of Methyl Betulinate (70):

To a solution of the crude betulinic acid (25 g) in ether (2 liter) a solution of diazomethane (prepared from 10 g of nitrosomethylurea) in ether (1 liter) was added. The solution was allowed to stand overnight. Excess of diazomethane was decomposed with acetic acid. The ether solution was washed with water, 10% sodium bicarbonate solution, and again with water till neutral and then dried (Na_2SO_4). Removal of ether left a gummy residue (20 g) which was dissolved in benzene (70 ml) and was placed over a column of alumina (1 kg; deactivated with 40 ml of 10% aqueous acetic acid) and the column was eluted with the following solvents (Table-II).

Table-II

Eluent	Fractions 250 ml each	Residue on evaporation
Petrol	1-4	Oil
Petrol: benzene (4:1)	5-8	Nil
Petrol: benzene (3:2)	9-25	Solid (8 g), m.p. 216-20°

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

The solid (8g), m.p. 216-20° obtained from the fractions 9-25 (Table-II) was crystallised from a mixture of chloroform and methanol to afford needle shaped crystals of methyl betulinate (70), m.p. 223-24°, $(\alpha)_D$ 5°, identical (mmp and IR) with an authentic specimen.

Found:	C, 78.79;	H, 10.52.
Calc. for $C_{31}H_{50}O_3$:	C, 79.10;	H, 10.71%

UV: No absorption in the region 220-300 nm.

IR : ν_{max}^{nujol} 3560 (OH), 1715 ($COOCH_3$), 1660 and 880 cm^{-1} (= CH_2)

Hydrogenation of Methyl Betulinate (70): Preparation of Methyl Dihydrobetulinate (71):

A solution of Methyl betulinate (7 g) in a mixture of ethyl acetate (150 c.c) and acetic acid (150 c.c) was shaken in an atmosphere of hydrogen under normal pressure in presence of PtO_2 catalyst (0.2 g) for 5 hours when the absorption of hydrogen ceased. A white solid separated out which dissolved on heating. The hot mixture was filtered. The filtrate was concentrated to a small volume and diluted with water whereby a white solid separated out. The solid was filtered and crystallised from a mixture of chloroform and methanol to give needle shaped crystals (5 g) of (71), m.p. 236-38°, which was found to be identical (mmp and IR) with an authentic sample of methyl dihydrobetulinate.

Found: C, 78.85; H, 11.16.

Calc. for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09%

U.V. : No absorption in the region 220-300 nm.

I.R ν_{max}^{nujol} 3560 (OH), 1710 cm^{-1} ($COOCH_3$)

Jones' Oxidation of Methyl Dihydrobetulinate (71): Preparation of Methyl Dihydrobetulonate (72):

To a solution of methyl dihydrobetulinate (71; 7.8 g) in acetone (700 ml) was added Jones' reagent dropwise with shaking at room temperature until a faint orange colour persisted. The mixture was kept at room temperature for 1 hour, diluted with water and the precipitated material extracted with ether. The ether layer was washed with water, dried (Na_2SO_4) and evaporated. The residue (6.5 g) dissolved in benzene (12 ml) was chromatographed over a column of active alumina (320 g). The chromatogram was eluted with the following solvents (Table-III).

Table-III

Eluent	Fractions 100 ml each	Residue on evaporation
Petrol	1-4	Nil
Petrol: benzene (4:1)	5-18	Solid (4.0 g), m.p. 189-90°
Petrol: benzene (3:2)	19-25	Solid (2.2 g), m.p. 236-37°

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

The solid (4.0 g) from fractions 5-18 (Table-III) on crystallisation from methanol gave needle shaped crystals of (72; 3.6 g), m.p. 191-193°, $(\alpha)_D$ 8°, identical (mmp and IR) with an authentic sample of methyl dihydrobetulonate.

Found: C, 79.21; H, 10.83.

Calc. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71%

IR ν_{max}^{nujol} 1730 ($COOCH_3$), 1705 cm^{-1} (CO).

Fig-1

The solid (2.2 g) from fractions 19-25 (Table-III) on crystallisation from a mixture of chloroform and methanol gave crystals (2.0 g), m.p. 236-38° which were found to be identical (mmp and IR) with the starting material methyl dihydrobetulinate (71).

Autoxidation of Methyl Dihydrobetulonate (72): Preparation of Diosphenol (73):

Methyl dihydrobetulonate (72; 2.0 g) suspended in potassium tertiary butoxide in tertiary butanol (prepared from 6 g of potassium and 160 ml of dry tertiary butanol) was shaken in a stream of dry oxygen for 75 minutes. The reaction mixture was diluted with water and acidified with 6N hydrochloric acid. The precipitated material was extracted with chloroform and the

chloroform solution was washed with water till neutral and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a yellowish gum (1.8 g), which was dissolved in benzene (10 ml) and placed over a column of alumina (120 g; deactivated with 4.8 ml of 10% aqueous acetic acid). The column was eluted with the following solvents (Table-IV).

Table-IV

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-4	Nil
Petrol: benzene (4:1)	5-15	Solid (1.2 g), m.p. 126-29°

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

The solid (1.2 g) from fractions 5-15 (Table-IV) on crystallisation from a mixture of chloroform and methanol yielded needle shaped crystals of (73), m.p. 131-33°, $(\alpha)_D$-1.96°, which gave a positive ferric chloride colouration and showed two spots on T.L.C. These were assumed to be due to the tautomeric forms, the diketone (73A) and the diosphenol (73B).

Found: C, 76.48; H, 10.01.

Calc. for $C_{31}H_{48}O_4$: C, 76.82; H, 9.98%

U.V. : λ_{max} 269 nm (ϵ , 7532)

I.R : ν_{max}^{nujol} 3460 (OH), 1730 (CO_2Me), 1670, 1650, 860 cm^{-1}

Fig-2

Hydrogen Peroxide Oxidation of Diosphenol (73): Preparation of Seco A-acid (74):

Hydrogen peroxide (100 vol; 6 ml) mixed with 10% aqueous sodium hydroxide solution (12 ml) was added in three equal portions at the intervals of 15 minutes to a solution of the diosphenol (73; 700 mg) in ethanol (25 ml) under refluxing condition. The mixture was kept at 20° for 2 hours, then diluted with water, and the precipitated material extracted with ether. The ether layer was washed with water till neutral. The aqueous layer was acidified with 6N hydrochloric acid and extracted with ether. The ether layer was washed with water till neutral and dried (Na_2SO_4). Removal of the solvent from the latter ether solution gave a solid residue (450 mg), which on crystallisation from a mixture of benzene and petrol afforded needle shaped crystals of (74), m.p. 175-77°.

Found: C, 72.06; H, 9.43.

Calc. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72%

I.R. : ν_{max}^{nujol} 1710, 1680 cm^{-1} (COOH).

Fig-3

CrO_3- Pyridine Oxidation of Methyl Dihydrobetulinate (71):

Preparation of Methyl Dihydrobetulonate (72):

To a magnetically stirred solution of dry methylene chloride (190.8 ml) and dry pyridine (12.3 ml) under anhydrous condition dry chromium trioxide (7.63 g) was added and the mixture stirred for 15 minutes. A solution of methyl dihydrobetulinate (71; 6 g) in dry methylene chloride (85 ml) was added in one lot whereby a black tarry deposit immediately separated out. The mixture was stirred for 15 minutes more and allowed to stand for further 15 minutes. The mixture was filtered through cotton, the residue washed with ether and the ether layer filtered through cotton. The combined methylene chloride-ether layer was evaporated to yield a black oily residue which was treated with hot ether (500 ml) and filtered through a flutted filter paper. The ether solution was washed with saturated $NaHCO_3$ solution and water till neutral and dried (Na_2SO_4). Removal of ether gave an oily residue (6 g) which was dissolved in benzene (12 ml) and chromatographed over a column of active alumina (280 g). The column was eluted with the following solvents (Table-V).

Table-V

Eluent	Fractions 100 ml each	Residue on evaporation
Petrol	1-4	Nil
Petrol: Benzene (4:1)	5-13	Solid (5.5 g) m.p. 188-90°
Petrol: Benzene (3:2)	14-18	Solid (0.3 g), m.p. 236-37°

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

The solid (5.5 g) from fractions 5-13 (Table-V) on crystallisation from methanol gave needle shaped crystals of (72; 5.0 g), m.p. 191-193°, $(\alpha)_D$ 8°, identical (mmp and IR) with an authentic sample of methyl dihydrobetulonate.

Found: C, 79.18; H, 10.80.

Calc. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71%

I.R. ν_{max}^{nujol} 1730 ($COOCH_3$), 1705 cm^{-1} (CO).

Fig. 1

The solid (0.3 g) obtained from fractions 14-18 (Table-V) on crystallisation from a mixture of chloroform and methanol gave crystals (0.2 g), m.p. 236-38° which were found to be identical (mmp and IR) with the starting material methyl dihydrobetulinate (71).

Fuming Nitric Acid-Ammonium Vanadate Oxidation of Methyl Dihydrobetulonate (72): Preparation of Seco A-Acid (74):

To a stirred mixture of ammonium vanadate (160 mg) and fuming nitric acid (151.5 ml) maintained at 0°C was added slowly a solution of methyl dihydrobetulonate (72; 5 g) in acetic acid (75 ml) during the course of 15 minutes. Then the reaction mixture was stirred for an additional hour at 0°C. The reaction mixture was poured in ice cold water whereby a white solid separated out. The solid was extracted with ether, and the ether solution was washed with water, 10% aqueous NaOH solution (3 x 200 ml) and with water till neutral and dried (Na_2SO_4).

The 10% NaOH solution (600 ml) was acidified with 6N hydrochloric acid and the precipitated acid was extracted with ether and the etherial solution was washed with water till neutral and dried (Na_2SO_4). Removal of the solvent gave a solid residue (3.6 g) which on crystallisation from a mixture of benzene and petrol afforded needle shaped crystals (3.2 g) of (74), m.p. 175-77°.

Found: C, 72.03; H, 9.61.

Calc. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72%

I.R. ν_{max}^{nujol} 1710, 1680 cm^{-1} (COOH)

Fig-3

Esterification of Seco A-Acid (74): Preparation of the Trimethyl Ester (68):

An ether solution (300 ml) of the Seco A-acid (74; 3 g) was esterified with an ether solution of diazomethane prepared from nitrosomethyl urea (3 g) in the usual way and the reaction mixture was kept overnight. Excess of diazomethane was destroyed with acetic acid (4 ml). The ether solution was washed with water, saturated $NaHCO_3$ solution and again with water till neutral and dried (Na_2SO_4). Evaporation of the solvent gave an oily mass (3.0 g), which was dissolved in benzene (10 ml) and placed over a column of alumina (180 g, deactivated with 7.2 ml of 10% aqueous acetic acid). The column was eluted with the following solvents (Table-VI).

Table-VI

Eluent	Fractions 100 ml each	Residue on evaporation
Petrol	1-5	Nil
Petrol: Benzene (4:1)	6-17	Solid (2.8 g), m.p. 143-46°

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

The solid (2.8 g) from fractions 6-17 (Table-VI) on crystallisation from methanol gave needle shaped crystals (2.7 g) of (68), m.p. 146-47° having identical m.p. and I.R. spectra reported in the literature for the trimethyl ester[3,12] of the Seco A-acid.

Found: C, 72.37; H, 9.88.

Calc. for $C_{33}H_{54}O_6$: C, 72.49; H, 9.95%

I.R. ν_{max}^{nujol} 1745, 1725 cm^{-1} (COOMe).

Fig. 4

Dieckmann Condensation of the Trimethyl Ester (68): Preparation of Methyl-2α-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-oate (66) and Methyl-2β-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-oate (67):

Potassium (0.28 g) was added to a mixture of dry tertiary butanol (20 ml) and dry benzene (20 ml) under nitrogen atmosphere. After all the potassium had dissolved, a solution of the trimethyl ester (68; 728 mg) in dry benzene (40 ml) was added. The mixture was then refluxed under nitrogen atmosphere for 24 hours, during which time about 30 ml of solvent was removed through a Dean-Stark trap. The orange coloured solution was cooled, diluted with water, acidified with cold 6N hydrochloric acid and extracted with ether. The organic layer was washed with water, 5% aqueous sodium bicarbonate solution and again with water till neutral and dried (Na_2SO_4). Evaporation of the solvent gave a gummy residue (500 mg) which was dissolved in benzene (5 ml) and placed over a column of alumina (30 g, deactivated with 1.2 ml of 10% aqueous acetic acid). The column was eluted with the following solvents (Table-VII).

Table-VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-3	Nil
Petrol: Benzene (9:1)	4-6	Nil
Petrol: Benzene (4:1)	7-9	Nil
Petrol: Benzene (7:3)	10-12	Nil
Petrol: Benzene (3:2)	13-16	Solid (180 mg), m.p. 189-91°
	17-22	Solid (205 mg), m.p. 173-75°

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

The solid (180 mg) from fractions 13-16 (Table-VII) on crystallisation from methanol afforded needle shaped crystals of (66), m.p. 191-93°, (TLC-homogenous) $(\alpha)_D$ 89° having identical m.p., rotation and I.R. spectra reported in the literature for dimethyl dehydrodihydroceanothate[22].

Found: C, 74.71; H, 9.72.

Calc. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79%

I.R ν_{max}^{nujol} 1755 (cyclopentanone), 1725 cm^{-1} (CO_2Me)

Fig. 5

The solid (205 mg) from fractions 17-22, (Table-VII) on crystallisation from methanol furnished needle shaped crystals of (67), m.p. 175-77° (TLC-homogenous) $(\alpha)_D$ 42°, having identical m.p, rotation and I.R. spectra reported in the literature for methyl-2β-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-oate[22].

Found: C, 74.61; H, 9.86.

Calc. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79%

I.R. ν_{max}^{nujol} 1750 (cyclopentanone),
1720 cm^{-1} (CO_2Me).

Fig. 6

Sodium Borohydride Reduction of Dimethyl Dehydrodihydroceanothate [Methyl-2α-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-oate] (66): Preparation of Dimethyl Dihydroceanothate [Methyl-3β-hydroxy-2α-methoxycarbonyl-A(1)-norlupan-28-oate] (75) and Its C-3 Epimer [Methyl-3α-hydroxy-2α-methoxycarbonyl-A(1)-norlupan-28-oate] (76):

Sodium borohydride (100 mg) was added to a solution of

dimethyl dehydrodihydroceanothate (66, 100 mg) in a mixture of methanol (30 ml) and dioxan (30 ml). The mixture was stirred for 5 hours and allowed to stand overnight at room temperature. Most of the solvents were removed under reduced pressure. The reaction mixture was diluted with water and extracted with ether. The etheral solution was washed with water till neutral and dried (Na_2SO_4). Removal of solvent gave a gummy residue (100 mg), which was dissolved in benzene (4 ml) and placed over a column of alumina (10 g, deactivated with 0.4 ml of 10% aqueous acetic acid). The column was eluted with the following solvents (Table-VIII).

Table-VIII

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-3	Nil
Petrol: Benzene (9:1)	4-6	Nil
Petrol: Benzene (4:1)	7-9	Nil
Petrol: Benzene (7:3)	10-12	Nil
Petrol: Benzene (3:2)	13-15	Nil

Contd..

Table-IX (Contd..)

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol: Benzene (3:2)	7-9	Nil
Petrol: Benzene (1:1)	10-12	Nil
Petrol: Benzene (2:3)	13-15	Solid (15 mg), m.p. 259-60°
	16-20	Solid (40 mg), m.p. 138-40°

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

The solid (15 mg) from fractions 13-15 (Table-IX) on crystallisation from methanol gave needle shaped crystals of (75), m.p. 261-63°, $(\alpha)_D$ 22°. This compound was found to be identical (mmp, Co-IR and Co-TLC) with an authentic sample of dimethyl dihydroceanothate [methyl-3β-hydroxy-2α-methoxycarbonyl-A(1)-norlupan-28-oate]. Kindly supplied by Professor P. de Mayo.

Found: C, 74.32; H, 10.17

Calc. for $C_{32}H_{52}O_5$: C, 74.38; H, 10.14%

IR ν_{max}^{nujol} 3540 (OH), 1730 (CO_2Me), 1710 cm^{-1} (CO_2Me)

Co-IR spectra shown in Fig. 7

TLC: Solvent used: Benzene, R_f = 0.34.

The solid (40 mg) obtained from fractions 16-20 (Table-IX) on crystallisation from methanol gave needle shaped crystals of methyl-3α-hydroxy-2α-methoxycarbonyl-A(1)-norlupan-28-oate, (76), m.p. 140-42°. Confirmation of the structure of (76) by NMR is in progress.

Found: C, 74.46; H, 10.21.

Calc. for $C_{32}H_{52}O_5$: C, 74.38; H, 10.14%

IR ν_{max}^{nujol} 3560 (OH), 1745 (CO_2Me), 1705 cm^{-1} (CO_2Me)

Fig. 8

T.L.C. : Solvent used: Benzene, R_f = 0.25

Sodium Borohydride Reduction of Dimethyl epi Dehydrodihydroceanothate / Methyl-2β-methoxycarbonyl-3-Oxo-A(1)-norlupan-28-Oate / (67): Preparation of Methyl-3α-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (77) and Methyl-3β-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (78):

To a solution of dimethyl epidehydrodihydroceanothate (67; 300 mg) in a mixture of methanol (35 ml) and dioxan (20 ml)

was added sodium borohydride (1.5 g) and 1N NaOH solution (2 ml) and the reaction mixture was stirred for 72 hours. The solvents were removed under reduced pressure, the residue diluted with water and the precipitated material was extracted with ether. The ether layer was washed with 10% NaOH solution then with water till neutral and dried (Na_2SO_4). Removal of ether gave an oily mass (240 mg), which showed two spots on TLC. It was dissolved in benzene (5 ml) and placed over a column of alumina (30 g, deactivated with 1.8 ml of 10% aqueous acetic acid). The column was eluted with the following solvents (Table-X).

Table-X

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-3	Nil
Petrol: Benzene (9:1)	4-6	Nil
Petrol: Benzene (4:1)	7-9	Nil
Petrol: Benzene (7:3)	10-12	Nil
Petrol: Benzene (3:2)	13-15	Nil

Contd..

Table-X (Contd.)

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol: Benzene (1:1)	16-22	Solid (80 mg), m.p. 197-201°
Petrol: Benzene (3:7)	23-32	Solid (130 mg), m.p. 168-72°

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

The solid (80 mg) obtained from fractions 16-22 (Table-X) on crystallisation from aqueous methanol afforded crystals of methyl-3 α-hydroxy-2 β-methoxycarbonyl-A(1)-norlupan-28-oate (77), m.p. 202-203°.

T.L.C. : Solvent Used	R_f
Benzene	0.184
Benzene: Methanol (4:1)	0.782

Found:	C, 74.44;	H, 10.17.
Calc. for $C_{32}H_{52}O_5$:	C, 74.38;	H, 10.14%

IR ν_{max}^{nujol} 3490 (OH), 1730 (CO_2Me), 1695 cm^{-1} (CO_2Me)

Fig. 9

^{1}H-NMR (80 MH_z) : Recorded using a solution of 1 mg of (77) in 15 μl of $CDCl_3$. Signals at δ 0.7 to 1.1 (seven CH_3 groups), 2.1 (1H, OH), 3.06 (1H, doublet, J = 7.0 H_z, C-2 proton), 3.65 (3H, singlet, $COOCH_3$), 3.7 (3H, singlet, $COOCH_3$), 4.18 (1H, doublet, J = 7.0 H_z, C-3 proton).

Fig. 10

^{13}C-NMR : Recorded using 4.5 mg of (77) in $CDCl_3$.

Fig. 11

The solid (130 mg) obtained from fractions 23-32 (Table-X) on crystallisation from aqueous methanol afforded crystals of methyl-3β-hydroxy-2β-methoxycarbonyl-A(1)-norlupan-28-oate (78), m.p. 174-76°.

T.L.C. :	Solvent Used	R_f
	Benzene	0.107
	Benzene: Methanol (4:1)	0.715

Found: C, 74.35; H, 10.11.

Calc. for $C_{32}H_{52}O_5$: C, 74.38; H, 10.14%

IR ν_{max}^{nujol} 3540 (OH), 1740 (CO_2Me), 1690 cm^{-1} (CO_2Me)

Fig. 12

^{1}H-NMR (80 MH_z) : Recorded using a solution of 5 mg of (78) in 200 μl of $CDCl_3$. Signals at δ 0.7 to 1.1 (seven CH_3 groups), 2.35 (1H, doublet, J = 7.2 H_z, C-2 proton), a doublet centred at 2.8 (1H, J = 4.5 H_z, OH), 3.65 (3H, singlet, $COOCH_3$), 3.7 (3H, singlet, $COOCH_3$), 4.02 (1H, multiplet, C-3 proton).

Fig. 13

^{13}C-NMR : Recorded using 6.5 mg of (78) in $CDCl_3$.

Fig. 14

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