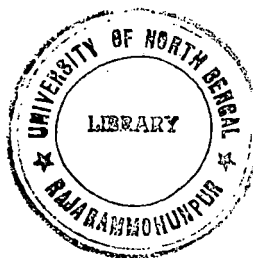


# **INVESTIGATION ON TERPENOIDS AND RELATED COMPOUNDS**

**Partial synthesis of Triterpenes, isolation and structure  
elucidation of Triterpenes and related compounds**

**Thesis submitted for the Degree of  
Doctor of Philosophy in Science ( Ph. D. )  
of the  
University of North Bengal  
1975**



*By*

**SHRI TAPAN KUMAR RAY, M. Sc.  
DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NORTH BENGAL**

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

The author takes this opportunity to record his grateful thanks to Dr. H. N. Khastgir, Professor and Head of the Department of Chemistry, University of North Bengal, Dist. Darjeeling, for his valuable suggestions, guidance and untiring interest throughout the research programme.

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The author's thanks are also due to Prof. J. N. Chatterjee, Patna Science College, Patna, to Dr. S. C. Pakrashi, Assistant Director, Indian Institute for Experimental Medicine, Calcutta for IR spectra, to Dr. A. K. Barua, Bose Institute, Calcutta for

some UV spectra, and to Dr. S. K. Sengupta, Research and Development Section, East India Pharmaceutical Works Ltd., Calcutta for optical rotations recorded in the thesis.

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The author expresses his warmest thanks to Dr. D. R. Misra, Mr. S. L. Dutta, Scientific Officer, his friends and colleagues for their cooperation and helpful discussions during the progress of the work.



The author is grateful to East India Pharmaceutical Works Ltd., Calcutta for the award of Research Fellowship and the authorities of University of North Bengal for extending Laboratory facilities.

Finally, the author expresses his deep feelings for the constant inspiration and encouragement he received from his beloved parents.

*Tapen Kumar Ray*

( Tapen Kumar Ray )

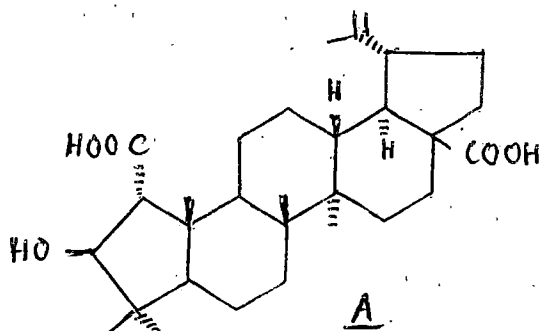
Department of Chemistry  
University of North Bengal  
Dist. Darjeeling  
September, 1975.

## SUMMARY

The work embodied in the present thesis has been divided into four parts :

### A. PART - I :

Chapter - I : In view of the fact that the present dissertation deals in part with a successful partial synthesis of dimethyl dihydroceanothate, it was felt pertinent to prepare an up-to-date survey on the isolation, structure and stereochemistry of ceanothic acid A. This constitutes the subject matter of sections A, B and C of Chapter -I.



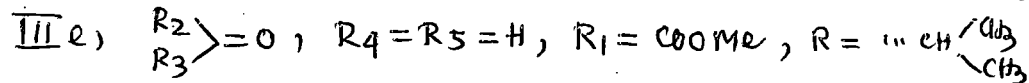
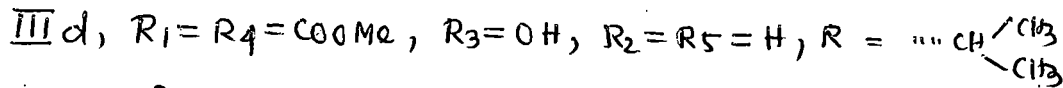
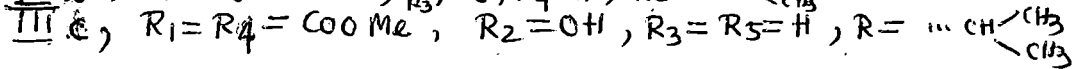
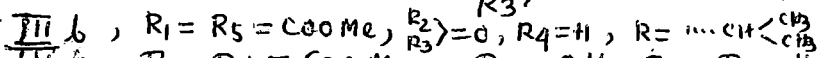
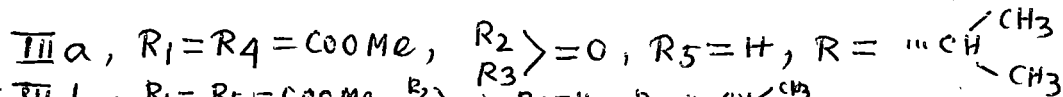
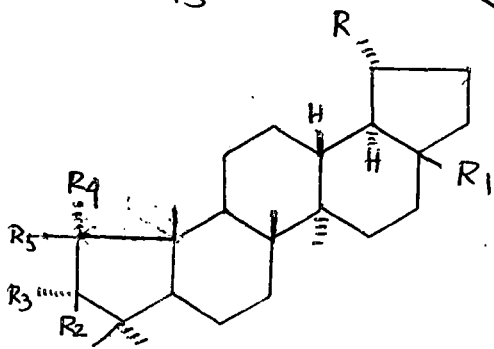
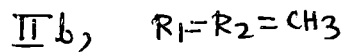
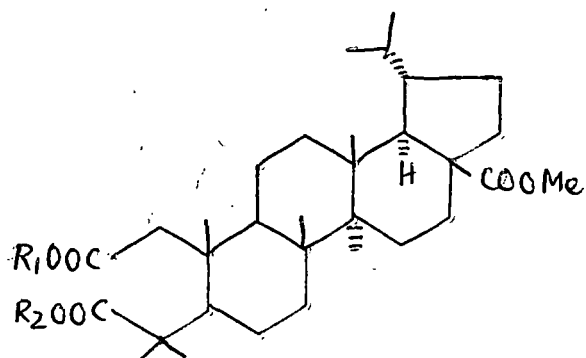
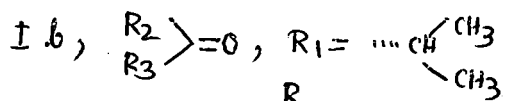
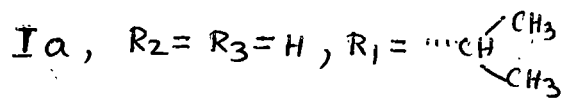
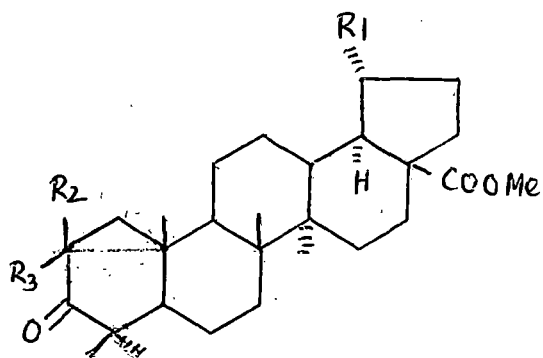
Chapter - II, Section A, deals with a short review on previous attempts toward the partial synthesis of ceanothic acid A. Section B describes a successful partial synthesis of dimethyl dihydroceanothate III C. Autoxidation of methyl dihydrobetulonate Ia by passing a stream of oxygen in presence of K-tert.butoxide in tert.butanol gave the diosphenol Ib, m.p. 131-33°,  $(\alpha)_D - 1.96^\circ$ . The diosphenol on oxidation with 30% hydrogen peroxide gave the seco-acid IIa, m.p. 175-77°.

(11)

ν  $\frac{\text{nujol}}{\text{max}}$  1710 and 1690 (COOH)  $\text{cm}^{-1}$ , which on methylation with diazomethane afforded the trimethyl ester Iib, m.p. 146-47°,

ν  $\frac{\text{nujol}}{\text{max}}$  1745, 1725 ( $-\text{CO}_2\text{Me}$ )  $\text{cm}^{-1}$ , identical with those reported in the literature for the trimethyl ester of the seco - A acid derived from dihydrobetulic acid (L. Ruzicka and O. Isler, *Helv. Chim. Acta*, 19 506, 1936). Dieckmann condensation of the trimethyl ester in presence of K-tert.butoxide in benzene solution under nitrogen blanket (B. Fuchs and H. J. E. Loewenthal, *Tetrahedron*, 11, 199, 1960; H. R. Nace and A. H. Smith, *J. Org. Chem*, 38, 1941, 1973) gave a gummy product which on chromatography over alumina gave methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate IIIa, m.p. 191-93°, ( $\alpha$ )<sub>D</sub> 89° and its epimer IIib, m.p. 175-77°, ( $\alpha$ )<sub>D</sub> 42° in the ratio of 17:1. The high yield of the  $\beta$ - ketoester IIIa with the desired stereochemistry in this reaction is significant and is at variance with the observation of Eade et al (R. A. Eade, P. K. Grant; M. J. A. McGrath, J. J. H. Simes and M. Wootton, *Aust. J. Chem*, 24, 621, 1971) who stated that methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlup-20(29)-en-28-oate (dimethyl dehydroceanothate) was rapidly epimerised by alkali to an equilibrium mixture containing 40% of the starting material and 60% of the isomer epimeric at C-2.

(111)



(iv)

The  $\beta$ -keto-ester IIIa on reduction with sodium borohydride in methanol-dioxan solution gave a mixture (TLC-two spots) which on chromatography over alumina first eluted (benzene : petroleum 3:2) a solid III C, m.p. 261-63°, ( $\alpha$ )<sub>D</sub> 22°,  $\nu$  <sub>max</sub> <sup>nujol</sup> 1755, 1725 cm<sup>-1</sup> identical with an authentic specimen of dimethyl dihydroceanothate supplied by Professor P. de Mayo. Further elution with the same solvent gave another solid, m.p. 140-42°,  $\nu$  <sub>max</sub> <sup>nujol</sup> 1750, 1725 cm<sup>-1</sup>. The yield of this material was not sufficient for NMR studies but by analogy with previous work (R. A. Eade, P. K. Grant, M. J. A. McGrath, J. J. H. Simes and M. Wootton, Aust. J. Chem, 24, 621, 1971) it is believed to be the C-3 epimer IIId.

Attempts were also made to prepare the  $\beta$ -keto ester by carbomethoxylation of the A-nor ketone IIIe at C-2. Carbomethoxylation reaction on the ketone with dimethyl carbonate was carried out in presence of sodium methoxide, K-tert.butoxide and sodium hydride in various solvents but so far the reaction did not meet with success (S. J. Rhoads, J. C. Gilbert, A. W. Decora, T. R. Garland, R. J. Spangler and M. J. Urbighkit, Tetrahedron, 19, 1665, 1963; J. A. Marshall and N. Cohen, J. Org. Chem., 30, 3475, 1965; E. J. Corey, Rajat B. Mitra and Hisashi Uda, 86, 485, 1964; H. O. House, Modern Synthetic Reactions, W. A. Benjamin Inc. 1972, page - 546 and references cited therein).

[A preliminary account of the work was presented at the Symposium sponsored by Indian National Science Academy and Calcutta University, 1975, held at Calcutta. (Abstract No.A27).

A short communication embodying the results of this work will be published in September issue, 1975 of Indian Journal of Chemistry (REPRINT NO. 4)].

**B. Part - II :**

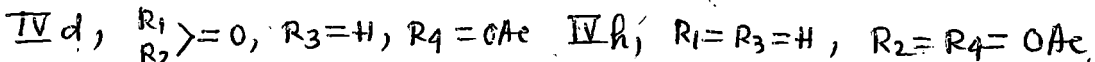
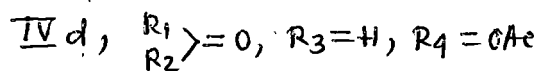
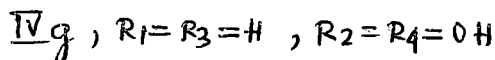
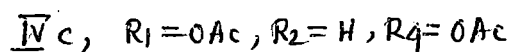
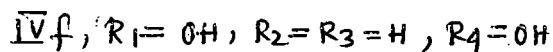
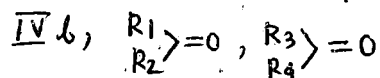
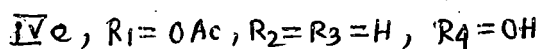
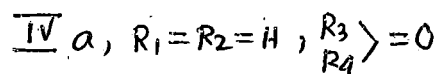
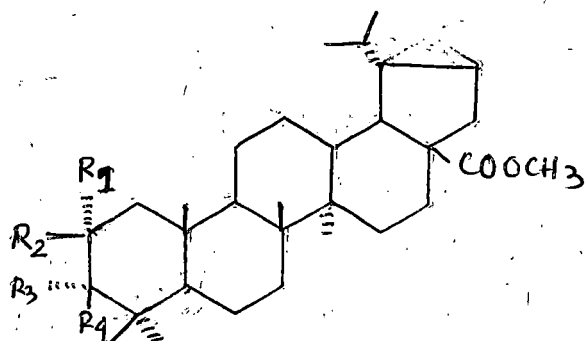
Chapter - I, Sections A-D deals with an up-to-date survey of the synthesis of 2,3-dibls of triterpenoids. Section E constitute a short review on autoxidation and isomerisation in ring A of triterpenoids. Section F deals with a short review on 2,3-dihydroxy triterpene acids from natural sources.

Chapter -II describes an unambiguous synthesis of methyl dihydroaliphitolate (Section B) and 2 $\beta$ , 3 $\beta$ -dihydroxy betulinate (Section C) starting from betulinic acid.

Methyl dihydrobetulonate IVa (Section B) on autoxidation gave the diosphenol IVb, m.p. 131-33 $^{\circ}$ , ( $\alpha$ )<sub>D</sub> - 1.96 $^{\circ}$ . Acetylation of the diosphenol followed by hydrogenation gave the corresponding 2 $\alpha$ -acetoxy - 3 Keto-dihydro-betulonate IVc, m.p. 223-25 $^{\circ}$ , ( $\alpha$ )<sub>D</sub> + 32.56 $^{\circ}$ . This compound was kept overnight on a basic column and after eluting it with benzene afforded a new product, m.p. 228-30 $^{\circ}$ , which has been assigned structure IVd, methyl-

( vi )

2-Keto-3- <sup>$\beta$</sup> -acetoxy dihydrobetulinate. This is formed by migration of the acyl group from 2 $\alpha$ -position to 3 $\beta$ -position. 2 $\alpha$ -acetoxy methyl dihydrobetulonate IVc on sodium borohydride reduction in methanol-dioxan solution at pH 8 to reduce isomerisation gave a crystalline solid IVe, m.p. 259-61°, ( $\alpha$ )<sub>D</sub> - 7.14°. The latter on hydrolysis with a 15% methanolic potassium hydroxide solution afforded a product which on chromatography gave a crystalline solid IVf, m.p. 230-32°, indistinguishable from an authentic sample of methyl dihydroalphitolate supplied by Prof. E. Ritchie. The infrared spectra of the two compounds were found to be identical throughout the entire range.



The diisphenol IVb (Section C) on sodium borohydride reduction in methanol solution gave a compound IVg, 261-63°, ( $\alpha$ )<sub>D</sub> 21.62°,  $\nu$ <sub>max</sub> 3525, 3360, 1720 cm<sup>-1</sup>; diacetate IVh, m.p. 186-87°,  $\nu$ <sub>max</sub><sup>KBr</sup> 1745, 1720, 1258 cm<sup>-1</sup>. Examination of the NMR spectrum of the diol showed a multiplet at about 3.20 ppm assigned to C3 - H and a broad unresolved multiplet at about 4.5 ppm (C2-H) which collapsed to a doublet (J = 3.7 Hz) and a multiplet ( $\Sigma J = 6$ Hz) respectively upon exchange of hydroxyl proton with D<sub>2</sub>O. Thus the hydroxyl group at C-3 is equatorial (Ha) and the one at C-2 is axial (He). In the NMR spectrum of the diacetate IVh these signals were shifted to a downfield to 4.8 ppm (doublet, J = 4Hz) and at about 5.4 ppm (broad multiplet). The signals for the ester group and the acetate group appeared at 3.65 ppm (3H, singlet) and at 2.06 (6H, singlet) ppm. Thus the assignment of the structure IVg for the NaBH<sub>4</sub> reduction product is consistent with NMR results. The mechanism for the exclusive formation of the 2 $\beta$ , 3 $\beta$ -diol has been discussed.

### C. PART - III :

Chapter-I describes the morphology of the fern Polypodium juglandifolium, Don.

Chapter-II gives a short review on the chemical constituents of ferns of Polypodiaceae family.



Chapter - III deals on the investigation on the neutral part of the benzene extract of the fern *Polypodium juglandifolium* and isolation of two new triterpenes - Polypodinol A,  $C_{30}H_{50}O$  and Polypodinol B,  $C_{30}H_{50}O$  along with fern-9(11)-ene, filicene, cyclolaudenol, nerifolliol and  $\beta$ -sitosterol and detection of a new triterpene,  $C_{32}H_{54}O$ , in the cyclolaudenol fraction from mass-spectrometry.

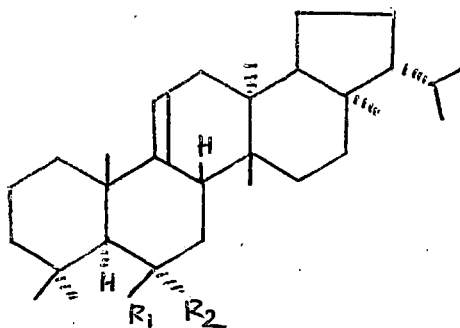
Chapter - IV describes the investigations on the structure elucidation of Polypodinol A,  $C_{30}H_{50}O$ .

Polypodinol A Va,  $C_{30}H_{50}O$ , m.p.  $223-25^{\circ}$ , gave a positive Libermann-Burchard test and reddish brown colour with TMM. IR spectrum of Va showed bands at  $3605\text{ cm}^{-1}$  ( $-OH$ ) and its NMR spectrum showed signals at  $\delta$  0.8 to 1.28, a multiplet centered at  $\delta$  5.40 (1H, trisubstituted double bond) and a broad diffused multiplet at  $\delta$  4.40 attributed to the proton attached to the carbon containing the  $-OH$  group. The coupling pattern indicates that this proton is attached to a carbon atom in the system  $-CH-CH(OH)-CH_2-$ . On treatment with acetic anhydride-pyridine it furnished a monoacetate Vb,  $C_{32}H_{52}O_2$  ( $M^+$  468), m.p.  $203-4^{\circ}$ ,  $(\alpha)_D - 83.72^{\circ}$ ,  $\nu_{\text{max}}^{\text{nujol}}$  1725 and 1245 ( $-OCOCH_3$ )  $\text{cm}^{-1}$ . Its NMR spectrum showed the presence of eight methyl groups between  $\delta$  0.8 to 1.16, a sharp peak at  $\delta$  2.00 (3H,  $-O.CO.CH_3$ ), a multiplet at  $\delta$  5.40 (1H, vinyl proton) and an unresolved multiplet

( 1x )

centered at  $\delta$  5.20 [  $\text{IH}_2 - \text{CH} - \text{CH}(\text{OAc}) - \text{CH}_2 -$  ]

Oxidation of Va with  $\text{CrO}_3$  - Pyridine complex gave a compound Vc,  $\text{C}_{30}\text{H}_{48}\text{O}$  ( $M^+$  424), m.p. 221-23°,  $(\alpha)_D + 16.66^\circ$ ,  $\nu_{\text{max}}^{\text{nujol}}$  1720 (six membered ring Ketone), NMR signals at  $\delta$  5.40 (IH, vinyl proton) and peaks between  $\delta$  0.8 to 1.04 for eight methyl groups. Wolff Kishner reduction of the Ketone under anhydrous condition furnished a hydrocarbon ( $M^+$  410), m.p. 168-69°,  $(\alpha)_D - 16.6^\circ$  and this was found to be identical with an authentic sample of fern-9(11)-ene (m.m.p., IR comparison). This experiment established that the triterpene was a hydroxy derivative of  $\Delta^{9-11}$ -fermene.



Va,  $R_1 = \text{OH}$ ,  $R_2 = \text{H}$ ; Vb,  $R_1 = \text{OAc}$ ,  $R_2 = \text{H}$

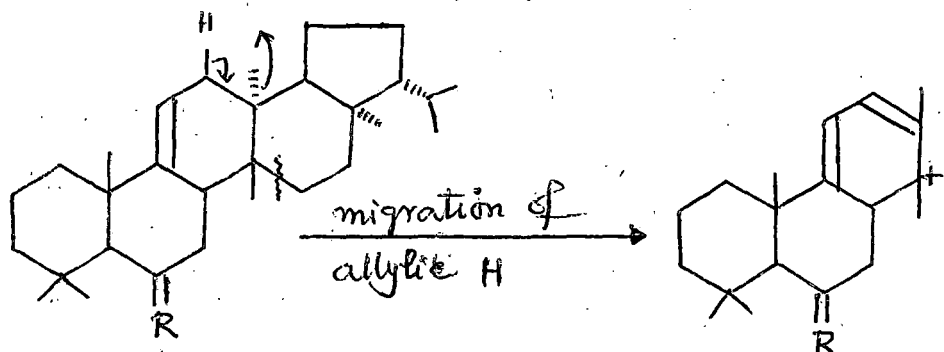
Vc,  $R_1 = \text{O}$ ,  $R_2 = \text{O}$ ; Vd,  $R_1 = R_2 = \text{H}$

( x )

The position of the oxygen function was established from the following observations. The position of the oxygen function may be at C-3, C-6, C-7, C-15 and C-16. Of these possibilities the first one was eliminated since the Ketone Vc did not respond to Zimmerman's colour test for 3-Keto group nor did it react with any carbonyl reagent. <sup>Moreover, it was not identical with 3-keto-farn-9(11)-ene.</sup> The study of the mass <sup>Spectra</sup> ~~spectra~~ of Vb and Vc indicated that the oxygen function was most probably present in ring B either at C-6 or C-7. The compounds Vb and Vc exhibited a mass peak (Chart - A) 257 K for Vc and 241 K for Vb (formed by the loss of acetic acid from the ion K). In addition to species K and its further decomposition products the spectra Vb and Vc showed a very abundant fragment at 271 L for Vc and 255 L for Vb. This type of fragmentation pattern and other evidences discussed earlier can be explained by assuming the presence of hydroxylic function at ring B either at C-6 or at C-7. A careful examination of the circular dichroism curve of Vc established the position of oxygen function at C-6. 300 MHz NMR of Vc was also taken which is also in conformity with the structure Va for the new triterpene.

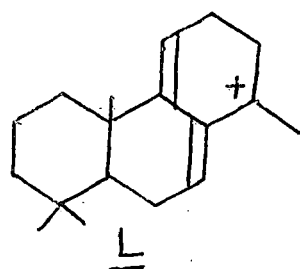
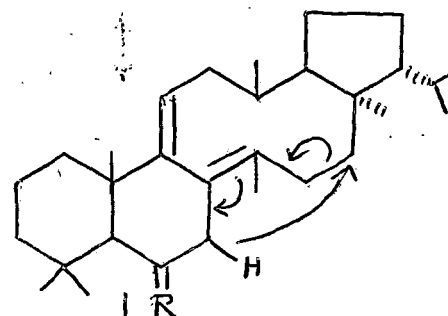
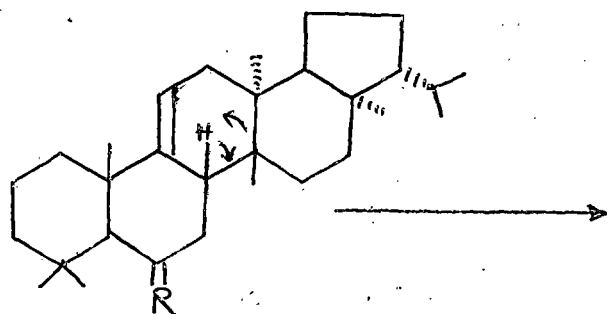
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CHART - A

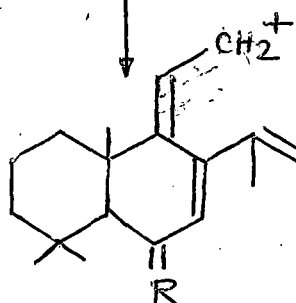


Va, R=OH( $\beta$ ), H  
Vb, R=OAc( $\beta$ ), H  
Vc, R=O

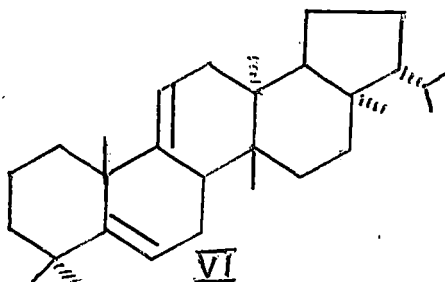
K  
 m/e 271 L (R=O)  
 m/e 255 L (K-AcOH)  
 R=OAc, (H)



m/e 257 K (R=O)  
 m/e 241 K (L-AcOH)  
 R=OAc, (H)



Dehydration of Va with  $\text{POCl}_3$  - Pyridine afforded a single product VI, m.p.  $157-58^\circ$ , no UV



absorption above 220 nm. NMR spectrum showed the presence of two vinyl protons (two multiplets, one centered at  $\delta$  5.28 and the other at  $\delta$  5.44). Compound VI on acid isomerisation afforded an oily compound which did not show any UV absorption above 220 m $\mu$ . The formation of a single product VI during the process of dehydration indicated the  $\beta$ -axial orientation of the hydroxylic function at C-6.  $\text{NaBH}_4$  reduction of the Ketone Vc gave the epimeric alcohol, m.p.  $200-1^\circ$ ; acetate, m.p.  $177-79^\circ$ . Oxidation gave back the original Ketone. This also established that the reduced alcohol has the  $6\alpha$ -equatorial conformation.

Chapter - V deals with investigation on the structure of the new triterpene polypodinol B,  $\text{C}_{30}\text{H}_{50}\text{O}$ .

In addition to Polypodinol A, a new triterpene Polypodinol B,  $\text{C}_{30}\text{H}_{50}\text{O}$ , m.p.  $164-66^\circ$  has been isolated. It gave a monoacetate  $\text{C}_{32}\text{H}_{52}\text{O}_2$  ( $M^+468$ ), m.p.  $207-8^\circ$ ,  $(\alpha)_D^{25}$   $38.00^\circ$ .

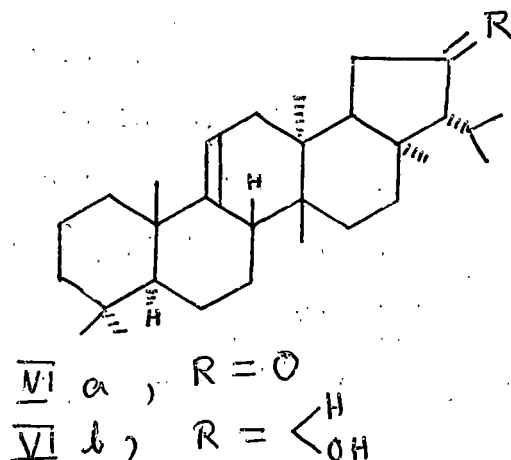
Oxidation of Polypodinol B gave a Ketone,  $C_{30}H_{48}O$  ( $M^+ 424$ ), m.p.  $174-75^\circ$  which on W.K. reduction gave a hydrocarbon, m.p.  $168-69^\circ$  identical with fern-9(11)-ene. Thus Polypodinol B is also a hydroxy derivative of  $\Delta^{9(11)}$ -fermene.

Mass spectrum of the Ketone showed the molecular ion peak at  $M^+ 424$  in addition to peaks at  $m/e 409$  ( $M^+-15$ ),  $m/e 243$  and  $m/e 231$  characteristic of a  $\Delta^{9(11)}$ -fermene system. The mass spectrum of the monoacetate was more informative and exhibited a mass fragmentation pattern having the following peaks :  $M^+ 468$ ,  $m/e 453$  ( $M^+-15$ ),  $m/e 393$  ( $M-15-HOAc$ ) in addition to peaks at  $m/e 231$  and  $m/e 243$ . The fragmentation pattern revealed that it was similar to that of  $\Delta^{9(11)}$ -fermene system. The presence of <sup>the</sup>base peak at  $m/e 243$  in both the Ketone and the acetate indicated that there is no substitution in rings A and B.

The other possible alternative positions for the (OH) group are at C-15, C-16 in ring D or some other position in ring E. CD curve of the Ketone showed a negative Cotton effect. Inspection of Drieding models of C-15 Ketone and C-16 Ketone suggested that both would show a positive Cotton effect. Since a negative Cotton effect is actually observed for the Ketone, positions C-15 and C-16 are ruled out. Therefore, we believe that the carbonyl group is most probably situated at some

( xiv )

position in ring E. From an examination of the 300 MHz NMR spectrum of the Ketone we have proposed a tentative structure Via for the Ketone and VIb for the triterpene Polypodinol B.



The stereochemistry of the -OH group has not been settled. CD curve and 300 MHz NMR spectrum of the Ketone has been discussed. Further work is in progress to arrive at a firm conclusion about the position of the (OH) group.

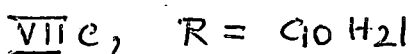
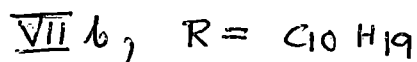
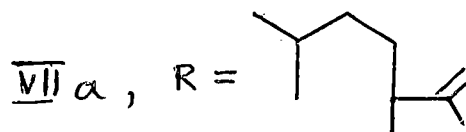
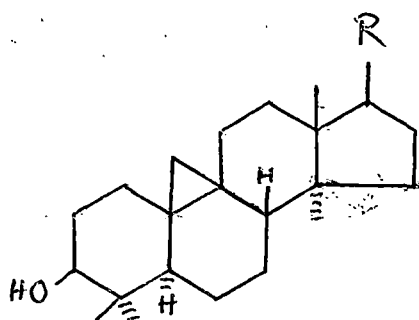
[A preliminary account of this work was presented at the "Science Congress Association" held in Nagpur, India (1974)]

Chapter - VI describes the detection and structure determination of a new triterpene - a cyclolaudenol homologue  $C_{32}H_{54}O$  by mass spectrometry.

In Chapter - III isolation and identification of cyclolaudenol has been mentioned. It has been shown that the

cyclolaudenol fraction was a mixture of two compounds (TLC). But by careful fractional crystallisation of their benzoates, one of the components was isolated as cyclolaudenol benzoate.

The original mixture (A) on acetylation gave an acetate (B), m.p.  $107-8^{\circ}$ ,  $(\alpha)_D$   $55.17^{\circ}$ . Hydrogenation of the mixture gave a compound (C), m.p.  $132-33^{\circ}$ , which on acetylation gave an acetate (D), m.p.  $127-28^{\circ}$ . Oxidation of the original mixture gave a mixture of Ketones (E), m.p.  $107-8^{\circ}$ . Mass spectra of the original mixture (A), its acetate (B), the dihydro-compound (C) and its acetate (D) were measured and it was observed that along with cyclolaudenol ( $M^{+} 440$ ) a second component having  $M^{+} 454$ , that is, a new triterpene alcohol was present. The mass spectrum of this new compound has been found to correspond very closely to <sup>that of</sup> Cyclolaudenol VIIa and are in agreement with earlier findings (P. Benveniste, L. Hirth and G. Ourisson, *Phytochem.*, 5, 31, 1966; H. E. Audier, R. Bengelmans and B. C. Das, *Tetrahedron Letters*, 4341, 1966; R. T. Aplin and G. M. Haraby, *J. Chem. Soc. (B)*, 1077, 1966)



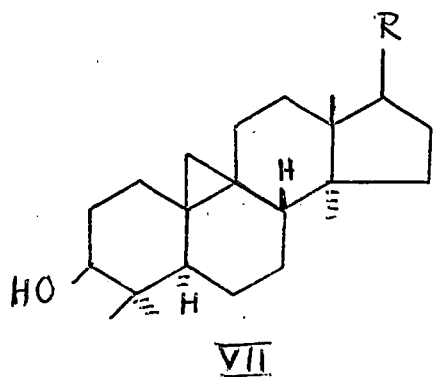


The unusual feature of the spectrum (Chart B) of Cyclolaudenol VIIa is the intense fragment (a) at  $m/e$  300 and have the composition  $C_{22}H_{36}$  and corresponds to the loss of ring A plus one hydrogen atom since it remains at  $m/e$  300 in the spectra of the corresponding Ketone. This is formed from the molecular ion  $m/e$  440 by a one step process. The other fragments, besides the loss of a methyl group ( $m/e$  425), water ( $m/e$  422) and methyl-plus-water ( $m/e$  407) arise from M-18 fragment and involve cleavage of the side chain - (b)  $m/e$  379 ( $M-18-C_3H_7$ ), (c)  $m/e$  353 ( $M-18-C_5H_9$ ). A further fragment (e)  $m/e$  175 common to all spectra, corresponds to the loss of both ring A and the side chain. Another significant peak (d)  $m/e$  315 ( $M-R$ ) is common to Cyclolaudenol VIIa, its homologue VIIb and its dihydroderivative VIIc which arises by elimination of R group from M. The most plausible mechanism (McLafferty, Chem. Commun., 78, 1966) for the formation of these fragments is depicted (path A) where initial ionisation of 9-10 bond relieves the strain imposed on ring B, fission of the activated 5-6 bond followed by transfer of one of the activated C-11 hydrogen via a "Mc Lafferty" type of rearrangement.

The mass spectra of the new C-32 triterpene VIIb ( $M^{+454}$ ) and its dihydrocompound VIIc has been found to contain all the peaks (Chart B) characteristic of Cyclolaudenol but in this case

( xvli )

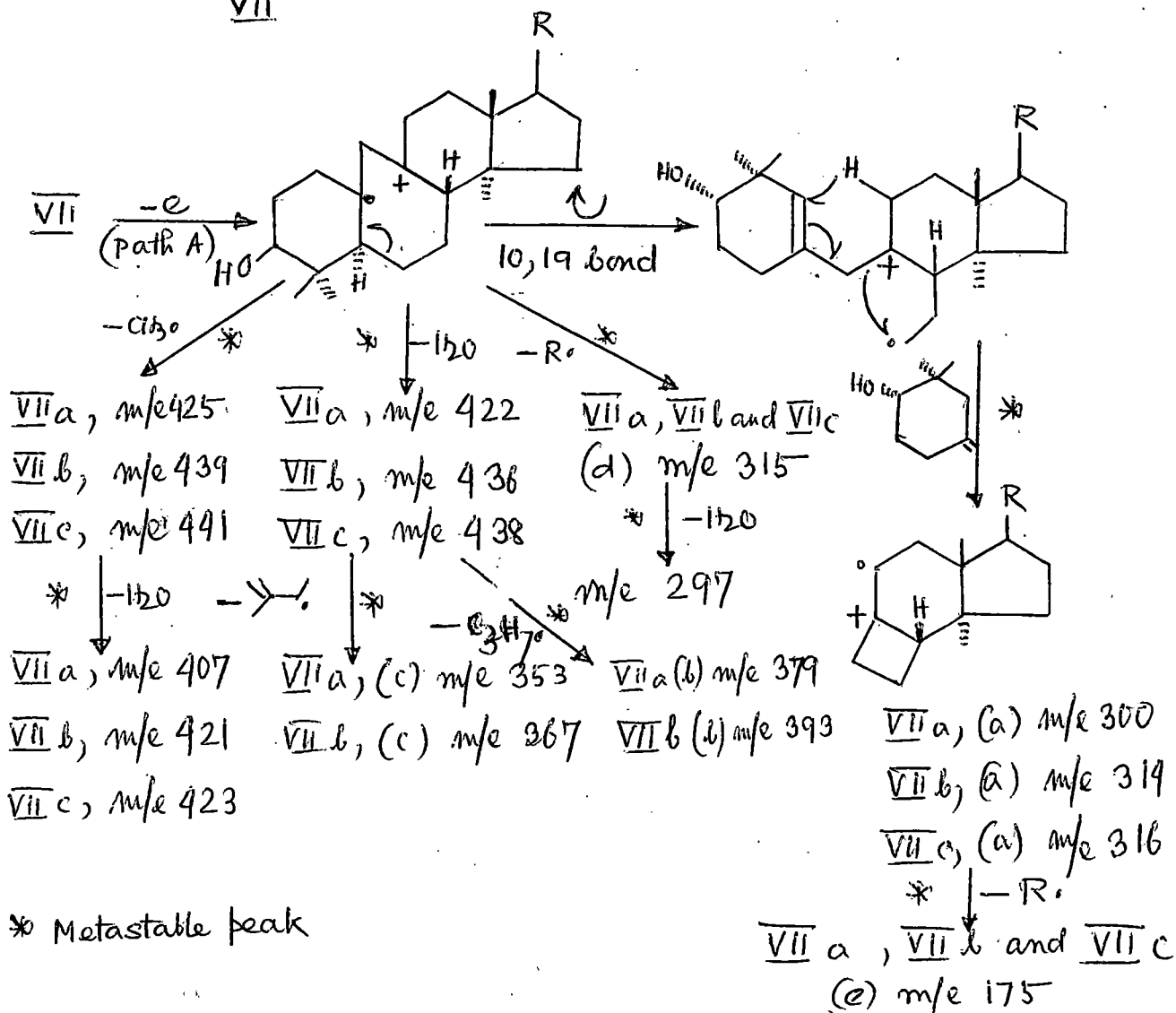
CHART - B



VII a, R = (M<sup>+</sup> 440)

VII b, R = C<sub>10</sub>H<sub>19</sub> (M<sup>+</sup> 454)

VII c, R = C<sub>10</sub>H<sub>21</sub> (M<sup>+</sup> 456)



\* Metastable peak

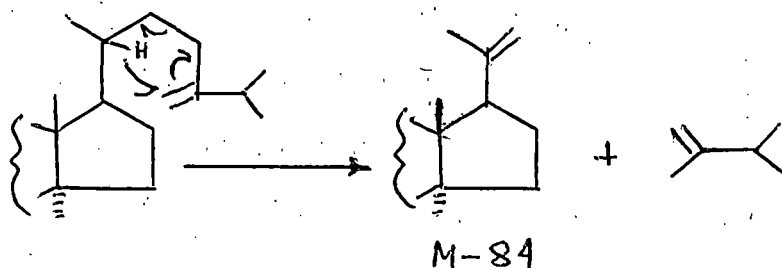
( xviii )

the peak found by loss of side chain  $M^+ - R$  for compounds VIib and VIic was at 315 indicating the presence of an extra carbon atom in the side chain. The intense fragment at  $m/e$  314 for VIib have the composition  $C_{23}H_{58}$  and corresponds to the loss of ring A and one hydrogen atom since it remains at 314 in the mass spectra of the corresponding acetate and is moved to  $m/e$  316 in the spectrum of the corresponding dihydrocompound VIic. A further fragment gave a sharp peak at  $m/e$  175 due to loss of ring A and side chain. This also corroborates the presence of the extra carbon atom in the side chain. In addition to these peaks, the peak at  $m/e$  315 ( $M^+ - R$ ) for VIib and VIic and at  $m/e$  357 for the acetates of VIib and VIic again established the presence of the extra carbon atom in the side chain (R) of the cyclolaudenol nucleus. The other major fragments, besides the loss of methyl radical ( $m/e$  439), water ( $m/e$  436) and methyl-plus-water ( $m/e$  421) are from M-18 fragment and involve cleavage of the side chain. These peaks are exhibited at  $m/e$  393 ( $M-18-C_3H_7\cdot$ ) and  $m/e$  367 ( $M-18-C_5H_9$ ). The appearance of these mass peaks has been explained.

The position of the double bond ( $=CH_2$ , NMR) in the side chain at 25 (26) position may be tentatively assigned from the mass spectrum of VIib and its acetate as the M-84 fragment observed in the "McLafferty" rearrangement involving 24-28

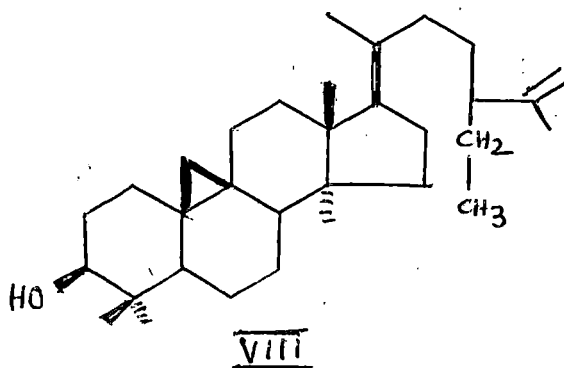
( xix )

double bond and the C-20 hydrogen has been found to be absent in the spectra of VIb and its acetate (J. Bergman, B.O. Lindgren and C.M. Svahn, Acta. Chem. Scand., 19, 1661, 1965)



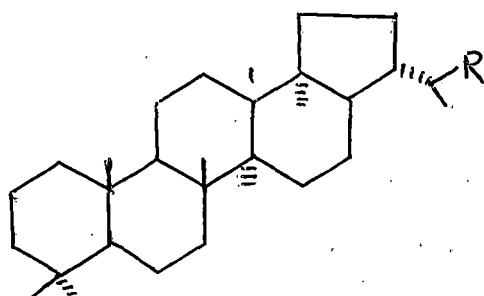
All these facts has been discussed in detail in Chapter - VI and established beyond doubt that the C-32 compound contains a cycloaudenol nucleus with the extra carbon atom in the side chain.

On biogenetic grounds the new triterpene has been tentatively assigned structure VIII. A mechanism for the biosynthesis of this new triterpene in the fern *Polypodium juglandifolium* has been proposed.



[A preliminary account of this work was presented at the "Convention of chemists, CSIR, India, 1974 held at Madurai, (REPRINT NO. 3)]

Chapter - VII : Mitra et al ( G.N. Pandey and C.R. Mitra, Tetrahedron Letters, 1353, 1967) converted nerifoliol IXa to the corresponding aldehyde by Sarret oxidation and recorded its m.p. as  $76^{\circ}$ . They reduced this aldehyde by Wolff - Kishner reduction and obtained a hydrocarbon m.p.  $190-92^{\circ}$ ,  $(\alpha)_D + 36^{\circ}$  which they reported was same as hopane. We repeated their experiment with hydrated  $\text{CrO}_3$ -Pyridine complex and the product obtained after chromatography was found to be identical with isoadiantone. The mother liquor after crystallisation showed the presence of both adiantone and isoadiantone (TLC). However, Sarret oxidation with anhydrous  $\text{CrO}_3$ -Pyridine complex according to the procedure of Ratchiffe and Rodehorst (Ronald Ratcliffe and Ronald Rodehorst, J. Org. Chem., <sup>1970</sup> 35, 4000) afforded a single compound, m.p.  $205-6^{\circ}$ ,  $\nu_{\text{max}}^{\text{nujol}}$  2720, 1730  $\text{cm}^{-1}$ ;  $M^+ 426$ . NMR spectrum of the compound showed a peak at  $\delta$  9.65 (1H) characteristic of one <sup>aldehydic</sup> proton. Wolff-Kishner reduction of the aldehyde IXb gave hopane, m.p.  $218-19^{\circ}$  identical with an authentic sample (IR).



IX a, R = CH<sub>2</sub>OH

IX b, R = CHO

IX c, R = CH<sub>2</sub>OTs

IX d, R = CH<sub>2</sub>Cl

The above observations are quite at variance with those reported by Mitra et al and suggest that the oxidation proceeds by different mechanisms depending on the reaction conditions. This reaction <sup>is</sup> being studied in detail to arrive at the correct mechanism of the reactions.

We had also planned to prepare nerefoliol tosylate IXc by the usual procedure with highly purified p-toluene sulfonyl chloride and pyridine at room temperature and then convert the tosylate to hopane by LAH reduction. But during this preparation we failed to isolate the desired tosylate but instead obtained a compound, m.p. 221-22<sup>o</sup>, which gave a positive test for halogen. Structure IXd has been assigned to it from elemental analysis, IR, NMR and mass spectral studies. In this case we find that the normal reaction is retarded and instead a different reaction takes place giving a product by substitution. A probable

mechanism for the formation of IXd has been suggested.

D. PART - IV :

Part - IV describes chemical investigation on some Indian plants.

[REPRINT NO.1 and REPRINT NO.2]

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PART - I

PARTIAL SYNTHESIS OF DIMETHYL DIHYDRO-  
CEANOTHATE STARTING FROM BETULINIC ACID

PART - 1

CHAPTER - 1

A short review on the isolation, structure and stereochemistry of ceanothic acid :

Section A : Isolation :

Ceanothic acid was originally isolated by Julian, Piki and Dawson<sup>1</sup> from the alcohol extract of the root bark of Ceanothus americanus and has since been isolated from a number of Australian Rhamnaceae species<sup>2,3</sup>. This substance was characterized by Julian et al<sup>1</sup> as a hydroxy dicarboxylic acid and attributed the formula  $C_{29}H_{44}O_5$ . It was characterized by the preparation of a dimethyl ester and of a dimethyl ester acetate. In 1958 Boyer et al<sup>2</sup> isolated emmolic acid from Emmenospermum alphitonioides F. Muell (Rhamnaceae). This acid was later found to be identical with ceanothic acid. by Birch and coworkers<sup>4</sup>. de Mayo and Starratt<sup>5</sup> in 1962 also isolated ceanothic acid from Ceanothus americanus but their method of isolation was not same as that used by Julian, Piki and Dawson<sup>1</sup>. They could not successfully isolate the acid by adopting the procedure used by Julian et al<sup>1</sup>. de Mayo and Starratt<sup>5</sup> remarked on the variability of the plant and subsequent workers<sup>6</sup> have also made similar observations. By adopting different procedures, de Mayo et al<sup>5</sup> were able to isolate different proportions of the various

acid constituents. The method leading to isolation of ceanothic acid is described here. The ground root bark of Ceanothus americanus was extracted continuously with ether in a Soxhlet apparatus for 33 hours. The residue was extracted with light petroleum under reflux for 3 hours, the light petroleum then being replaced, and the process repeated four times. The residue in ethereal solution, was extracted exhaustively with 2% potassium hydroxide solution. A solid, which separated at the interface, 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 acid mixture was added, in benzene solution, to a column of silica. Elution with benzene-ether (20:1) gave an acid, m.p. 350-54° (decomp.),  $(\alpha)_D^{25}$  39° (C, 1.23 ; pyridine). Further elution with the same solvent mixture then gave betulinic acid, m.p. 270-85°. Further elution with benzene-ether (10:1) then gave material which after two crystallisations from ether-methanol gave pure ceanothic acid, m.p. 356-57° ( gas evolution ) ( lit.<sup>1</sup> 354° ),  $\nu_{\text{max}}$  <sup>nujol</sup> 3480, 1720, 1641 and 983 cm<sup>-1</sup>.

Section B : Structure of Ceanothic acid :

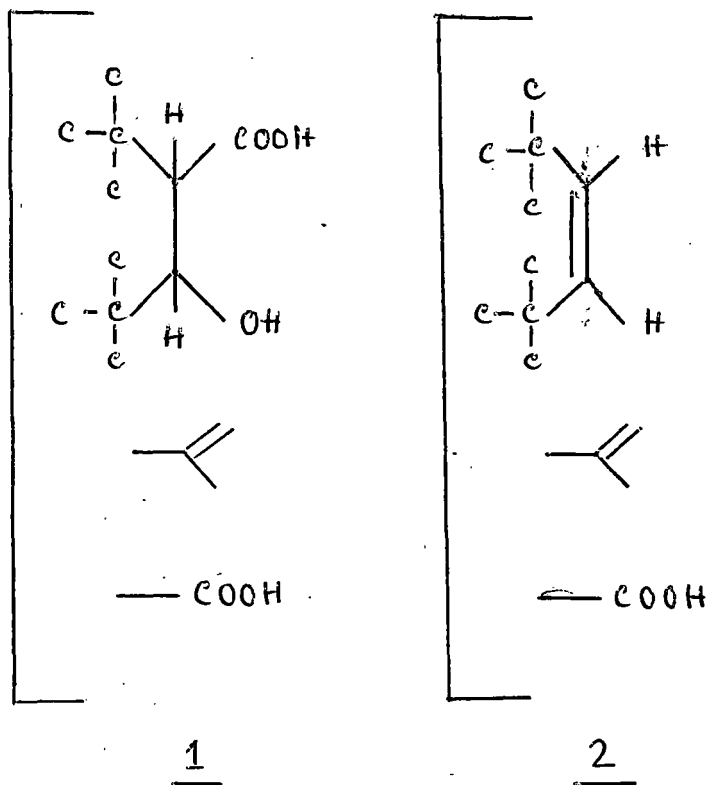
The systematic study for the determination of the structure of ceanothic acid was mostly due to de Mayo and Starratt<sup>5</sup>.

Their work is summarised below.

The infrared spectra of ceanothic acid and of its dimethyl ester showed bands  $\gamma_{\text{max}}^{\text{nujol}}$  833 and  $\gamma_{\text{max}}^{\text{nujol}}$  889  $\text{cm}^{-1}$  respectively. These bands indicated the presence of an exo methylene group. This interpretation was supported by the disappearance of this band on catalytic hydrogenation both of the acid and of the ester to their respective saturated dihydro compounds. The NMR spectrum of the ester showed a doublet at  $\tau$  5.37 ( $J \sim 7.7$  cps) (2 hydrogens) attributed to the methylene group and a singlet (3 hydrogens) at  $\tau$  8.36 attributed to vinylic methyl group<sup>7</sup>. These observations, in the absence of any other double bond, indicated the presence of an isopropenyl group. The NMR spectrum, further, showed singlets (1 hydrogen) at  $\tau$  5.98 and  $\tau$  7.51, indicating that both the hydroxyl group and one of the carboxyl groups were attached to carbon atoms bearing one hydrogen atom. The above bands are in appropriate position for the respective methine hydrogens<sup>7</sup>. Evidence for the proximity of the hydroxyl group and the secondary carboxyl group was obtained by an examination of the "lactone" obtained by Julian, Piki and Dawson<sup>1</sup>. This substance was shown to be an unsaturated acid by its conversion, with diazomethane, to the corresponding ester. In addition to the bands at  $\tau$  5.40 and  $\tau$  8.46 in the NMR spectrum, indicative of the continuing presence of the isopropenyl group, two hydrogens producing an AB pattern (doublets at  $\tau$  4.16 and  $\tau$  4.66,



$J_{AB} \sim 5.4$  cps) were present. The formation of this pattern was due to the conversion of 1 to 2, that is, the dehydration-decarboxylation of a  $\beta$ -hydroxy acid.

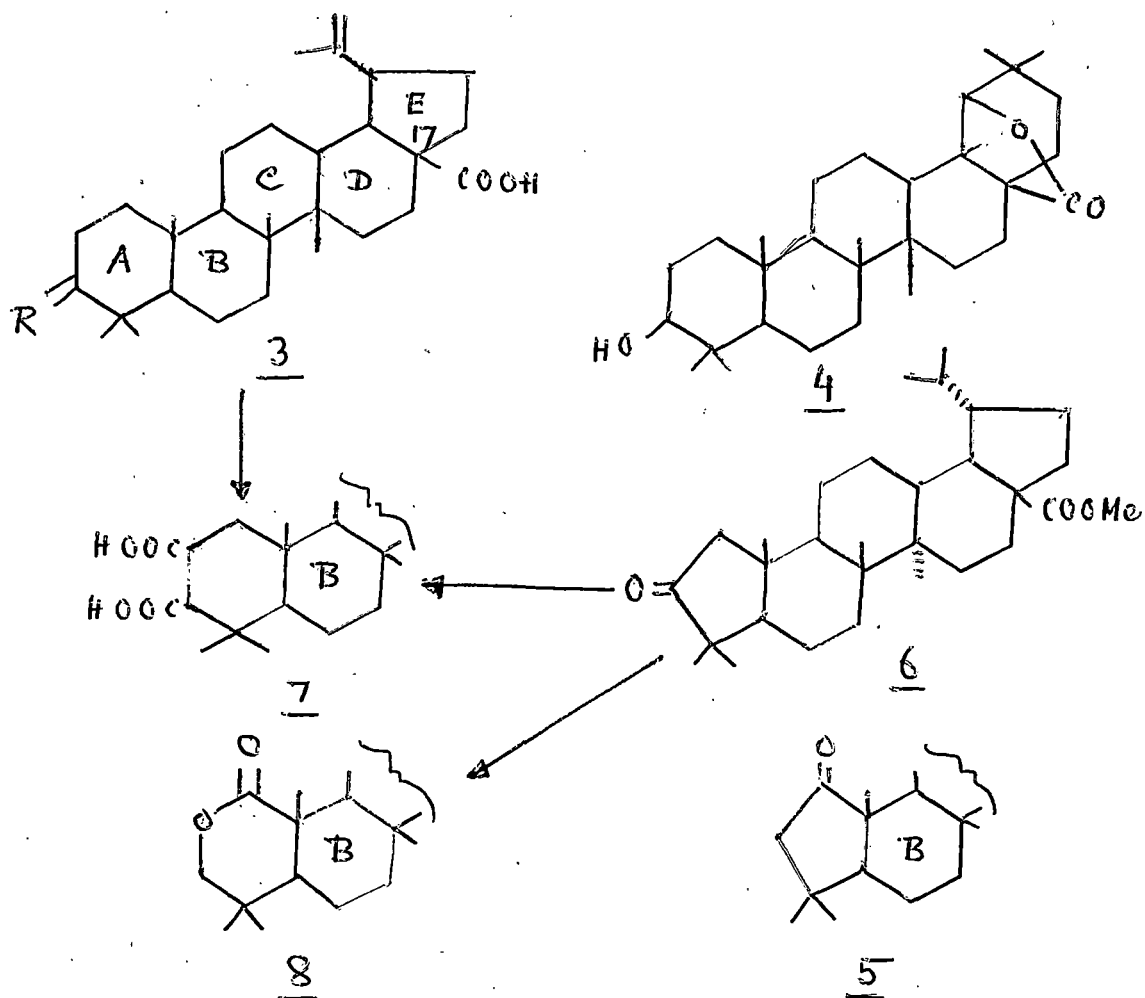


de Mayo et al confirmed this on the basis of the following experiments. Dimethyl ceanothate was converted into the benzoate which was isolated as an oil and the latter was pyrolysed at  $330^\circ$  with the elimination of benzoic acid. The isolated anhydro-acid showed the expected ultraviolet end absorption for an isolated isopropenyl group and an  $\alpha\beta$ -unsaturated ester. Furthermore, a band (singlet, 1 hydrogen) at  $\tau$  3.9 in the NMR spectrum

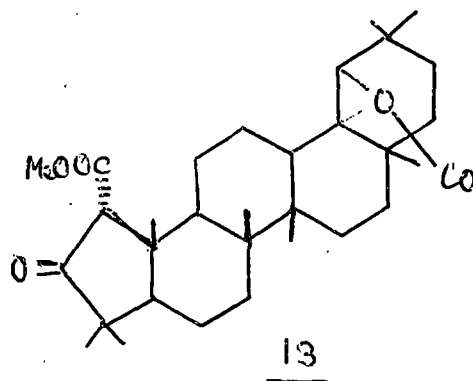
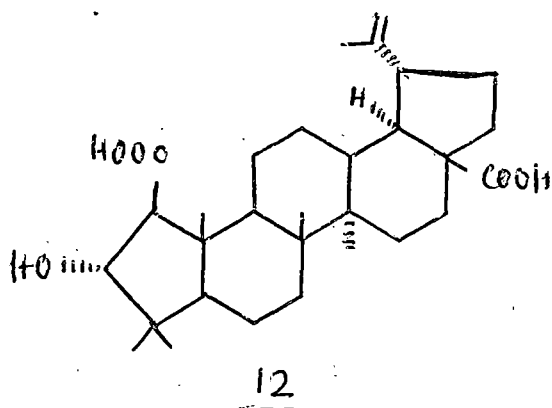
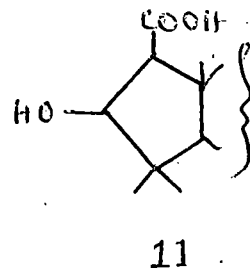
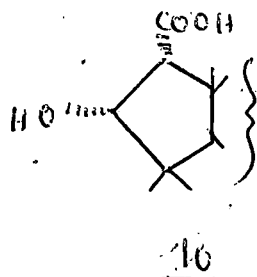
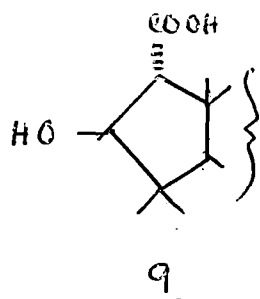
indicated the presence of the expected vinyl hydrogen in addition to those of the isopropenyl group<sup>7</sup>. Since dihydro-ceanothic acid and its dimethyl ester showed no absorption in the ultraviolet spectrum in the 200-210  $m\mu$  region, they were, presumably saturated. It followed that ceanothic acid was, therefore, pentacarbocyclic. They assumed that ceanothic acid was probably related to the lupeol-betulin-betulinic acid (3, R=H,OH) in view of the presence of the isopropenyl group and this view was strengthened by the presence of betulinic acid itself amongst the root constituents. One of the characteristic transformations of lupeol-betulin group of substances is the ready acid catalysed expansion of the terminal E-ring to give derivatives of the  $\beta$ -amyrin series<sup>8</sup>. In those substances having a carboxyl function at C<sub>17</sub> concomitant lactonisation occurs : betulinic acid (3, R = H,OH) for instance, is converted into 4. When subjected to suitable conditions - refluxing formic acid for 3 hours - ceanothic acid was converted into a  $\gamma$ -lactone,  $\nu_{\max}$  1696 (carboxyl) and 1762  $\text{cm}^{-1}$  ( $\gamma$ -lactone) with the simultaneous disappearance of the isopropenyl group. The resulting monocarboxylic acid lactone was further characterized as the acetate, indicating the non-participation of the hydroxyl group in the lactonisation process, and as the methyl ester. In order to accommodate the functional groups indicated in 1, they 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 gibberelllic acid<sup>9</sup> and of the aldehyde in magnamycin<sup>10</sup>. Oxidation of methyl dihydroceanothate with sodium dichromate gave the corresponding ketone. Alkaline hydrolysis afforded a ketonic monoester with the loss of carbon dioxide expected of a  $\beta$ -keto ester. The substance showed an unresolved band in the infrared spectrum,  $\nu_{\text{max}}$   $1738 \text{ cm}^{-1}$  for the cyclopentanone and ester, while its precursor showed bands at  $\nu_{\text{max}}^{\text{CCl}_4}$   $1750$  (cyclopentanone) and  $\nu_{\text{max}}^{\text{CCl}_4}$   $1727 \text{ cm}^{-1}$  (ester) (~~1727~~).

From the above experiments de Mayo et al proposed two structures 5 and 6 for the ketone, but its properties suggested that it was 6, a substance previously prepared by Ruzicka et al<sup>11,12</sup> from betulonic acid by hydrogenation, nitric acid oxidation to 7, followed by pyrolysis and esterification.



Direct preparation of this substance from methyl dihydrobetulonate and comparison of it, the derived 2:4 - dinitrophenyl hydrazone, and the anhydride 8 obtained by selenium dioxide oxidation showed them to be identical in every respect. Therefore, they proposed four possible structures 9 , 10 , 11 and 12 for ceanothic acid.



They noted that, in dimethyl ceanothate, the methine hydrogens adjacent to the carbomethoxyl and hydroxyl groups were singlets, that is, although the hydrogens were on contiguous carbon atoms, the coupling constant was close to zero. In contrast, the ketone 13, derived from methyl ceanothate lactone, on reduction with sodium borohydride gave an epimeric lactone, called as methyl isoceanothate lactone. This substance showed, in its NMR spectrum, a doublet at  $\tau$  7.0, the methine hydrogens being coupled, and a quartet at  $\tau$  5.9<sup>7</sup>, the hydrogen on the carbon

bearing oxygen being split by the adjacent methine and by the hydroxyl hydrogen. This suggested that in dimethyl ceanothate the hydrogen atoms were at an angle of about  $90^\circ$  <sup>13,14</sup> and, therefore, in the trans relationship.

Further evidence supporting this view was obtained from a study of infrared spectra. Treatment of methyl dehydroceanothate lactone 13, 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. The results recorded by de Mayo and Starratt are given below (Table - 1).

Table-I

| Compound                     | $\nu$ ester ( $\text{cm}^{-1}$ ) * | $\nu$ hydroxyl ( $\text{cm}^{-1}$ ) * | Concentration (%) <sup>†</sup>       |
|------------------------------|------------------------------------|---------------------------------------|--------------------------------------|
| Dimethyl dihydroceanothate   | 1733                               | 3634 (sharp)                          | 2.5                                  |
|                              |                                    | ~ 3510 (broad)                        |                                      |
|                              |                                    | 3634 (sharp)                          | 1.2                                  |
|                              |                                    | 3640 (sharp)                          | 0.2                                  |
| Methyl isoceanothate lactone | 1718                               | 3582 (sharp)                          | 0.7                                  |
|                              |                                    | 3580 (sharp)                          | 0.1                                  |
| Methyl epiceanothate lactone | 1717                               | 3682 (sharp),                         | 1.9 ( $\text{CHCl}_3$ ) <sup>‡</sup> |
|                              |                                    | 3508 (broad)                          |                                      |
|                              |                                    | 3634 (sharp),                         | 0.3                                  |
|                              |                                    | 3508 (broad)                          |                                      |

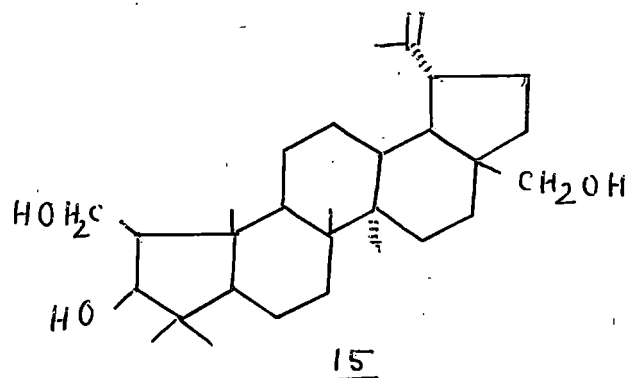
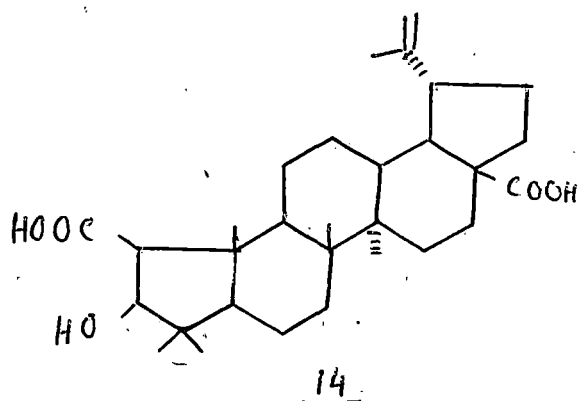
\* Spectra taken in  $\text{CCl}_4$  (Fisher spectranalyzed) unless specified otherwise

<sup>†</sup> Maximum concentration limited by compound solubility.

<sup>‡</sup> Chloroform washed and dried to remove alcohol.

Methyl dihydroceanothate showed a normal unbonded ester and, in agreement, the hydroxyl group showed intermolecular bonding only in the most concentrated solution. In contrast, the epimeric methyl isoceanothate lactone showed a bonded ester group and a bonded hydroxyl band even in dilute solution. These results supported the trans and cis configurations, respectively, and they assumed that methyl epiceanothate was, also, cis.

Therefore, they proposed structures 9 and 12 for ceanothic acid. Inspection of models indicated that the  $\beta$ -carboxy group was under more severe non-bonded interaction than the  $\alpha$ -epimer. In view of the ready epimerisation of methyl dehydro ceanothate lactone, they preferred the stereostructure 12 for ceanothic acid. In 1961, just prior to the publication of this paper, Mechoulam<sup>15</sup> published a paper in which he assigned the  $\beta$ -configuration to the carboxyl group in ring A and a cis relationship to this carboxyl and the adjacent hydroxyl group 14. The reasons which led<sup>him</sup> to propose this stereochemical assignment were based on the following grounds.





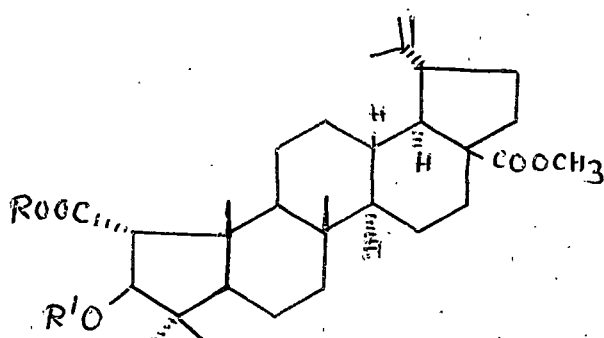
The hindrance of the secondary as well as tertiary - COOCH<sub>3</sub> group was shown by the fact that dimethyl ceanothate did not undergo basic hydrolysis with 10% potassium hydroxide solution. Examination of the model showed that a 1 $\beta$  - carboxyl group was considerably hindered but little hindrance was expected from 1 $\alpha$  - configuration. This study led them to propose  $\beta$  - carboxyl configuration for the natural product. Again lithium aluminium hydride reduction of dimethyl 3-oxo-ceanothate gave a triol<sup>2</sup> 15, m.p. 226-28°, ( $\alpha$ )<sub>D</sub> 46° in EtOH, which was shown to be identical with a triol obtained by lithium aluminium hydride reduction of dimethyl ceanothate. They argued that, since for steric reasons, the hindered 3-oxo group in dimethyl - 3-oxo-ceanothate would be expected to give the 3 $\beta$  - ol by this type of reduction the natural product was in  $\beta$ -OH grouping. They further reported that this assignment was supported by a broad hydrogen - bonded hydroxyl IR absorption in the neighbourhood of 3500 cm<sup>-1</sup> of a very dilute solution of triol 15. Later Eade, Kornis and Simes<sup>16,17</sup> and Mechoulam<sup>18</sup> himself negated the idea of  $\beta$  - carboxyl group and cis relationship of the carboxyl and hydroxyl group with the supporting evidence in favour of the structure proposed by de Mayo and Starratt<sup>5</sup>.

Eade et al<sup>16</sup> found that with lithium fluoride optics, a 0.05 M solution ( CCl<sub>4</sub>, 1 mm cell ) of dimethyl ceanothate

showed strong absorption at  $3629\text{ cm}^{-1}$  due to free hydroxyl group and medium absorption at  $3513\text{ cm}^{-1}$  due to bonded hydroxyl group whereas a 0.01 M solution (  $\text{CCl}_4$ , 5 mm cell ) had the same strong absorption at  $3629\text{ cm}^{-1}$  but showed no absorption at  $3513\text{ cm}^{-1}$ . They concluded that these results are consistent with the presence of intermolecular hydrogen bonding and absence of intramolecular hydrogen bonding. A study of molecular models indicated that intramolecular hydrogen bonding would be expected to occur where the methoxycarbonyl and hydroxyl group were cis<sup>19</sup>.

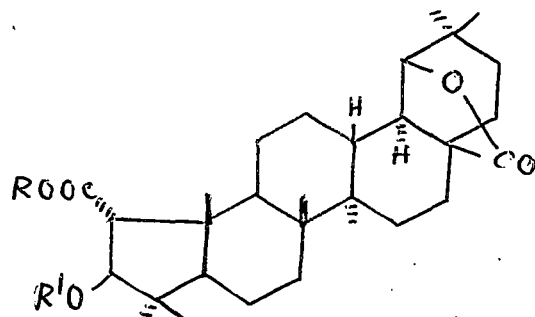
They also reported that lithium aluminium hydride reduction of dimethyl dihydroceanothate and the corresponding ketone dimethyl -3-oxo- dihydroceanothate gave different triols ( The dimethyl -3-oxo-dihydro ceanothate gave a mixture, but only one product was isolated). This result was in contrast to that obtained by Mechoulam<sup>15</sup> who isolated the same triol on lithium aluminium hydride reduction of dimethyl ceanothate and the corresponding ketone dimethyl - 3-oxo- ceanothate. Eade, in his paper<sup>16</sup>, refuted this conclusion drawn from this reduction on the basis of two possible factors ; (i) the reducing agent could chelate with 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<sup>2</sup> reported

that dimethyl ceanothate was stable to boiling 10% ethanolic potassium ethoxide for 2-hours. But Eade *et al*<sup>16</sup> found that with 20% methanolic potassium hydroxide and heating under reflux for 48 hours dimethyl ceanothate could be converted into a mixture containing very little or no starting material. The major product isolated in 50% yield was a monomethyl ester 16A. Methylation of 16A gave dimethyl ester 16B which was isomeric with dimethyl ceanothate. The corresponding acid isomeric with ceanothic acid was named isoceanothic acid. Lactonisation of compound 16A ( $\text{H}_2\text{SO}_4$  /  $\text{AcOH}$  / Benzene) gave the lactone 17B indicating that it was the methoxycarbonyl group which had been hydrolysed by alkali. Both lactone 18A (normal series; prepared by lactonisation of dimethyl ceanothate) and lactone 17A (iso-series) was converted into the corresponding oxo-esters having different physical constants; lactone 18B (normal series; prepared by lactonisation of ceanothic acid) and 17A (iso-series) yielded the same nor-ketone 19



16A ,  $R=R'=H$

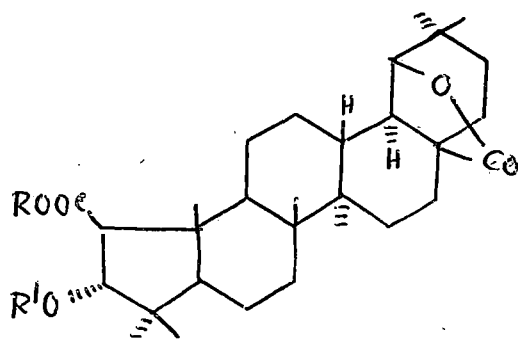
16B ,  $R=Me, R'=H$



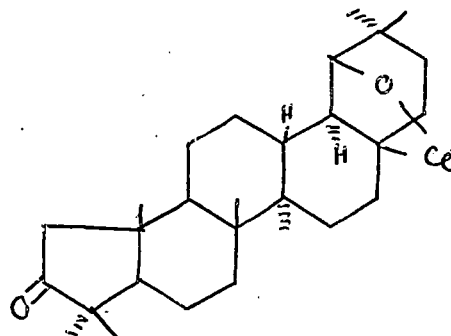
17A ,  $R=R'=H$

17B ,  $R=H, R'=CO_2Me$

17C ,  $R=Me, R'=COMe$



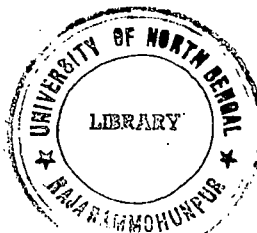
18A , R = Me, R<sup>1</sup> = CO Me  
18B , R = H , R<sup>1</sup> = CO Me



19

Thus the methoxycarbonyl group in ceanothic acid and that in isoceanothic acid were epimeric. These authors reported, from the examination of models, that if the methoxycarbonyl group in ring A had the  $\beta$  - configuration it would be under considerable non-bonded interaction. Such steric strain would be virtually absent if it possessed the  $\alpha$  - configuration. Thus, they stated that the carboxyl group in ring A of ceanothic acid, probably possessed the more strained  $\beta$  - configuration leading to the  $\alpha$  - assignment for the hydroxyl group. The ring A constituents of isoceanothic acid had been assigned a trans relationship because the infrared spectra of the

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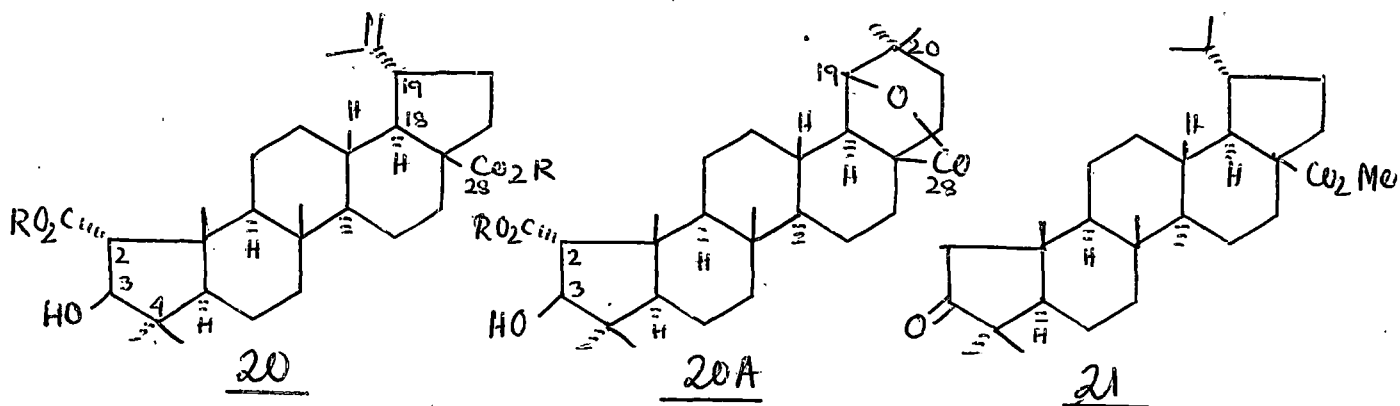


dimethyl ester 16B showed evidence of intermolecular hydrogen bonding. Consequently, formation of isoceanothic acid required opening of ring A in a reverse aldol type reaction to an A - seco derivative with subsequent reclosure.

Crowley<sup>20</sup>, in 1962, reported the natural occurrence of a 2,3 - seco triterpene and this supported the suggested biogenesis<sup>3</sup> of ceanothic acid by ring closure of a similar 2,3 - seco derivative derived from 2 - hydroxy betulinic acid; the latter has recently been isolated along with ceanothic acid from Alphitonia whitei Braid<sup>3</sup>. Eade et al<sup>16</sup> found that the  $\beta$  - keto acid derived from the iso series either from 16 or 17 was quite stable. Oxidation of 16A or 17A gave the corresponding  $\beta$  - keto acid which could be dried in vacuo at 100° without decomposition. Conversion of the  $\beta$  - keto acid derived from 17A into the corresponding nor-ketone 19 was effected by heating in vacuo at 220°C. This stability, according to them, was due to difficulty in finding a cyclic planar transition state. In this connection, they cited the example of camphor-3-carboxylic acid which, according to them, is stereochemically similar and a stable  $\beta$  - keto acid. They could not isolate the  $\beta$  - keto acid derived from the normal series, that is, carboxyl with  $\beta$  - configuration which, they explained, was due to its ready decarboxylation.

Section C : Stereochemistry of Ceanothic Acid :

Final convincing proof for the stereochemistry of ceanothic acid was published in 1971 by Eade et al<sup>21</sup>. These authors<sup>17</sup>, in 1964, observed that the ester group at C - 2 in dimethyl ceanothate 20 ( R = Me ) could be epimerised only with great difficulty, whereas in the corresponding ester, dimethyl ceanothate lactone 20A ( R = Me ) with a modified ring E area, it could be comparatively easily epimerised and was accompanied by simultaneous hydrolysis. They suggested<sup>17</sup> that this considerable difference in reactivity might be caused by a long-range conformational transmission effect. In 1971, Eade et al published a paper<sup>21</sup> in which they stated that a comparison of the coupling constants of the C - 2 and C - 3 protons of the four possible C 2 - CO<sub>2</sub>Me / C 3 -OH isomers and their acetates in the dimethyl ceanothate series ( carbon skeleton type 20 ) with those of the corresponding compounds in the lactone series ( carbon skeleton type 20A ) would reveal any significant differences in the shape of ring A between the relevant compounds.



They also expected that explanation might be found of the signal reported<sup>5</sup> for the C - 3 proton in the NMR spectrum of dimethyl ceanothate, viz. a singlet, which was not in agreement with the structure then assigned to ceanothic acid even if ring A existed in the  $\alpha$  - envelope conformation<sup>22, 23</sup>.

They further expected that the preparation of the complete group of four isomers in each series would resolve the controversy whether the "methyl epiceanothate" of de Mayo and Starratt has a cis or trans relationship of the ring A substituents<sup>5, 17</sup>.

Eade et al<sup>24</sup> from some chemical and physical studies, described below, finally proposed<sup>24</sup> structure 20 ( R = H ) for ceanothic acid in which the ring A substituents, while still having a trans relationship, possess the configurations opposite to those originally put forward by de Mayo and Starratt<sup>5</sup>.

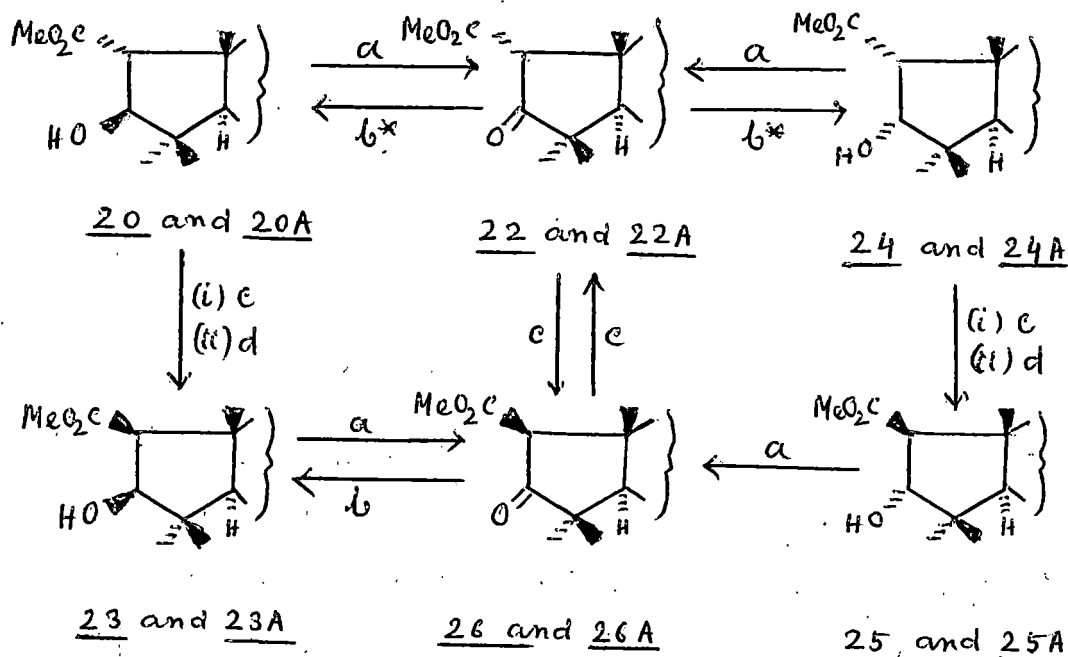
#### Preparation of the Dimethyl Ceanothate Series :

Of the four possible isomers in the dimethyl ceanothate series, two have been previously prepared. One is dimethyl ceanothate itself 20 ( R = Me ) and the other is its C - 2 epimer 23 that is , methyl 3/ $\beta$  - hydroxy - 2/ $\beta$  -methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate (Scheme 1) reported by Eade, Kornis and Simes<sup>16</sup> who incorrectly formulated it as the trans isomer

of dimethyl ceanothate epimerised at both C - 2 and C - 3. This isomer was originally given the name dimethyl epiceanothate in accordance with the nomenclature used by de Mayo and Starratt. This isomer 23 was first prepared by epimerisation of the C - 2 methoxycarbonyl group by prolonged treatment of dimethyl ceanothate with concentrated methanolic sodium methoxide to give a mixture from which the half ester of 23, methyl  $2\beta$  - carboxy -  $3\beta$  - hydroxy - A(1) - norlup-20(29)-en-28-oate could be isolated after a lengthy purification procedure; methylation with diazomethane then gave 23. Eade *et al*<sup>21</sup> prepared this compound according to the procedure used by de Mayo and Starratt<sup>5</sup> for the analogous compound in lactone series. Thus oxidation of dimethyl ceanothate with Jones reagent gave the known methyl  $2\alpha$  - methoxycarbonyl-3-oxo-A(1) - norlup-20(29)-en-28-oate, that is, dimethyl dehydroceanothate 22 which was rapidly epimerised by alkali to an equilibrium mixture containing 40% of starting material and 60% of the isomer, epimeric at C - 2, methyl  $2\beta$  - methoxycarbonyl-3-oxo-A(1) - norlup-20(29)-en-28-oate 26, that is, dimethyl epidehydroceanothate.



scheme - 1



$a$ , Jones reagent;  $b$ , Sodium borohydride;  $c$ , Sodium methoxide;  $d$ , Diazomethane.  $\times$  22  $\rightarrow$  20 + 24 but 22A  $\rightarrow$  24A only

Eade et al, in 1964 described<sup>17</sup> the corresponding half-ester, methyl 2 $\beta$ -carboxy-3-oxo-A(1)-norlup-20(29)-en-28-oate, that is, monomethyl epidehydroceanothate. Reduction of 26 with sodium borohydride gave the cis isomer 23 as sole product; on oxidation with Jones reagent it gave back the parent ketone 26 quantitatively.

Reduction of dimethyl dehydroceanothate 23 with sodium borohydride gave a 1 : 1 mixture of dimethyl ceanothate

20 ( R = Me ) and its C - 3 epimer 24 which were readily separated by column chromatography. Oxidation of 24 with Jones reagent gave back the original ketone 22 quantitatively.

The fourth isomer, methyl 3 $\alpha$ -hydroxy-2 $\beta$ -methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate 25, <sup>was prepared</sup> ~~was prepared~~ by long heating of a solution of methyl 3 $\alpha$ -hydroxy-2 $\alpha$ -methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate 24 in methanolic sodium methoxide whereupon partial epimerisation of the C 2 - methoxycarbonyl group occurred. After remethylation of the total crude product, they separated the isomer 25 from starting material by column chromatography; the identity of 25 was confirmed by its oxidation with Jones reagent to the ketone 26.

Eade et al have also reported the conversion of the above alcohols into their corresponding acetates.

#### Preparation of the Lactone Series :

Three of the four isomers in this series have previously been reported<sup>5,16</sup>. Methyl ceanothate lactone 20 A ( R = Me ) has been prepared from ceanothic acid by formic acid catalysed lactonisation followed by hydrolysis and methylation<sup>5</sup> and also by lactonisation of dimethyl ceanothate in acetic acid and sulphuric acid followed by deacetylation<sup>17</sup>.

The compounds 23 A and 24 A have been prepared by borohydride reduction of the corresponding ketones 22 A and 26 A. Eade et al also prepared the above three isomers using the same procedure described above. The fourth and previously unknown isomer,  $3\alpha$ -hydroxy- $2\beta$ -methoxycarbonyl-A(1)-nor-18 $\alpha$ -olean-28  $\rightarrow$  19 $\beta$ -olide 25 A, was prepared by isomerisation of  $3\alpha$ -hydroxy- $2\alpha$ -methoxycarbonyl-A(1)-nor-18 $\alpha$ -olean-28  $\rightarrow$  19 $\beta$ -olide 24 A with sodium methoxide in methanol, followed by remethylation with diazomethane and purification by chromatography on alumina. Its identity was confirmed by oxidation to the ketone 26 A.

The acetates of all four isomeric alcohols in the lactone series were also prepared by Eade et al.

These authors also reported that the epimerisations of both the ketones in each series exist in reversible equilibrium; that is, if either 22 or 26 is treated with sodium methoxide in methanol a mixture of 60% 26 and 40% 22 results and a similar mixture of 26 A and 22 A is obtained from either 22 A or 26 A.

#### N. M. R. Results :

Eade et al<sup>21</sup> gave a list of coupling constants between the protons on C - 2 and C - 2 and C - 3 for each isomer and its

acetate in both series and also a list of the vicinal coupling constants ( $J_{2,3}$ ) of the four isomers calculated for the three possible ring A conformations from the dihedral angles measured from Drieding models. Of these three possible conformations, the  $\alpha$ -envelope seemed least likely since examination of the models showed strong  $10\beta$ -methyl /  $4\beta$ -methyl interaction which would be expected to destabilise ~~this~~ this conformation; in addition, Fishman<sup>25</sup> in his investigation on analogous 16,17-disubstituted steroids lacking these methyl / methyl interaction did not consider the  $\alpha$ -envelope. The only significant difference between the three conformations were the two trans couplings in the  $\alpha$ -envelopes<sup>25</sup> and therefore these results, although excluding the  $\alpha$ -envelope, did not distinguish between the other two conformations. The observed couplings taken by Eade et al<sup>21</sup> were clearly consistent with their proposed formula for ceanothic acid 20 (R = H) and do not conform with the original stereochemistry assigned by de Mayo and Starratt<sup>5</sup>.

Table - II shows that there are no significant differences between the coupling constants of the C - 2 and C - 3 protons in members of dimethyl ceanothate series compared with those of the analogous compounds in the lactone series and hence this investigation did not yield any evidence about the long range conformational transmission effect which had been proposed<sup>17</sup> to explain differences in reactivity in the

ring A area between the two series. The coupling constants in Table - II, together with interconversions summarized in Scheme - 1, established unequivocally that in the epiceanothate series the ring A substituents are cis to one another, each having the  $\beta$ - configuration.

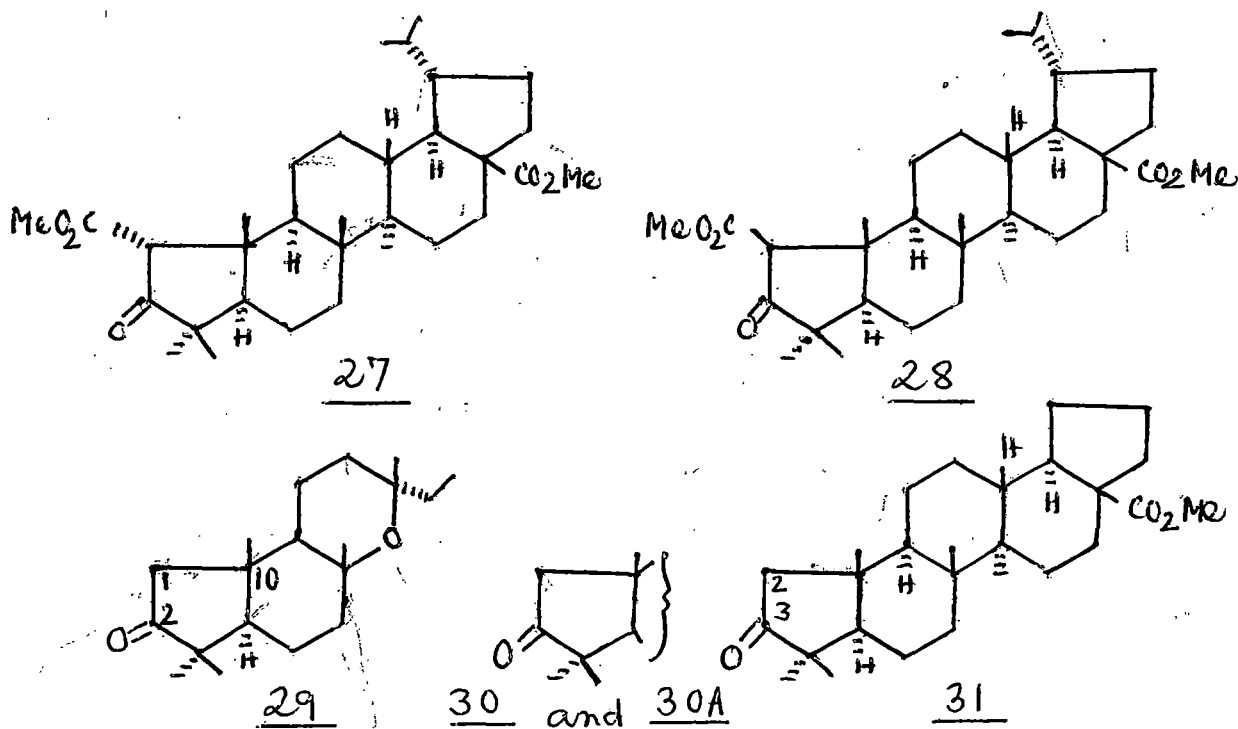
Table - II

Coupling constants of C - 2 and C - 3 protons

|                              | Configurations of Ring A Substituents |                  |                    |                   |
|------------------------------|---------------------------------------|------------------|--------------------|-------------------|
|                              | $2\alpha, 3\beta$                     | $2\beta, 3\beta$ | $2\alpha, 3\alpha$ | $2\beta, 3\alpha$ |
| <hr/>                        |                                       |                  |                    |                   |
| Observed $J_{2,3}$ (Hz)      |                                       |                  |                    |                   |
| Dimethyl ceanothate series : |                                       |                  |                    |                   |
| C 3 - OH                     | 1.0                                   | 7.3              | 7.0                | 9.0               |
| C 3 - OAc                    | 0.2                                   | 7.6              | 7.6                | 9.5               |
| Lactone series :             |                                       |                  |                    |                   |
| C 3 - OH                     | 1.0                                   | 7.4              | 7.0                | 9.0               |
| C 3 - OAc                    | 0                                     | 7.6              | 7.7                | 9.5               |
| Calculated $J_{2,3}$ (Hz)    |                                       |                  |                    |                   |
| $\alpha$ - Envelope          | 1.8-2.9                               | 8.2              | 8.2                | 1.8 - 2.9         |
| $\beta$ - Envelope           | -0.3-0.0                              | 6.2-6.9          | 5.7-6.7            | 5.9 - 7.1         |
| Half-chair                   | 0.3-0.8                               | 7.8-8.0          | 7.5-7.9            | 3.5 - 5.0         |
| <hr/>                        |                                       |                  |                    |                   |

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 (40) coupling between the 10 $\beta$ -methyl group and the C - 2 proton provides further support for their assignment. This coupling was studied using the following three pairs of isomers ; (i) methyl dehydroceanothate lactone 22 A and its 2 $\beta$ -epimer 26 A ; (ii) dimethyl dehydroceanothate 22 and its 2 $\beta$ -epimer 26 ; (iii) dimethyl dihydrodehydroceanothate, that is, methyl 2 $\alpha$ -methoxycarbonyl - 3-oxo-A(1)-norlupan-28-oate 27 and its 2 $\beta$ -epimer 28. The last pair of isomers were prepared from dimethyl dihydroceanothate by methods identical with those used to prepare 22 and 26 from dimethyl ceanothate. In each of these six compounds the C - 2 proton appeared as a singlet at approximately  $\delta$  3.0 ; and, this signal, they observed for each of the 2 $\beta$ -methoxycarbonyl (epi) derivatives was broad (  $W_{1/2}$  2.5 Hz ) compared with that in each of the 2 $\alpha$ -methoxycarbonyl compounds (  $W_{1/2}$  1.5 Hz ). This broadening was confirmed by spin-spin decoupling; decoupling of the C - 2 proton signal at  $\delta$  3.08 in dimethyl epidehydroceanothate 26 increased the intensity of the C - 10 methyl signal at  $\delta$  1.06 markedly, while irradiation of this methyl frequency resulted in a sharpening of the C - 2 signal (  $W_{1/2}$  became 1.6 ). They also observed similar sharpening of the C - 2 proton signal from  $W_{1/2}$  2.5 Hz to  $W_{1/2}$  1.6 Hz when the relevant methyl signal in

both 26 A and 28 was irradiated. Similar long range ( 46 ) coupling between the  $1\alpha$ -proton and the  $10\beta$  - methyl was observed in the nor-diterpene colensan - 2-one 29<sup>26</sup>. The observed couplings, they stated, was due to the pseudo-axial character of the  $2\alpha$  - hydrogens in 26, 26 A, and 28 and consequently supported the half-chair or  $\beta$  - envelope conformation of ring A for these derivatives when the  $2\alpha$  - proton and  $10\beta$  - methyl possessed some degree of co-planarity.

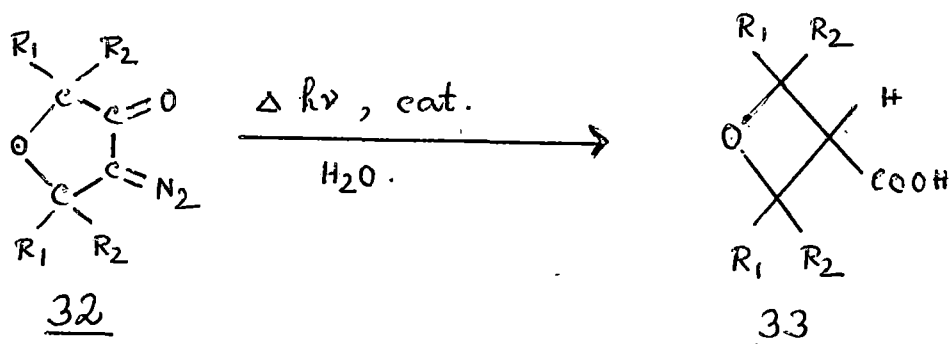


C. D. Results : The c. d. of the two epimeric ketones 22 and 26 together with that of the corresponding norketone 30 and also the corresponding sets of three ketones in the lactone series 22A, 26A and 30A and in the dimethyl dihydroceanothate series 27, 28 and 31 have been studied. But the changes in c. d. between members of each set did not yield any information about the configuration of the C-2 methoxycarbonyl group.

## CHAPTER - II

### Section A : Previous attempts toward the partial synthesis of ceanothic acid.

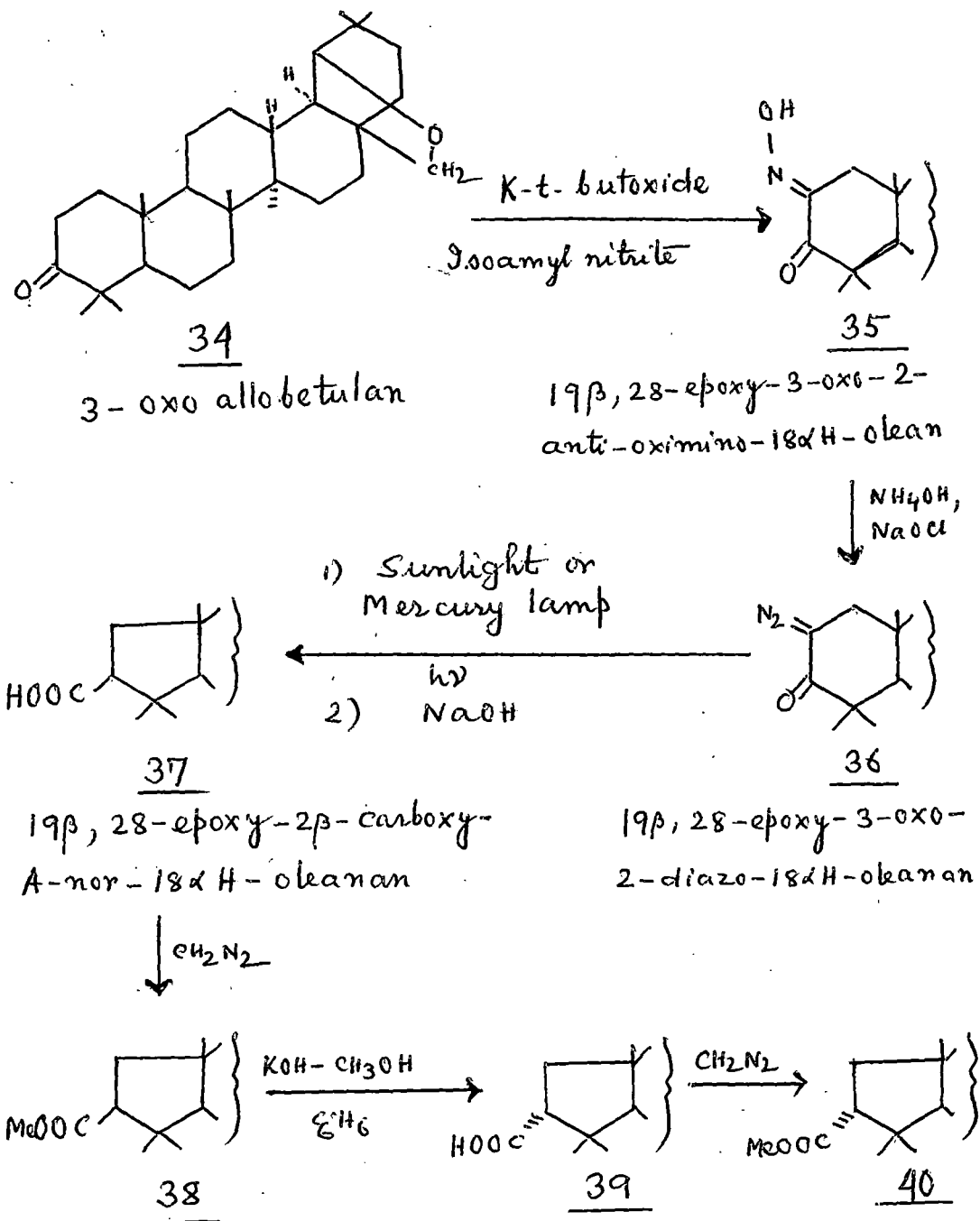
In 1965 Huneck<sup>27</sup> with a view to devising a method for the partial synthesis of ceanothic acid developed a procedure for contraction of Ring A in normal triterpenoid in order to achieve the synthesis of suitable A-nor carboxylic acids, a structural feature present in ceanothic acid. As originally demonstrated by Horner<sup>28</sup> and Sus *et al*<sup>29</sup>, Wolff rearrangement can be implemented with cyclic diazo-ketones resulting in ring contraction under photochemical conditions. The photochemical variant proves very suitable for producing strained systems also<sup>28c, 30</sup>. The formation of the oxetane<sup>31, 32</sup> 33 can be considered as an example.



The first publication of Huneck<sup>27a</sup> reported the conversion of 3-oxo-allobetulan 34 to 19 $\beta$ , 28-epoxy-2 $\beta$  -

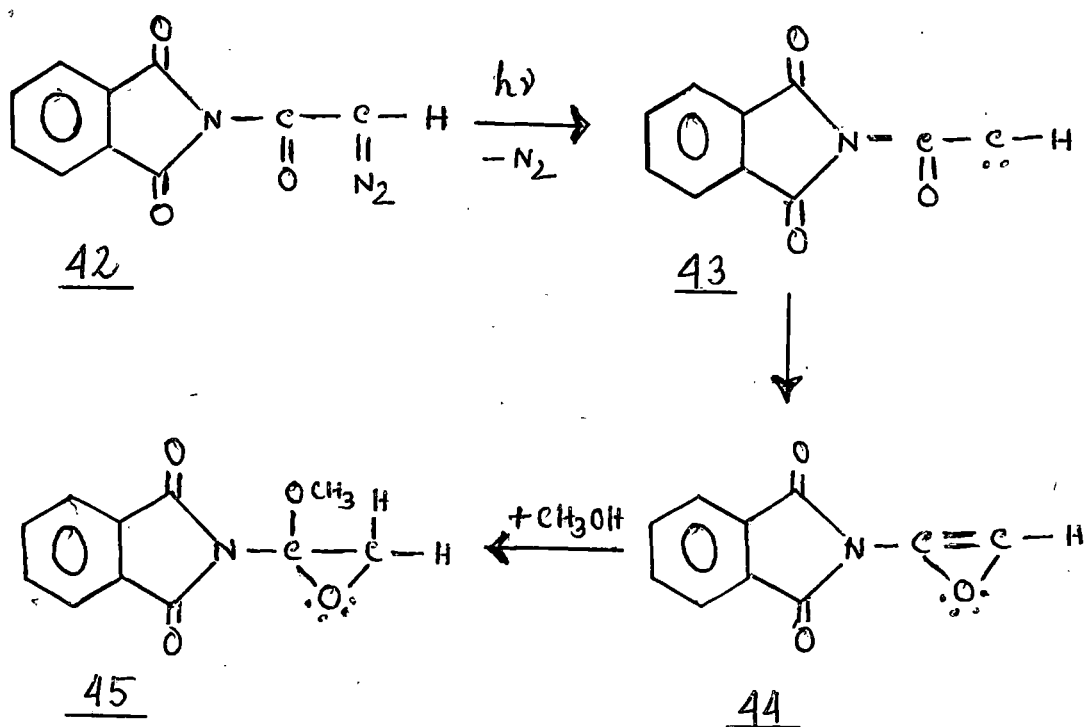


methoxycarbonyl-A-nor-18 $\alpha$ H-oleanan 38 according to the following sequence

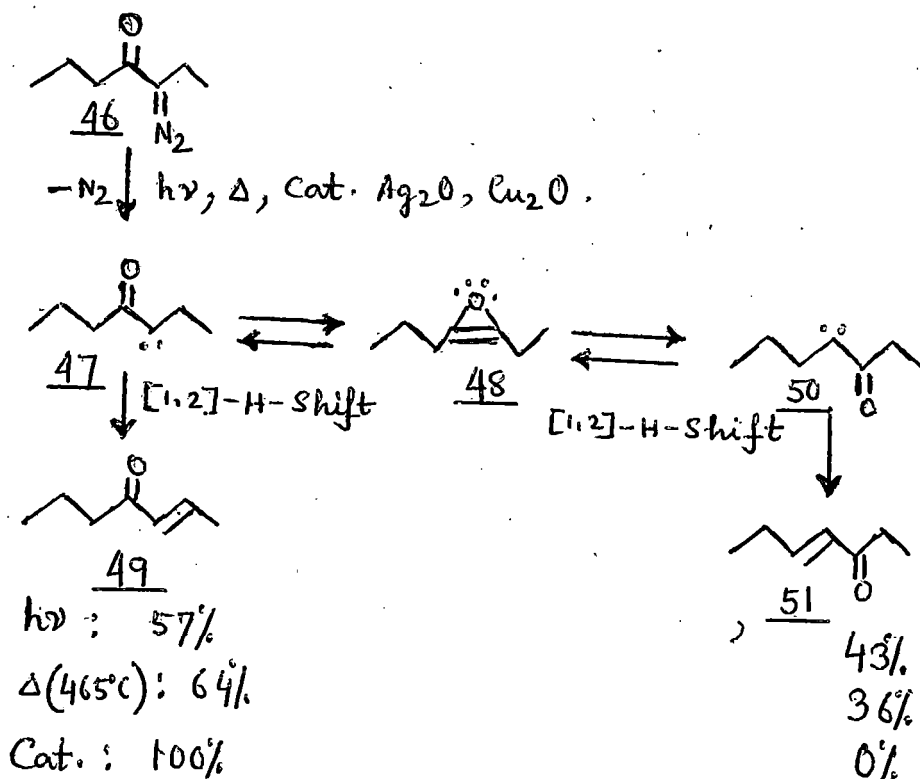




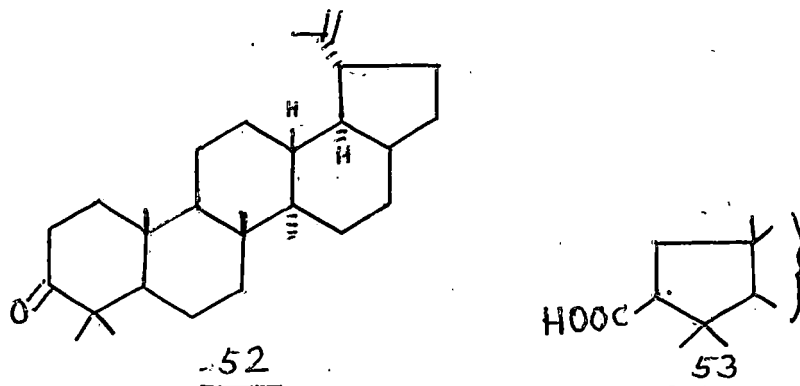
The extent of oxirene formation depends upon the conditions e.g. on the concentration of water<sup>37, 38</sup>. The carbene-oxirene equilibrium makes it difficult to reach any conclusion regarding the migratory aptitude of the groups  $R_1$  and  $R_2$ . In all systems investigated so far<sup>39</sup>, however, the initial carbene formed after nitrogen elimination undergoes reaction. Oxirene participation could be ruled out on sensitised photolysis, on catalysis and on thermolysis in solution<sup>37-41</sup>. The trapping reaction yielding 45 strongly suggests that the oxirenes are true intermediates and not merely transition states<sup>42</sup>.



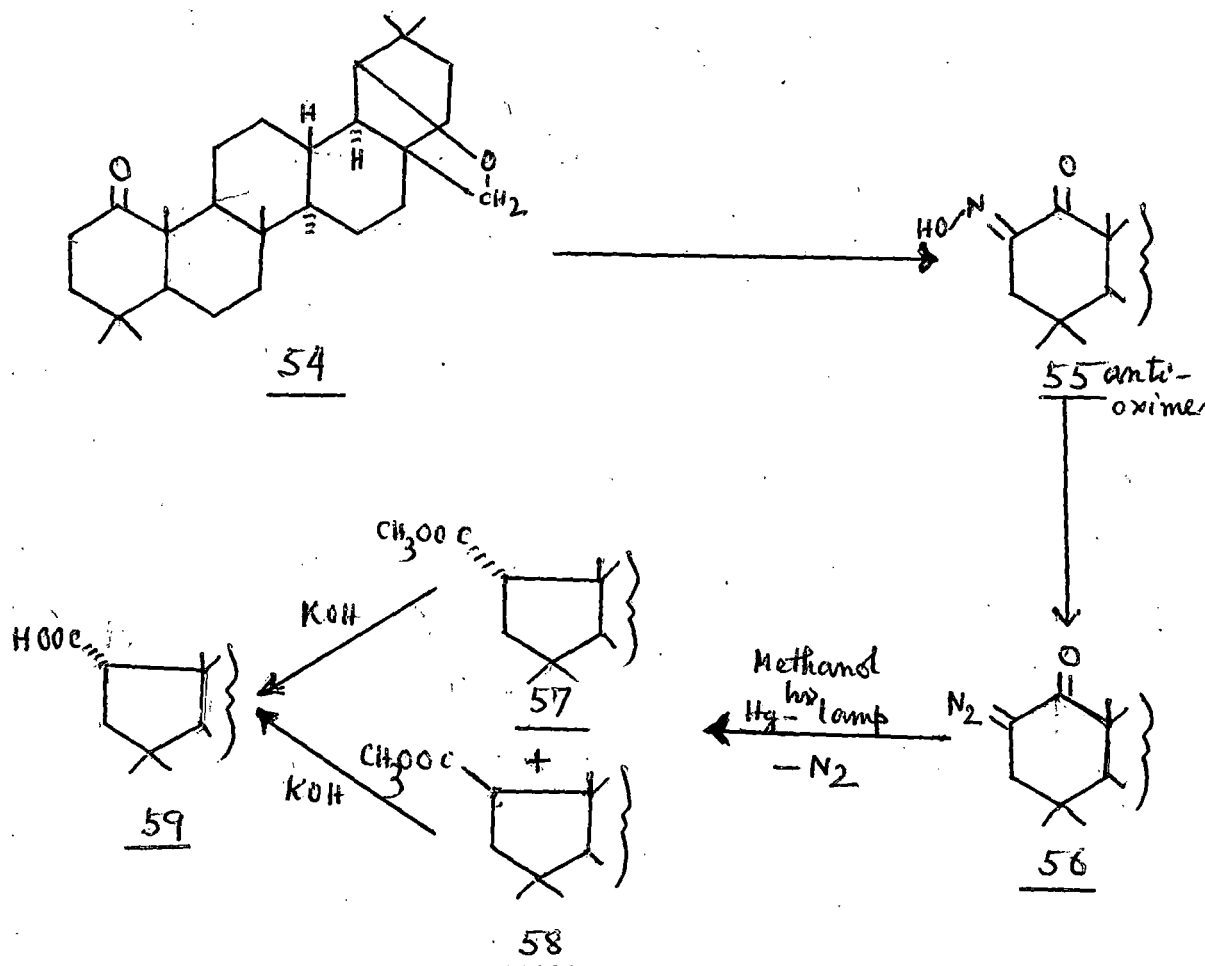
Proof of oxirene formation that is completely independent of the Wolff rearrangement was obtained with unsymmetrical  $\alpha$ -dialko dialkyl ketones<sup>43</sup> and a number of special aryl substituted  $\alpha$ -dialko ketones<sup>44</sup>



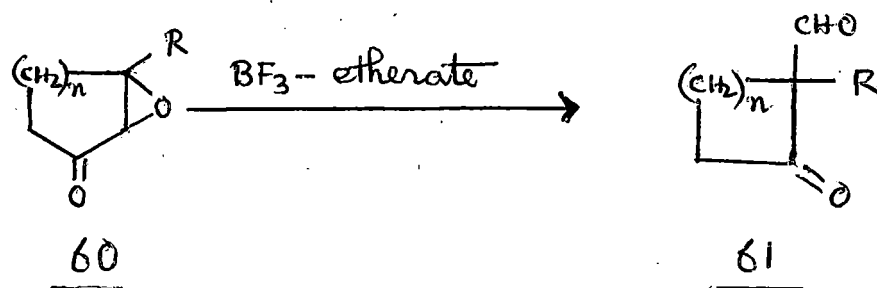
In another paper, Huneck<sup>27b</sup> reported the conversion of 3-oxo lupen-20(29) 52 to 2 $\beta$ -carboxy-A-nor-lupen-20(29) 53 following the same sequence of reactions described before.



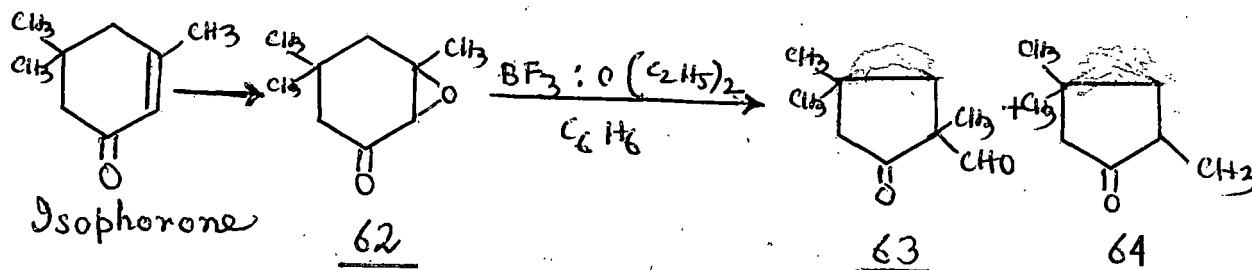
Methyl oleanonoic ester 54<sup>27b</sup> by similar reactions gave a mixture of 2 $\beta$  - methoxycarbonyl-A-nor-  $\Delta^{12}$ -oleanene-17-methylester and its 2 $\alpha$ - epimer. They<sup>27c</sup> have also shown that the appropriate 1-oxo-triterpenoids can be converted to the A-nor-1- carboxy compounds by application of the same procedure. Thus 19 $\beta$  , 28-epoxy-1-oxo-18 $\alpha$  H-oleanan 54 gave a mixture of 1 $\alpha$  - methoxy carbonyl - 19 $\beta$  , 28 - epoxy-A-nor-18 $\alpha$  H-oleanan 57 and 1 $\beta$  - methoxycarbonyl- 19 $\beta$  , 28-epoxy-A-nor-18 $\alpha$  H-oleanan 58. The reaction path is shown below.



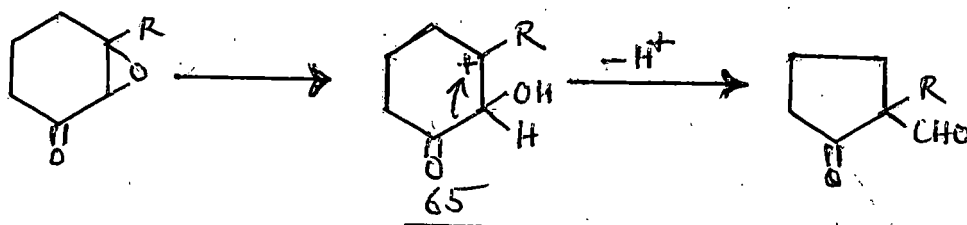
House and Wasson<sup>45</sup> have shown that, epoxy ketones of type 60 in the presence of  $\text{BF}_3$ -etherate undergo rearrangement with reduction in ring size of the cyclic ketone, and produced the keto - aldehyde 61



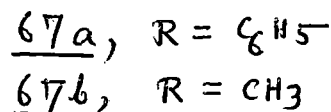
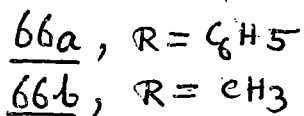
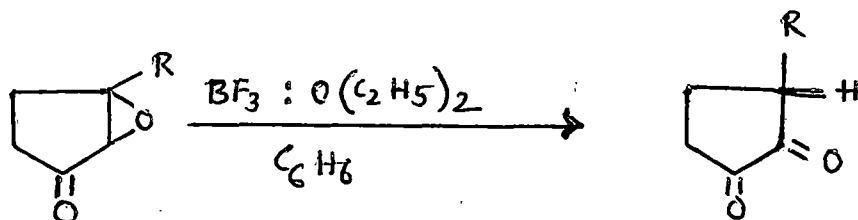
Thus isomerisation of a benzene solution of isophorone oxide 62 in the presence of boron-trifluoride etherate produced the keto aldehyde 63 in 33% yield accompanied by the ketone 64. The ketone 64 was presumably formed by the deformylation of 63 during the isolation



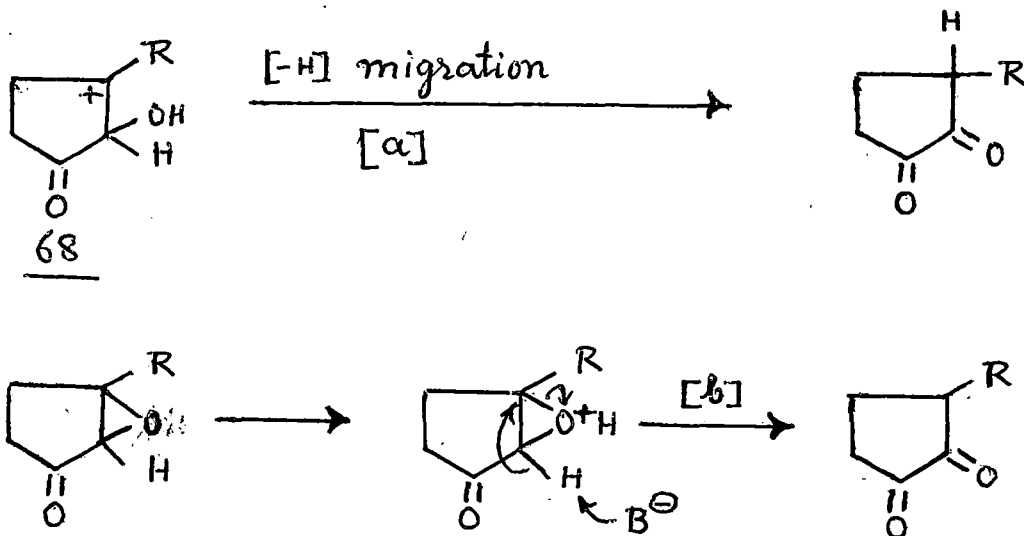
The reaction proceeds via the carbonium ion 65



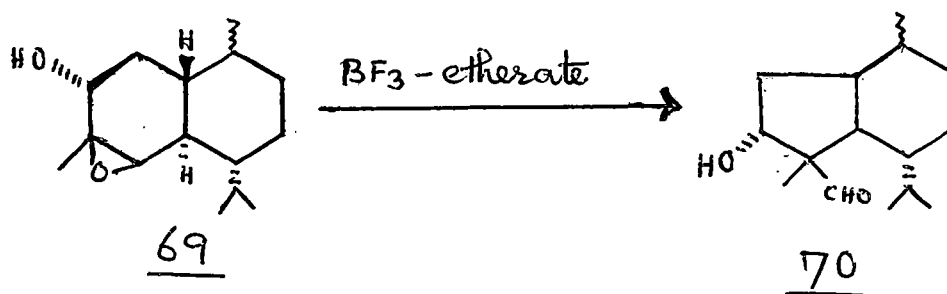
In order to study whether similar ring contractions could be achieved in the cyclopentanone series, the isomerisation of cyclopentenone oxide 66 was investigated. However, the only products which could be isolated were the enol forms of the  $\alpha$  - diketone 67



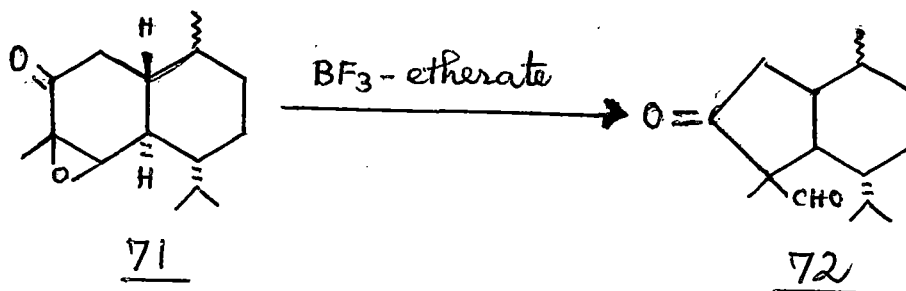
These products could arise either by the formation of a carbonium ion 68 followed by hydrogen migration ( path a ) or by a concerted elimination reaction ( path b )



Kartha and Chakravarti<sup>46</sup> studied the action of  $\text{BF}_3$ -etherate on some sesquiterpene oxides and found that an aldehyde was formed through contraction from a six-membered ring to a five-membered ring. Dihydrokhusinol epoxide 69 on treatment with freshly distilled  $\text{BF}_3$ -etherate is converted to the crystalline hydroxy aldehyde 70.

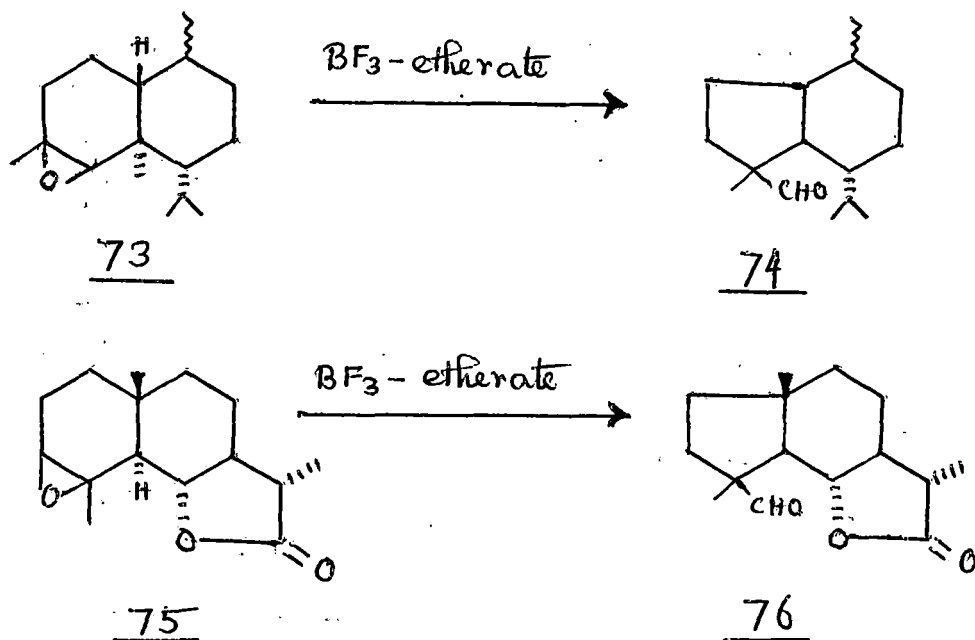


They studied the same reaction on the epoxy ketone 71 and observed that it rearranged to the keto aldehyde 72.

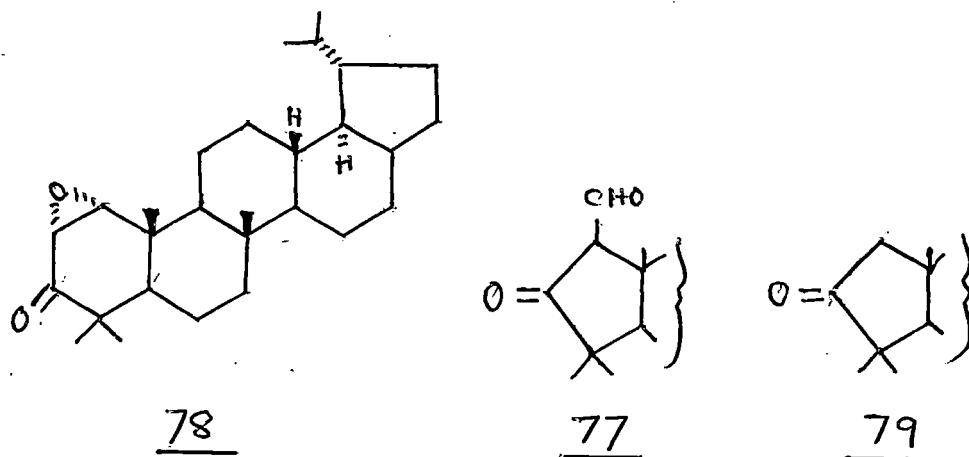


The epoxide of ( - ) dihydro -  $\gamma$  - cadinene 73 undergoes ring contraction to the aldehyde 74.  $\alpha$  - Santenolide epoxide 75 also gave the aldehyde 76 by the action of  $\text{BF}_3$ - etherate.

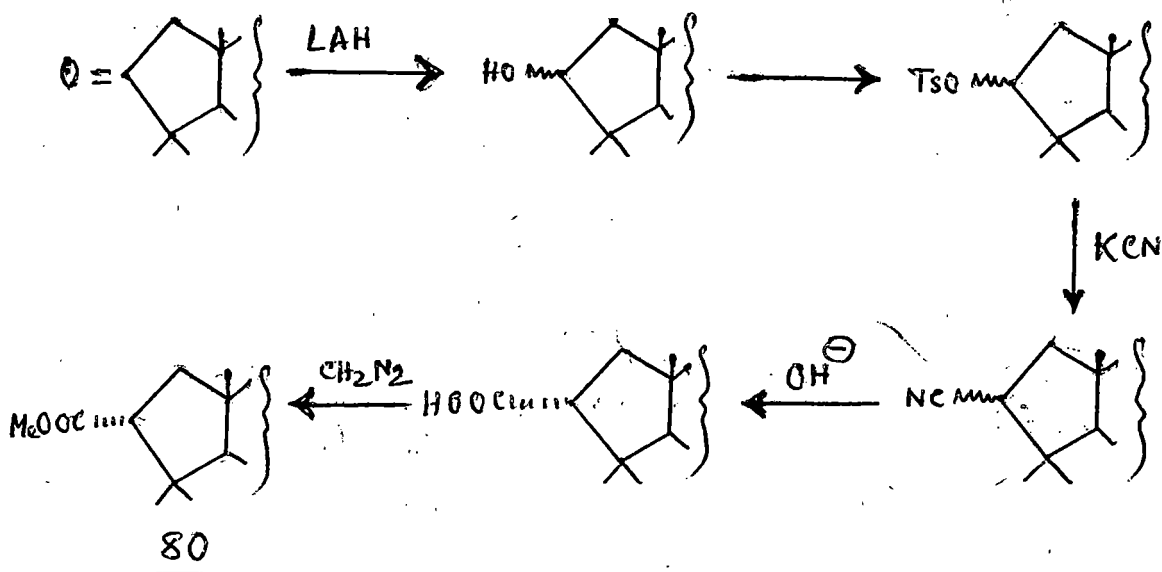




In 1968, Chatterjee and colleagues<sup>47</sup> attempted to prepare the keto aldehyde 77 by ring contraction of  $1\alpha,2\alpha$ -epoxy lupanone (3) 78 by following the same procedure as described by House et al<sup>45</sup> and Kartha and coworkers<sup>46</sup>



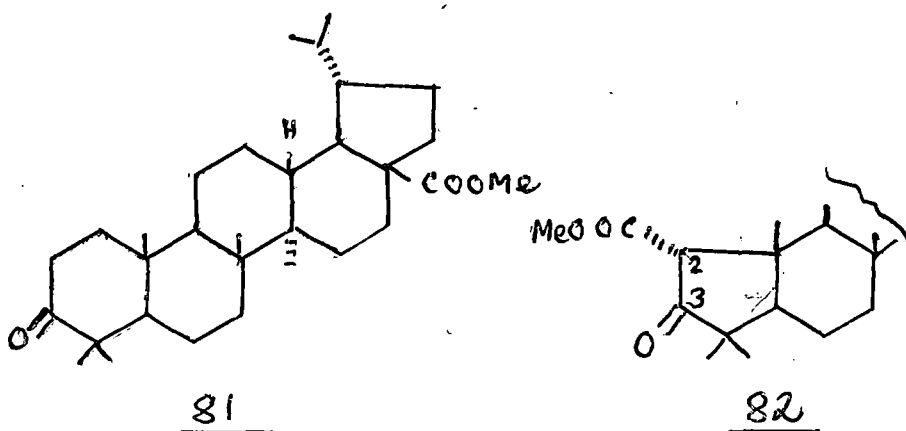
However, in this reaction, they could not isolate the keto aldehyde but obtained the ketone, A-nor-lupanone (2), 79 in good yield. Most probably the intermediate keto-aldehyde that is formed, easily loses the formyl group during the reaction. The object of their work was to develop a method for the partial synthesis of ceanothic acid starting from the keto-aldehyde 77. They, however, finally achieved the synthesis of 2 $\alpha$ -methoxycarbonyl-A-nor-lupan 80 by following the sequence of reactions shown below.



Section B : Partial synthesis of dimethyl dihydroceanothate starting from betulinic acid.

The objective of the present study was the design of a partial synthesis of dimethyl dihydroceanothate starting

from methyl dihydrobetulinate. It was our intention to introduce the substituents in ring A ( centres of asymmetry ) step by step. Therefore, we were to devise a synthetic scheme involving an intermediate possessing only a single substituent ( a single centre of asymmetry ) in correct stereochemistry in the five membered ring A. This intermediate must be converted into dimethyl dihydroceanothate in which the additional ring substituent in ring A is present in one particular "natural" configuration. With these basic requirements in view we explored two methods for the conversion of the six-membered ring A of methyl dihydrobetulonate 81 to the the  $\beta$  - keto ester - <sup>dimethyl</sup> ~~dehydro dihydro~~ ~~methoxy~~ ceanothate 82.

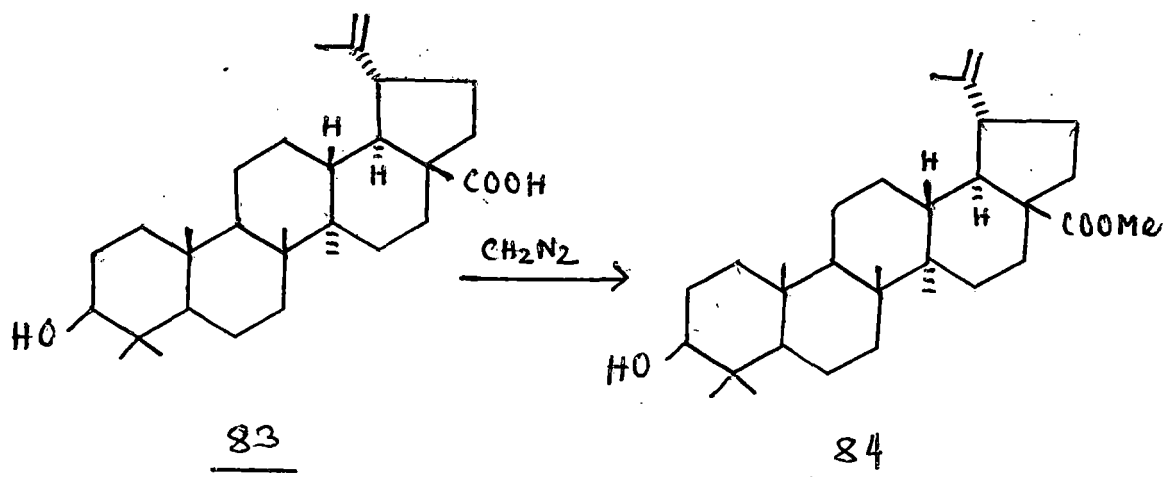


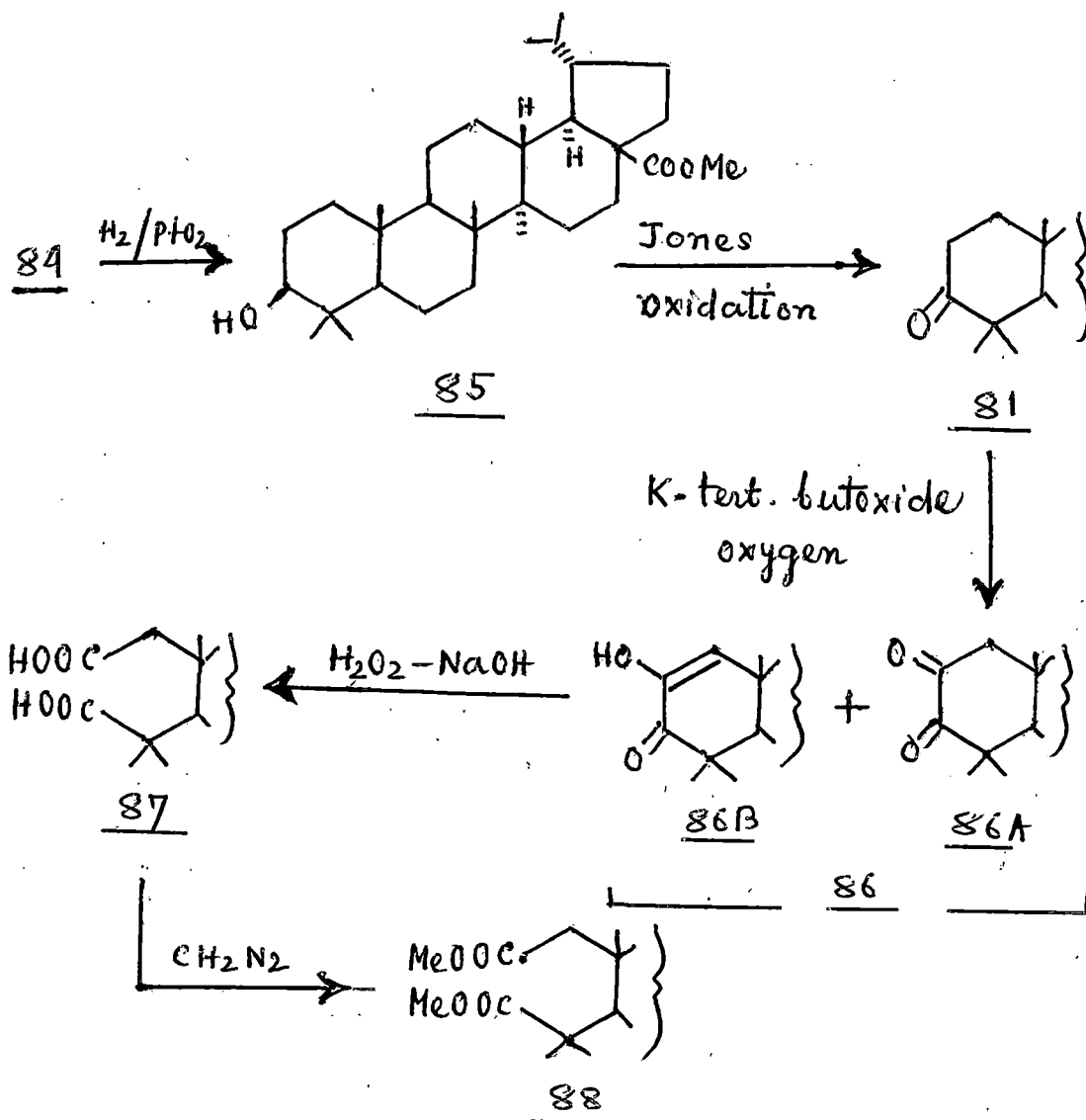
where the methoxy carbonyl group is in the natural  $\alpha$  - configuration. The next step in the synthesis is to introduce a hydroxyl group at C - 3 in proper stereochemistry that is, with  $\beta$  - configuration. Eade et al<sup>21</sup> have already shown that

dimethyl dehydroceanothate on reduction with sodium borohydride gives a 1 : 1 mixture <sup>of</sup> dimethylceanothate and its C - 3 epimer which could be separated by column chromatography. We, therefore, thought that once we can achieve the successful development of a method for construction of skeleton 82, the next step, that is, accommodation of a  $\beta$  -OH group at C - 3 would not be very difficult.

The present work describes in detail the result of our synthetic work and successful development of a method for the partial synthesis of dimethyl dihydroceanothate.

The simplest synthetic pathway for the preparation of the  $\beta$  - ketoester would be Dieckmann condensation of the 2,3-seco-trimethyl ester 88 which was prepared by following the synthetic sequence shown below. The details are described in the following lines.



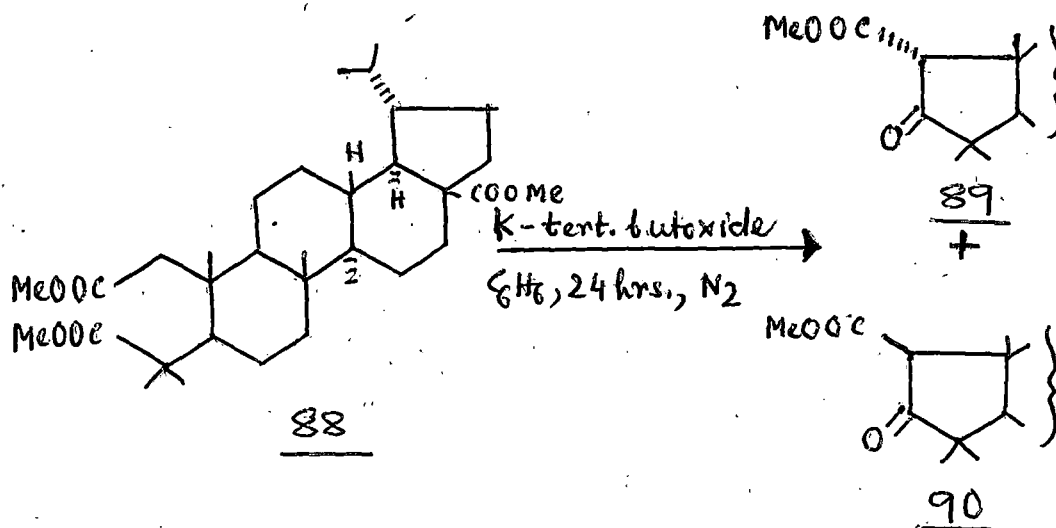


Betulinic acid 83 extracted from the bark of Bischofia javanica Blume on esterification with diazomethane gave methyl betulinate 84, m.p. 223-24°, ( $\alpha$ )<sub>D</sub> 5°,  $\nu$  <sub>max</sub> nujol 3560 (OH), 1715 (COOCH<sub>3</sub>), 1660 and 880 cm<sup>-1</sup> (= CH<sub>2</sub>), which on hydrogenation in presence of PtO<sub>2</sub> - catalyst afforded methyl dihydrobetulinate 85, m.p. 236-38°,  $\nu$  <sub>max</sub> nujol 3560 (OH), 1715 cm<sup>-1</sup> (COOCH<sub>3</sub>).

of  
Jones oxidation methyl dihydrobetulinate gave methyl dihydrobetulonate 81, m.p. 202-4°, ( $\alpha$ )<sub>D</sub> 8°,  $\gamma$  <sub>max</sub> nujol 1730 (COOCH<sub>3</sub>), 1708 cm<sup>-1</sup> (CO). Autoxidation of 81 by passing a stream of oxygen in presence of potassium tertiary butoxide in tert. butanol furnished a product m.p. 131-33°, ( $\alpha$ )<sub>D</sub> -1.96°,  $\gamma$  <sub>max</sub> nujol 3460 (OH), 1730 (COOCH<sub>3</sub>), 1670, 1650, 860 cm<sup>-1</sup>,  $\lambda$  <sub>max</sub> 269 m $\mu$  ( $\epsilon$ , 7532). It gave a positive ferric chloride coloration for diosphenol and showed two spots on chromatoplate indicating the presence of two tautomeric forms of the  $\alpha$ -diketone 86 A and diosphenol 86 B. The diosphenol 86 B on oxidation with hydrogen peroxide<sup>48</sup> in presence of sodium hydroxide gave the seco-acid 87, m.p. 175-77°,  $\gamma$  <sub>max</sub> nujol 1710 and 1690 cm<sup>-1</sup> (COOH). The latter on esterification with diazomethane gave the desired trimethyl ester 88, m.p. 146-47°,  $\gamma$  <sub>max</sub> nujol 1745 (COOCH<sub>3</sub>) and 1725 cm<sup>-1</sup> (COOCH<sub>3</sub>) identical with those reported in the literature<sup>3,12</sup>.

Dieckmann condensation of the trimethyl ester 88 was carried out by refluxing it for 24 hours in presence of potassium tertiary butoxide in benzene solution under nitrogen blanket<sup>49,50</sup>. perfectly anhydrous condition was maintained and during the time of reflux a portion of benzene was removed with a Dean-stark trap. After the reaction was over the gummy product obtained after usual work up was chromatographed over deactivated alumina. The first three fractions

of the petroleum - benzene (3 : 2) eluate afforded a solid A, m.p. 190-92° and the next two fractions gave a solid B, m.p. 174-75°.

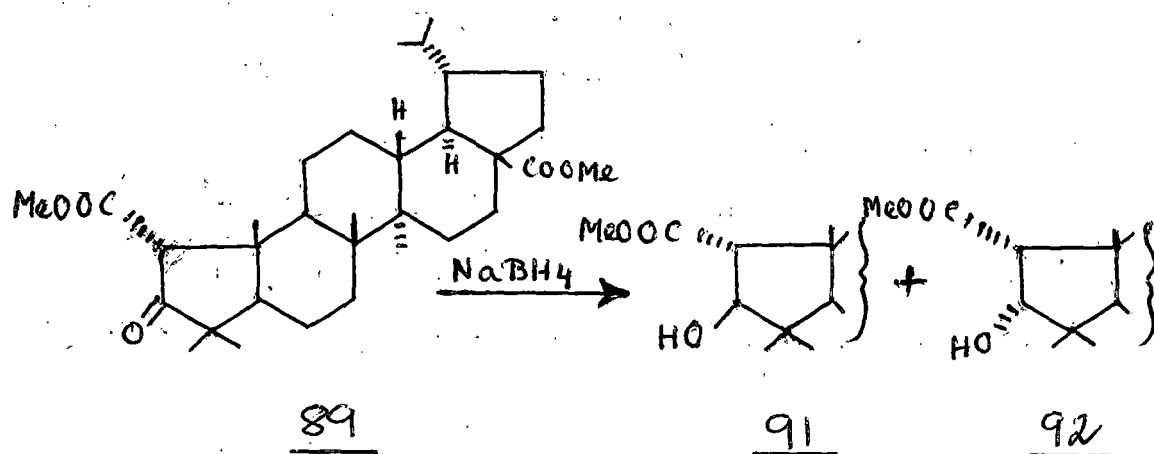


The solid A on crystallisation from methanol afforded fine colourless needles, m.p. 191-93° (TLC-homogeneous),  $(\alpha)_D^{25} 89^\circ$ ,  $\nu_{\text{max}}^{\text{nujol}}$  1755 (cyclopentanone), 1725  $\text{cm}^{-1}$  ( $\text{CO}_2\text{Me}$ ) having the same m.p., rotation and IR spectra reported in the literature<sup>21</sup> for dimethyl dehydrodihydroceanothate, i.e. methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate 89. The solid B on crystallisation from methanol furnished needle shaped crystals, m.p. 175-77° (TLC homogeneous),  $(\alpha)_D^{25} 42^\circ$ ,  $\nu_{\text{max}}^{\text{nujol}}$  1750 (cyclopentanone), 1730  $\text{cm}^{-1}$  ( $\text{CO}_2\text{Me}$ ), has been assigned structure 90 as it had the same m.p., rotation and IR spectra as those reported in the literature<sup>21</sup> for methyl 2 $\beta$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate.

The most significant point which we observed in this reaction was the high yield of the  $2\alpha$ -methoxycarbonyl isomer compared to its  $2\beta$ -isomer. In a Dieckmann condensation starting from 273 mg of the seco-trimethyl ester 88 we obtained 170 mg of the  $2\alpha$ -methoxycarbonyl isomer 89 and only 10 mg of the  $\beta$ -isomer 90. This observation was at variance with those reported by Eade et al<sup>21</sup> who have stated that methyl  $2\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlup-20(29)-en-28-oate (dimethyl dehydroceanothate) was rapidly epimerised by alkali to an equilibrium mixture containing 40% of starting material and 60% of the isomer epimeric at C - 2, methyl  $2\beta$ -methoxycarbonyl-3-oxo-A(1)-norlup-20(29)-en-28-oate (dimethyl epidehydroceanothate). Thus the most important step in our synthetic goal, the preparation of  $\beta$ -ketoester 89, having been achieved, we next turned our attention to the next step in the synthesis, that is, introduction of the  $\beta$ -(OH) at C - 3.

The  $\beta$ -ketoester 89 on reduction with sodium borohydride in methanol-dioxan solution afforded an oily residue. The latter was chromatographed over deactivated alumina. The first two fractions of the petroleum-benzene (2:3) eluate gave a solid C, m.p.  $257-59^{\circ}$  and further elution with the same solvent gave another solid D, m.p.  $140-42^{\circ}$

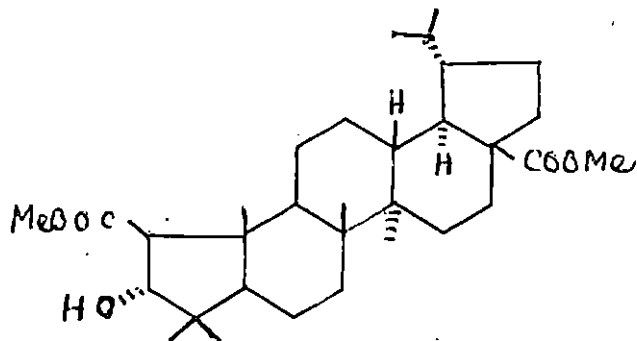




The solid C, m.p.  $257-59^\circ$  on crystallisation from methanol afforded colourless needles, m.p.  $261-63^\circ$ ,  $(\alpha)_D^{22} 22^\circ$ ,  $\gamma_{\text{max}}^{\text{nujol}}$   $3540$  ( $\text{OH}$ ),  $1730$  ( $\text{CO}_2\text{Me}$ ),  $1710\text{ cm}^{-1}$  ( $\text{CO}_2\text{Me}$ ), and was found to be identical with an authentic sample of dimethyl dihydroceanothate<sup>5</sup> [methyl-~~3 $\beta$~~ -hydroxy-2 $\alpha$ -methoxycarbonyl-A(1)-norlupan-28-oate] 91 supplied by Professor P. de Mayo (m, m, p, Co-TLC and IR comparison).

The solid D, on crystallisation from methanol gave crystals, m.p.  $140-42^\circ$ ,  $\gamma_{\text{max}}^{\text{nujol}}$   $3560$  ( $\text{OH}$ ),  $1745$  ( $\text{CO}_2\text{Me}$ ) and  $1705$  ( $\text{CO}_2\text{Me}$ ). The yield of this compound was not sufficient for NMR studies. But by analogy with previous work<sup>21</sup>, it is believed to be the C - 3 epimer of dimethyl dihydroceanothate

\* Addendum : NMR spectrum of (E), m.p.  $205-6^{\circ}$  exhibited signals at  $\delta$  4.2 (1 H, d after  $D_2O$ , J.8.0 Hz,  $\underline{CHDH}$ ), 3.65 (3 H, s, COOMe), 3.7 (3 H, s, COOMe), 2.25 (1 H, d, J.8.5 Hz, C-2 proton), the splitting pattern being almost similar to those recorded for methyl  $3\alpha$ -hydroxy- $2\beta$ -methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate<sup>21</sup>. The above NMR evidence permits the assignment of structure 92A for (E)



92A

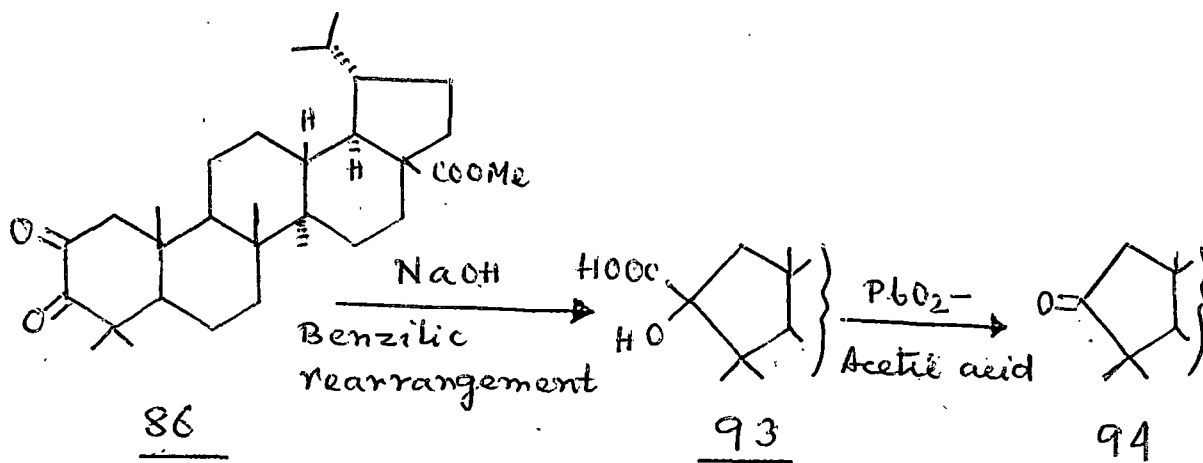
i.e. methyl-3 $\alpha$ -hydroxy-2 $\alpha$ -methoxycarbonyl-A(1)-norlupan-28-oate 92. Further work is in progress to prepare this isomer in sufficient quantity in order to enable us to study its NMR spectrum and establish its stereochemistry.

The  $\beta$  - ketoester [methyl 2 $\beta$ - methoxycarbonyl-3-oxo-A (1)- norlupan - 28 - oate], m.p. 175-77° was too insufficient for any further work.

In another run, Dieckmann condensation product of the trimethyl ester, was chromatographed over silica gel (E. Merck; Kieselgel-60) but separation of the isomeric  $\beta$ - ketoesters could not be achieved. We, therefore, reduced the mixture of  $\beta$  - ketoesters with sodium borohydride. The reduced product on chromatography over deactivated alumina first eluted a solid E, m.p. 203-4° (Petroleum : Benzene 2 : 3). Further elution with the same solvent gave a solid F which was seen to be a mixture of two compounds by TLC examination. On further chromatography we obtained two compounds 91 and 92 which were previously isolated directly from dimethyl dehydrodihydro-ceanothate 89. The solid E on crystallisation from acetone gave platelets, m.p. 205-6°,  $\nu$   $\begin{matrix} \text{nujol} \\ \text{max} \end{matrix}$  3520 (OH), 1740 (CO<sub>2</sub>Me) and 1705 cm<sup>-1</sup> (CO<sub>2</sub>Me).\*

Investigation were also initiated in this laboratory for the preparation of the  $\beta$  - ketoester 91 and 92 by a second route. The method involved the preparation of the A-nor ketone

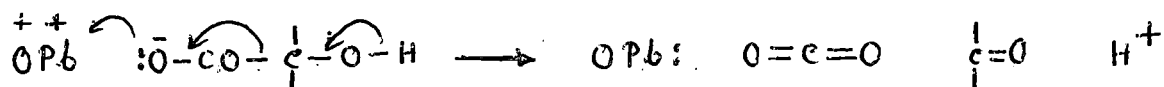
94, carbomethoxylation of which at C-2 would be expected to afford the  $\beta$  - ketoester 91 in proper stereochemistry. Since our previous results showed that in the Dieckmann condensation of the trimethyl ester 88, the 2 $\alpha$ - methoxycarbonyl derivative 89 was obtained in high yield in the equilibrium mixture, we thought that, in the carbomethoxylation reaction, under the similar basic condition of the reaction, if successful, would also give the 2 $\alpha$ - isomer 89 in the same ratio. With this end in view we prepared the A-nor ketone 94 starting from the diosphenol 86 by following the sequence shown below.



The diosphenol 86 in ethanol on refluxing with 10% alcoholic sodium hydroxide<sup>48</sup> undergoes a benzil-benzilic acid

type of rearrangement and gave a product, m.p. 255-56°,  $\nu$  max nujol 3440, 3340, 1720  $\text{cm}^{-1}$  which has been assigned  $\alpha$ -hydroxy carboxylic acid structure 93. The latter on treatment with lead dioxide ~~51~~ <sup>51, 52</sup> in glacial acetic acid solution under mild reflux yielded a product 94, m.p. 179-80°,  $(\alpha)_D$  75.4°,  $\nu$  max nujol 1735  $\text{cm}^{-1}$  (unresolved band for cyclopentanone and ester) and on expansion of the spectrum exhibited peaks at 1734 (cyclopentanone) and 1728  $\text{cm}^{-1}$  ( $\text{CO}_2\text{Me}$ ) having identical m.p., rotation and IR spectra as those reported in the literature for methyl decarboxydehydrodihydroceanothate<sup>5</sup>, or A-nor- dihydrobetulonic acid methyl ester<sup>12</sup>.

Probable mechanism<sup>49</sup> for the reaction is shown below.



Carbomethoxylation<sup>53</sup> of the A-nor ketone 94 with dimethyl carbonate was attempted using different bases, e. g. sodium methoxide, potassium tert. butoxide, sodium hydride under different experimental conditions (including different solvents). But in all cases the reaction was unsuccessful and we could isolate the starting material only, (see experimental). Further work is in progress in search of conditions for successful introduction of the  $-\text{COOMe}$  group at C-2.

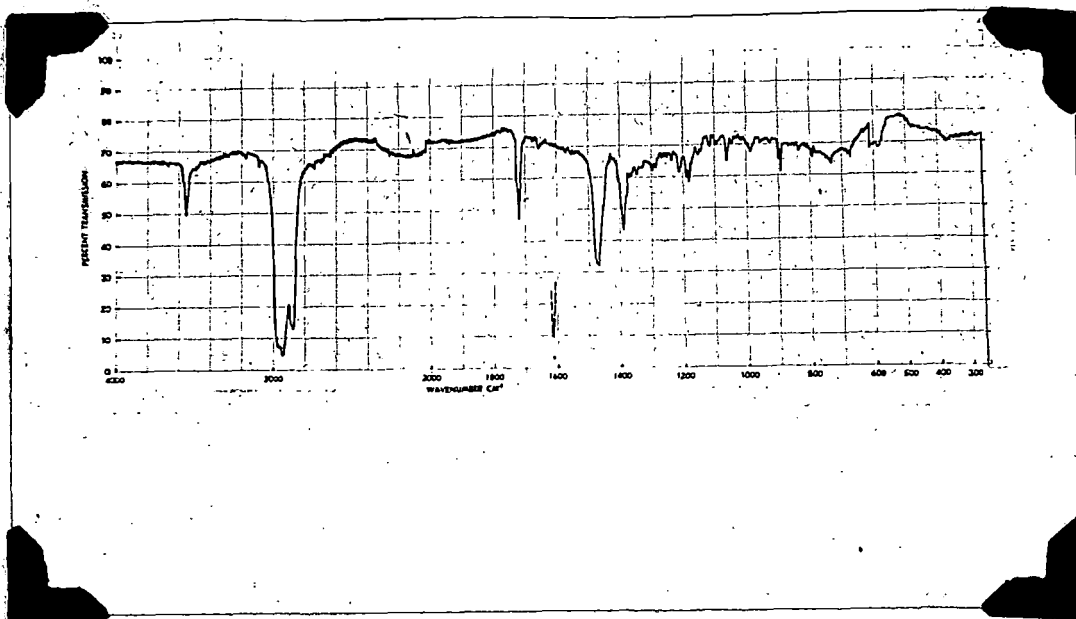


**Fig. 1 :** IR spectrum of methyl betulinate 84

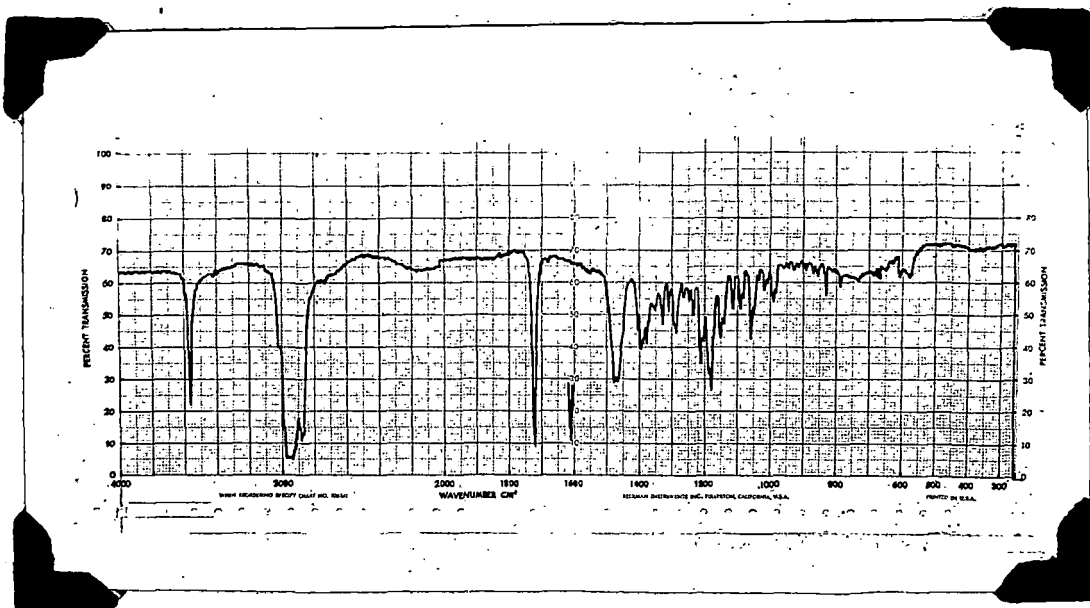


**Fig. 2 :** IR spectrum of methyl dihydrobetulinate 85

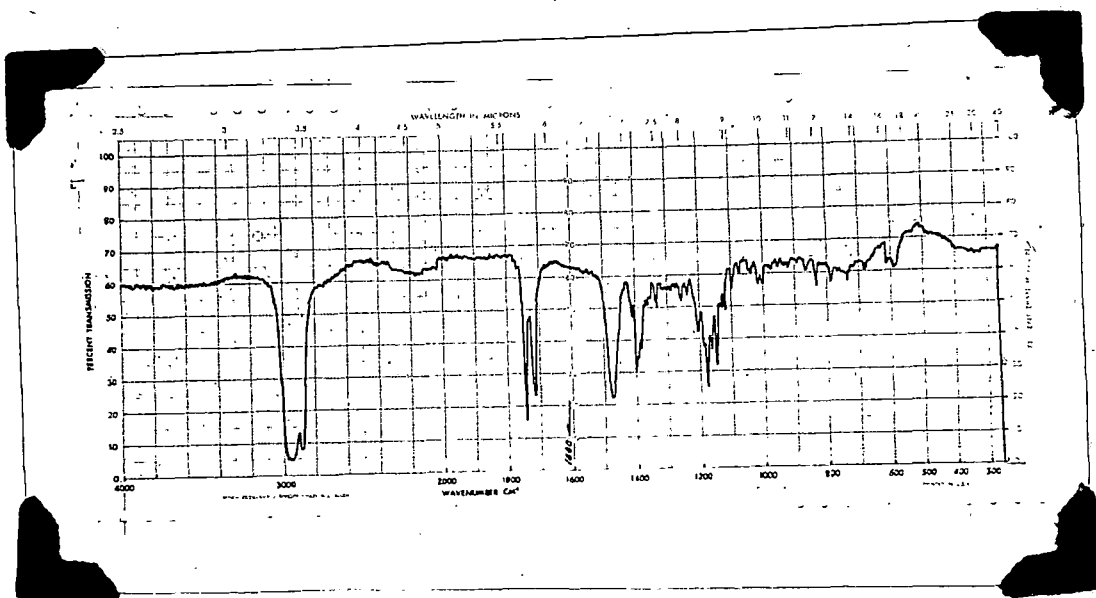


Fig. 3 : IR spectrum of methyl dihydrobetulonate 81

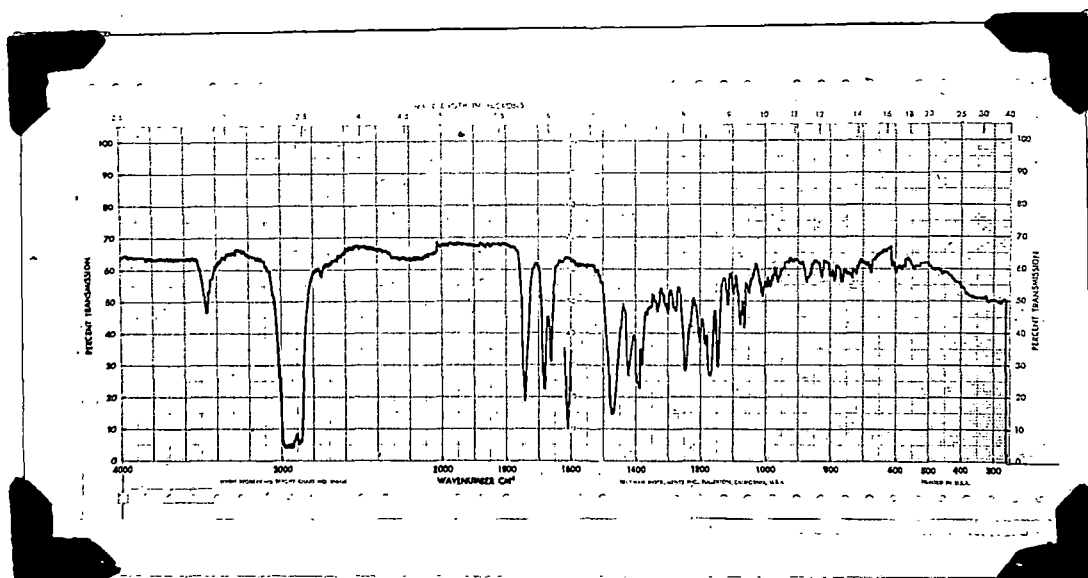


Fig. 4 : IR spectrum of diosphenol 86

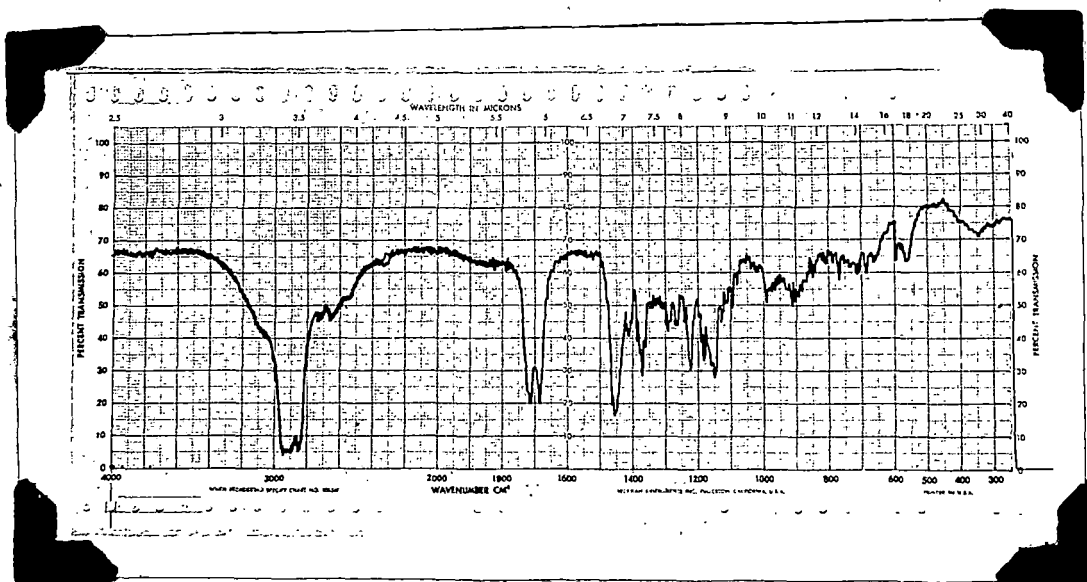


Fig. 5: IR spectrum of seco A-acid 27

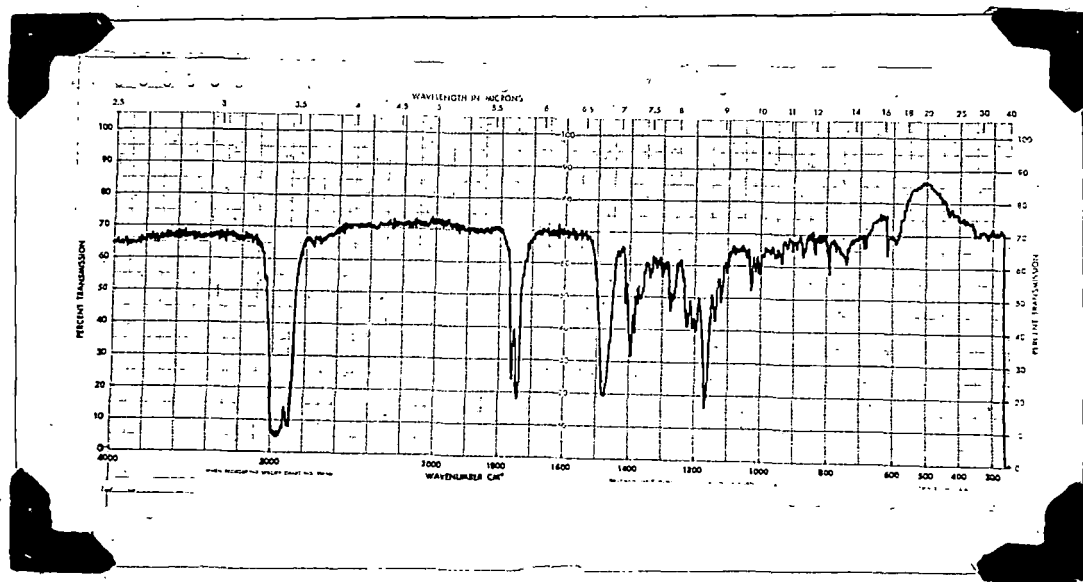


Fig. 6: IR spectrum of trimethyl ester 28



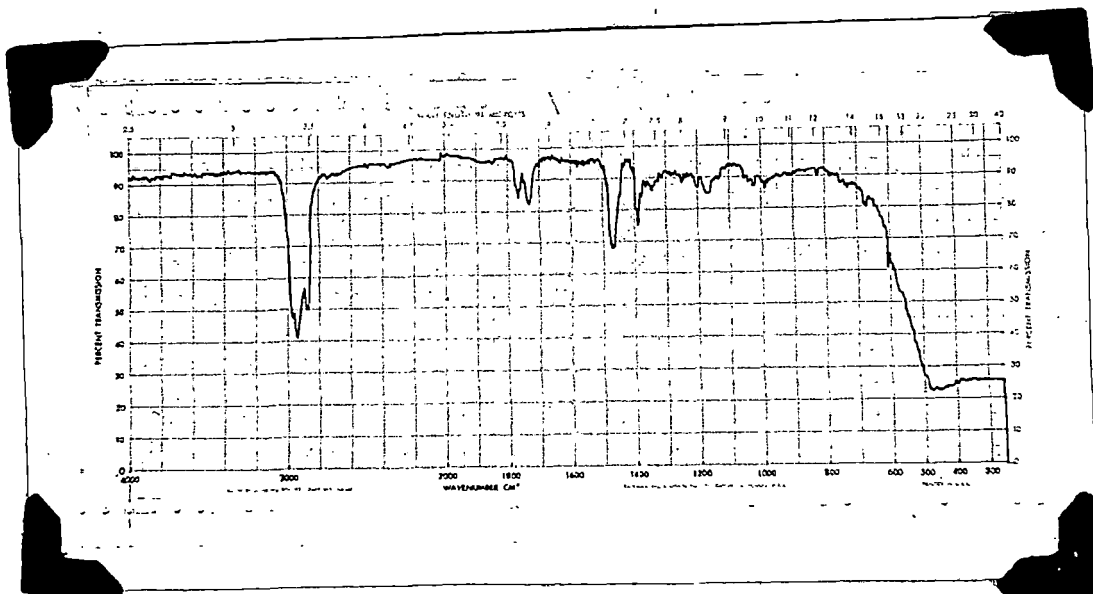


**Fig. 7:** IR spectrum of methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate 89 (Sample insufficient).

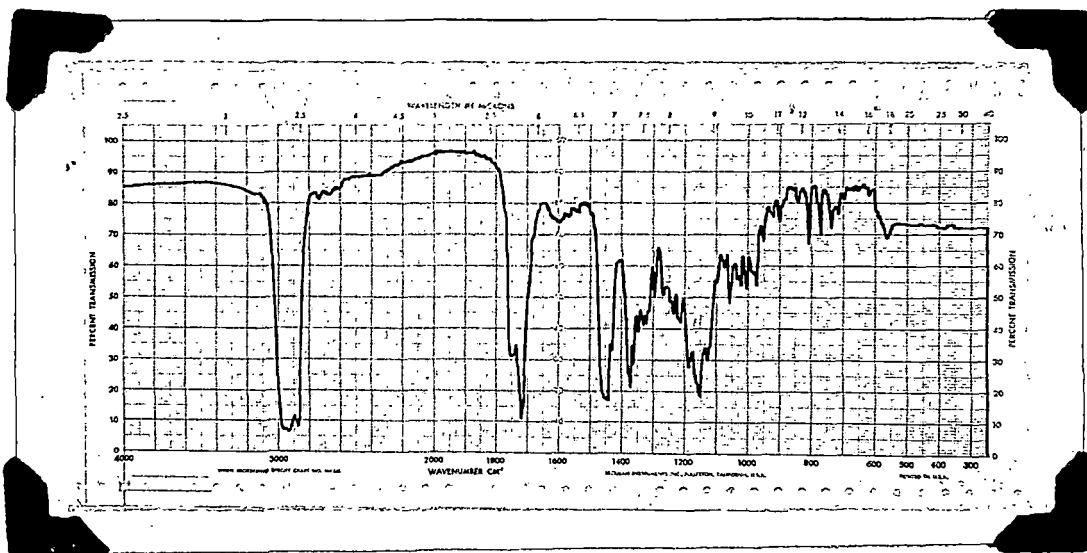


**Fig. 8:** IR spectrum of methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate 89

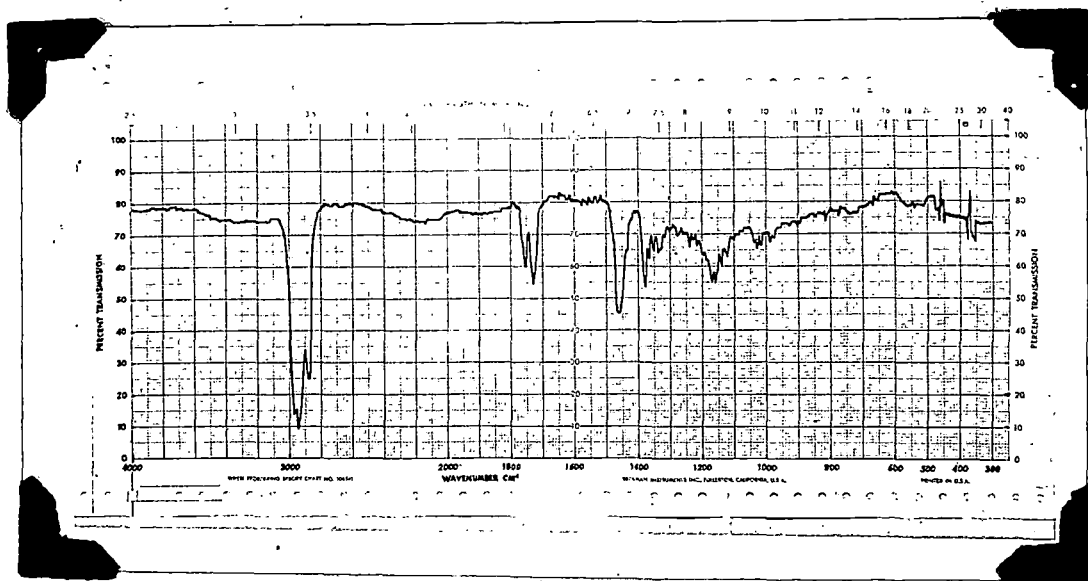


**Fig. 9 :** IR spectrum of methyl 2 $\beta$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate 90

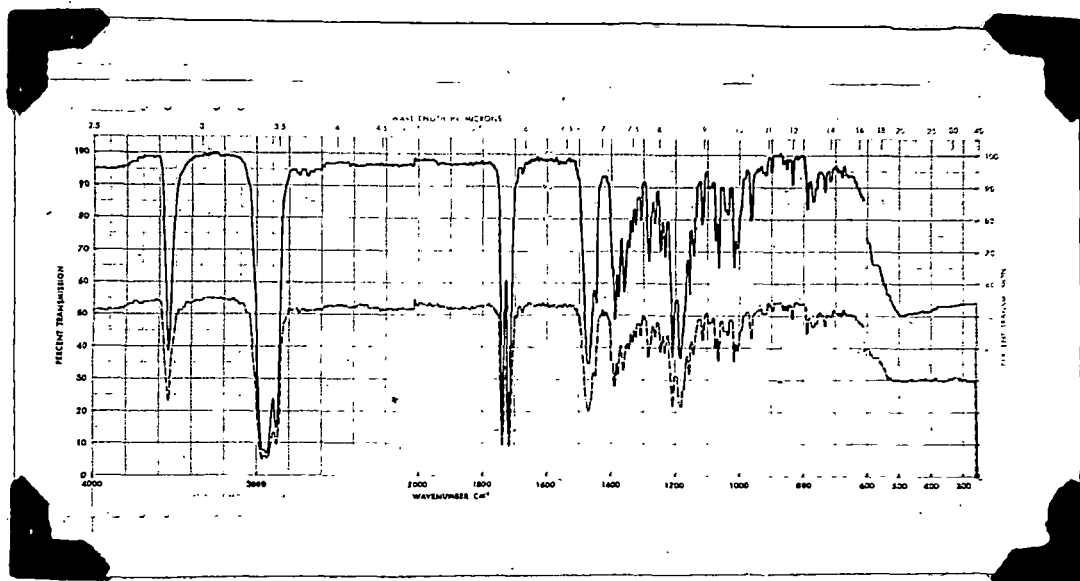


**Fig. 10 :** IR comparison of dimethyl dihydroceanothate 91 (solid line) with an authentic specimen (dotted line)

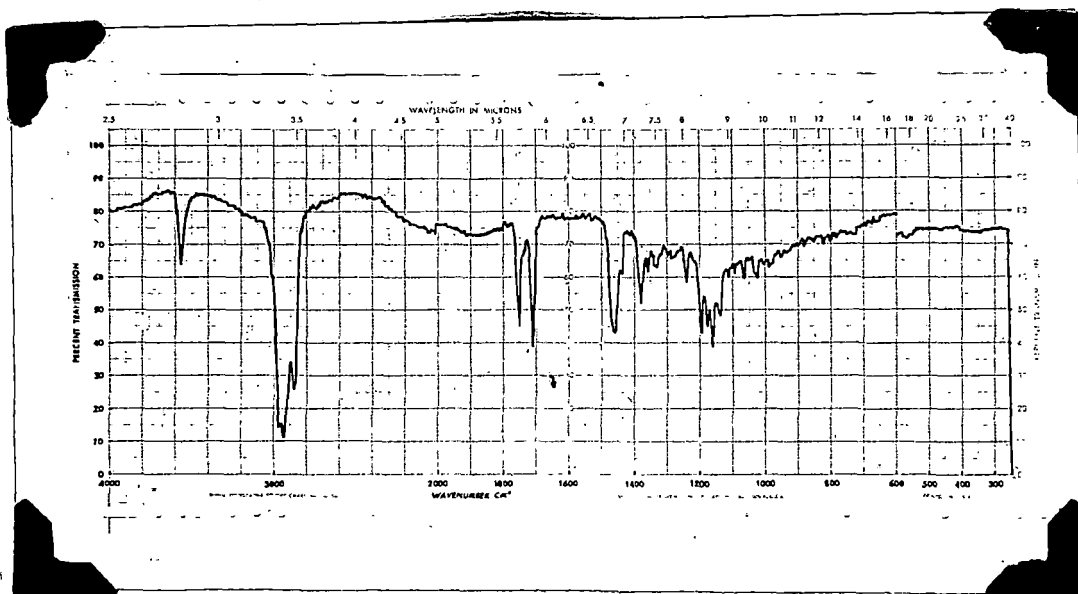


**Fig. 11:** IR spectrum of methyl 3 $\alpha$ -hydroxy-2 $\alpha$ -methoxycarbonyl-A(1)-norlupan-23-oate 92

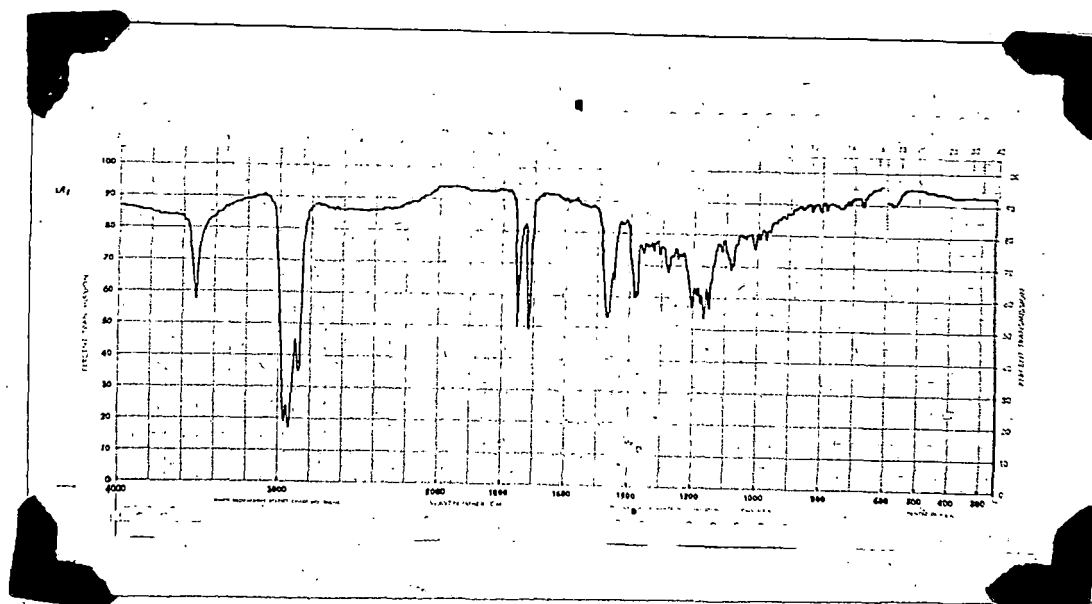


**Fig. 12:** IR spectrum of another epimer derived from  $\beta$ -ketoesters.

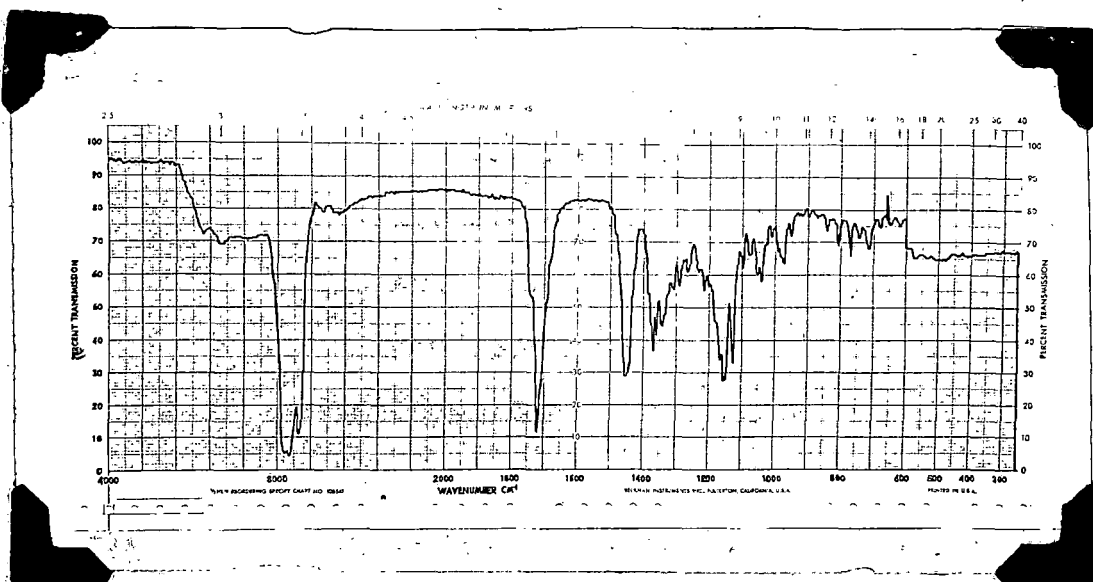


**Fig. 13:** IR spectrum of  $\alpha$ -hydroxy carboxylic acid 22

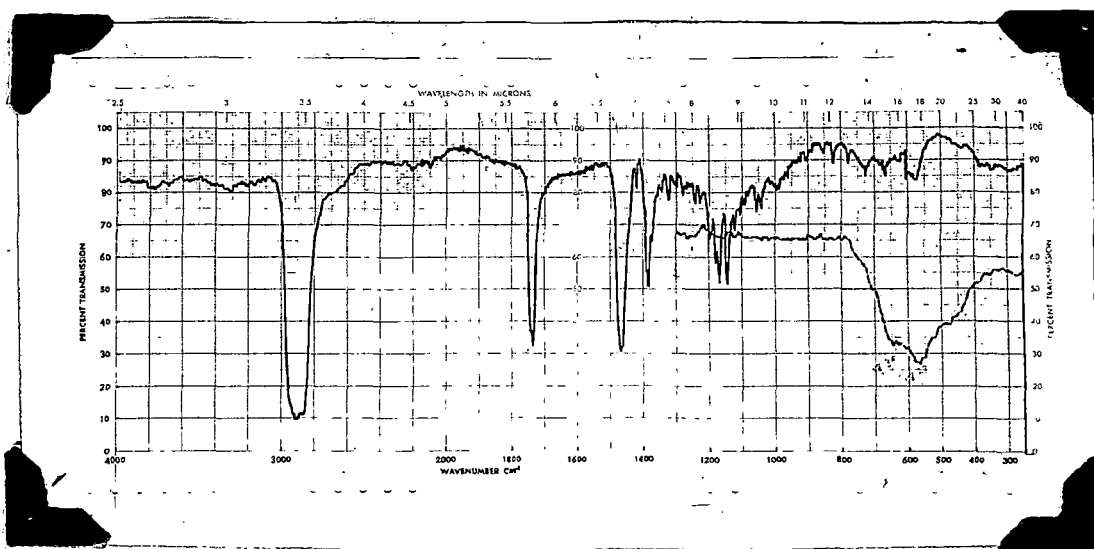


**Fig. 14:** IR spectrum of methyl decarboxydehydro-dihydroceanothate 24

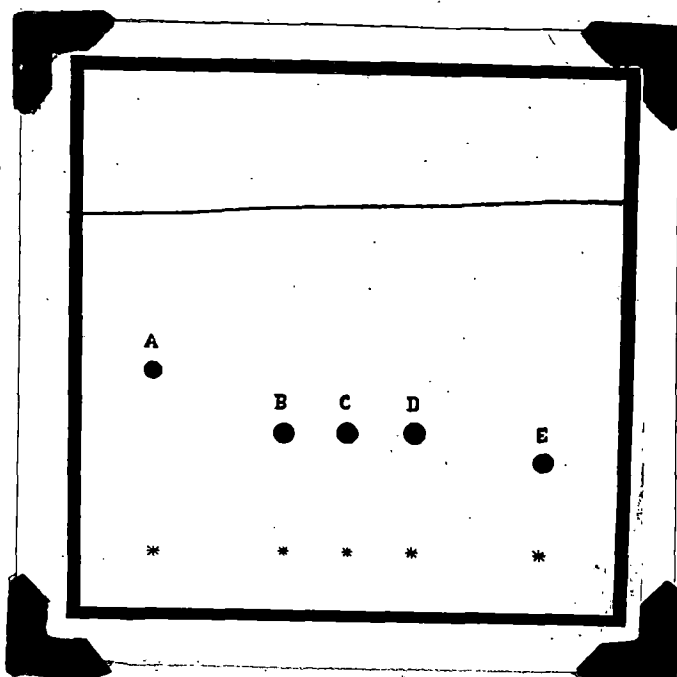


**Fig. 15 :** TLC of Dimethyl dihydroceanothate and its epimers on 12%  $\text{AgNO}_3$  impregnated Silica gel (Kieselgel G; E. Merck) Plate :

Solvent used : Benzene

Developing agent :  $\text{Ac}_2\text{O}-\text{H}_2\text{SO}_4$  (9:1)

- A : A compound having m.p.  $205-6^\circ$  (Rf 0.53)
- B : Dimethyl dihydroceanothate 91 (Rf 0.34)
- C : Authentic Dimethyl dihydroceanothate (Rf 0.34)
- D : Mixture of B and C (Rf 0.34)
- E : Methyl -  $3\alpha$ - hydroxy- $2\alpha$ -methoxycarbonyl-A(1)-norlupan-28-oate 92 (Rf 0.25)

### EXPERIMENTAL

Melting points are uncorrected. The petroleum used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution unless stated otherwise. NMR spectra were determined on Varian A-60 and HA-100 spectrophotometer using chloroform-d solution containing tetramethyl silane as reference. The IR spectra were recorded on Beckmann IR-20 spectrophotometer. UV absorption spectra were taken Ziess VSU-1 and UV Beckmann DU-2 spectrophotometers in 95% ethanol solution unless stated otherwise. TLC was done on chromatoplate of Silicagel G (E. Merck) and the spots were developed with  $H_2SO_4-Ac_2O$  (9:1) mixture.

#### Extraction of Bischofia javanica Blume ;

#### Isolation of betulinic acid 93 ;

Dried and powdered trunk bark of Bischofia javanica Blume (2 kg) was extracted with benzene in a Soxhlet apparatus for twenty hours. Benzene was distilled off and the gummy residue (9 gm) was taken up in ether (1 liter). The ether solution was washed with 10% aqueous sodium hydroxide solution (3x300 ml). The aqueous alkaline layer was thoroughly shaken with ether to remove any neutral material that might be present. The aqueous layer was acidified (1 liter) when some insoluble

solids separated out. The acidified portion was extracted with ether, washed with water till neutral and then dried ( $\text{Na}_2\text{SO}_4$ ) of betulinic acid 83. Ether was removed when a gummy residue (2gm) was obtained.

Esterification of betulinic acid 83 : Preparation of methyl betulinate 84 :

To the crude acid 83 (3gm) dissolved in ether (250 ml) was added a solution of diazomethane in ether prepared from nitrosomethyl urea (1.5gm) and was kept overnight. Next day excess of diazomethane was destroyed with acetic acid (1 ml). The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and then dried ( $\text{Na}_2\text{SO}_4$ ). Evaporation of the ether yielded a gummy residue (1.5gm). This crude ester (1.5gm) dissolved in benzene (12 ml) was placed over a column of alumina (100 gm, deactivated with 4 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents, (Table - III)

Table - III

| Eluent                      | Fractions<br>50 ml each | Residue on<br>evaporation     |
|-----------------------------|-------------------------|-------------------------------|
| Petroleum                   | 1 - 4                   | Oil                           |
| Petroleum;<br>benzene (4:1) | 5 - 8                   | Nil                           |
| Petroleum;<br>benzene (3:2) | 9 - 14                  | Solid (1.2gm)<br>m.p. 217-19° |

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

Solids obtained from the fractions 9-14, m.p. 217-19° (Table-III) were combined (1.2 gm), and crystallised from a mixture of chloroform and methanol to afford colourless needles of methyl betulinate 84, m.p. 223-24°,  $(\alpha)_D + 5^\circ$ , identical with authentic sample (m.m.p and IR)

Found : C, 78.79; H, 10.52%

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

UV : No absorption in the region 220-3000 m $\mu$

IR(nujol): 3560 (-OH), 1715(-COOCH<sub>3</sub>), 1660 and 680 cm<sup>-1</sup>

(= CH<sub>2</sub>)

Fig. 1

NMR:  $\delta$  4.8-4.9 (two doublets; = CH<sub>2</sub>),

$\delta$  3.75 (singlet - COOCH<sub>3</sub>),

$\delta$  2.01 (singlet, - CHOH),

$\delta$  1.75 (sharp singlet, H<sub>2</sub>C = C - ) and  
 $\begin{matrix} 1 \\ \text{CH}_3 \end{matrix}$

$\delta$  1.00 ( a tall singlet accounting for  
 15 protons, 5 CH<sub>3</sub>)

Hydrogenation of methyl betulinate 84 : Preparation of methyl dihydrobetulinate 85 :

Methyl betulinate (3 gm), dissolved in a mixture of ethyl acetate and acetic acid (100 ml each) was shaken in an



atmosphere of hydrogen in presence of  $\text{Pt} \cdot \text{O}_2$  catalyst (0.2 gm) for three hours until absorption of hydrogen ceased. Ethyl acetate was removed by distillation and the solution was diluted with water whereby a white solid (7.5gm) separated out which was collected by filtration. Crystallisation from a mixture of chloroform and methanol furnished colourless needles of 85, m.p.  $236-38^\circ$ . This compound 85 was found to be identical with an authentic sample of methyl dihydrobetulinate (m.m.p. and IR)

Found : C, 78.92; H, 11.23%

Calculated for  $\text{C}_{31}\text{H}_{52}\text{O}_3$ : C, 78.76; H, 11.09%

UV: transparent in the region 220 to 300  $\text{m}\mu$

IR: 3560 (OH), 1715  $\text{cm}^{-1}$  ( $\text{CO}_2\text{Me}$ ) Fig-2

Jones oxidation of methyl dihydrobetulinate 85 : Preparation of methyl dihydrobetulonate 81 :

To a solution of methyl dihydrobetulinate 85 (7gm) in pure acetone ( 1 liter ) was added Jones reagent dropwise with shaking until a faint orange colour persisted. The mixture was kept at room temperature for 1 hour, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried ( $\text{Na}_2\text{SO}_4$ ) and the ether evaporated. The residue (5.9gm) dissolved in benzene (15ml) was chromatographed

over a column of active alumina (360 gm). The chromatogram was developed with petroleum and then eluted with the following solvents (Table - IV)

Table - IV

| Eluent                      | Fractions<br>50 ml each. | Residue on<br>evaporation       |
|-----------------------------|--------------------------|---------------------------------|
| Petroleum                   | 1 - 4                    | Nil                             |
| Petroleum;<br>benzene (4:1) | 5-25                     | Solid, (5.7 gm)<br>m.p. 197-99° |

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

Fractions 5-25 (5.7 gm) were combined and on crystallisation from methanol furnished needle shaped crystals of 81, m.p. 202-4°, ( $\alpha$ )<sub>D</sub> + 8°, identical with an authentic sample of methyl dihydrobetulonate (m.m.p and IR comparison)

Found : C, 79.32; H, 10.86%

Calculated for C<sub>31</sub>H<sub>50</sub>O<sub>3</sub>: C, 79.10; H, 10.71%

IR : 1730 (CO<sub>2</sub>Me), 1708 cm<sup>-1</sup> (CO).

Fig - 3

Autoxidation of methyl dihydrobetulonate 81 : Preparation of diosphenol 86 :

Methyl dihydrobetulonate 81 (2 gm) suspended in potassium tertiary butoxide in tertiary butanol (prepared from 6 gm potassium and 160 ml of tertiary butanol) was shaken in a stream of oxygen for 75 minutes. The reaction mixture was diluted with water and then 6 N hydrochloric acid was added till the solution was acidic. It was then extracted with chloroform (150 ml), washed with water till neutral and dried ( $\text{Na}_2\text{SO}_4$ ). On removal of the solvent under reduced pressure, a yellowish gummy residue (1.8 gm) was obtained. This residue dissolved in benzene (10 ml) was placed over a column of alumina (120 gm, deactivated with 4.8 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - V)

Table - V

| Eluent                      | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------------------------|-------------------------|--------------------------------|
| Petroleum                   | 1 - 4                   | Nil                            |
| Petroleum;<br>benzene (4:1) | 5 - 15                  | Solid (1.6 gm)<br>m.p. 126-29° |

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

forms of the diketone 86A and diosphenol 86B.

Found :

Calculated for  $C_{31}H_{48}O_4$  :

UV :  $\lambda_{\text{max}}$  269 m $\mu$  (E, 7532)

IR : 3460 (OH), 1730 (CO<sub>2</sub>Me), 1670

1650 860  $\text{cm}^{-1}$  Fig-4

DIR : 7) ~~XXXXXXXXXXXXXX~~

Hydrogen peroxide oxidation of diosphenol 86 : Preparation of  
seco A - acid 87 :

Hydrogen peroxide (100 vol; 6 ml) mixed with 10% potassium hydroxide solution (12 ml) were added in three equal portions at intervals of 15 minutes to a solution of diosphenol 86 (700 mg) in ethanol (25 ml) in refluxing condition. After the mixture had been kept at 20° for 2 hours it was diluted with water, extracted with ether and washed with water till neutral. The aqueous portion was acidified with 6 N hydrochloric acid.

and extracted with ether and washed with water till neutral and dried ( $\text{Na}_2\text{SO}_4$ ). On evaporation of the solvent it gave a solid residue (550 mg). Crystallisation of the product from a mixture of benzene - petroleum afforded colourless needles of 87, m.p.  $175-7^\circ$ .

Found : C, 72.06 ; H, 9.43%

Calculated for  $\text{C}_{21}\text{H}_{50}\text{O}_6$  : C, 71.78 ; H, 9.72%

IR : 1710 and  $1690\text{ cm}^{-1}$  (COOH) Fig - 5

Esterification of seco A-acid 87 :

Preparation of trimethyl ester 88 :

The above acid 87 (500 mg) dissolved in ether (50 ml) was added a solution of diazomethane in ether prepared from nitrosomethylurea (500 mg) and was kept overnight. Next day excess of diazomethane was destroyed with acetic acid (1 ml). The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and then dried ( $\text{Na}_2\text{SO}_4$ ). Evaporation of the solvent yielded a gummy residue (480 mg). This product dissolved in benzene (5ml) was placed over a column of alumina (30 gm, deactivated with 1.2 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - VI)

Table - VI

| Eluent                       | Fractions<br>50 ml each | Residue on<br>evaporation        |
|------------------------------|-------------------------|----------------------------------|
| Petroleum                    | 1 - 3                   | Nil                              |
| Petroleum ;<br>benzene (4:1) | 4 - 12                  | Solid (450 mg)<br>m.p. 145 - 46° |

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

Fractions 4 -12 (450mg) were combined and on crystallisation from methanol afforded colourless needles of 88, m.p. 146-47°, ~~(88)~~ having identical m.p., ~~rotation~~ and IR spectra reported in the literature for the trimethyl ester<sup>312</sup> of the seco-A acid.

Found : C, 72.32; H, 9.97%

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

IR : 1745, 1725  $cm^{-1}$  ( $Co_2Me$ )

Fig - 6

Dieckmann condensation of trimethyl ester 88 : Preparation of methyl 2 $\alpha$ -methoxy carbonyl-3-oxo-A(1)-norlupan-28-oate 89 and methyl 2 $\beta$ -methoxy carbonyl-3-oxo-A(1)-norlupan-28-oate 90:

Potassium (0.1 gm, 2.6 mg atom) was added to 10 ml of tertiary butanol and 10 ml of benzene (solvents dried for 24 - hours over calcium hydride) under nitrogen blanket and after all the potassium had reacted, a solution of 273 mg (0.5 m.mol) of the seco ester 88 in 10 ml of benzene was added with a syringe with stirring. The solution was then boiled under reflux (nitrogen atmosphere) with a Dean-Stark trap for 24-hours, during which time 10 ml of solvent was removed. The orange coloured solution was cooled, diluted with water, acidified with 6N hydrochloric acid and extracted with ether. The organic layer was washed with water, 5% sodium bicarbonate and water to neutrality and dried ( $\text{Na}_2\text{SO}_4$ ). Evaporation of the solvent gave a gummy residue (200 mg). The product dissolved in benzene (5 ml) was placed over a column of alumina (20 gm, deactivated with 0.8 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - VII)

Table - VII

| Eluent                      | Fractions<br>50 ml each | Residue on<br>evaporation    |
|-----------------------------|-------------------------|------------------------------|
| Petroleum                   | 1 - 2                   | Nil                          |
| Petroleum;<br>benzene (9:1) | 3 - 4                   | Nil                          |
| Petroleum;<br>benzene (4:1) | 5 - 6                   | Nil                          |
| Petroleum;<br>benzene (7:3) | 7 - 8                   | Nil                          |
| Petroleum;<br>benzene (3:2) | 9 - 11                  | Solid (170 mg), m.p. 190-92° |
|                             | 12 - 13                 | Solid (10 mg); m.p. 174-75°  |

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

Fractions 9 - 11 (170 mg) were combined and on crystallisation from methanol afforded colourless needles of 89, m.p. 191-93°, ( $\alpha$ )<sub>D</sub> 89°, having identical m.p., rotation and IR spectra reported in the literature for dimethyl dehydrodihydroceanothate.<sup>21</sup>



Found : C, 74.69; H, 9.73%

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

IR : 1755 (cyclopentanone), 1725  $cm^{-1}$  ( $CO_2Me$ ) Fig - 7 and

~~IR~~  
(Sample taken was insufficient)

1750 (cyclopentanone), 1720  $cm^{-1}$  ( $CO_2Me$ ) Fig - 8

Spectrum recorded for the compound obtained from the mother-liquor of the above sample.

Fractions 12-13 (10 mg) were combined and on crystallisation from methanol furnished needle shaped crystals of 90, m.p. 175-77°, ( $\alpha$ )<sub>D</sub> + 42°, having similar m.p., rotation and IR spectra reported in the literature for methyl 2 $\beta$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate.

Found : C, 74.53; H, 9.92%

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

IR : 1750 (cyclopentanone), 1730 ( $CO_2Me$ ) Fig - 9

Sodium borohydride reduction of dimethyl dehydrodihydroceanothate

[methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate] 89 :

Preparation of dimethyl dihydroceanothate [methyl 3 $\beta$ -hydroxy-2 $\alpha$ -methoxycarbonyl-A(1)-norlupan-28-oate] 91 and its epimer 92 :

Sodium borohydride (70 mg) was added to a solution of dimethyl dehydrodihydroceanothate 89 (70 mg) in methanol (20 ml).

and dioxan (20 ml), 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 then extracted with ether. The ethereal layer was washed till neutral and dried ( $\text{Na}_2\text{SO}_4$ ). On removal of the solvent, a gummy residue (70 mg) was obtained. The product dissolved in benzene (3 ml) was placed over a column of alumina (10 gm, deactivated with 0.4 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents. (Table - VIII)

Table - VIII

| Eluent        | Fractions   | Residue on                  |
|---------------|-------------|-----------------------------|
|               | 50 ml. each | evaporation                 |
| Petroleum     | 1 - 2       | Nil                         |
| Petroleum :   | 3 - 4       | Nil                         |
| Benzene (9:1) |             |                             |
| Petroleum :   | 5 - 6       | Nil                         |
| benzene (4:1) |             |                             |
| Petroleum :   | 7 - 8       | Nil                         |
| benzene (7:3) |             |                             |
| Petroleum :   | 9 - 10      | Nil                         |
| benzene (3:2) |             |                             |
| Petroleum :   | 11- 12      | Nil                         |
| benzene (1:1) |             |                             |
| Petroleum :   | 13-19       | Solid (50 mg)               |
| benzene (2:3) |             | ( Mixture of two compounds) |

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

Fractions 13-19 (50 mg) were subjected to TLC separately and the first five fractions 13-17 showed two spots on chromatoplate indicating the mixture of two compounds and the last two fractions were homogeneous. However, all the fractions 13-19 were combined and rechromatographed through a column of alumina (20 gm) deactivated with 0.8 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - IX)

Table - IX

| Eluent                       | Fraction<br>50 ml each | Residue on<br>evaporation  |
|------------------------------|------------------------|----------------------------|
| Petroleum                    | 1 - 2                  | Nil                        |
| Petroleum :<br>benzene (9:1) | 3 - 4                  | Nil                        |
| Petroleum :<br>benzene (4:1) | 5 - 6                  | Nil                        |
| Petroleum :<br>benzene (7:3) | 7 - 8                  | Nil                        |
| Petroleum :<br>benzene (3:2) | 9 - 10                 | Nil                        |
| Petroleum :<br>benzene (1:1) | 11-12                  | Nil                        |
| Petroleum :                  | 13-14                  | Solid (10mg), m.p. 257-59° |
| benzene (2:3)                | 15-20                  | Solid (30mg), m.p. 138-40° |

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

Fractions 13-14 (10 mg) were combined and on crystallisation from methanol afforded colourless needles of 91, m.p. 261-63°, ( $\alpha$ )<sub>D</sub> + 22°. The compound was found to be identical with an authentic sample of dimethyl dihydroceanothate [methyl-3 $\beta$ -hydroxy-2 $\alpha$ -methoxycarbonyl-A(1) norlupan-28-oate] supplied by de Mayo and Starrett (m.m.p.) IR and co- TLC)

|                                                                 |                                                                                                         |                 |
|-----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-----------------|
| Found :                                                         | C, 74.32; H, 10.22%                                                                                     |                 |
| Calculated for C <sub>32</sub> H <sub>52</sub> O <sub>5</sub> : | C, 74.38; H, 10.14%                                                                                     |                 |
| IR :                                                            | 3540 (OH), 1730 (CO <sub>2</sub> Me), 1710 <sup>cm<sup>-1</sup></sup> <sub>^</sub> (CO <sub>2</sub> Me) | <u>Fig - 10</u> |
| TLC :                                                           | Rf 0.34                                                                                                 | <u>Fig - 15</u> |

Fractions 15-20 (30 mg) were combined and on crystallisation from methanol gave crystals, m.p. 140-42°, believed to be an epimer of dimethyl dihydroceanothate - methyl 3 $\alpha$ -hydroxy-2 $\alpha$ -methoxycarbonyl-A(1)-norlupan-28-oate 92. Further studies for establishing its stereochemistry by NMR could not be done for want of sufficient material.

|                                                                 |                                                                                                         |                 |
|-----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-----------------|
| Found :                                                         | C, 74.57; H, 10.32%                                                                                     |                 |
| Calculated for C <sub>32</sub> H <sub>52</sub> O <sub>5</sub> : | C, 74.38; H, 10.14%                                                                                     |                 |
| IR:                                                             | 3560 (OH), 1745 (CO <sub>2</sub> Me), 1705 <sup>cm<sup>-1</sup></sup> <sub>^</sub> (CO <sub>2</sub> Me) | <u>Fig - 11</u> |
| TLC :                                                           | Rf 0.25                                                                                                 | <u>Fig - 15</u> |

sodium borohydride reduction could not be performed on methyl 2 $\beta$ -methoxycarbonyl-3-oxo- $\Delta$ (1)-norlupan-28-oate 90 due to paucity of sample.

Dieckmann condensation of trimethyl ester 88 :

Trimethyl ester 88 (273 mg) was subjected to Dieckmann condensation using the procedure described above (page 57 ). The reaction mixture after usual work up gave a gummy residue (220 mg). The product dissolved in benzene was placed over a column of silica gel (25 gm) (Merck; Kieselgel-60) developed with petroleum and was eluted with the following solvents, (Table - X).

Table - X

| Eluent                        | Fractions<br>50 ml each | Residue on<br>evaporation |
|-------------------------------|-------------------------|---------------------------|
| Petroleum                     | 1 - 2                   | Nil                       |
| Petroleum ;<br>benzene (4:1)  | 3 - 4                   | Nil                       |
| Petroleum ;<br>benzene (3:2)  | 5 - 6                   | Nil                       |
| Petroleum ;<br>benzene (2 :3) | 7 - 8                   | Nil                       |
| Petroleum ;<br>benzene (1:4)  | 9 - 10                  | Nil                       |
| Benzene                       | 11-12                   | Nil                       |
| Benzene ;<br>Ether (4:1)      | 13-14                   | Semisolid (190 mg)        |

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



Fractions 13-14 (19 mg) were combined but this material could not be induced to crystallisation. The product showed two spots on chromatoplate indicating the presence of a mixture of two epimers 89 and 90.

Sodium borohydride reduction of the above mixture obtained in the Dieckmann condensation reaction ( page 65 ) :

The Dieckmann Condensation product (mixture of 89 and 90)  
(150 mg) was reduced with sodium borohydride<sup>(150mg)</sup> following the procedure described before ( page ). The reaction mixture after usual work up gave a gummy residue (125 mg). This product dissolved in benzene was placed over a column of alumina (20 gm, deactivated with 0.3 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - XI).

Table - XI

| Eluent                       | Fractions<br>50 ml each | Residue on<br>evaporation                   |
|------------------------------|-------------------------|---------------------------------------------|
| Petroleum                    | 1 - 2                   | Nil                                         |
| Petroleum :<br>benzene (4:1) | 3 - 4                   | Nil                                         |
| Petroleum :<br>benzene (3:2) | 5 - 6                   | Nil                                         |
| Petroleum :<br>benzene (2:3) | 7 - 8                   | Solid (40 mg) .m.p. 203-4°                  |
|                              | 9 - 10                  | Nil                                         |
|                              | 11-17                   | Solid (60 mg)<br>(Mixture of two compounds) |

Further elution with more polar solvent did not  
(~~Mixture of two compounds~~) yield any solid material.

Fractions 7-8 (40 mg) were combined and on crystallisation from acetone afforded colourless needles, m.p.  $205-6^{\circ}$ ,

|                                    |                                                             |                 |
|------------------------------------|-------------------------------------------------------------|-----------------|
| Found :                            | C, 74.28; H, 10.27%                                         |                 |
| Calculated for $C_{32}H_{52}O_5$ : | C, 74.38; H, 10.14%                                         |                 |
| IR :                               | 3520 (OH), 1740 ( $CO_2Me$ ), $1705_{cm^{-1}}$ ( $CO_2Me$ ) | <u>Fig - 12</u> |
| TLC :                              | Rf 0.53                                                     | <u>Fig - 15</u> |
| (homogeneous)                      |                                                             |                 |

Fractions 11-17 (60 mg) were subjected to TLC separately and first five fractions 11-15 showed two spots on chromatoplate indicating the mixture of two compounds and the last two fractions 16-17 were homogeneous. However, all the fractions 11-17 were combined and rechromatographed over a column of alumina (20 gm, deactivated with 0.8 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - XII).

Table - XII

| Eluent                       | Fractions<br>50 ml. each | Residue on<br>evaporation   |
|------------------------------|--------------------------|-----------------------------|
| Petroleum                    | 1 - 2                    | Nil                         |
| Petroleum ;<br>benzene (3:2) | 3 - 4                    | Nil                         |
| Petroleum ;<br>benzene (1:1) | 5 - 6                    | Nil                         |
| Petroleum ;                  | 7 - 8                    | Solid (12 mg), m.p. 258-60° |
| benzene (2:3)                | 9 - 12                   | Solid (35 mg), m.p. 139-41° |

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

Fractions 7-8 (12 mg) on crystallisation gave a product, m.p. 261-63° identical with a sample of methyl dihydroceanothate. Fractions 9-12 (35 mg) on crystallisation from methanol gave crystals m.p. 140-42°, identical with the product obtained before in the sodium borohydride reduction of the  $\beta$ -keto ester 89. (Page 59 )

Benzilic acid rearrangement of diosphenol 86 : Preparation of  $\alpha$ -hydroxy - carboxylic acid 93 :

The diosphenol 86 (500 mg) in ethanol (15 ml) was heated under reflux with 10% alcoholic sodium hydroxide (10 ml) for 5 hours. Most of the ethanol was removed under reduced pressure. The reaction mixture was cooled, diluted with water and then acidified with 6N hydrochloric acid. This acidified portion was taken up in ether and the ethereal solution was extracted with 1% sodium bicarbonate solution. The alkaline extract was acidified with 6N hydrochloric acid and extracted with ether and then with ethyl acetate. The combined extracts afforded a residue (425 mg) which was further purified by dissolution in 2% sodium bicarbonate solution followed by filtration, acidification and extraction. The resulting product (300 mg) on crystallisation from a mixture of chloroform and methanol furnished colourless needles of 93, m.p. 255-56°.

Found : C, 74.23; H, 9.88%

Calculated for  $C_{31}H_{50}O_5$  : C, 74.06; H, 10.02%

IR : 3440, 3340, 1720  $cm^{-1}$

Fig - 13

Lead dioxide oxidation of  $\alpha$  - hydroxy carboxylic acid 93 :

Preparation of methyl decarboxydehydrodihydroceanothate 94 :

$\alpha$ - Hydroxy carboxylic acid 93 (1 gm) and Lead dioxide (1.0 gm) were suspended in glacial acetic acid (7 ml). The mixture was heated under mild reflux for 3-hours with occasional stirring. The excess of lead dioxide was filtered and the clear, straw-colored liquid was diluted with water. This aqueous portion was extracted with ether, washed with 10% sodium bicarbonate solution, washed with water till neutral and then dried ( $Na_2SO_4$ ). On removal of the solvent a solid residue (0.75 gm) was obtained which on crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals of 94, m.p. 179-80°, ( $\alpha$ )<sub>D</sub> 75.4°. This compound was found to be identical in all respect with the sample prepared by de Mayo and Starratt<sup>5</sup>.

Found : C, 78.96; H, 10.48%

Calculated for  $C_{30}H_{48}O_3$  : C, 78.90; H, 10.59%

IR : 1735  $cm^{-1}$  (unresolved band for cyclopentanone and ester)

On expansion : Two peaks at 1734 (cyclopentanone) Fig-14

and 1728  $cm^{-1}$  ( $CO_2Me$ )

Attempted carbomethoxylation of methyl decarboxydehydro-  
dihydroceanothate 94 using sodium methoxide :

A solution of 94 (456 mg, 1m mol) in dry benzene (3 ml) was added dropwise over 3-hour under nitrogen to a stirred mixture of .054 gm sodium methoxide (1 m.mol. prepared from .023 gm sodium and methanol in anhydrous condition) and 0.23 gm dimethyl carbonate in 5 ml dry benzene in a mild refluxing condition. After the addition of the ketone, the mixture was stirred for a further 2-hour in a refluxing condition. The reaction mixture was cooled in an ice-water bath and acidified with slight excess of aqueous acetic acid. The solvent was removed under reduced pressure and the residue was diluted with water and extracted with ether. The ether extract was washed with aqueous sodium bicarbonate to remove acetic acid, then with saturated brine, dried ( $\text{Na}_2\text{SO}_4$ ) and freed from ether. A solid residue (400 mg) was obtained. This product on crystallisation from a mixture of chloroform and methanol furnished colourless needles, of m.p.  $179-80^\circ$ . This compound was found to be identical with the starting material (m.m.p and IR comparison). Carbomethoxylation was also attempted using different solvents such as dioxane, toluene and dimethyl carbonate itself but in every case the reaction gave the same result.

Attempted carbomethoxylation<sup>53</sup> of methyl decarboxy dehydro-  
dihydroceanothate 94 using potassium tert butoxide:

A solution of 0.3 gm (.0006 gm mol) of the ketone 94 in 0.5 ml tert. butanol was added under nitrogen to a mixture of 0.73 gm potassium tert.butoxide (.0065 gm. mol; prepared from 0.5 ml tert.butanol and 0.25 gm potassium in anhydrous condition) and 1 gm dimethyl carbonate. The whole solution was refluxed under nitrogen for 24-hours. The reaction mixture was cooled with an ice-water bath and acidified with a slight excess of acetic acid. The solvent was removed in vacuo and the residue was diluted with water and extracted with ether. The ether extract was washed with aqueous sodium bicarbonate to remove acetic acid, then with saturated brine, dried ( $\text{Na}_2\text{SO}_4$ ) and solvent removed. A solid residue (250 mg) was obtained which on crystallisation from a mixture of chloroform and methanol gave crystals, m.p.179-80°, identical in all respect with the starting material (m.m.p and IR)

Attempted carbomethoxylation<sup>53</sup> of methyl decarboxy  
dehydrodihydroceanothate using sodium hydride :

A solution of 0.5 gm of the ketone 94 in 30 ml benzene was added, under nitrogen, to a refluxing mixture of 0.15 gm sodium hydride and 1 gm of dimethyl carbonate. The rate of addition of the benzene solution of the compound was so



adjusted that it corresponded to the rate of distillation by a distilling arrangement. This procedure was continued for 3-hours. The resulting slurry was cooled in an ice-water bath and acidified with a slight excess of aqueous acetic acid. The solvent was removed under vacuo and the residue was diluted with water and extracted with ether. The ether extract was washed with aqueous sodium bicarbonate to remove acetic acid, then with saturated brine, dried ( $\text{Na}_2\text{SO}_4$ ). Evaporation of ether gave a solid residue (465 mg) which on crystallisation from a mixture of chloroform and methanol gave crystals, m.p.  $179-80^\circ$ , identical in all respect with the starting material (m.m.p and IR). This reaction was also attempted in dry dimethylsulfoxide as well as in dimethylcarbonate alone but in each case the same result was obtained.

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PART - II

AN UNAMBIGUOUS PARTIAL SYNTHESIS OF METHYL  
DIHYDROALPHITOLATE FROM BETULINIC ACID.



PART - II

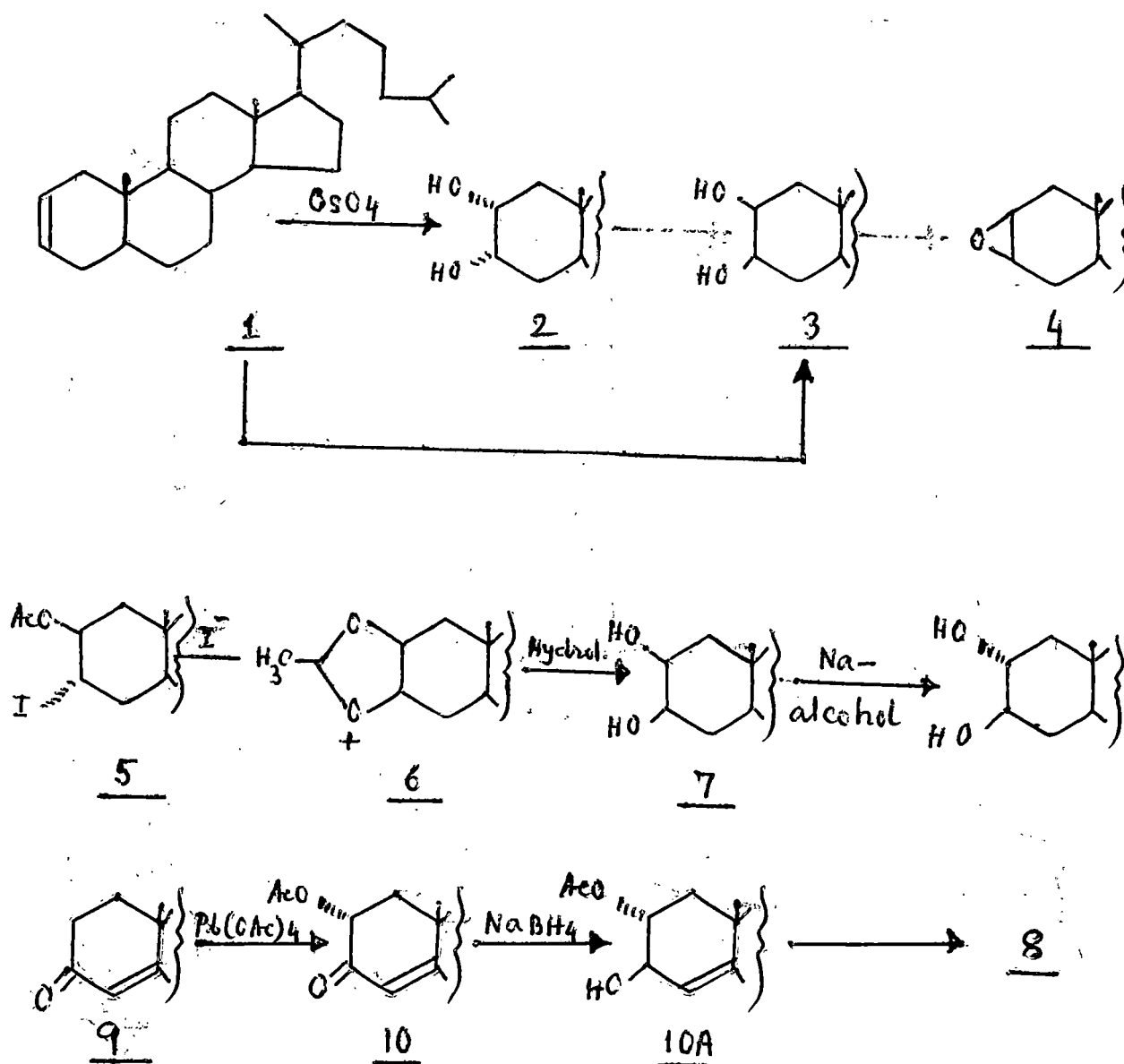
CHAPTER - I

A short review of synthesis of isomeric 2,3-diols  
of triterpenoids ;

Section A : Introduction:

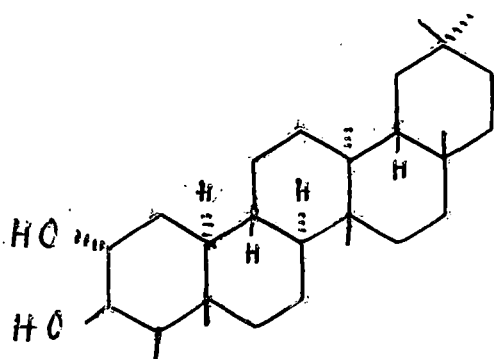
In the course of elucidation of the configurations of sapogenins containing a 2,3-diol system, methods were developed for production of the four possible isomers in this series. The four cholestane 2,3-diols have also been prepared by the methods developed in this connection<sup>1,2</sup>.  $\Delta^2$ -Cholestene 1 on reaction with osmium tetroxide gives the  $2\alpha,3\alpha$ -diol 2 whereas peracetic acid oxidation and subsequent hydrolysis affords  $2\beta,3\alpha$ -diol<sup>1-3</sup> 3.  $2\alpha,3\alpha$ -or  $2\beta,3\beta$ -oxido-cholestanes 4 on diaxial opening also gives the same diol 3. The  $2\beta,3\beta$ -diol 7 has also been prepared<sup>1,2</sup> according to the procedure of Winstein and Buckles<sup>4</sup> by treatment of  $\Delta^2$ -cholestene 1 with silver acetate, iodine and moist acetic acid. The reaction probably involves formation of a cyclic  $2\alpha,3\beta$ -iodonium ion which on acetolysis with inversion at C - 2 gives 5. Expulsion of iodide ion with inversion at C - 3 forms a resonant oxonium - carbonium ion 6 which leads to a mono-acetate which on hydrolysis gives 7. As the diol 7 contains one axial substituent at C - 2, it is epimerized by treatment with sodium

in ethanol at  $180^{\circ}$  to the diequatorial  $2\alpha, 3\beta$ -diol 8. Diol 8 was also obtained from cholestenone 9 which reacts with lead-tetracetate to give in about 10% yield a product 10 having  $2\alpha$ -acetoxy group<sup>5,6</sup>. Reduction of 10 with sodium borohydride followed by hydrogenation gives the  $2\alpha, 3\beta$ -diol 8.

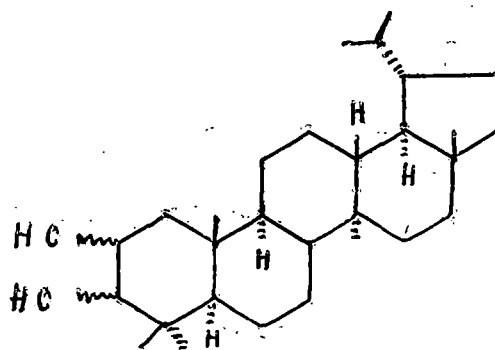


Section B : Synthesis of 2,3-diols of lupane and friedelane series:

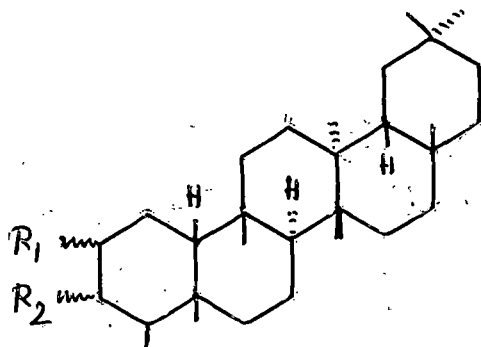
McGinnis<sup>7</sup> et al have reported the preparation of all the four possible stereoisomeric lupane 2,3-diols 12. Samson et al<sup>8</sup> have synthesised  $2\alpha, 3\alpha$ -13 A;  $2\beta, 3\beta$ -13 B;  $2\alpha, 3\beta$ -13 C friedelane diols and shown that the last named isomer is identical with natural pachysandiol A<sup>9</sup> 11.



11



12 (A,B,C,D)



13 A

$R_1 = R_2 = \alpha\text{-OH}$

13 B

$R_1 = R_2 = \beta\text{-OH}$

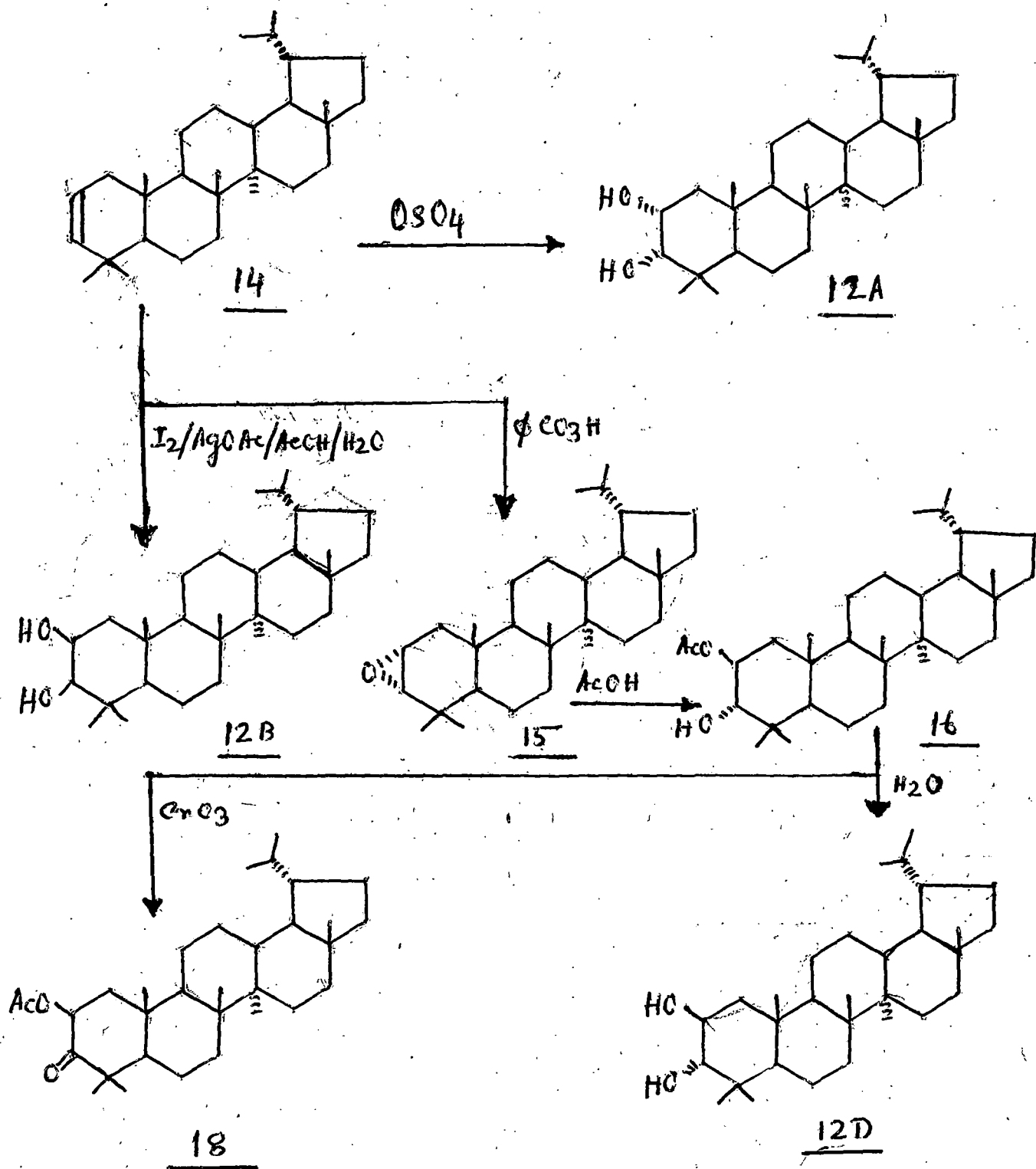
13 C

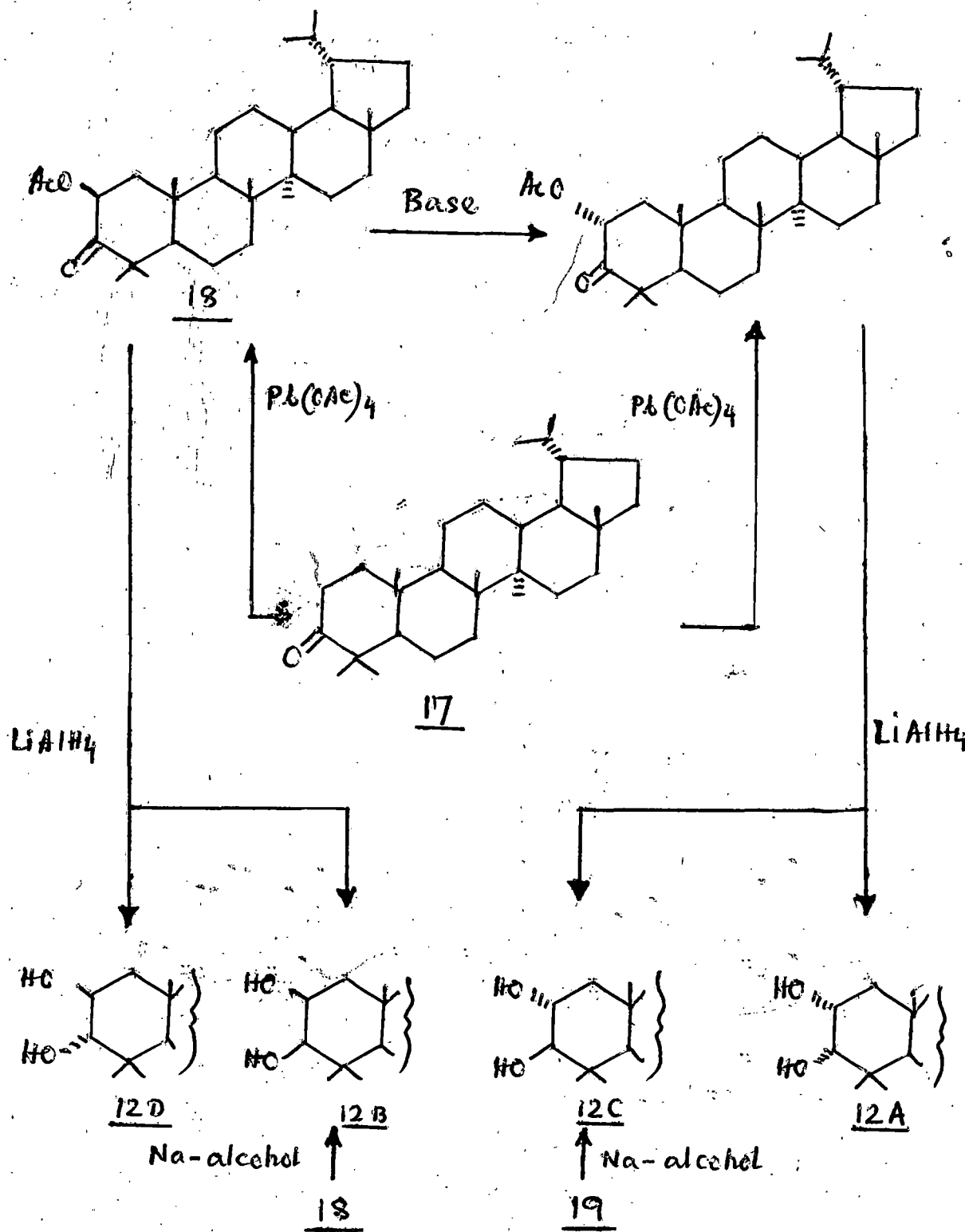
$R = \alpha\text{-OH} ; R_2 = \beta\text{-OH}$

Synthesis of Lupane-2,3-diols<sup>7</sup> 12 (Chart I)

Lup-2-ene 14 was converted to lupane-2 $\alpha$ ,3 $\alpha$ -diol 12 A, lupane-2 $\beta$ ,3 $\beta$ -diol 12 B and lupane-2 $\alpha$ ,3 $\alpha$ -epoxides 15 by the action of osmium tetroxide, iodine-silveracetate-acetic acid and perbenzoic acid respectively. The epoxide 15 was opened with acetic acid to give 2 $\beta$ -acetoxy-lupane-3 $\alpha$ -ol 16 which on mild alkaline hydrolysis yielded the diaxial trans diol, lupane 2 $\beta$ ,3 $\alpha$ -diol 12 D. In order to increase the poor yield of the 2 $\beta$ ,3 $\beta$ -diol 12 B obtained by the above procedure and also to prepare the remaining isomer, lupane 2 $\alpha$ ,3 $\beta$ -diol 12 C, lupane-3-one 17 was treated with lead tetracetate to give 2 $\beta$ -acetoxy-lupane-3-one 18 and 2 $\alpha$ -acetoxy-lupane-3-one 19 as the major and minor products respectively. The 2 $\beta$ -acetoxy isomer 18 could also be prepared by the chromic acid oxidation of 2 $\beta$ -acetoxy-lupane-3 $\alpha$ -ol 16. This acetoxy ketone 18 upon equilibration with base furnished the 2 $\alpha$ -acetoxy-3-ketone 19 which was then reduced with lithium aluminium hydride mainly to lupane-2 $\alpha$ ,3 $\alpha$ -diol 12 A and with sodium and isopropanol to lupane-2 $\alpha$ ,3 $\beta$ -diol 12 C. The 2 $\beta$ -acetoxy-3-ketone 18 was similarly reduced with lithium aluminium hydride to give the 2 $\beta$ ,3 $\alpha$ -diol 12 D as the main product and with sodium and isopropanol to the 2 $\beta$ ,3 $\beta$ -diol 12 B.

CHART I

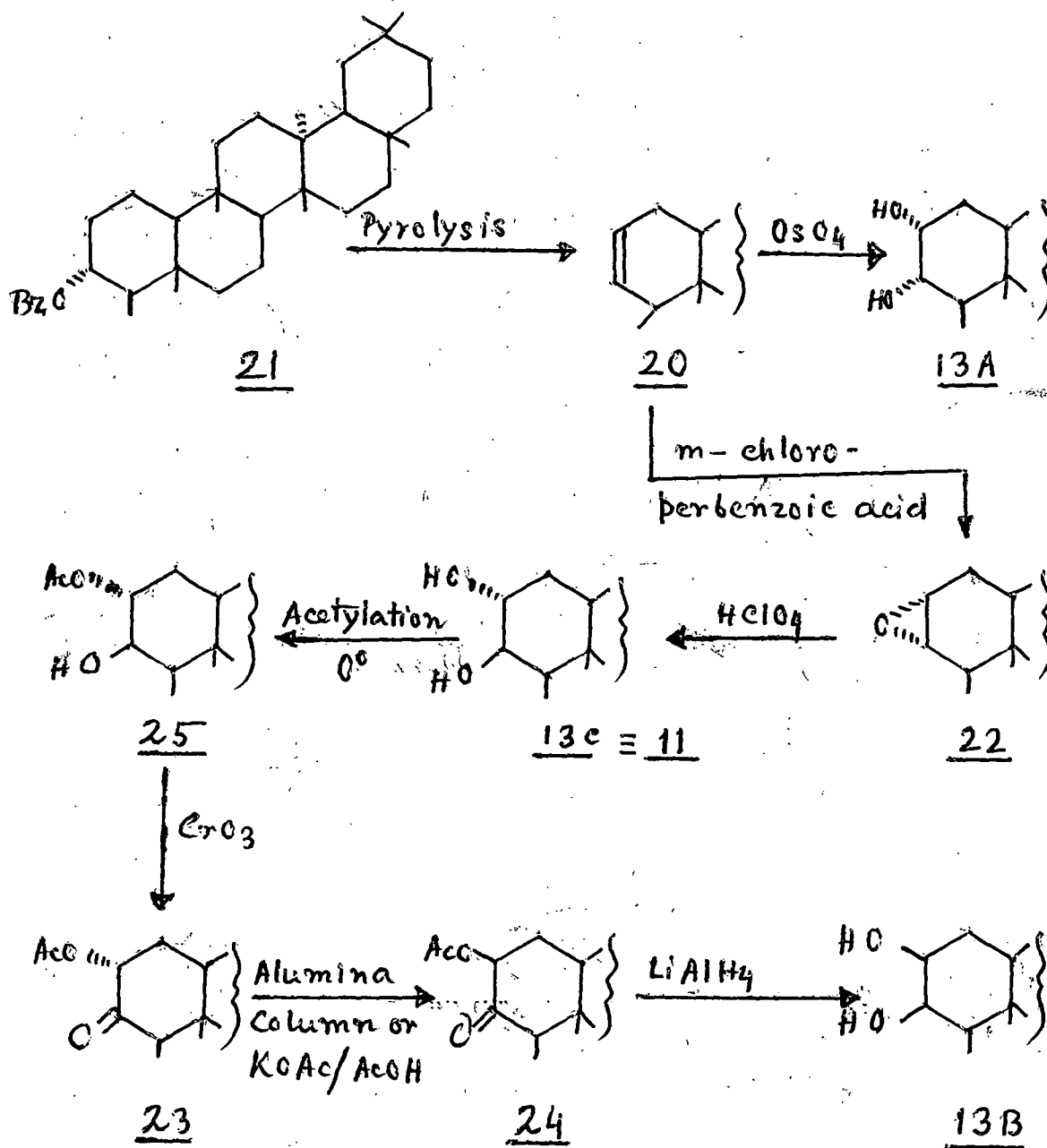




synthesis of Friedelane-2 $\alpha$ , 3 $\alpha$  13 A; 2 $\beta$ , 3 $\beta$  - 13 B; 2 $\alpha$ , 3 $\beta$  - 13 C - diols<sup>8</sup> : ( Chart II )

Friedel-2-ene 20, obtained by the pyrolysis of friedelanol benzoate 21 was converted to friedelane- 2 $\alpha$ , 3 $\alpha$  -diol 13 A by the action of osmium tetroxide and a 2, 3-epoxide 22 by the action of *m*-chloroperbenzoic acid. The 2,3-epoxide 22 was opened with perchloric acid to yield friedelane 2 $\alpha$ , 3 $\beta$  - diol 13 C identical with naturally occurring pachysandiol A<sup>9</sup> 11. Kikuchi and Toyoda<sup>9</sup> has suggested that cerin acetate was 2 $\alpha$  -acetoxy friedelan-3-one 23, contrary to its previous formulation as 2 $\beta$  -acetoxy-friedelan-3-one 24 on the following grounds; Pachysandiol A-2-monoacetate 25 obtained by the acetylation of Pachysandiol A 11 at 0°, could be oxidised to cerin acetate 23 with chromic acid. The cerin acetate so obtained, on prolonged absorption on alumina, was isomerized to another 2-acetoxy-3-ketone 24 which must hence be the more stable 2 $\beta$  -(e, equatorial)-acetoxy isomer. Consequently the original cerin acetate must be less stable 2 $\alpha$  -(axial) acetoxy isomer 23. Samson et al<sup>8</sup> isomerized cerin acetate 23 with potassium acetate in acetic acid and reduced the resulting 2 $\beta$  -acetoxy-friedelan-3-one 24 with lithium aluminium hydride and thus synthesised friedelane-2 $\beta$ , 3 $\beta$  -diol 13 B. (Chart II)

CHART - II





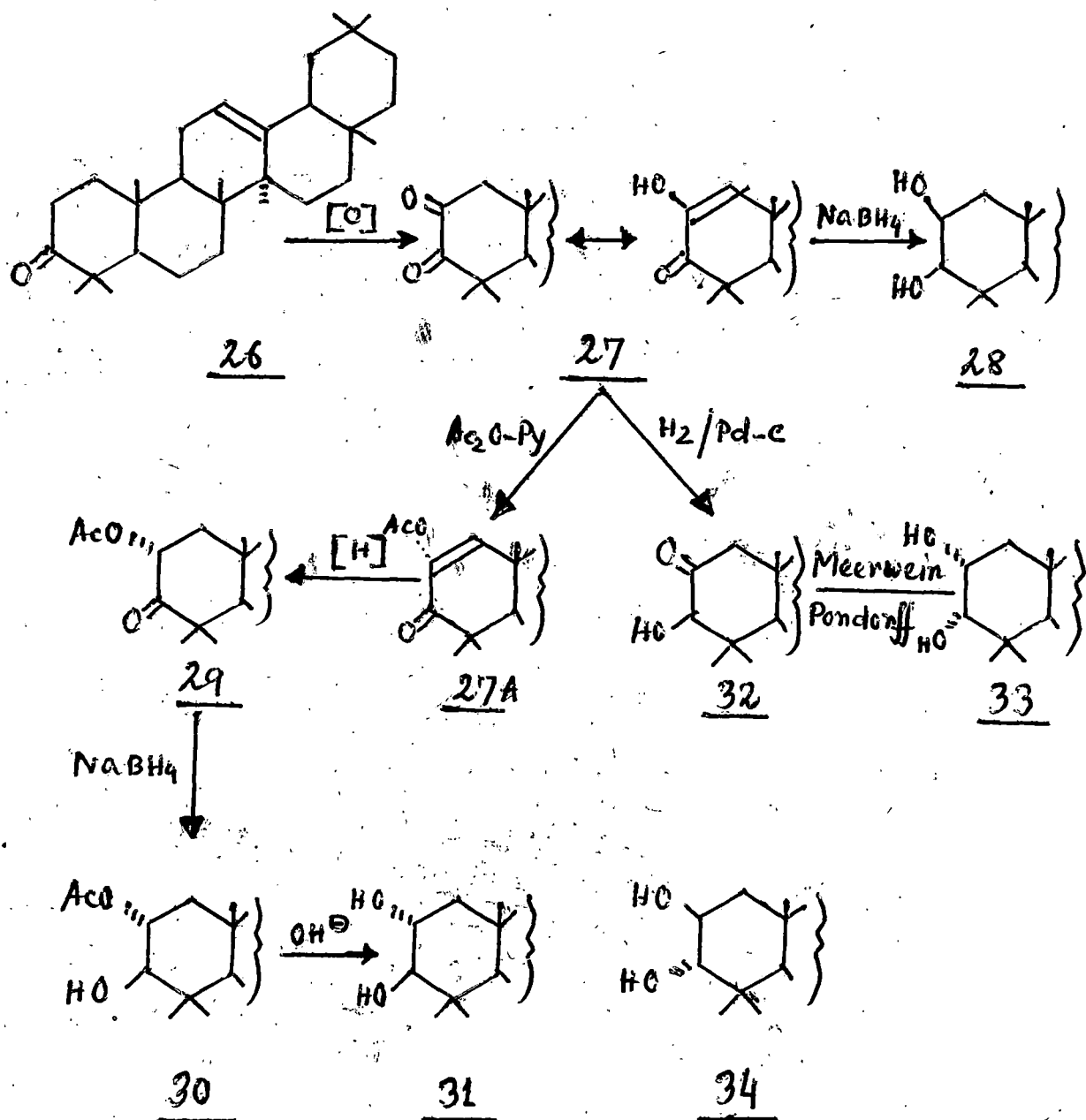
Section C: A short review of synthesis of isomeric  $\Delta^{12}$ -Oleanene 2,3-diols<sup>10a</sup>:

A number of 2,3-diols of triterpenoids have been isolated from natural sources. Recently Khastgir et al<sup>10a</sup> have synthesised three out of the four isomeric 2,3-diols (Chart IV) by using diosphenol 27 as the intermediate, <sup>prepared</sup> from  $\beta$ -amyrone 26.  $\Delta^{12}$ -oleanene 2 $\beta$ , 3 $\beta$ -diol<sup>28</sup> m.p. 240-42°, ( $\alpha$ )<sub>D</sub>+101.88° 28 was obtained by sodium borohydride reduction of 27. Acetylation of 27 followed by hydrogenation gave 2 $\alpha$ -acetoxy- $\beta$ -amyrone 29 which on sodium borohydride reduction at pH-8 gave 2 $\alpha$ -acetoxy- $\beta$ -amyrin 30 which on hydrolysis gave the most stable  $\Delta^{12}$ -oleanene 2 $\alpha$ , 3 $\beta$ -diol 31. Hydrogenation of 27 gave 32 which on Meerwein Ponderff reduction afforded  $\Delta^{12}$ -oleanene-2 $\alpha$ , 3 $\alpha$ -diol, 33 having m.p. 278-81°, ( $\alpha$ )<sub>D</sub> 71.28°. The sterically most unstable 2 $\beta$ , 3 $\alpha$ -diol 34 was also synthesised. The configurations assigned have been confirmed from NMR spectral evidence. The melting points and rotations of the isomeric diols, their diacetates and their acetonide derivatives have been shown in Chart III.

Chart III

|                         | DIOL    |                           | DIACETATE         |                           | ACETONIDE DERIVATIVE |                           |
|-------------------------|---------|---------------------------|-------------------|---------------------------|----------------------|---------------------------|
|                         | m.p.    | ( $\alpha$ ) <sub>D</sub> | m.p. <sup>o</sup> | ( $\alpha$ ) <sub>D</sub> | m.p.                 | ( $\alpha$ ) <sub>D</sub> |
| 2 $\beta$ , 3 $\beta$   | 240-42° | 101.88°                   | 221-22°           | 83.63°                    | 180-2°               | 102.50°                   |
| 2 $\alpha$ , 3 $\alpha$ | 278-82° | 71.28°                    | 180-82°           | 40.77°                    | 199-201°             | 97°                       |
| 2 $\alpha$ , 3 $\beta$  | 202-4°  | 60.00°                    | 216-18°           | 73.42°                    | 173-4°               | -                         |
| 2 $\beta$ , 3 $\alpha$  | 250-52° | 12°                       | 161-63°           | -                         | -                    | -                         |

Chart IV

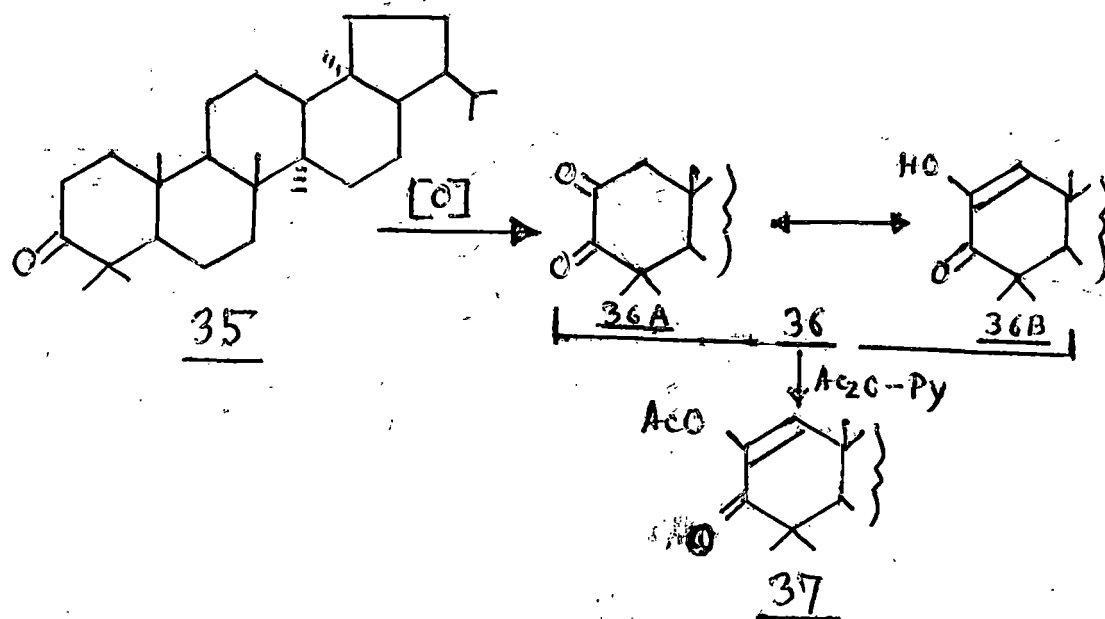


Section D : A short review on the synthesis of isomeric 2,3-diols of isohopane ( moretane ) <sup>10b</sup>;

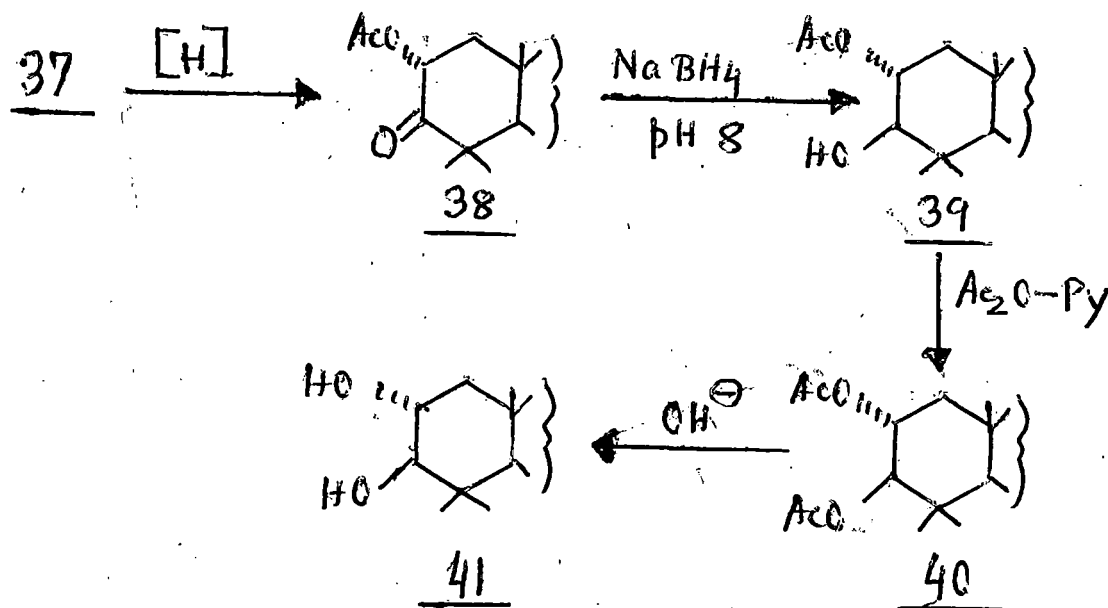
Khastgir et al <sup>10b</sup> have been able to prepare all the four isomeric diols using diosphenol obtained by the autoxidation of moretanone.

1. Synthesis of 2 $\alpha$ , 3 $\beta$ - dihydroxy isohopane ( moretane ) 41 :

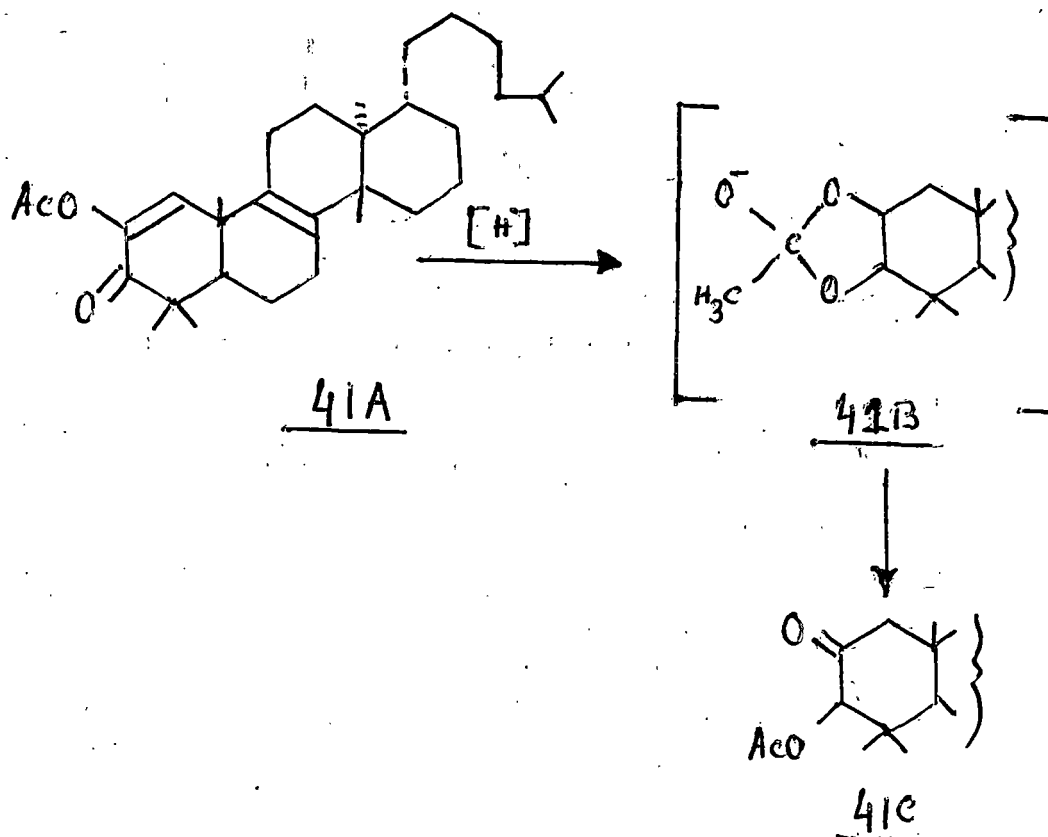
Moretanone 35 obtained by hydrogenation of moretenone <sup>10c</sup> was oxidised by passing oxygen through a suspension of 35 in dry t-butanol containing potassium tertiary butoxide <sup>11, 12, 13, 14</sup>. The product,  $\alpha$ -diketone derivative 36, m.p. 190-2°, ( $\alpha$ )<sub>D</sub> 40.00° showed two spots on chromatoplate indicating the presence of a mixture of two compounds. The compound 36 showed positive ferric chloride test. The assignment of structures 36 A and 36 B are consistent with the UV and IR spectral data.



Acetylation of 36 with acetic anhydride and pyridine gave the corresponding acetate 37 ( single spot in TLC). The diosphenol acetate 37 on hydrogenation in presence of 10% palladium-on-charcoal catalyst in ethanol solution gave 38, m.p. 179-81°, ( $\alpha$ )<sub>D</sub> 86.31°,  $\lambda_{\text{max}}$  276 m $\mu$  ( $\epsilon$  82 ) NMR spectrum was consistent with the structure 38. Sodium borohydride reduction of 38 in dioxan solution buffered at pH-8 to reduce isomerisation gave the crystalline 2 $\alpha$ -acetoxy-3 $\beta$ -hydroxy compound 39, m.p. 199-200°, ( $\alpha$ )<sub>D</sub> 95.35°. The latter on acetylation with pyridine and acetic anhydride gave the 2 $\alpha$ , 3 $\beta$ -diacetate 40, m.p. 228-30°, ( $\alpha$ )<sub>D</sub> 50.60° which on alkaline hydrolysis afforded the corresponding 2 $\alpha$ , 3 $\beta$ -diol 41, m.p. 242-3°, ( $\alpha$ )<sub>D</sub> 82.86°. From examination of the NMR spectrum of the diol 41 and its diacetate 40, they confirmed unequivocally the diequatorial 2 $\alpha$ , 3 $\beta$  configuration of the hydroxyl groups in the diol 41.



In this connection it is necessary to mention that Lavie and co-workers during their studies on autoxidation in euphol series claimed that hydrogenation of diosphenol acetate of dihydroeuphone 41 A gave a product which was identical with C - 3 acetate 41 C i. e., a 2-keto-3-acetoxy derivative. Migration of the acetoxy group from C - 2 to C - 3 position was proposed through cyclic intermediate 41 B<sup>15</sup>. These results are contrary to the observations reported in the  $\beta$ -amyrone series<sup>10a</sup> and also in the moretanone series<sup>10b</sup> where they obtained the corresponding 2 $\alpha$ -acetoxy-3-keto compounds with 1,2-addition of hydrogen.

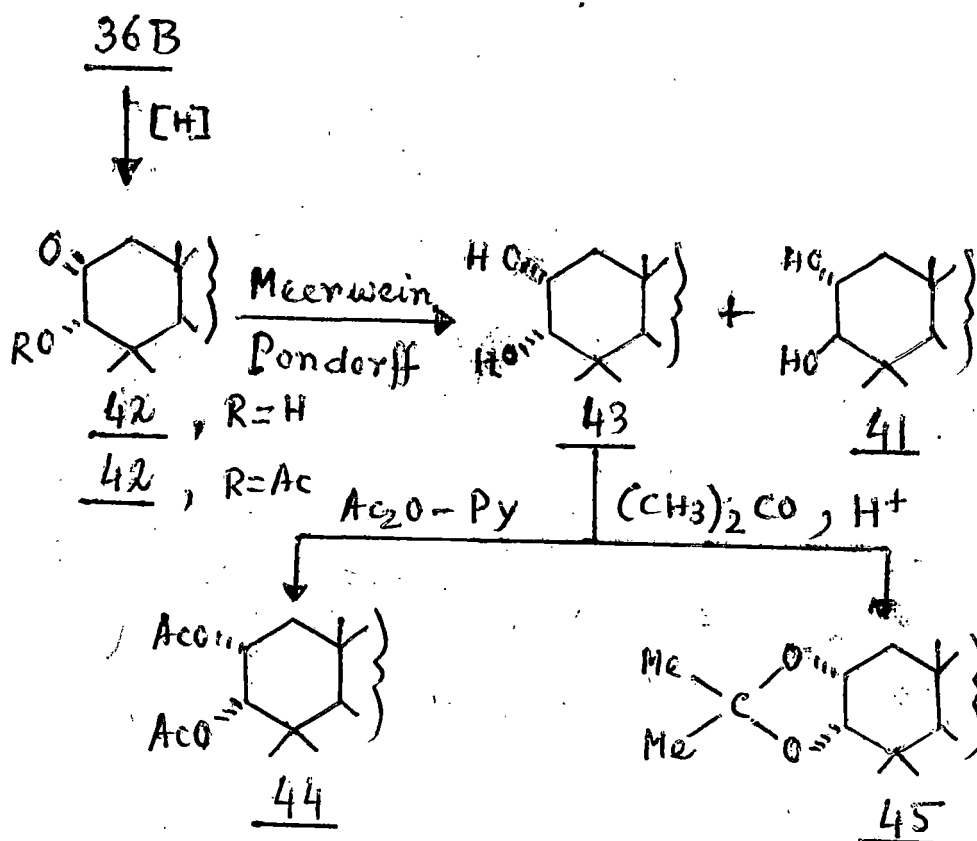


In order to provide a reasonable explanation for the different behaviour shown by these compounds (dihydroeuphone,  $\beta$ -amyrone and moretanone) they examined the Drieding models of euphol,  $\beta$ -amyrine and moretane derivatives. In the euphol series the presence of a double bond in 8,9 position causes deformation<sup>16</sup> and has a modified chair conformation which confers additional strain in the molecule whereas in the  $\beta$ -amyrin series as well as in moretane (isohopane) series (having A/B chair-chair conformation) this strain is not present. They assumed that the 2 $\alpha$ -acetoxy-3-keto compound which presumably is formed at first on hydrogenation of 41 A isomerises to 41 C via 41 B to release additional strain in the molecule. Therefore, they proposed 38 for the structure of 2 $\alpha$ -acetoxy moretanone the acetoxy group being at C - 2 with  $\alpha$ -configuration.

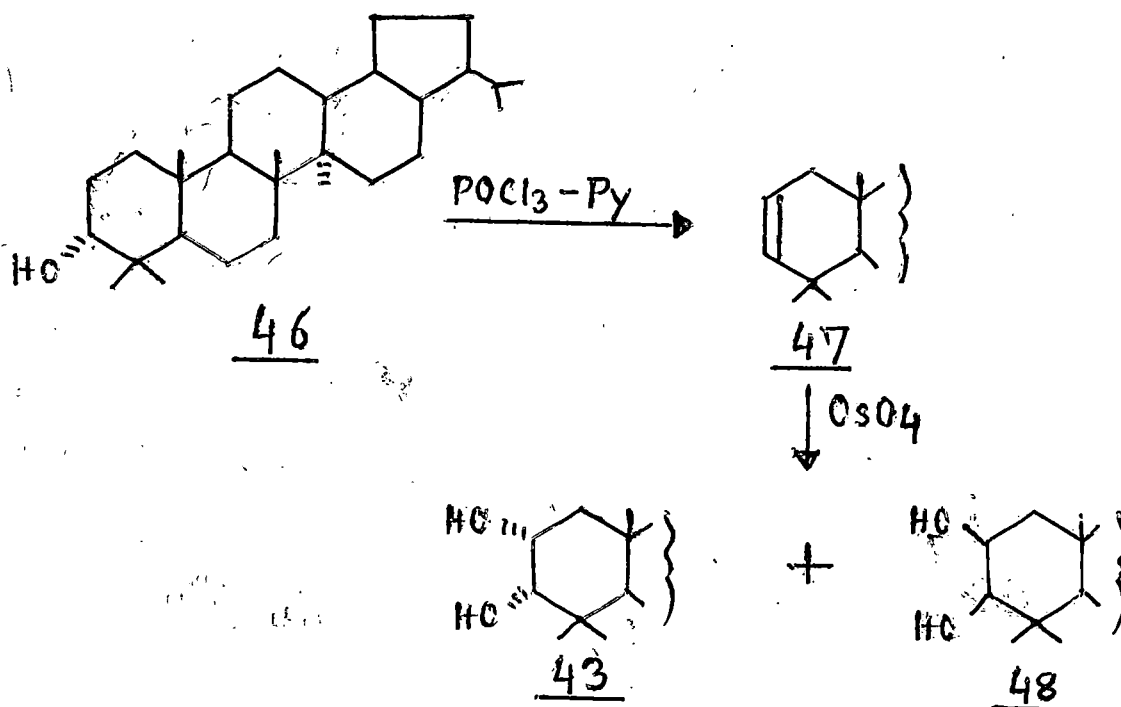
2. Synthesis of 2 $\alpha$ , 3 $\alpha$ -dihydroxy isohopane (Moretane) 43 :

Diosphenol 36 B on hydrogenation in presence of 10% palladium-on-charcoal catalyst in ethanol solution afforded 2-keto-moretanol 42, m.p. 181-3°, ( $\alpha$ )<sub>D</sub> 29.41°, which on acetylation with pyridine and acetic anhydride afforded the corresponding acetate 42 A, m.p. 264-7°, ( $\alpha$ )<sub>D</sub> 82.61°. They reported that the NMR spectra was consistent with the structures 42 and 42 A assigned to them. Meerwein-Ponndorff reduction of 2-keto

moretanol 42 furnished a crystalline solid which exhibited two distinct spots on chromatoplate. Chromatography of the solid, first eluted a solid compound which after crystallisation from methanol and chloroform mixture afforded a crystalline solid 43, m.p.  $250-51^{\circ}$ ,  $(\alpha)_D$   $9.37^{\circ}$ ,  $\gamma_{\max}$   $3420, 2960, 1450, 1370, 1350, 1042 \text{ cm}^{-1}$  in 92% yield. The latter on acetylation with pyridine and acetic anhydride afforded an acetate 44, m.p.  $185-7^{\circ}$ . The more polar solid obtained from the chromatogram in about 5% yield had the m.p.  $242-3^{\circ}$  and was found to be identical with  $2\alpha, 3\beta$ -diol 41, by m.m.p. and IR comparison. The assignment of stereochemistry of the hydroxyl groups in the diol 43 as  $2\alpha, 3\alpha$  was based on the following chemical and physical evidence.



$\Delta^2$ -moretane 47 prepared by dehydration of epi-moretanol 46 with phosphorous oxychloride and pyridine, was treated with osmium tetroxide in pyridine solvent and the product obtained after chromatography melted at  $235-40^\circ$  and showed two spots on chromatoplate. The separation of the two compounds could not be successfully accomplished by column chromatography. However, on careful fractional crystallisation ( chloroform - methanol mixture ) they first obtained a diol, m.p.  $250-51^\circ$ ,  $(\alpha)_D 9.83^\circ$  as the major component (87%). From the mother-liquor a second diol, m.p.  $261-3^\circ$ ,  $(\alpha)_D 23.68^\circ$  was isolated as a minor product (8%). They identified the latter as the  $2\beta, 3\beta$ -diol 48 from its m.m.p. and IR comparison with an authentic sample of  $2\beta, 3\beta$ -diol ( described on page 98 ). The major diol  $250-51^\circ$ ,  $(\alpha)_D 9.83^\circ$  afforded a diacetate 44, m.p.  $185-87^\circ$ . The diol and the diacetate were found



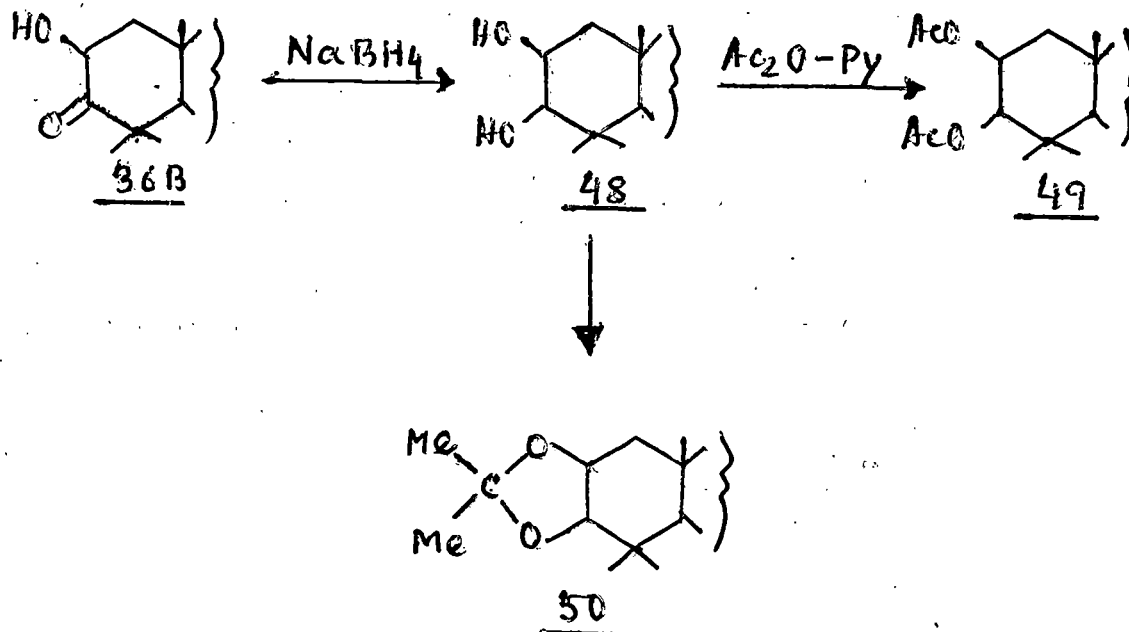


to be identical with the diol and diacetate respectively obtained by the Meerwein - Ponderoff reduction of 2-keto-moretanol 42 described above. They argued that since osmylation could afford only two cis isomers, the stereochemical assignments of the diol 43 having m.p.  $250-51^{\circ}$  and its diacetate 44, m.p.  $185-87^{\circ}$  must have  $2\alpha$ ,  $3\alpha$ - configuration as depicted in 43 and 44 respectively. NMR spectra of the diol 43 and its diacetate 44 are in good agreement with their stereochemical assignments 43 and 44.

The diol 43 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetonide derivative 45, m.p.  $186-88^{\circ}$ ,  $(\alpha)_D$   $19.04^{\circ}$ .

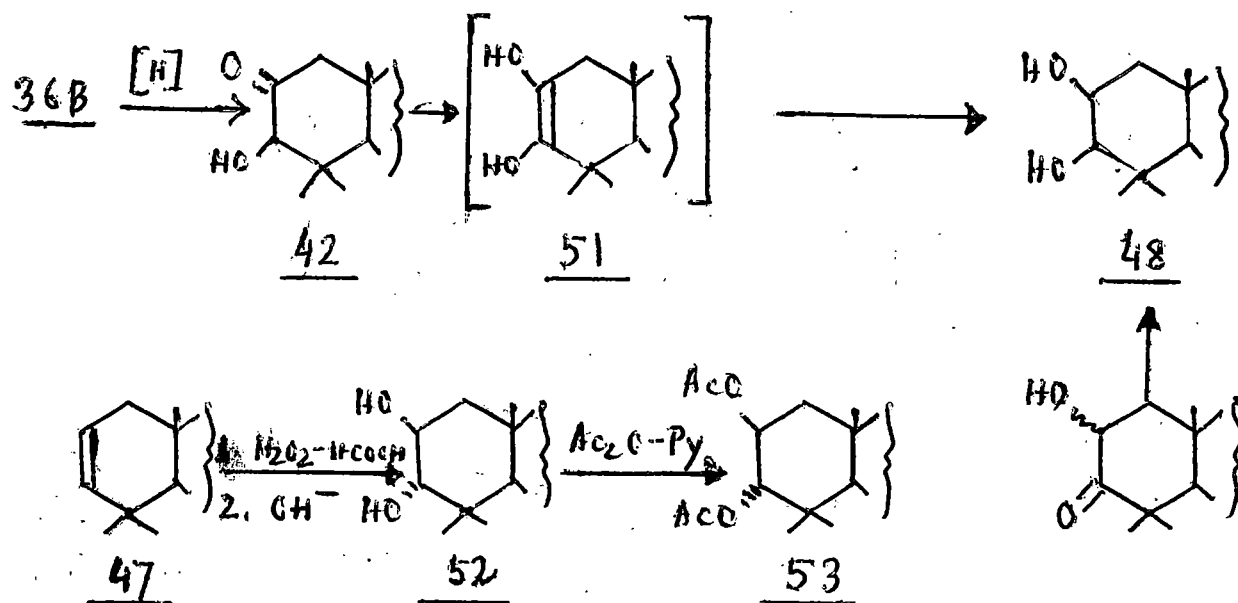
### 3. Synthesis of $2\beta$ , $3\beta$ -dihydroxy isochopane ( moretane ) 48 :

Diosphenol 36 B on sodium borohydride reduction gave a compound 48, m.p.  $262-64^{\circ}$ ,  $(\alpha)_D$   $23.68^{\circ}$ ,  $\nu_{\text{max}}^{\text{nujol}}$  3420, 2950.



1460, 1275, 1250  $\text{cm}^{-1}$ . The latter on acetylation with acetic anhydride and pyridine gave the diacetate 49, m.p. 214-15°, ( $\alpha$ )<sub>D</sub> 31.25°. NMR of 48 showed a multiplet at 3.23 ppm and a broad unresolved multiplet at about 4.60 ppm which collapsed to a doublet ( $J = 3.6 \text{ Hz}$ ) and a multiplet ( $J = 6 \text{ Hz}$ ) respectively upon exchange of hydroxyl proton with  $\text{D}_2\text{O}$ . This data was in good agreement with the equatorial orientation ( $\text{H}_a$ ) of the hydroxyl group at C - 3 and the axial orientation ( $\text{H}_e$ ) at C - 2. In the NMR spectra of the diacetate 49 these signals were shifted downfield to 4.97 ppm (doublet,  $J = 3\text{Hz}$ ) and at about 5.25 ppm (broad multiplet).

Compound 48 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetamide derivative 50, m.p. 239-41°, ( $\alpha$ )<sub>D</sub> 29.85°. They explained the formation of the 2 $\beta$ , 3 $\beta$ -diol 48 by the following mechanism via intermediate 51.



4. Synthesis of 2 $\beta$ , 3 $\alpha$ -dihydroxy isohopane ( moretane ) 52 :

The sterically most unstable 2 $\beta$ , 3 $\alpha$ -diol ( axial-axial ) was prepared by them by the known method<sup>1,2,3</sup> described in the literature. The method involved the oxidation of  $\Delta^2$ -moretane 47 with performic acid and subsequent hydrolysis of the ester with alkali solution. By following the above procedure they obtained a crystalline diol 52, m.p. 221-24 $^{\circ}$ , ( $\alpha$ )<sub>D</sub> 21.18 $^{\circ}$ . IR spectrum of the diol showed peaks at 3560, 2980, 1455 cm<sup>-1</sup>. Acetylation of the diol with acetic anhydride and pyridine afforded the crystalline 2 $\beta$ , 3 $\alpha$ -diacetate 53, m.p. 145-47 $^{\circ}$ , ( $\alpha$ )<sub>D</sub> 31.40 $^{\circ}$ . NMR spectrum of the diacetate was consistent with the structure and stereochemical assignment as in 52 and also by analogy with the previous work reported in the literature<sup>10a,17</sup>.

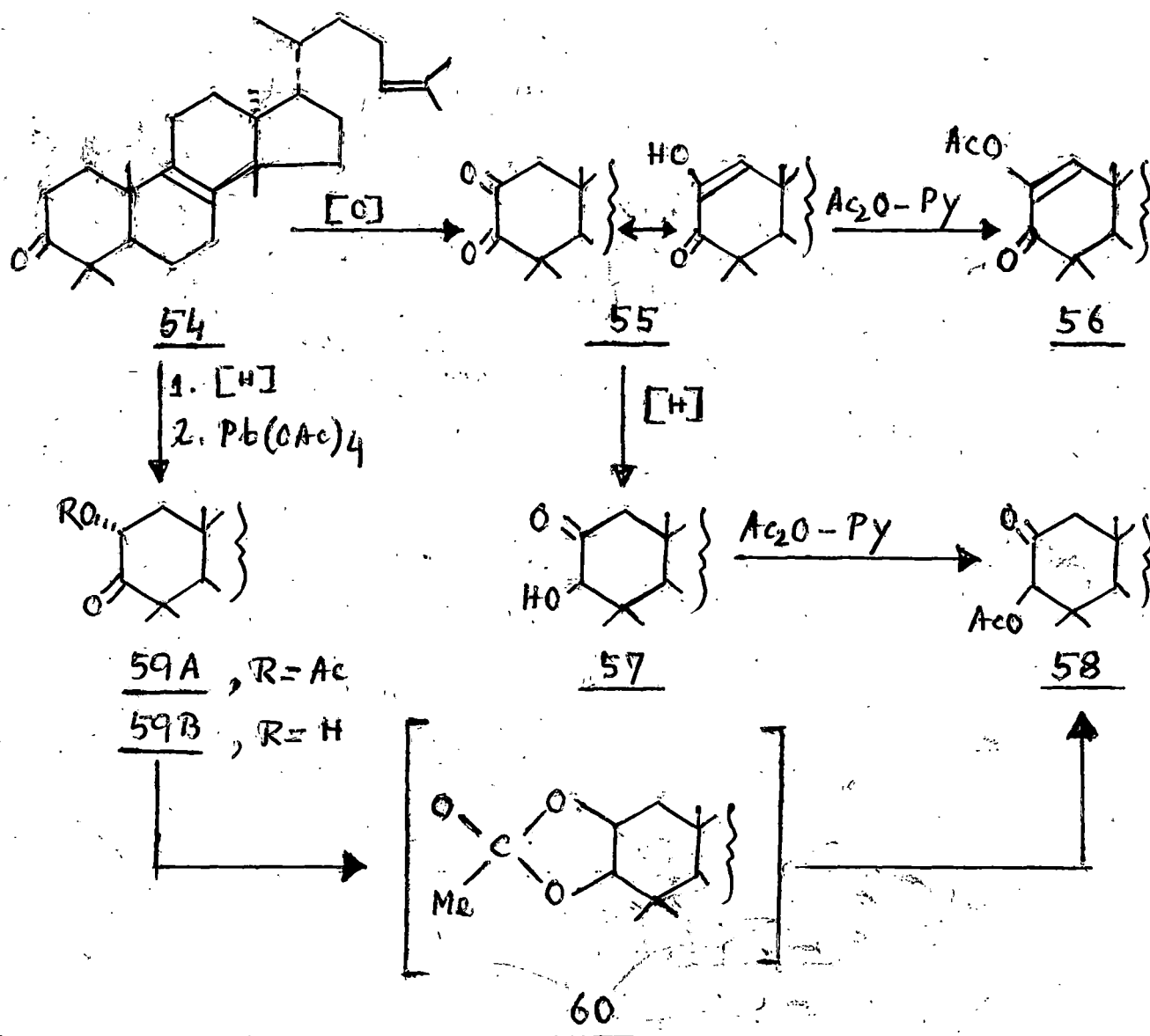
Section E: A short review on autoxidation and isomerisation in ring A in triterpenoids :

1. Oxidation in ring A in Euphol.

Lavie and co-workers<sup>11</sup> studied the autoxidation of euphadiene-3-one 54 and the results of their work is summarized in the following lines ( Chart - V ). Euphadiene-3-one 54 was oxidised by shaking in oxygen in t-butanol saturated with potassium-t-butoxide<sup>12,13</sup>. A tautomeric mixture of diketone and the

corresponding diosphenol 55 ( two spots in chromatoplate ) was produced by absorbing one mole of oxygen; UV,  $\lambda_{\text{max}}$  269  $m\mu$  ( $\epsilon$ , 7900).  $\nu_{\text{max}}$  1715, 1672, and 1653  $\text{cm}^{-1}$ . NMR of the compound 55 showed a singlet at  $\tau$  3.60 due to vinylic proton at C - 1. Acetylation gave the corresponding acetate 56, UV  $\lambda_{\text{max}}$  236  $m\mu$  ( $\epsilon$ , 9000).  $\nu_{\text{max}}$  1764  $\text{cm}^{-1}$ . NMR showed a singlet at  $\tau$  3.02 due to C - 1 proton. On hydrogenation of the diosphenol 55 over palladium on charcoal ( two moles of hydrogen were absorbed - one mole to reduce the side chain double bond and the second mole to reduce the enolic double bond ) a noncrystallisable homogeneous solid  $\nu_{\text{max}}$  1712  $\text{cm}^{-1}$ , NMR singlet at  $\tau$  5.95 accounting for one hydrogen and two AB type doublets centered at  $\tau$  7.69 and 7.35 accounting for two hydrogens, was obtained. Upon acetylation a crystalline ketoacetate was obtained,  $\nu_{\text{max}}$  1742 and 1730  $\text{cm}^{-1}$ , NMR singlet at  $\tau$  4.95 for one hydrogen and a broad peak at  $\tau$  7.50 accounting for two hydrogens. From the above spectral properties structures 57 and 58 were proposed for hydroxy ketone and keto acetate respectively. 2 $\alpha$ -acetoxy ( equatorial ) derivative 59 A was prepared by the reaction of dihydroderivatives of 54 with lead tetraacetate in acetic acid in presence of boron-trifluoride<sup>18</sup>. The product 59 A showed IR bands at 1742 and 1730  $\text{cm}^{-1}$  and the NMR spectra showed a quartet of lines centered at  $\tau$  4.3 (  $J_{\text{ae}}$  = 6.5 cps and  $J_{\text{aa}}$  = 13.0 cps ) for the C - 2 proton but no signals for

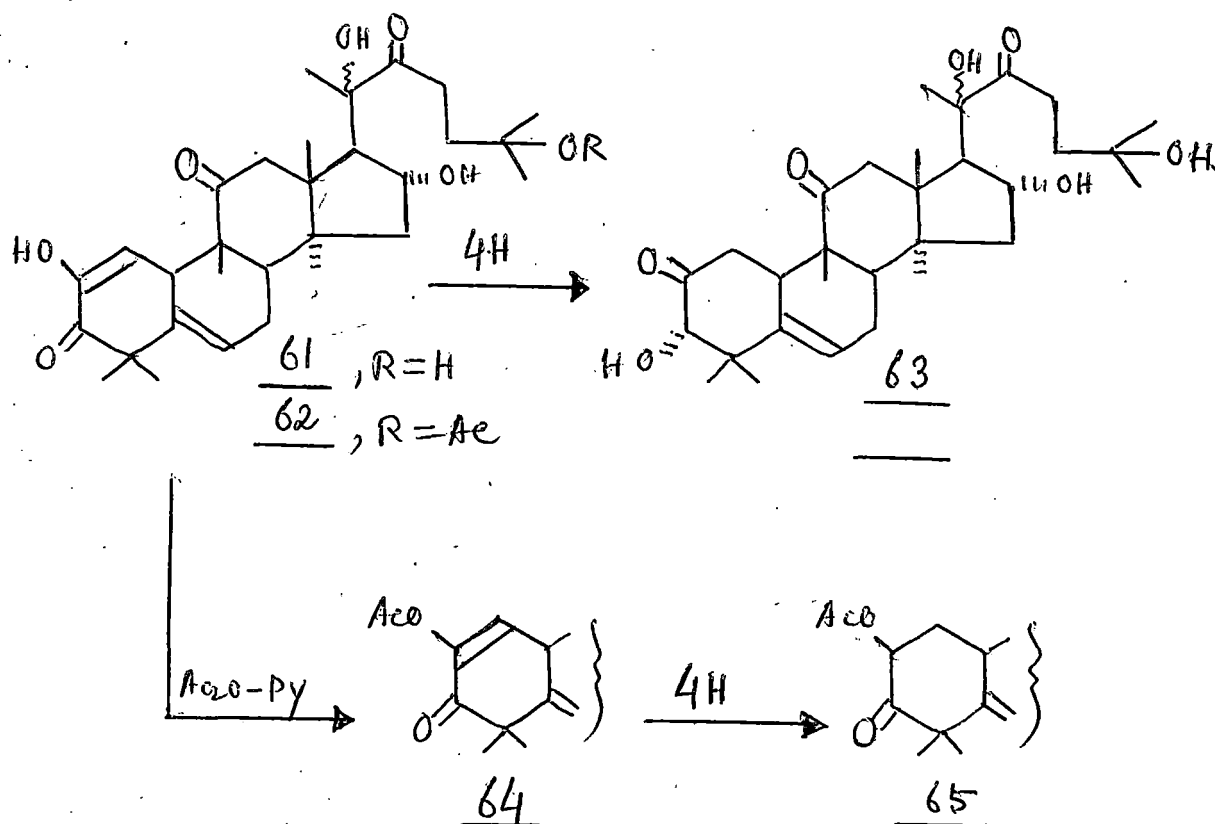
CHART - V



protons  $\alpha$  to a keto-function. The isomerisation of 2 $\alpha$ -equatorial acetoxy ketone 59 A into the isomer 58 was also observed and they proposed that the migration proceeded through the cyclic intermediate 60.<sup>15</sup> Acid hydrolysis of 59 afforded a compound which has been assigned the 2 $\alpha$ -equatorial hydroxy 3-keto derivative 59 B on the basis of its IR,  $\nu_{\text{max}}$  1718  $\text{cm}^{-1}$ .

## 2. Isomerisation in ring A of the Cucurbitacins

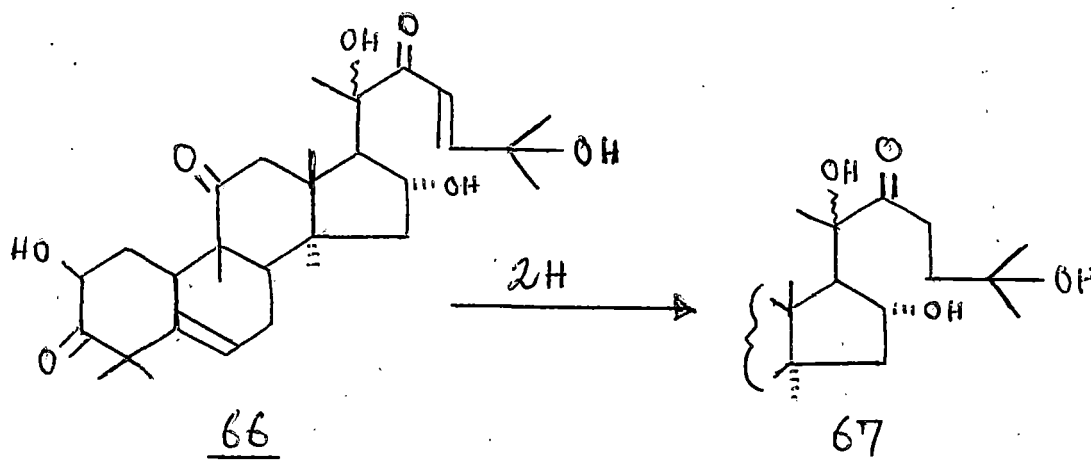
Lavie and co-workers<sup>19,20</sup> reported that hydrogenation of the diosphenol containing cucurbitacins namely elatericin B 61 and elaterin 62 resulted in 1,4 addition of hydrogen during the process of hydrogenation. The NMR spectrum of hydrogenated product of elatericin B was found to show a singlet at  $\tau$  6.02 and that of its diacetate a sharp one at  $\tau$  5.00.



This observation clearly pointed to the fact that the proton linked to the carbon to which the acetoxy group is also attached had no neighbouring protons and can not therefore be at C - 2. The NMR spectra could be explained, if it was considered that 1,4 addition of hydrogen to the diosphenol system took place, resulting in the conversion of  $\Delta^1$ -2-hydroxy-3keto to a 2 keto-3-hydroxy system 63.

Elatericin B diacetate 64 on hydrogenation formed the  $2\beta$  - equatorial - acetoxy - 3keto derivative 65 by abnormal 1,2 addition of hydrogen. This compound showed a quartet of lines related to the  $2\alpha$ -axial proton which is centered at  $\tau$  4.4 (J<sub>aa</sub> = 13.5 cps; J<sub>ae</sub> = 5.1 cps ). The isomerisation of 2 -acetoxy-3-keto derivative 65 on a basic column of alumina as well as on an acidic column was studied. In both the cases the material recovered from the columns showed that it had remained unchanged.

The ORD curves of dihydro elatericin A 67 and tetrahydro elatericin B 65 were also interpreted, cotton effect curves of both 2 and 3-keto derivatives were found to be positive with the



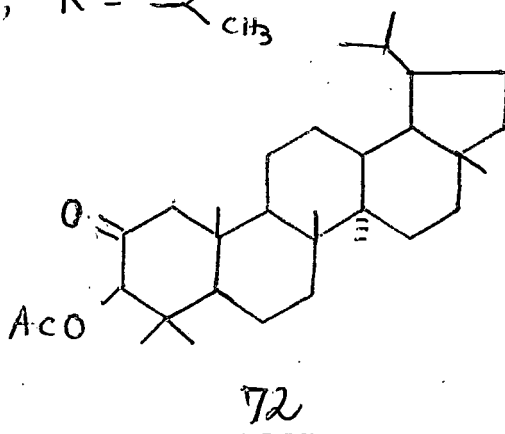
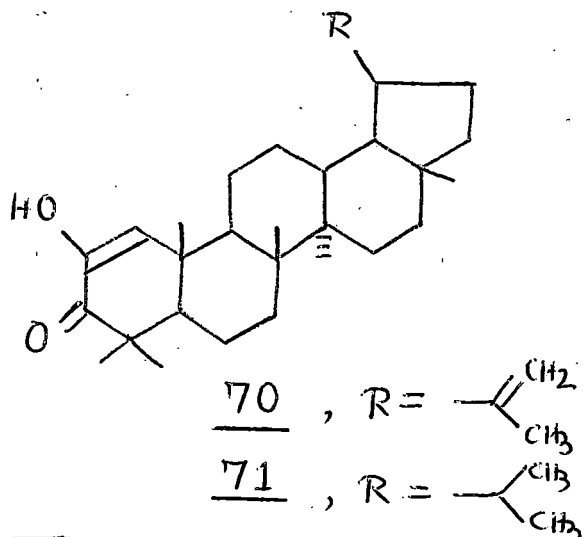
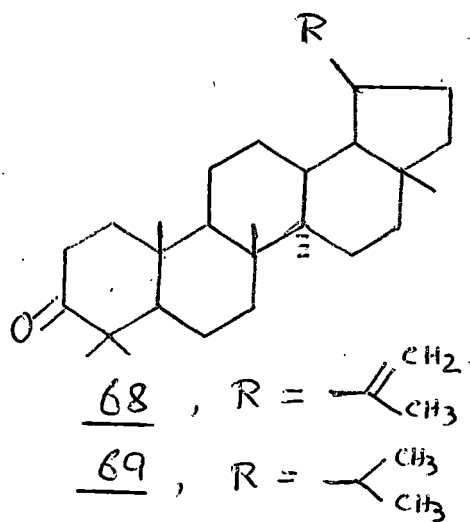
amplitude of the 2-keto-derivative being larger than that of the 3-keto form. ORD studies on 2 and 3 ketosteroids<sup>21</sup> and the oxomanoyl oxide series<sup>22</sup> also revealed the same result. The inverted stereochemistry of cucurbitacins at C - 10 resulting in a mirror image of the C - 10,  $\beta$  - analog should give rise to a negative cotton effect but instead the two compounds displayed positive curves. This can be interpreted as due to the presence of two additional carbonyl chromophore, one in particular at C - 11 displaying a large amplitude, which counteracts thereby the inverted rotation of the keto group in ring A as should be expected. The result is a lower positive value instead of a negative one. The peak for dihydro elatericin A 67 (3 keto) at  $(\alpha)_{325} + 2200^\circ$  is larger, than that of tetrahydroelatericin B 63 (2 keto)  $(\alpha)_{325} + 1558^\circ$ . In both the cases the keto group was flanked by an equatorial (OH) substituent which is either likely to increase the cotton effect or to render no change at all.

In order to obtain pure tetrahydroelatericin B 64, alkaline hydrolysis of tetrahydroelatericin B diacetate 65 was attempted but the reaction resulted in the formation of dihydroelatericin B<sup>23</sup> 66  $\lambda_{\max} 267 \text{ m}\mu (\epsilon, 5700)$ , positive ferric chloride coloration (characteristic of diosphenol). Tetrahydroelatericin B diacetate 65 on alkaline hydrolysis yielded the same dihydroelatericin B 66. The alkali induced autooxidation of  $\alpha$  -hydroxy ketone in elatericin was also studied<sup>24</sup> and was found to occur at much slower rate.



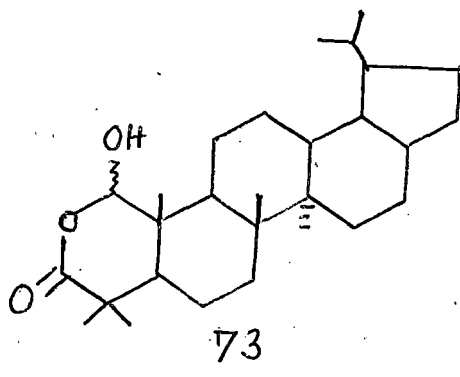
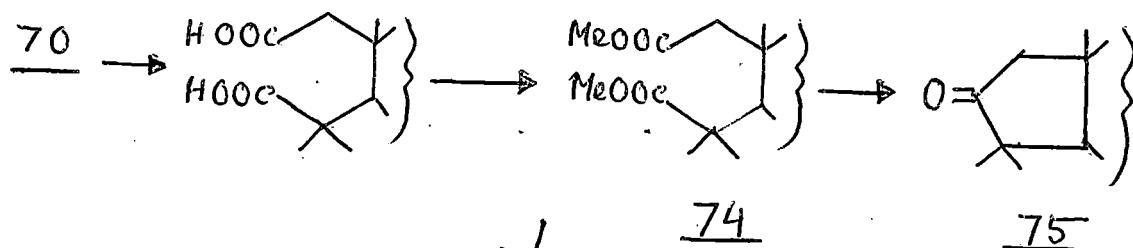
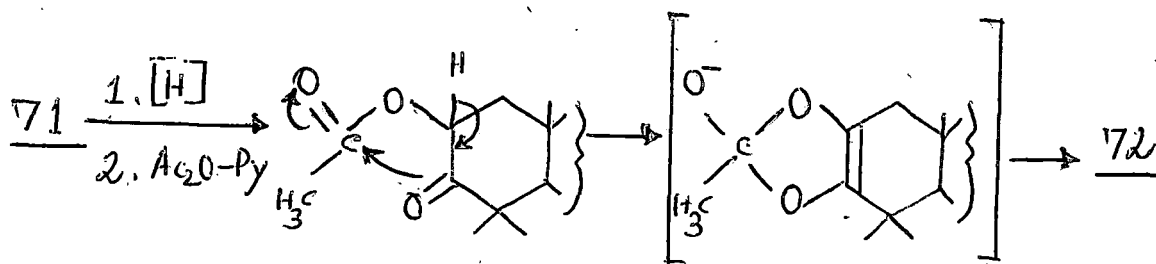
### 3. Oxidation in ring A in Lupeol

Ganguly and co-workers<sup>14</sup> carried out the oxidation of lupenone 68 and lupanone 69 to the corresponding diosphenols 70 and 71 respectively by passing oxygen in dry t-butyl alcohol containing potassium tertiary butoxide. Diosphenol 71 on hydrogenation afforded a non-crystalline alcohol which on acetylation yielded the keto acetate 72. The structure 72 was assigned to the keto-acetate by examining its NMR spectra ( a sharp singlet at  $\delta$  4.95 ) ascribed to the C - 3 proton.



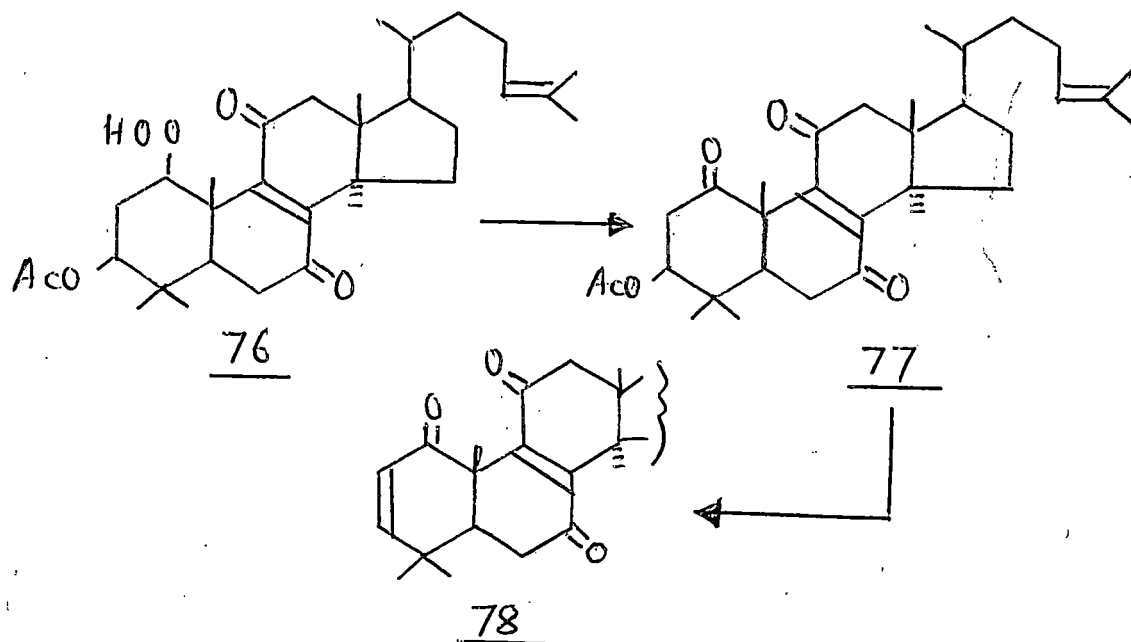
Formation of 72 from 71 was explained by the mechanism shown in chart V. Diosphenol 71 on ozonisation gave a neutral compound  $C_{29}H_{48}O_3$ , whose structure was assigned as 73 on the basis of mode of formation, spectral characteristics and elemental composition. Diosphenol 70 was cleaved by alkaline hydrogen peroxide to the dicarboxylic acid  $C_{30}H_{48}O_4$ . The acid was converted into the dimethyl ester, 74 which on refluxing with alcoholic alkali yielded a neutral crystalline compound 75.

Chart - V



#### 4. Autooxidation of Lanostenyl acetate

Horn and Ilse<sup>25</sup> stated that lanostenyl acetate in ethyl acetate was extensively converted into a mixture of 7-hydroperoxy and 7,11 - dihydroperoxy lanostenyl acetates by treatment with gaseous oxygen at 50° for 48 - hours. After that Scotney and Truter<sup>26</sup> found that the autooxidation of lanostenyl acetate in ethyl acetate at 50° after 14 days was a mixture of at least eight peroxides ( laminar chromatography ). The two most plentiful peroxides were recovered and shown to be 7 $\beta$  - and 11 $\beta$  - hydroperoxy lanostenyl acetates. The structure of 7 $\beta$  - hydroperoxy - lanostenyl acetate was obtained by reducing it with sodium borohydride to 7 $\beta$  - hydroxy lanostenyl acetate. The structure of 11 $\beta$  - hydroperoxide was proved by converting it to 11-oxo-lanostenyl acetate with ferrous ion. Furthermore, lithium aluminium hydride reduction of the 11-hydroperoxide afforded one product, which was identical with 11 $\beta$  - hydroxy-lanostenol.



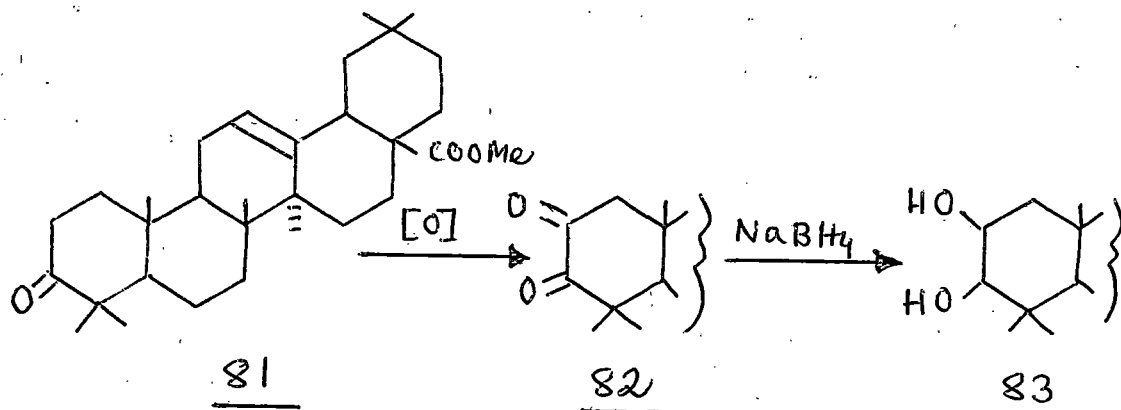
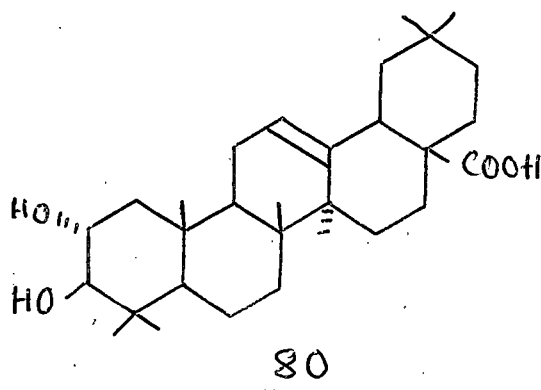
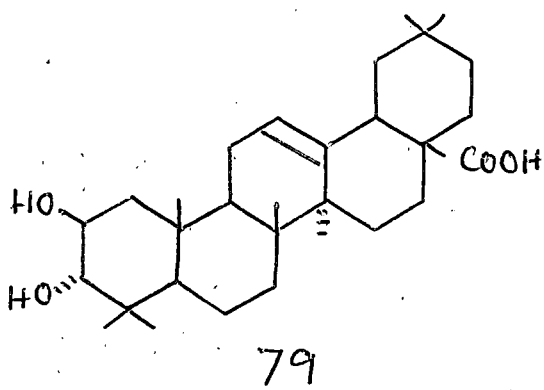
Autoxidation of 7,11-dioxolanost-8-enyl-3 $\beta$ -acetate in cyclohexane at 40° proceeded via 1 $\beta$ -hydroperoxy - 7, 11-dioxolanostenyl acetate to 1,7,11-trioxolanost-8-enyl acetate<sup>27</sup>. The location of ketone at 1-position was deduced from the behaviour of the trione acetate with alkali. With alkali 1,7,11-trioxolanost-8-enyl acetate yielded 1,7,11-trioxolanosta-2,8-diene and it had been derived from the trione acetate by elimination of the 3 $\beta$ -acetate group and the formation of a conjugated unsaturated grouping ( 76, 77, 78 ). That the precursor for the trione is a mono-hydroperoxide of 7,11-dioxolanostenyl acetate was established by the fact it was decomposed by ferrous ion to 1,7,11-trioxolanostenyl acetate,

In an experiment a solution of lanost-3-en-3 $\beta$ -yl acetate in cyclohexane at 40° was oxidised by passing oxygen through it<sup>28</sup>. After twelve months treatment the neutral fraction was examined and was found to contain at least sixteen components. From the Rf values several components have been identified e.g. 1,7,11-trioxolanostenyl acetate, 1,7,11-trioxolanosta-2,8-diene. Besides these 15 $\beta$ -hydroxy-7-oxo, 15 $\alpha$ -hydroxy-7-oxo, 7,15-dioxo- and 11-15-dioxolanostan 3 $\beta$ -yl acetate were also identified.

##### 5. Oxidation of ring A in Oleanolic acid

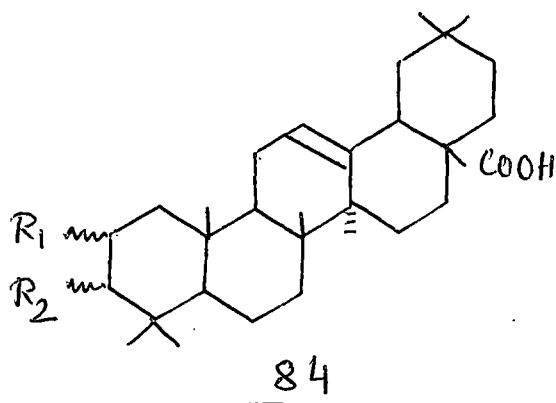
In connection with their work to confirm the structure of bredemolic acid 79 and crategolic acid 80 Teschesche and

co-workers<sup>29, 30</sup> performed the autoxidation of ring A in methyl oleanolate 81. Methyl oleanolate was stirred in t-butanol containing potassium metal at 25-50° with simultaneous introduction of oxygen. The reaction mixture on acidification and usual working up gave an amorphous solid for which structure 82 was proposed. The diosphenol 82 m.p. 130-35°,  $(\alpha)_D$  104  $\pm$  4° on sodium borohydride reduction gave 2 $\beta$ , 3 $\beta$ -dihydroxy-12-en-olean-28-oate 83 which on oxidation with kilani solution gave a mixture of several compounds in which 10% of 82 was found to be present as was shown by its UV spectrum.



Section F : A short review on 2,3-dihydroxy triterpene acids from natural sources :

The four stereo-isomeric 2,3-dihydroxy olean-12-en-28-oic acids are known to occur in nature : (1) the  $2\alpha$ ,  $3\alpha$ -dihydroxy-olean-12-en-28-oic acid 84 A<sup>31</sup> (2) the  $2\alpha$ ,  $3\beta$ -dihydroxyolean-12-en-28-oic acid ( crategolic acid / maslinic acid ) 84 B<sup>32</sup> (3) the  $2\beta$ ,  $3\beta$ -dihydroxy olean-12-en-28 oic acid<sup>33</sup> 84 C (4) the  $2\beta$ ,  $3\alpha$ -dihydroxy olean-12-en-28-oic acid 84 D<sup>30</sup> ( bredemolic acid )



84A ,  $R_1 = R_2 = \alpha-OH$

84B ,  $R_1 = \alpha-OH, R_2 = \beta-OH$

84C ,  $R_1 = R_2 = \beta-OH$

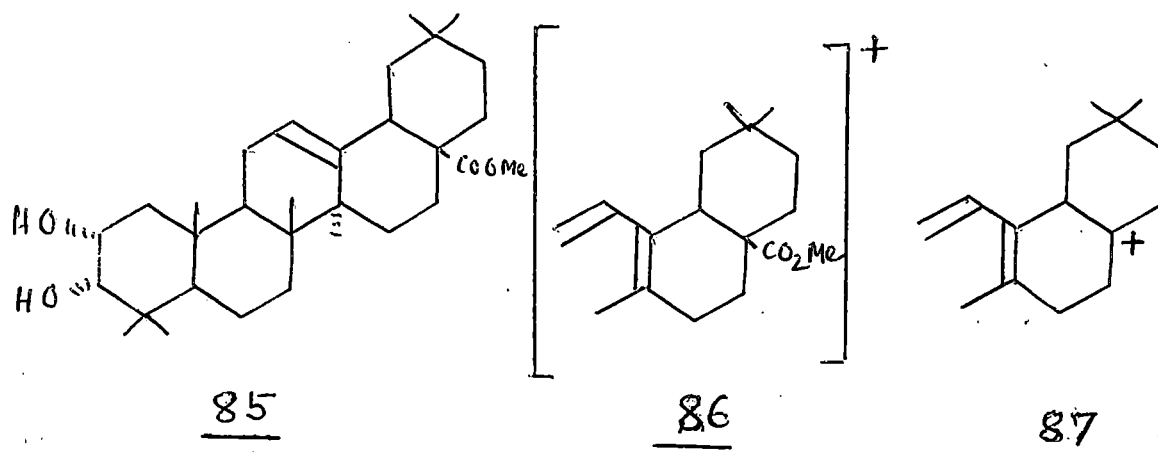
84D ,  $R_1 = \beta-OH, R_2 = \alpha-OH$

Alphitolic acid<sup>34</sup> in the lupeol series and  $2\alpha$ -hydroxy ursolic acid<sup>35</sup> in the ursane series are known to occur in nature.

1. The 2 $\alpha$ , 3 $\alpha$ - dihydroxy olean-12-en-28-oic acid :

Cheung et al<sup>31</sup> isolated a triterpene acid 84 A as its methyl ester m.p. 296-99° from shorea accuminata resin, which has been shown to be the 2 $\alpha$ , 3 $\alpha$ - dihydroxyolean-12-en-28-oic acid. The methyl ester 85 ( $\nu$  max 3340, 1725 cm<sup>-1</sup>) formed a diacetate, a monoacetate and a O, O - isopropylidene derivative indicating the presence of two hydroxyl groups. All these compounds showed NMR signals due to methyl ester group ( 3H, singlet  $\delta$  3.6 ) and a triplet for an olefinic proton ( J 4H<sub>2</sub>,  $\delta$  5.3 ).

The mass spectrum of the diol methyl ester or its acetonide showed intense peaks at m/e 262, 203 corresponding to ions 86 and 87<sup>36</sup>

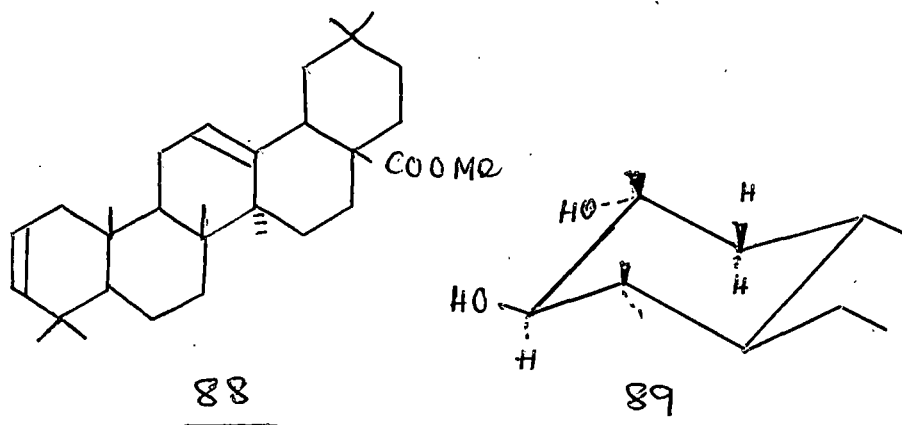


The mass fragmentation pattern established that the diol methyl ester belongs to 12-oleanene or 12-Ursene series with a 28 - methoxy-carbonyl group. They concluded from the NMR signal<sup>37</sup> of the allylic  $18\beta$  - hydrogen at  $\delta$  2.8 ( AB quartet ), that the diol ester belonged to oleanene group. Further evidence for assignment of a  $2\alpha$ ,  $3\alpha$ - configuration of the diol is supported by the following observations. In this diol, the C - 3 proton showed a doublet ( J  $3H_2$  ) at  $\delta$  3.35 due to vicinal coupling of equatorial and axial protons. Upon saturation ( by double irradiation ) of this signal, the multiplet near  $\delta$  3.9 due to the proton at C - 2 simplified to a four-line signal characteristics of X part of an ABX type spectrum.

The cis isomers,  $2\alpha$ ,  $3\alpha$ -diol and  $2\beta$ ,  $3\beta$ -diol were first prepared by Djerassi et al<sup>38</sup> by osmium tetroxide oxidation of methyl-oleana-2,12-diene-28-oate 88. Cheung et al<sup>1</sup> also repeated the oxidation and obtained two cis-diols and the one with higher melting point was identical to the methyl ester of m.p.  $296-99^\circ$ , isolated from shorea accuminata. Tschesche et al<sup>29,30</sup> assigned a  $2\beta$ ,  $3\beta$  - configuration to this diol and an  $2\alpha$ ,  $3\alpha$ - configuration to the one with lower melting point ( $258-60^\circ$ ), from consideration of the infrared absorption due to O-H stretching. Cheung et al demonstrated that the configurations assigned by Tschesche et al should be reversed. By comparing the



methyl resonance frequencies from published substitution effects<sup>37</sup> with those observed for the two cis diols and their acetate derivatives Cheung et al suggested that, contrary to the views of Tschesche et al<sup>29,30</sup>, the diol m.p. 296-99° must have the 2 $\alpha$ , 3 $\alpha$ - and the diol m.p. 258-60° the 2 $\beta$ , 3 $\beta$ - configuration

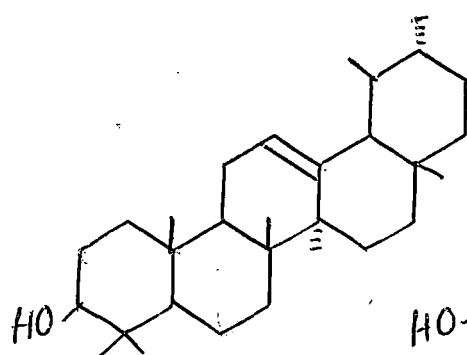


In the 2 $\alpha$ , 3 $\alpha$ - diol, with a chair ring A, 89, the 2 $\beta$ - proton is axial and is expected<sup>39</sup> to be subject to a large ax-ax coupling with the 1 $\alpha$ - proton and to small ax-eq. coupling with the 3 $\beta$  and 1 $\beta$ -protons. Of the two cis diols from osmium tetroxide only one with m.p. 296-99°, showed a signal due to C - 2 proton of sufficient width at half-height (wh/2 21H<sub>2</sub>) to be compatible with a 2 $\alpha$ , 3 $\alpha$ -diol structure. The other diol m.p. 258-60° having a corresponding signal of wh/2 8Hz should have a 2 $\beta$ , 3 $\beta$ -arrangement.

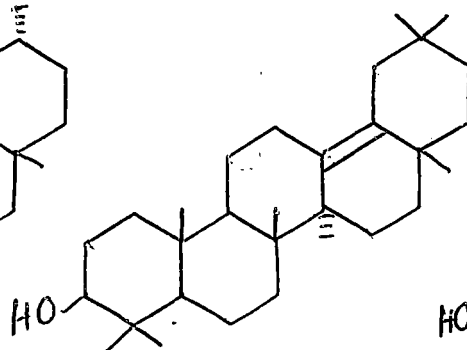
2. The 2 $\alpha$ , 3 $\beta$ - dihydroxy olean - 12-en-28-oic acid  
( Crategolic / maslinic acid ) ;

Bachler<sup>40</sup> was the first to isolate an amorphous acid " Crategus acid " from the leaves of Crategus Oxycantha L. He, however, erroneously assigned the molecular formula  $C_{32}H_{54}O_4$  to it. The acid was also observed to occur in the leaves of Psidium guajava by Arthur and Hui.<sup>40</sup> This acid was subsequently studied by a number of workers<sup>40</sup>. However a more detailed study of the acid was made by Tschesche et al<sup>40,41</sup>, who succeeded in obtaining the acid in crystalline form and establishing the correct molecular formula  $C_{30}H_{48}O_4$ . They designated the acid as crategolic acid, established the presence of a double bond resistant to catalytic hydrogenation and suggested the presence of two hydroxyl groups, although they could not prepare a diacetate. From a consideration of the behaviour of the acid towards acylation, decarboxylation and lactonisation, they erroneously concluded that crategolic acid was an  $\alpha$ - amyrin derivative and even suggested the revision of the accepted structure 90 of  $\alpha$ - amyrin to the  $\delta$ -amyrin structure 91. On the basis of their proposed new formula of  $\alpha$ - amyrin 91, they suggested without much valid reason that crategolic acid had the structure 92. However, they themselves later showed<sup>41</sup> that their " crategolic acid "

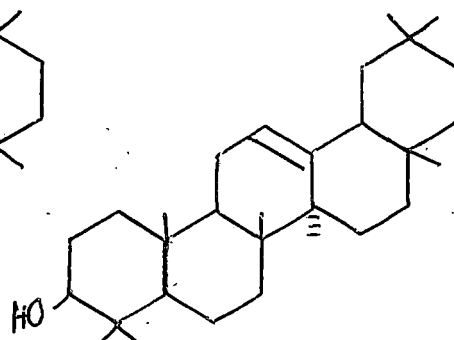
was impure, being



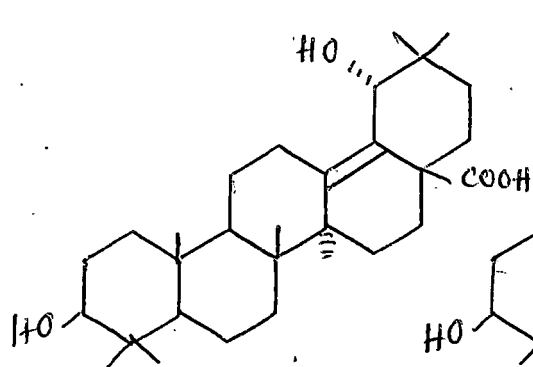
90



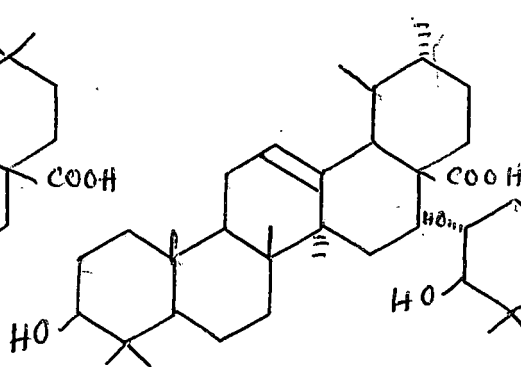
91



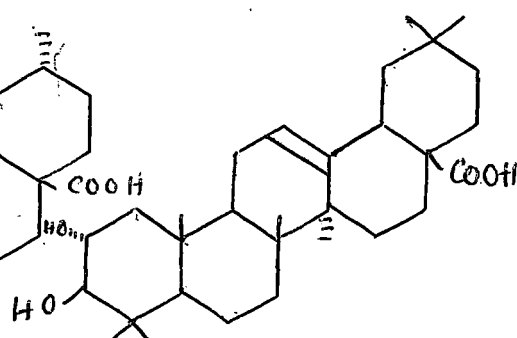
95



92



93



94

contaminated with 60 - 65 % of ursolic acid 93 which could not be easily separated. Arthur et al<sup>42</sup> drew attention to this fact

and suggested further work. Tschesche et al in a subsequent paper<sup>43</sup> correctly recognised crategolic acid 94 as a derivative of  $\beta$  - amyirin 95. The impure acid mixture could be resolved by them by paper chromatography or column chromatography of the methyl esters derived from it. They were also able to prepare a diacetate, a monoacetate and a keto-monoacetate from methyl ester of crategolic acid 94.

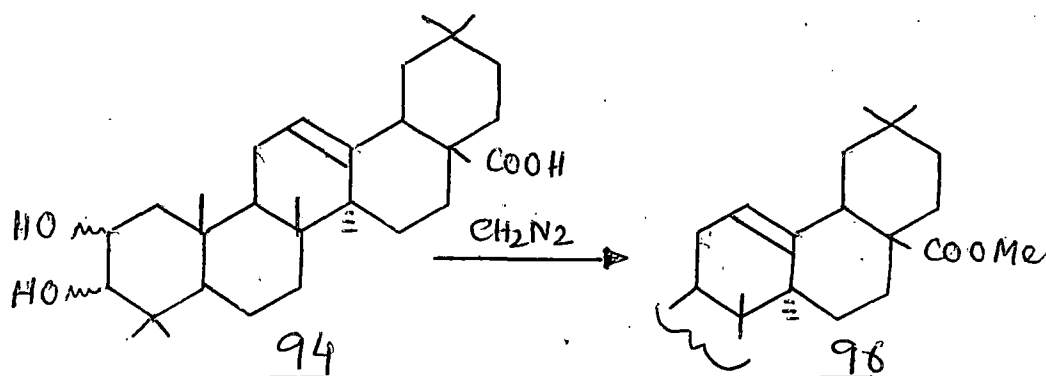
However, in the mean time, Caglioti et al reported<sup>32</sup> the isolation from the cakes of Olean europa of a new acid, mastinic acid, which latter proved identical with Crategolic acid 94 of Tschesche et al. The Italian workers<sup>32</sup> were able to show that mastinic acid was a pentacyclic triterpenic acid, probably belonging to the  $\beta$  - amyirin group, containing two acylable hydroxyl groups and a non-hydrogenizable double bond  $\gamma$  - to the carboxyl group.

In their subsequent investigations<sup>44,45</sup> Caglioti et al were able to elucidate the complete structure of the acid as a 2,3- dihydroxy olean-12-en-28-oic acid. Their work of structure elucidation of crategolic acid 94 is shown schematically in chart.V. Crategolic acid 94 formed a methyl ester 95, a diacetate 97, and a methyl ester diacetate 98. The diacetoxo acid 97 with bromine gave a bromolactone 99 which on treatment with zinc and acetic acid regenerated the diacetoxo acid 97. The latter 97 with hydrogen peroxide gave a hydroxy-diacetoxo-

lactone 100 A which gave a triacetoxylactone 100 B on acetylation. Selenium dioxide oxidation of the methyl ester diacetate gave a conjugated diene ester 101 showing UV absorption maxima at 260, 251, 243  $m\mu$ , characteristics of  $\beta$ -amyrin derivatives. The presence of an  $\alpha$ -glycol system was shown by the consumption of one mole of periodic acid of methyl crategolate 96.

Finally crategolic acid 94 was correlated with the  $\beta$ -amyrin group by its elegant conversion into methyl oleanonate 103 and methyl olean-12-en-28-oate 104. This conversion inter alia settled the position of the two hydroxyl group at C - 2 and C - 3. When methyl crategolate 96 was acetylated at  $0^\circ$ , the major product was the 2-acetate 101A, which could be oxidised with chromic acid to give the 3-keto-2-acetate 102. The latter 102 on treatment with calcium in liquid ammonia was converted to methyl oleanonate 103, a substance of known structure. Methyl crategolate 96, itself on oxidation with chromic acid followed by Huang Minlon reduction furnished the known ester 104. The transformations are shown schematically in chart VI

Chart - VI



- 119 -  
Chart-VI

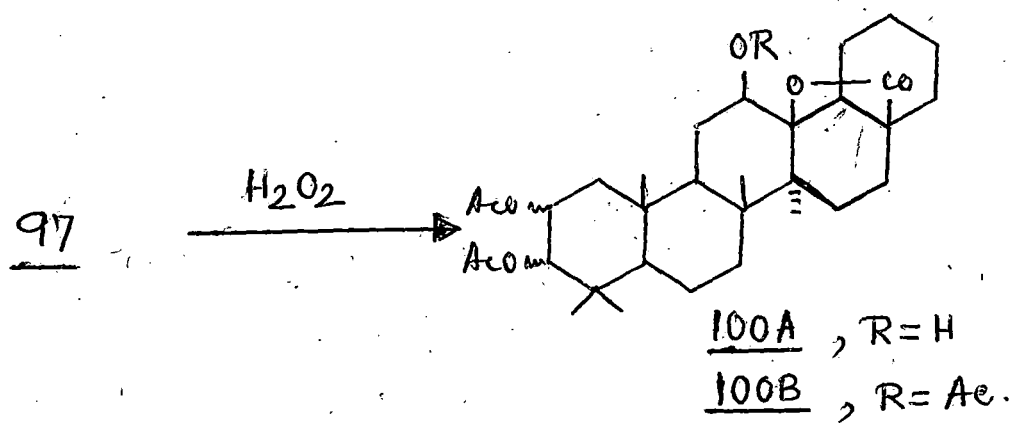
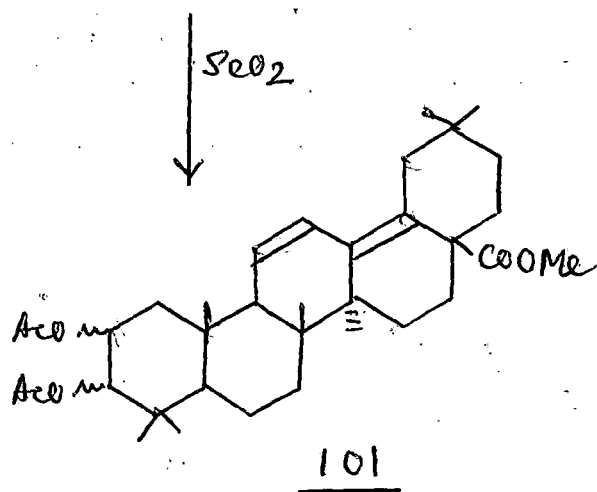
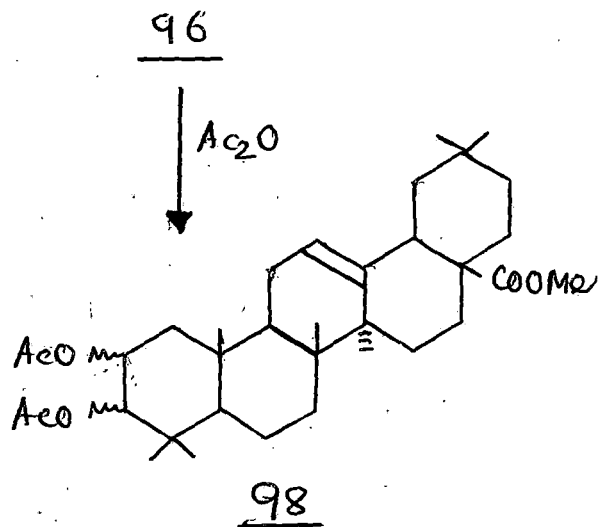
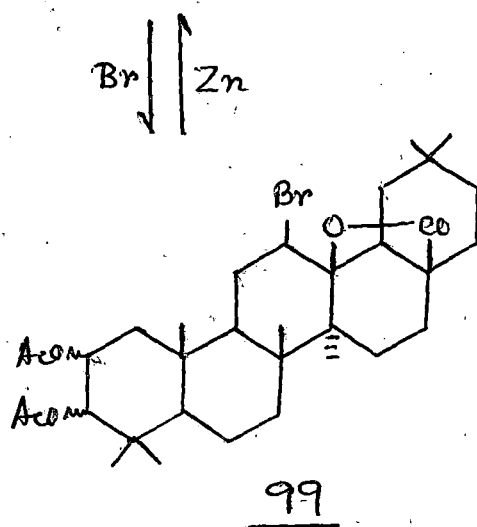
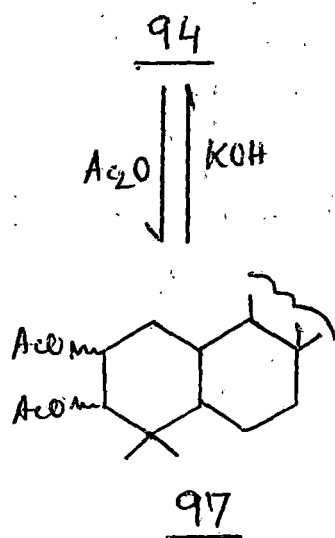
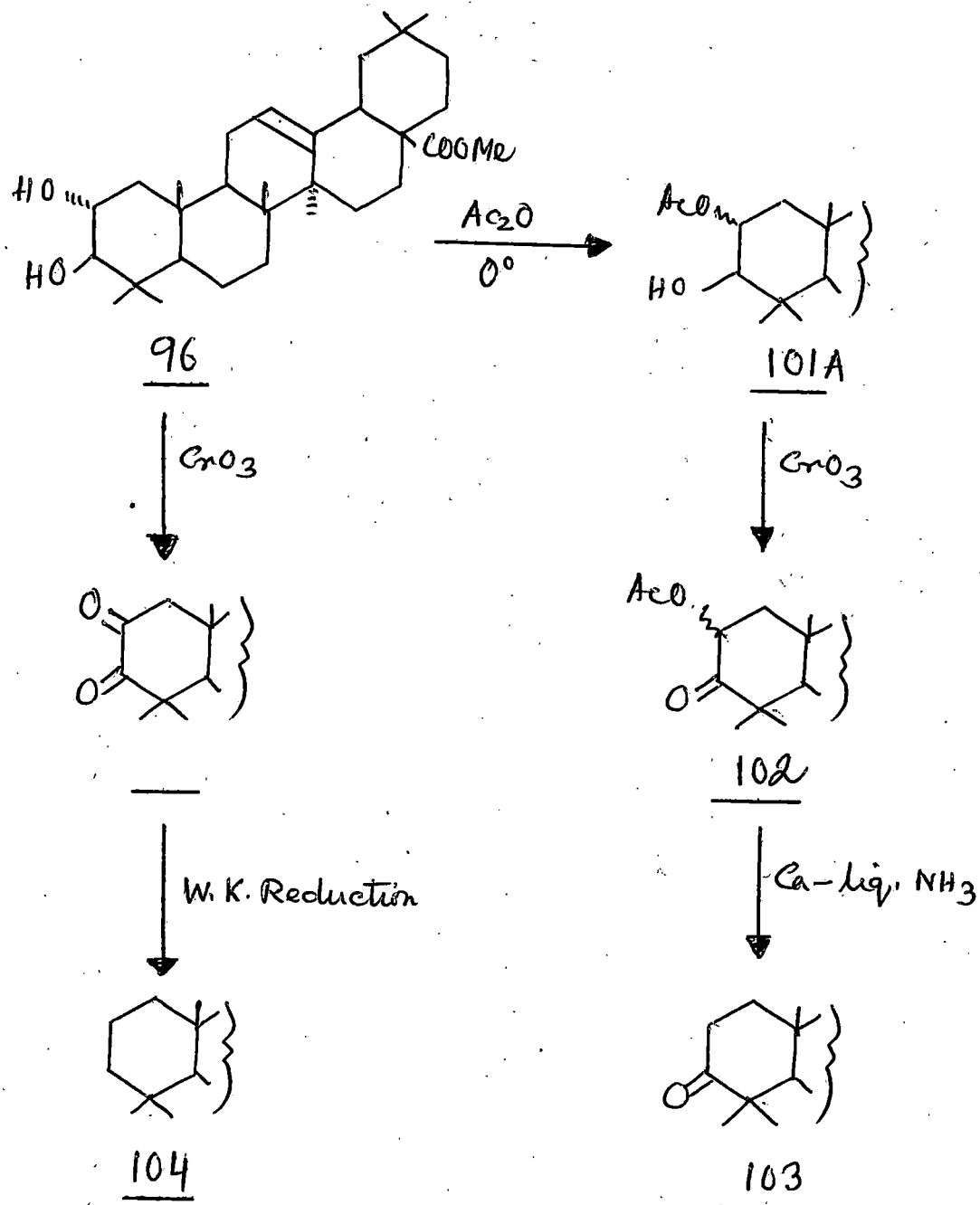
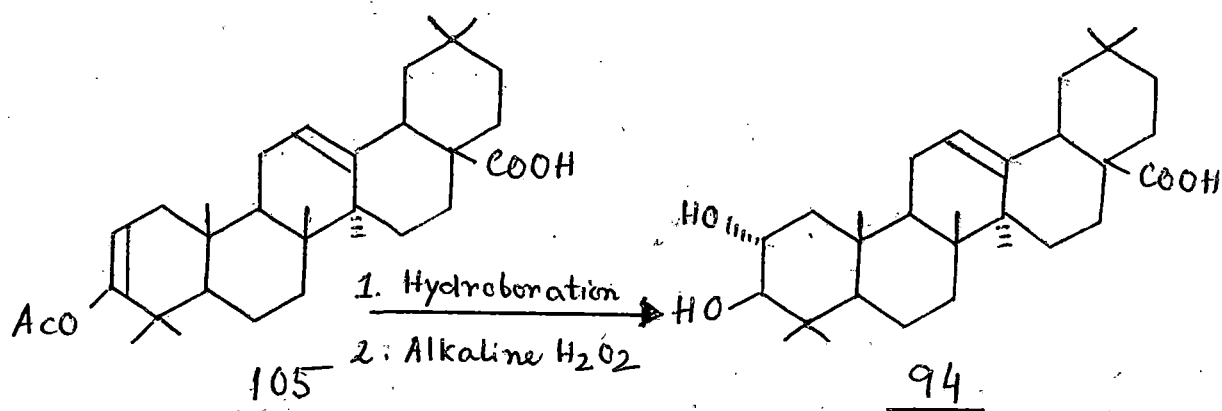


Chart - VI



Subsequently, the configuration of the two hydroxyl groups was elucidated by Caglioti *et al*<sup>46</sup>. Preferential formation of the 2-monoacetate 101 suggested the 2  $\alpha$ , 3  $\beta$ -trans diequatorial configuration of the diol moiety, which was confirmed by successful synthesis<sup>46</sup> of crategolic acid 94 from the enol-acetate 105 of oleanonic acid by hydroboration, which was known to be a stereospecific process<sup>47,48</sup>.



The correctness of the above assignment of configuration of the two hydroxyl group of crategolic acid 94 was further supported by the work of Tschesche *et al*<sup>38</sup> on the structure of bredemolic acid ( discussed in page 124 , Part II of this thesis )



Sengupta et al<sup>49</sup> has also isolated Crategolic acid (maslinic acid) 94 from the flowers of Eugenia jambolana Lam as its methyl ester along with oleanolic acid.

3. The  $2\beta$ ,  $3\beta$ -dihydroxy olean-12-en-28-oate.

Bannon et al<sup>33</sup> recently reported the isolation of  $2\beta$ ,  $3\beta$  dihydroxyolean-12-en-28-oic acid 106 from the sapogenin mixture prepared from the extract of the wood of Castanospermum australe Cunn and Frus. These authors established the identity of 106 ( $2\beta$ ,  $3\beta$ -dihydroxyolean-12-en-28-oic acid) by a high yielding stereospecific synthesis (chart VII) from methyl crategolate 108.

Bannon et al isolated 106 as its methyl ester 107. The structure was suggested by its IR, NMR, mass spectrum<sup>31b</sup>. The melting point of the methyl ester  $276-80^\circ$ , 107 was in agreement with that published previously for methyl  $2\beta$ ,  $3\beta$ -dihydroxyolean-12-en-28-oate (lit  $278-82^\circ$ <sup>38</sup> and  $276-84^\circ$ <sup>30</sup>). These authors carried out a stereospecific synthesis of methyl  $2\beta$ ,  $3\beta$ -dihydroxyolean-12-en-28-oate 107 in high yield from methyl crategolate 108 and thereby concluded that the product isolated from C. australe is in fact methyl  $2\beta$ ,  $3\beta$ -dihydroxy-olean-12-en-28-oate.

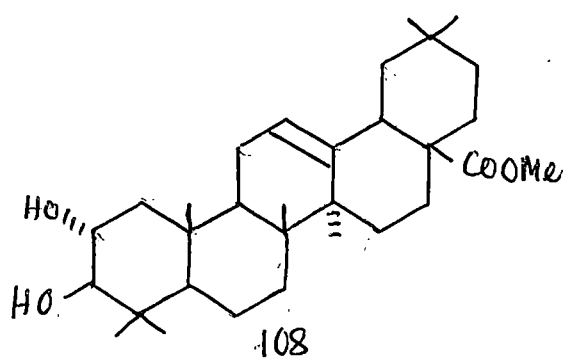
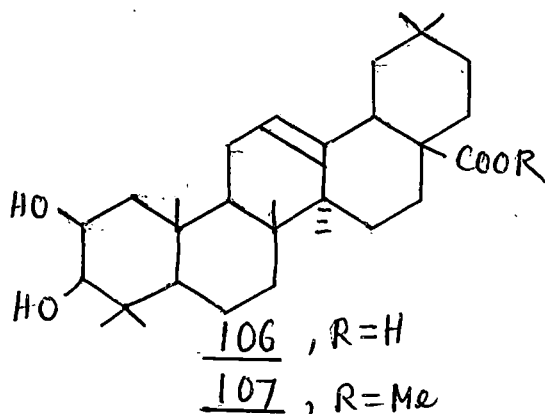
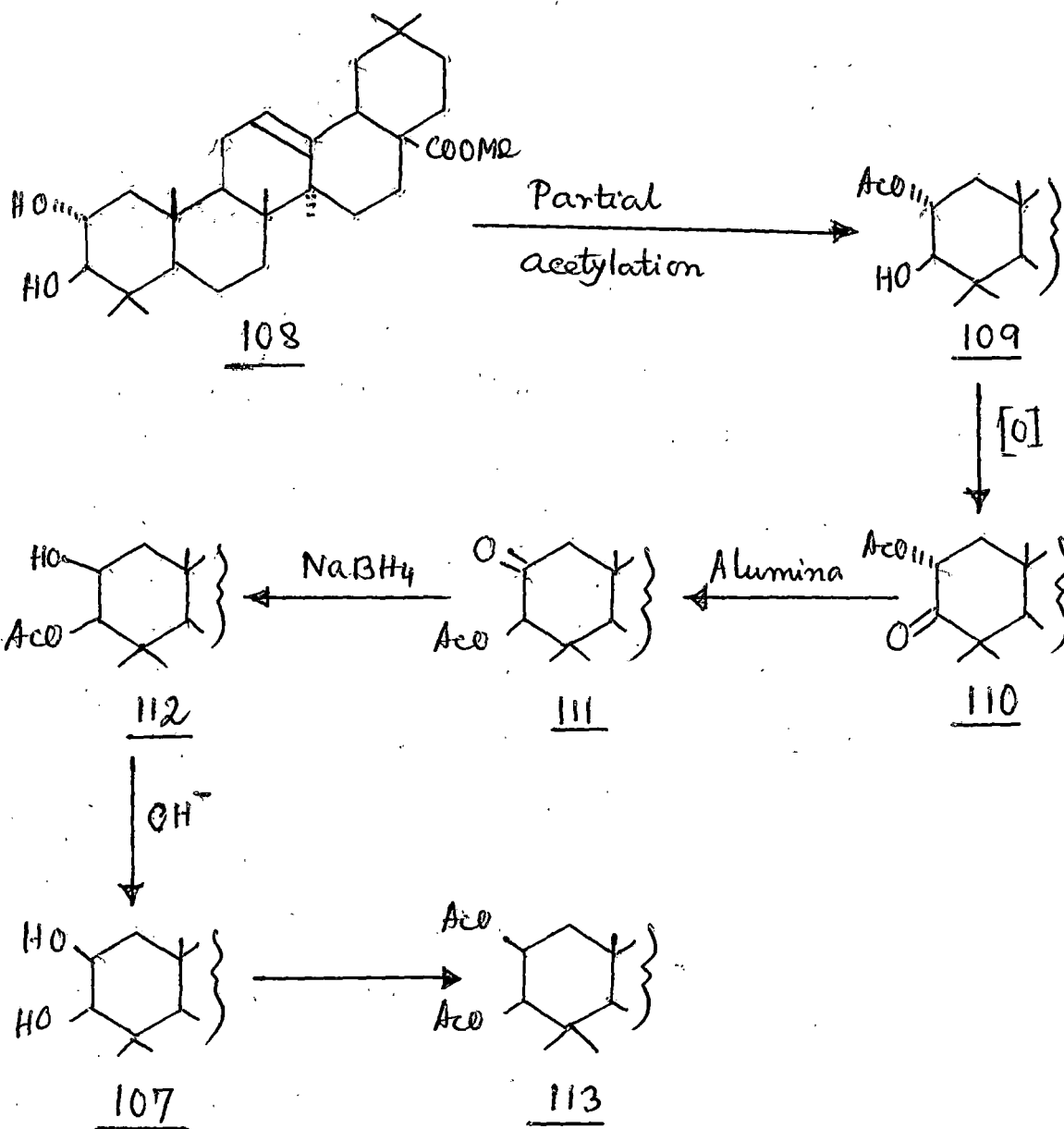


Chart VII



Methyl crategolate 108 on partial acetylation yielded the 2 $\alpha$ -acetoxy-3 $\beta$ -alcohol<sup>43</sup> 109 which was oxidised with dimethyl sulfoxide in acetic anhydride to give 2 $\alpha$ -acetoxy-3-ketone 110. The latter 110 on isomerisation on alumina gave the 3 $\beta$ -acetoxy-2-ketone 111. The structure of this rearranged acetoxy ketone followed from analogy with the rearrangements of similar groups in lupane<sup>50</sup>, lanostane and 4,4-dimethyl cholestane derivatives<sup>51</sup> and from its IR and NMR spectra. Reduction of the 3 $\beta$ -acetoxy-2-ketone 111 with sodium borohydride proceeded quantitatively to give a single product 112 in which the introduced hydroxyl group at C - 2 could be assigned the  $\beta$  - configuration on the assumption that attack has occurred from less hindered  $\alpha$ -side of the molecule. Mild alkaline hydrolysis of 3 $\beta$ -acetoxy-2 $\beta$ -hydroxyolean-12-en-28-oate 112 gave the diol 107 m.p. 278-80°, ( $\alpha$ )<sub>D</sub> 88° (lit. m.p. 258 - 60°, ( $\alpha$ )<sub>D</sub> 97°<sup>43</sup>, m.p. 258-62°<sup>30</sup>, m.p. 258-62° ( $\alpha$ )<sub>D</sub> 85°<sup>38</sup>, m.p. 258-61°<sup>31b</sup>). 107 on acetylation afforded the diacetate, methyl 2 $\beta$ , 3 $\beta$ - diacetoxyolean-12-en-28-oate 113, m.p. 232-40°, ( $\alpha$ )<sub>D</sub> 82°, (lit<sup>31b</sup> m.p. 227-31°).

4. The 2 $\beta$ , 3 $\alpha$ -dihydroxyolean-12-en-28-oic acid  
( bredemolic acid ) :

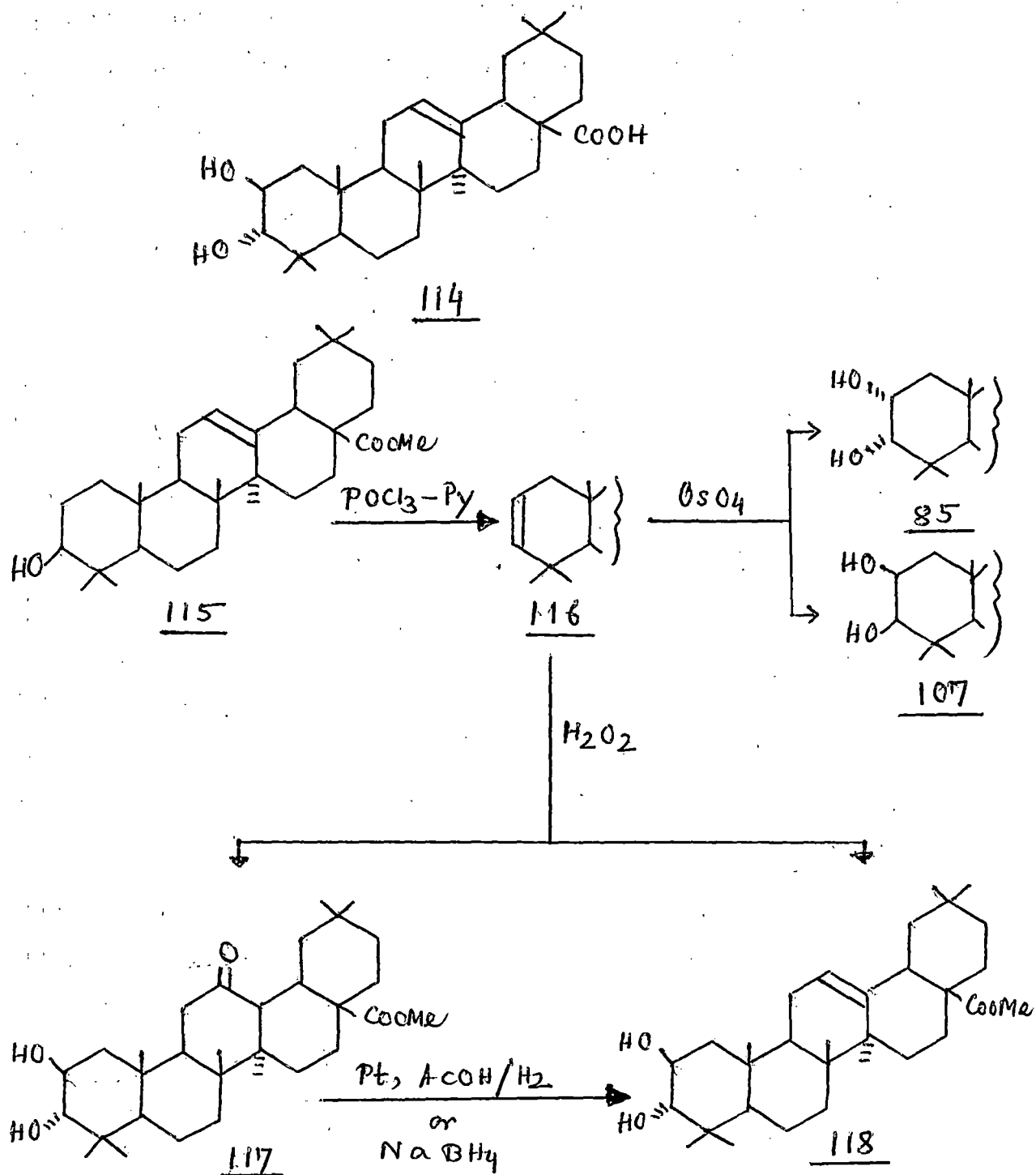
Bredemolic acid 114, isolated from *Bredemeyera floribunda* Willd., was also a 2, 3 - dihydroxyolean-12-en-28-oic acid and

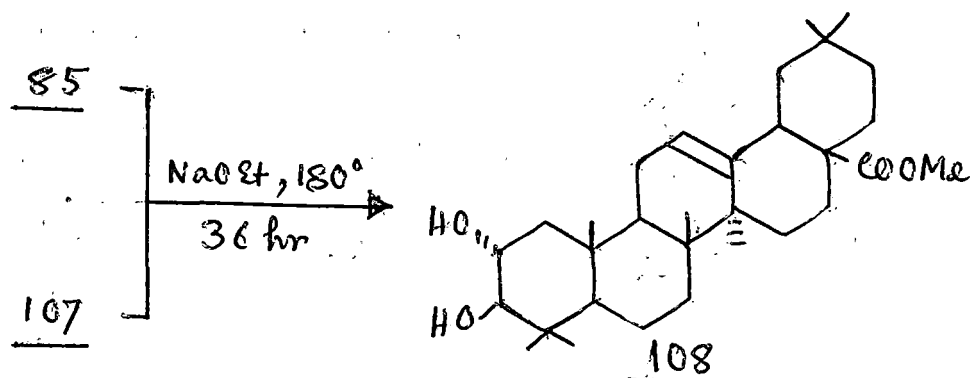
hence must be an epimer of crategolic acid 94. Since the  $2\beta$ ,  $3\beta$ -84, and the  $2\alpha$ ,  $3\alpha$ -84 A isomers were already known by synthesis<sup>38</sup> and crategolic acid 94 was shown to be the  $2\alpha$ ,  $3\beta$ -  
 $\text{dihydroxyolean-12-en-28-oic acid}$  by Caglioti et al<sup>44</sup> bredemolic acid 114 must then be the remaining  $2\beta$ ,  $3\alpha$ -dihydroxy isomer. However bredemolic acid 114 was found to form an acetonide, a somewhat unexpected behaviour on the part of a normal 1,2-diaxial trans cyclohexane diol derivative. Therefore, Tschesche et al synthesised<sup>29, 30</sup> unambiguously all the four stereoisomeric 2,3-dihydroxy olean-12-en-28-oic acids as their respective methyl esters. The synthetic work of Tschesche et al is shown in chart, VIII.

The key compound in the synthesis of the above epimeric methyl esters chart VIII; methyl oleana-2,12-dien-28-oate 116 was prepared from methyl oleanolate 115 by dehydration with phosphorous oxychloride and pyridine. The diene-ester 116 gave two cis diols : the  $2\alpha$ ,  $3\alpha$ - diol 85 and the  $2\beta$ ,  $3\beta$ -diol 107 by treatment with osmium tetroxide. On the otherhand treatment of the diene-ester 116 with hydrogen peroxide gave methyl 12-keto- $2\beta$ ,  $3\alpha$ -dihydroxyolean-28-oate 117 as the major product and only a trace of the desired  $2\beta$ ,  $3\alpha$ - diol 118. The above 12-keto-ester 117, however, was converted by reduction into methyl bredemolate 118. Finally, all the above three diol esters 85, 107 and 118 on equilibration with base gave methyl crategolate 108, which must

consequently have the stablest diequatorial  $2\alpha$ ,  $3\beta$ -configuration of the two hydroxyl groups.

Chart VIII





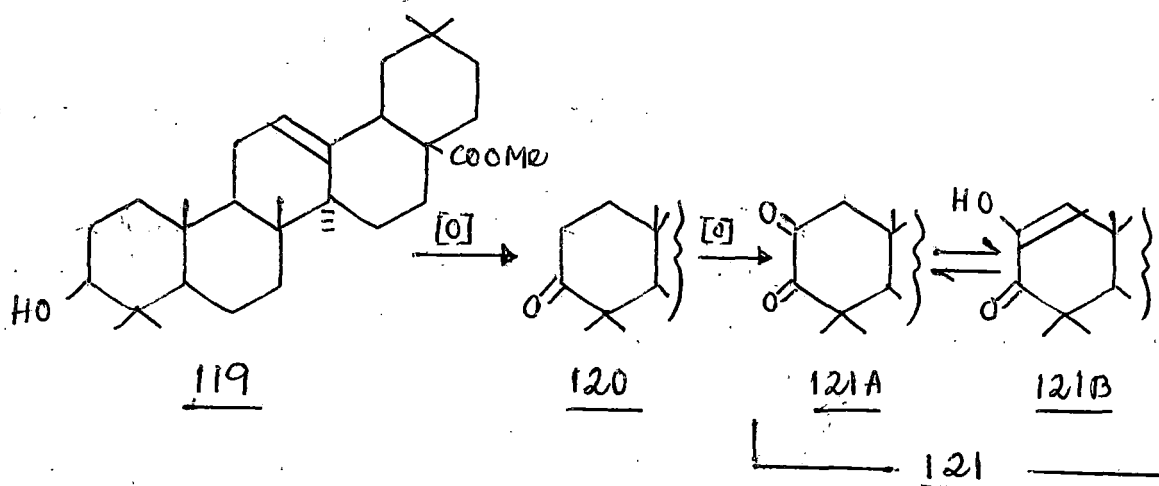
Synthesis of isomeric 2,3-diols of methyl olean-12-en-28-oate.

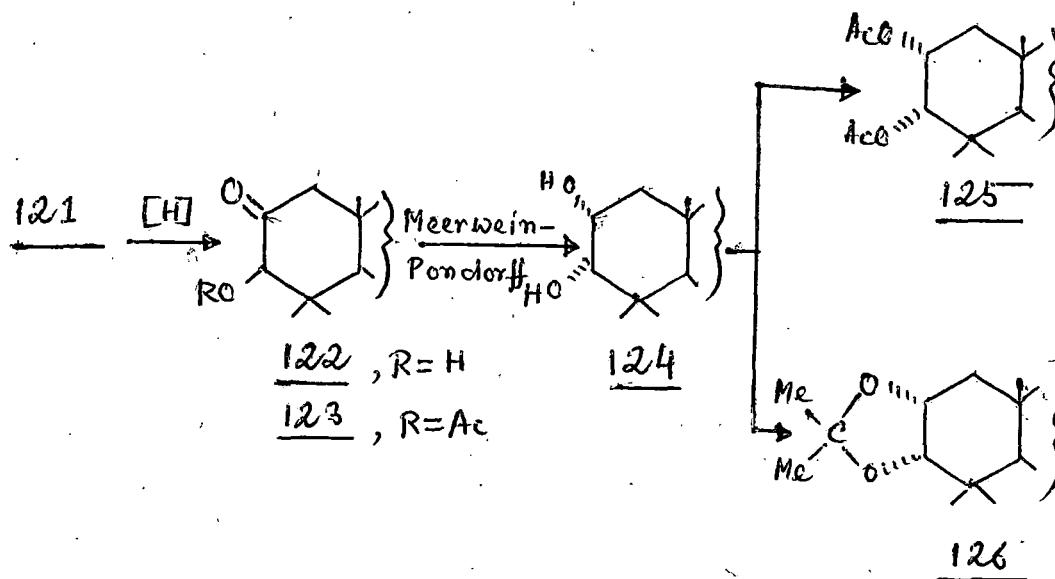
Khastgir et al<sup>10b</sup> also reported the synthesis of the three isomeric diols ( 2 $\alpha$ ,3 $\alpha$ -; 2 $\beta$ ,3 $\beta$ -; 2 $\alpha$ , 3 $\beta$ - ) out of the four possible isomers, using diosphenol obtained by the autoxidation of methyl oleanonate.

1. Synthesis of methyl 2 $\alpha$ , 3 $\alpha$ -dihydroxy-olean-12-en-28-oate 124 :

Methyl oleanonate 120 m.p. 182-4°, ( $\alpha$ )<sub>D</sub> 89° prepared by Jones' oxidation of methyl oleanolate<sup>58</sup> 119, was oxidised by passing oxygen through a suspension of 120 in dry t-butanol containing potassium tertiary butoxide<sup>11-14</sup>. Hydrogenation of diosphenol 121, m.p. 130-5°, ( $\alpha$ )<sub>D</sub> 104  $\pm$  4°, in presence of 10% palladium - on-charcoal catalyst gave the corresponding reduced

product 122, m.p.  $129-31^{\circ}$ ,  $(\alpha)_D 109^{\circ}$ . During this hydrogenation 1,4-addition of hydrogen took place giving the ketol 122 (TLC homogeneous). Acetylation of 122 with acetic anhydride and pyridine gave the corresponding acetate 123, m.p.  $182-4^{\circ}$ ,  $(\alpha)_D 85^{\circ}$ . Meerwein-Ponndorf reduction of 122 furnished a crystalline solid, m.p.  $286-7^{\circ}$ ,  $(\alpha)_D 71^{\circ}$ ,  $\nu_{\max}$  3340 (-OH), 1725 (-COOMe)  $\text{cm}^{-1}$ , which was shown to be identical with methyl 2 $\alpha$ , 3 $\alpha$ -dihydroxy-olean-12-en-28-oate. Acetylation of 124 with acetic anhydride and pyridine gave the diacetate 125 m.p.  $226-8^{\circ}$ ,  $(\alpha)_D 95.20^{\circ}$ . NMR spectrum of the diacetate was in good agreement with the structure 125. The diol 124 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetonide derivative 126, m.p.  $235-9^{\circ}$ .



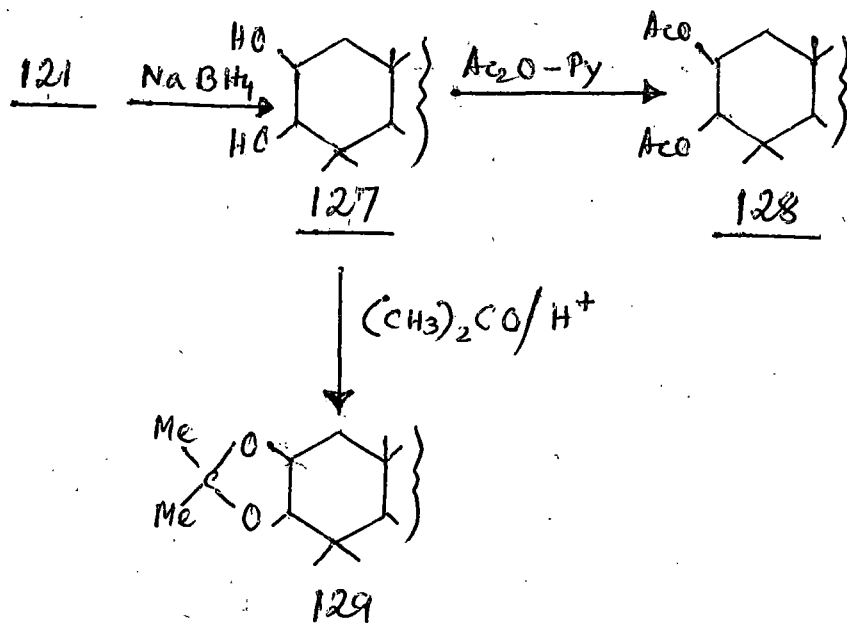


2. Synthesis of methyl 2 $\beta$ , 3 $\beta$ -dihydroxy-olean-  
12-en-28-oate 127:

Diosphenol 121 on sodium borohydride reduction in methanol solution gave a compound 127, m.p. 269-72°. ( $\alpha$ )<sub>D</sub> 98.85° (lit. 31b, 38, 43 m.p. 258-60°, ( $\alpha$ )<sub>D</sub> 97°; m.p. 258-62°, ( $\alpha$ )<sub>D</sub> 85°, m.p. 258-61°), no UV absorption in region 220-300 m $\mu$ ,  $\nu$  max 3525, 3360, 1720 cm<sup>-1</sup>. Treatment of 127 with pyridine and acetic anhydride gave the diacetate 128, m.p.

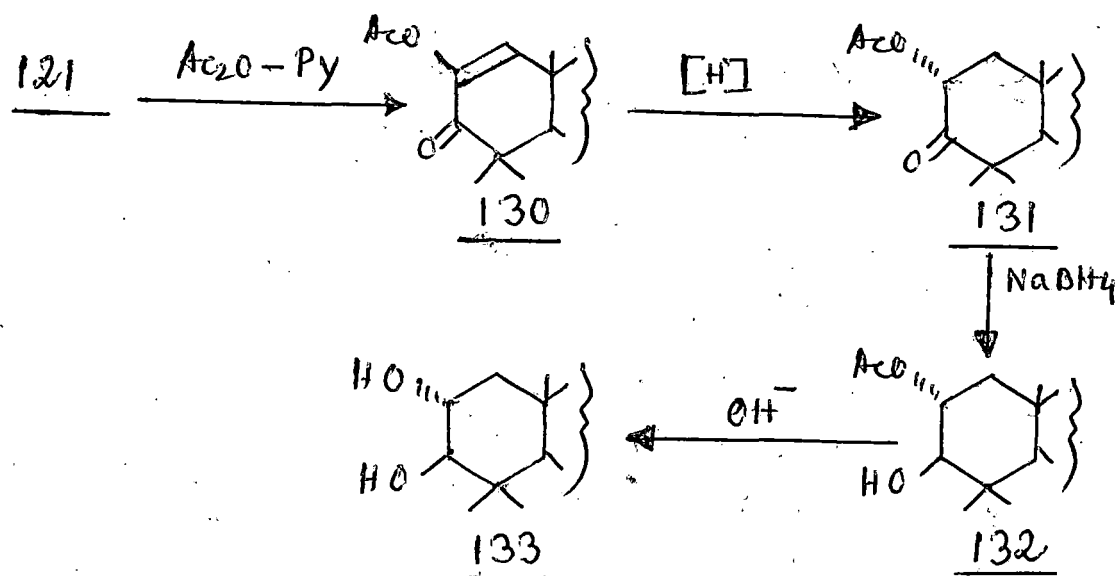


220-22°, ( $\alpha$ )<sub>D</sub> 36.20°,  $\nu$  <sup>KBr</sup><sub>max</sub> 1745, 1720 (C = O), 1258 (C - C) cm<sup>-1</sup>. Examination of the NMR spectrum of 127 exhibited two unresolved multiplets, one at 3.15 ppm assigned to C - 3H and the other at about 4.4 ppm (C - 2H) in addition to the olefinic proton at 5.15 ppm. Thus the hydroxyl group at C - 3 was axial (H<sub>e</sub>) and the one at C - 2 was equatorial (H<sub>a</sub>). In the NMR spectrum of its diacetate, these signals were shifted downfield to 4.6 (J = 4Hz) ppm and at about 5.4 ppm (broad multiplet). The signal for the ester group showed a singlet at 3.65 and that for the acetate groups at 2.06 (singlet 6 H) ppm. The diol 127 on treatment with acetone in presence of catalytic amount p-toluene sulfonic acid gave an acetonide derivative 129, sintering at 75-80°.



3. Synthesis of methyl-2 $\alpha$ , 3 $\beta$ -dihydroxy-olean-12-en-28-oate 133:

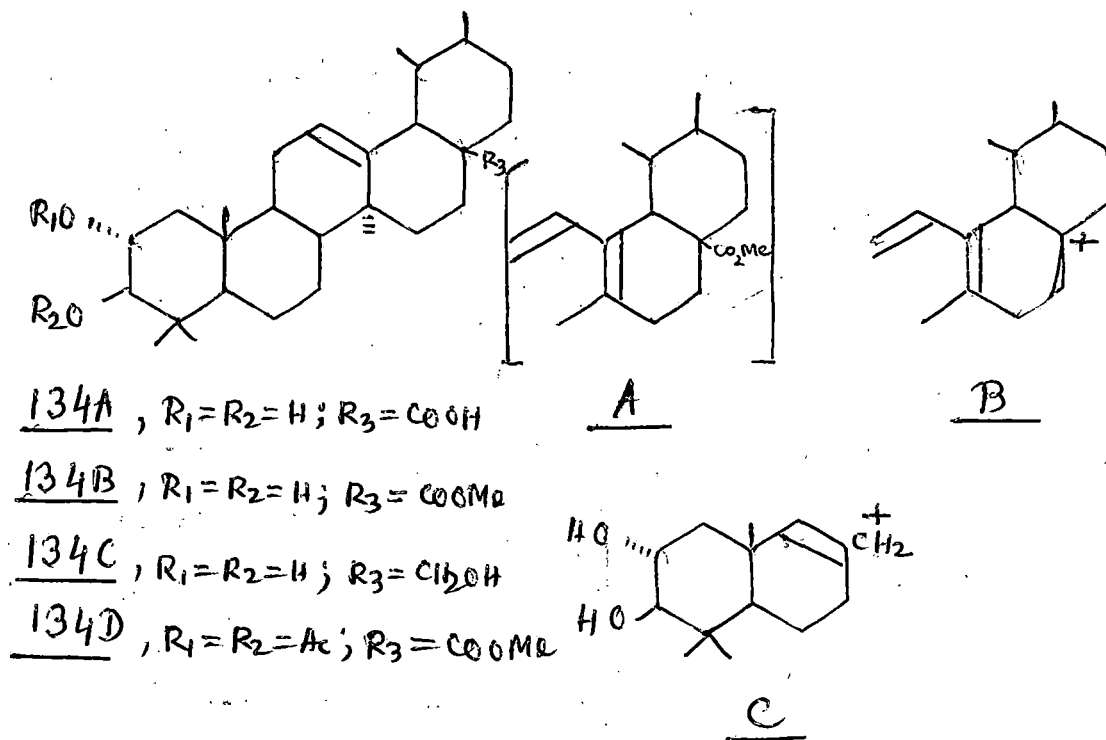
Diosphenol 121 on acetylation with acetic anhydride-pyridine gave the corresponding acetate 130 m.p. 168-70°, ( $\alpha$ )<sub>D</sub> 93°, having  $\lambda_{\text{max}}$  237 m $\mu$  ( $\epsilon$  .8500),  $\nu_{\text{max}}$  nujol 1205, 1685, 1720, 1738 cm<sup>-1</sup>. Hydrogenation of 130 with 10% palladium-on-charcoal catalyst gave a solid 131 m.p. 208-9°, ( $\alpha$ )<sub>D</sub> 52°,  $\nu_{\text{max}}$  1225, 1730, 1750 cm<sup>-1</sup>. The ketoacetate 131 on reduction with sodium borohydride at pH 8 to reduce isomerisation in methanol solution gave a solid 132 m.p. 199-204°, ( $\alpha$ )<sub>D</sub> 27.9°. The latter 132 was directly hydrolysed by 10% sodium hydroxide solution to afford a solid 133 m.p. 220-22°, ( $\alpha$ )<sub>D</sub> 36°. The solid <sup>133</sup> was shown to be identical with an authentic sample of methyl 2 $\alpha$ , 3 $\beta$ -dihydroxy-olean-12-en-28-oate (methyl crategolate) by m.m.p and Co - TLC.



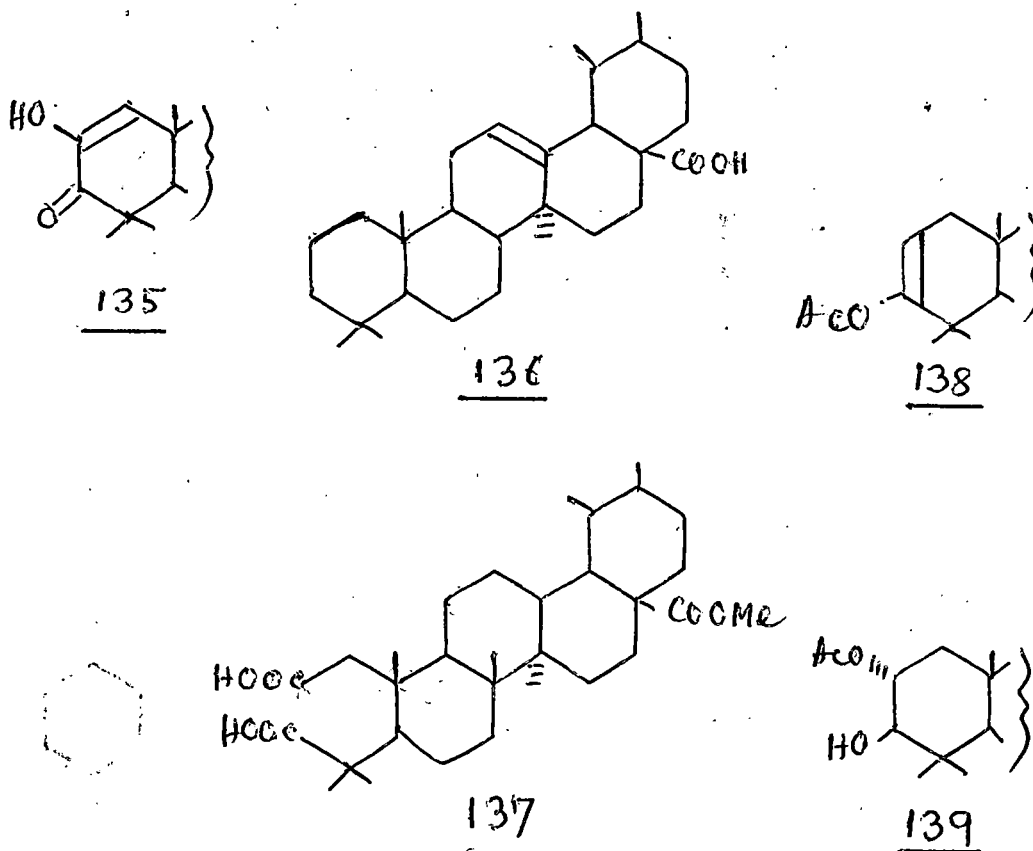
2 $\alpha$ -hydroxy ursolic acid .

Glen et al<sup>35</sup> reported the isolation of 2 $\alpha$ -hydroxy ursolic acid 134 A as its methyl ester from the leaves of Rose-bay Willowherb (Chamaecrista angustifolium). That the methyl ester 134 B was an ursane derivative was indicated by the presence of three peaks in the region of 1400-1350  $\text{cm}^{-1}$  and two peaks in the region 1320-1240  $\text{cm}^{-1}$  in the infrared spectrum<sup>52</sup>. NMR spectrum revealed the presence of one olefinic proton ( $\tau$  4.73) and one methyl ester group ( $\tau$  6.38). Further evidence came from mass spectrum of 134 B which showed intense peaks at  $m/e$  263, 203 and at 223 corresponding to ions A, B and C, a pattern frequently associated with  $\Delta^{12}$ -triterpenoids<sup>36</sup>. A similar mass fragmentation pattern was observed for the 2 $\alpha$ -hydroxy-uvazol 134 C formed by lithium aluminium hydride reduction of the dihydroxy methyl ester 134 B. The methyl ester 134 B formed a diacetate 134 D (two distinct peaks in the NMR at  $\tau$  7.97 and 8.04<sup>53</sup>). This physical evidence for the presence of a 2,3-diol system in 134 B was further substantiated by the preparation of an isopropylidene derivative and also by the formation of the diosphenol 135 by chromium trioxide-pyridine oxidation. The presence of an ursane skeleton in the ester 134 B was shown by the Wolff Kishner reduction of the diosphenol 135 to give

urs-12-en-28-oic acid 136 identical with an authentic sample.



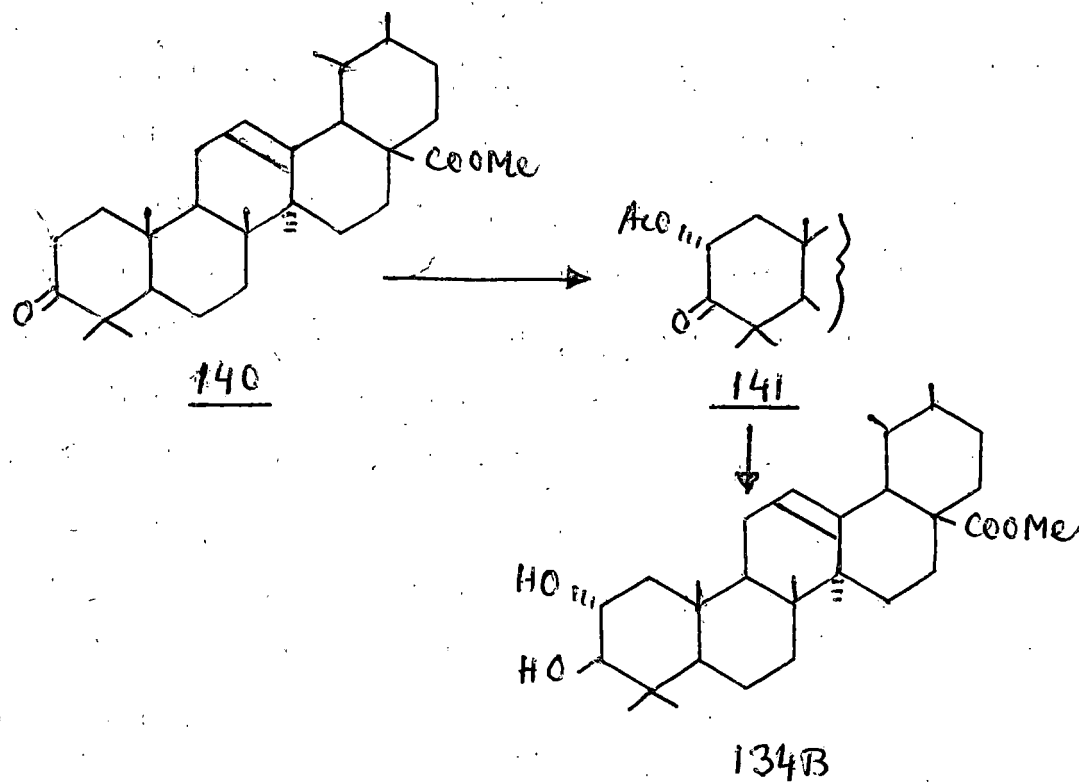
Cleavage of the diosphenol 135 with alkaline hydrogen peroxide gave 2,3 seco-urs-12-en-2,3,28-trioic acid-28-methyl ester 137 which confirmed the presence of the ursane skeleton in the ester 134 B. The configuration of the hydroxyl group in the dihydroxy ester 134 B was settled by the synthesis of the trans diequatorial diol 134 B. The enol acetate 138 on treatment with diborane and then subsequent oxidation of the intermediate



afforded three products viz. methyl ursonate, methyl ursolate and methyl  $2\alpha, 3\beta$ -dihydroxy urs-12-en-28-oate 134 B, the latter was identical with that obtained from the natural dihydroxy acid 134 A.

Further support for the assignment of the  $\alpha$ -configuration to the hydroxyl group at C - 2 in 134 B was revealed by the NMR spectrum of the monoacetate, methyl  $2\alpha$ -acetoxy- $3\beta$ -

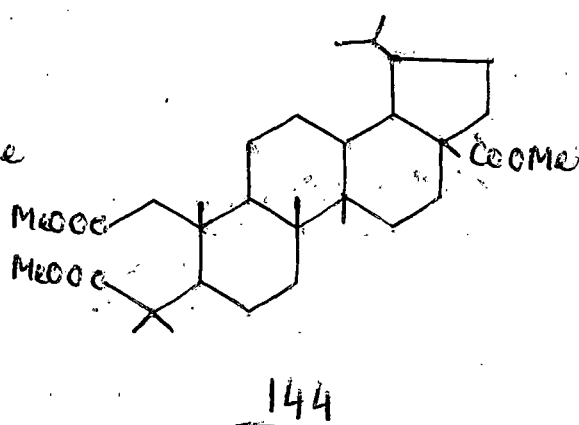
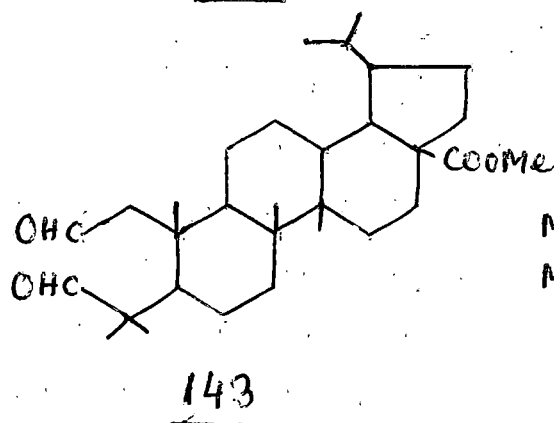
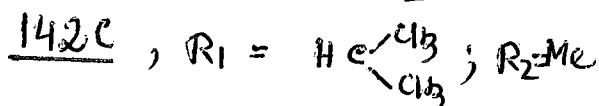
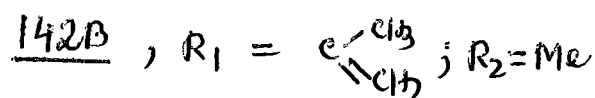
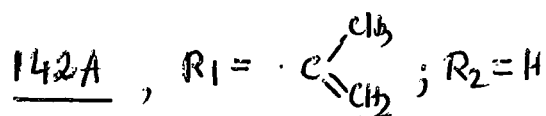
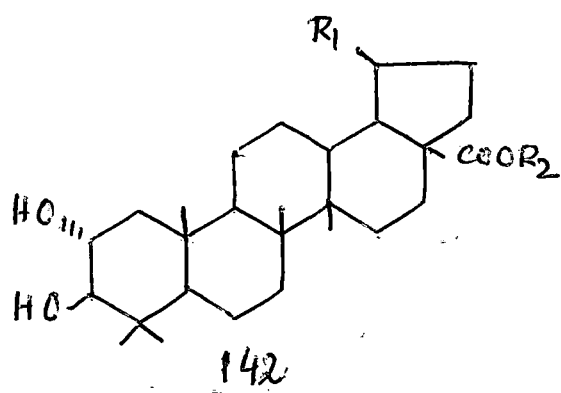
hydroxy urs-12-en-28-oate 139. The NMR spectrum showed signals in the region  $\tau$  4.9 - 5.4 due to C - 2 proton. The breadth of the resonance indicated that at least two couplings of about 8 c/sec are present and this can only occur when the proton at C - 2 occupies an axial conformation allowing an axial interaction with the proton at C - 3 and a similar interaction with the axial proton at C - 1. In addition, there is an axial - equatorial interaction with the other proton at C - 1. This indicated that the proton at C - 2 has the axial conformation and acetoxy group at C - 2 has the equatorial ( $\alpha$ ) conformation. Rastogi et al<sup>54</sup> repeated Glen's<sup>35</sup> work and obtained the enol acetate m.p. 198° in 46% yield but subsequent steps of hydroboration and oxidation yielded only the starting material methyl ursolate, Rastogi et al employed lead tetraacetate oxidation on methyl ursolate 140 and obtained 2 $\alpha$ -acetoxy-3-keto derivative 141 as an amorphous powder, the latter was characterised by NMR spectrum. Reduction of 140 with sodium borohydride gave the methyl 2 $\alpha$ -hydroxy ursolate 134 B in an overall yield of 26.7%, characterised by IR and NMR data.



Alphitolic acid :

Guise et al<sup>34</sup> isolated alphitolic acid **142 A** as its methyl ester, m.p. 233 - 35° from the wood of Alphitonia petrici Braid and white. The ester, methyl alphitolate **142 B** ( $\nu_{\text{max}}$  3634, 3587, 1735, 887  $\text{cm}^{-1}$ ) formed a diacetate (no hydroxyl absorption in the infrared) and a dihydro-derivative **142 C** (no absorption in the infrared at 887  $\text{cm}^{-1}$ ) namely methyl dihydro-alphitolate. The ester **142 B** consumed one mole of lead tetraacetate. Guise et al converted dihydro-methyl alphitolate **142 C** to a dialdehyde **143** by treatment with 1 mole equivalent of sodium metaperiodate. The latter on oxidation with chromic anhydride in acetic acid followed by

methylation gave a trimethyl ester 144 identical with trimethyl ester of the Seco-A-acid derived from dihydrobetulinic<sup>ni</sup> acid<sup>55</sup>. From the above physical and chemical evidences Guise et al confirmed the structure of methyl alphitolate as depicted in 142 B.



The stereochemistry of 1,2-glycol grouping in 142 B was based on quantitative lead-tetraacetate titrations under the defined conditions of Djerassi and Ehrlich<sup>56</sup>, where a value  $k = 2.7 \times 10^{-3} \text{ L mole}^{-1} \text{ sec}^{-1}$  was obtained, identical for triterpene 2 $\alpha$ , 3 $\beta$ -glycols.

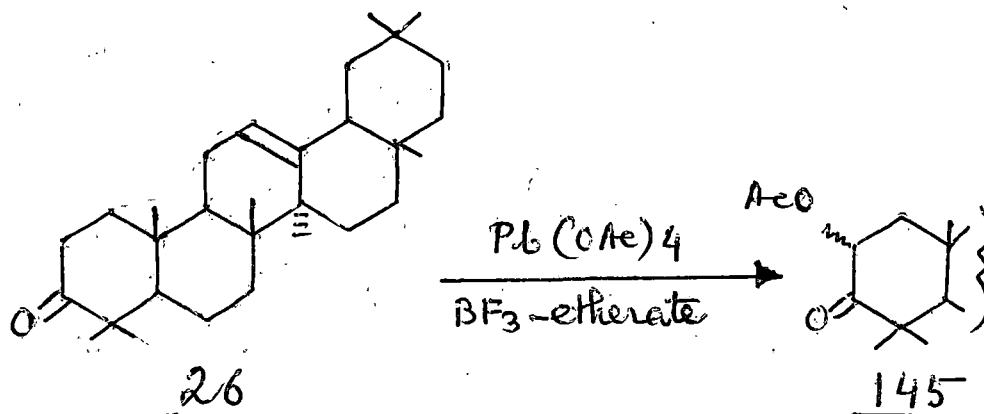


CHAPTER - II

Studies on autoxidation : Unambiguous synthesis of methyl dihydro-alphitolate (section B) and  $2\beta$ ,  $3\beta$ -dihydroxy betulinate (section C) from betulinic acid.

Section A : Introduction :

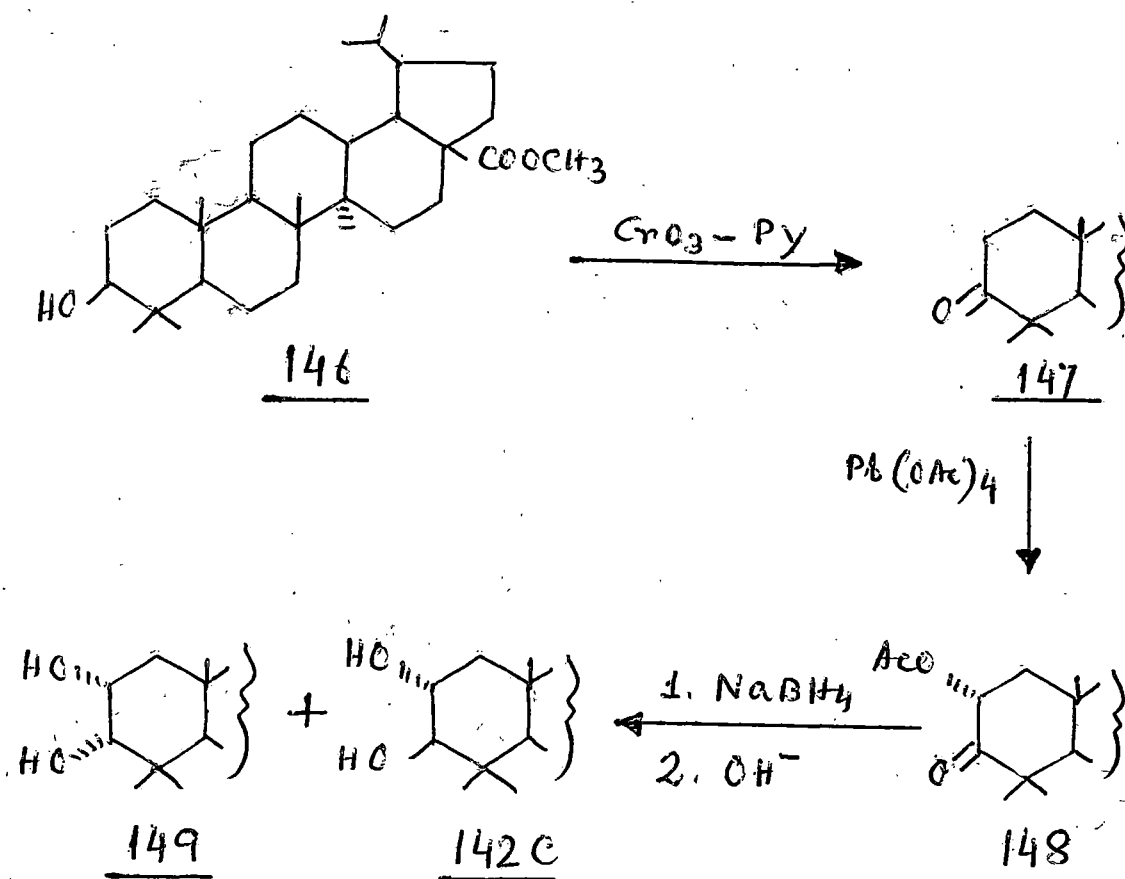
During the course of studies on autoxidation in  $\beta$  - amyron series Khastgir et al<sup>106</sup> found that the  $2\alpha$ -acetoxy- $\beta$ -amyron 29 ( page 92) obtained by acetylation of diosphenol 27 and subsequent hydrogenation of the diosphenol acetate differed from that of the  $2\alpha$ -acetoxy- $\beta$  -amyron 145 obtained by direct acetoxylation of  $\beta$  -amyron 26 by lead tetra acetate in presence of  $\text{BF}_3$  - etherate in benzene solution. Acetoxylation at the  $\alpha$ -position to



the 3 - keto group in ring A of triterpenoids with lead tetraacetate leading to 2 $\alpha$ -acetoxy-3-keto compounds is well documented <sup>18,57,58</sup>. This reaction, thought to proceed via the enol <sup>22</sup> is expected to yield a product resulting by attack from the less hindered  $\alpha$  - side. They reported the isolation of an acetoxy ketone 145 having the same melting point and m.m.p. 158 - 60° as 29 prepared by hydrogenation of diosphenol acetate but their IR spectra revealed significant deviations in the region 1225 - 1245 cm<sup>-1</sup> and 1070 - 1095 cm<sup>-1</sup>. This observation led them to believe that most probably the lead tetraacetate acetoxylation product 145 could be a mixture of conformational isomers. Relevant to this observation is the paper of Jones et al <sup>59</sup> in which they stated that acetoxylation of 3-keto triterpenoids can cause conformational changes in ring A. Khastgir et al <sup>60</sup> also studied the NMR spectra of the two compounds 29 and 145 and they concluded that the lead tetraacetate acetoxylation product 145 was a mixture of conformational isomers. The NMR spectrum of the compound 29 showed a multiplet at 5.62 ppm for the proton at C - 2 <sup>39</sup>. As the X part of an ABX pattern with a width of 20 Hz ( sum of J ) the proton must be axial with an axial-axial and an axial-equatorial coupling. Thus the acetoxy group at C - 2 in 29 is equatorial and predominantly contained one isomer. However, the NMR spectrum

of 145, showed a doubling of several signals. The region of peaks associated with C - 2 proton in 145 was complex and showed several additional peaks. From these observations they stated that 145 must be a mixture of isomers.

Furthermore, Row and co-workers<sup>61</sup> attempted the synthesis of methyl dihydroaliphitolate 142 C starting from methyl dihydrobetulinate 146 according to the following scheme:



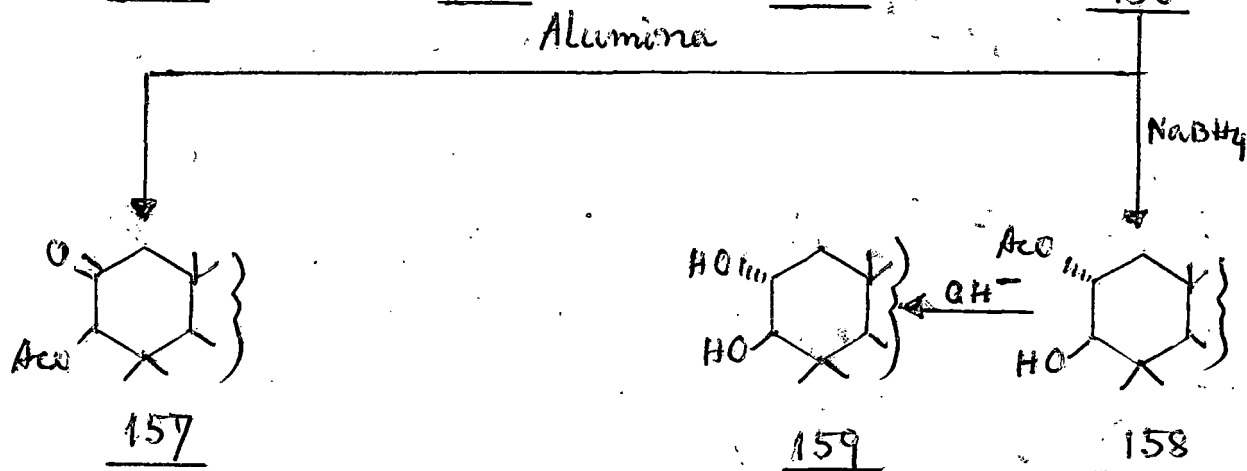
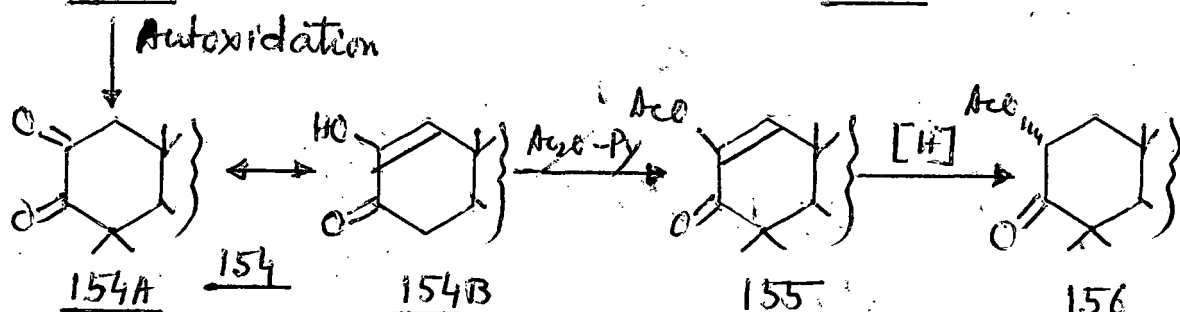
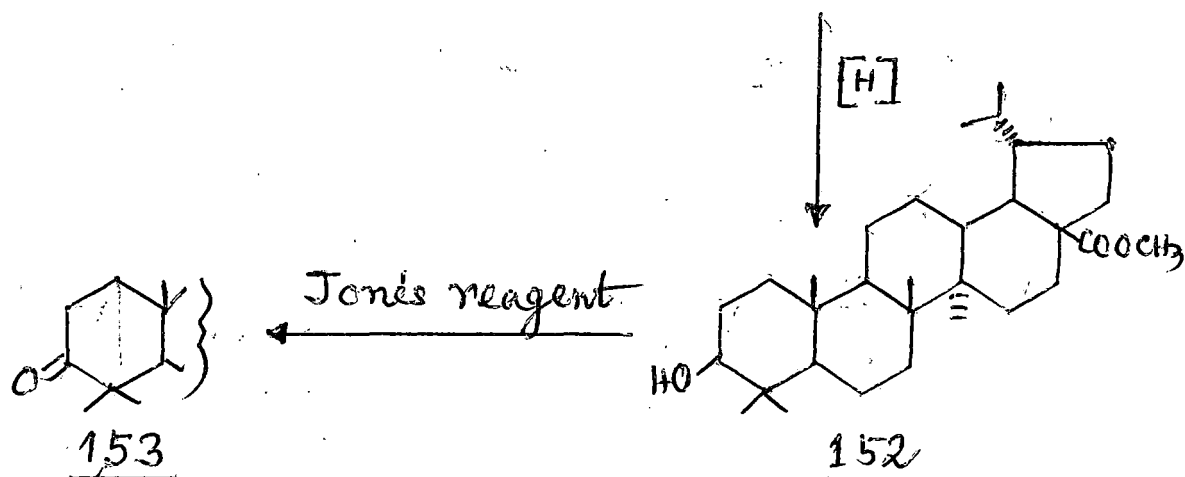
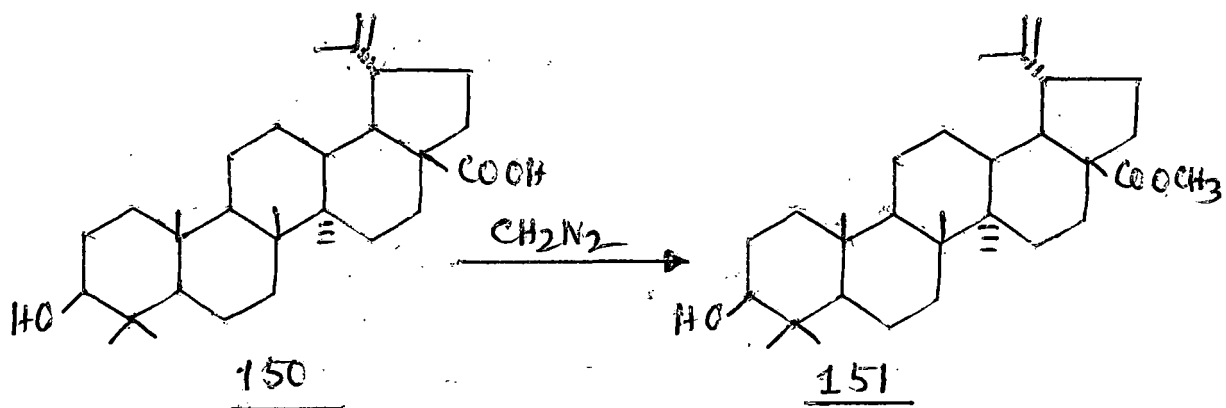
The compound 146 was prepared by catalytic reduction of methyl betulinate in ethanol using Raney nickel-hydrogen at 100°/400 lb. or Adams' catalyst. Oxidation of 146 with chromium trioxide - pyridine furnished 3-keto methylester 147 as colourless plates, m.p. 203-5°,  $(\alpha)_D^{30} + 9^\circ$  which was subjected to acetoxylation with lead tetraacetate under varying conditions and the best yield of 2 $\alpha$ -acetoxy-3-keto ester 148, m.p. 215-16°,  $(\alpha)_D^{30} + 4^\circ$ ,  $\nu_{\max}$  1735 and 1720  $\text{cm}^{-1}$  was obtained when 3-keto ester 147 was heated at 100° with freshly prepared lead tetraacetate in glacial acetic acid. Reduction of 148 with sodium borohydride followed by mild alkaline hydrolysis gave a mixture of glycols ( 142 C and 149 ) which was separated by chromatography on alumina. The minor fraction was found to be a mixture ~~of~~ and the major fraction crystallised from methanol as colourless needles, m.p. 228-30°,  $(\alpha)_D^{30} + 1.2^\circ$ ,  $\nu_{\max}$  3600, 1720  $\text{cm}^{-1}$ . It consumed 1.15 moles of lead tetraacetate within 8-hr indicating the presence of a trans-1,2 - glycol system. It was therefore, regarded to be methyl 2 $\alpha$ , 3 $\beta$ -dihydroxy-lupan-28-carboxylate 142 C. Row *et al*<sup>61</sup> reported that the compound 142 C showed no depression ~~of~~ in melting point on admixture with an authentic sample but the IR comparison showed a significant difference. This is in accord with the observations stated by Khastgir *et al*<sup>10a</sup>.

In 1968 Cheung and Feng<sup>57</sup> reported the partial synthesis of methyl dihydro\_alphitolate by following essentially the same sequence of reactions as described by Row et al. Although the melting point of their product was same as that recorded in the literature<sup>34</sup> for natural dihydroalphitolate we suspect that their synthetic product was probably a mixture. They have not recorded the IR spectra of the two compounds. These anomalies prompted us to synthesise methyl dihydro\_alphitolate starting from betulinic acid as described in section B.

Section B : An unambiguous synthesis of methyl dihydro-alphitolate from betulinic acid.

Betulinic acid 150 obtained by the extraction of *Bischofia javanica* Blume with benzene was esterified with excess diazomethane. The crude ester on chromatography and crystallisation from chloroform - methanol mixture afforded colourless needles of 151, m.p. 222 - 24°, ( $\alpha$ )<sub>D</sub> + 4°,  $\nu$ <sub>max</sub> 3520, 1735, 1660 and 876 cm<sup>-1</sup>. 151 was found to be identical with an authentic sample of methyl betulinate by m.m.p. determination and I.R. comparison. Hydrogenation of methyl betulinate 151 in presence of Adams' catalyst gave methyl dihydrobetulinate 152, m.p. 236 - 38°, ( $\alpha$ )<sub>D</sub> - 18.6°  
Jones' oxidation of methyl dihydrobetulinate furnished a

product 153, m.p.  $202-4^{\circ}$ ,  $(\alpha)_D + 8^{\circ}$ , which was found to be identical in all respects with methyl dihydrobetulonate. Oxidation of methyl dihydrobetulonate 153, by passing oxygen through a suspension of it in tertiary butanol containing potassium tertiary butoxide ( IN ) afforded a product, 154, m.p.  $131-3^{\circ}$ ,  $(\alpha)_D - 1.96^{\circ}$ . The compound showed a positive ferric chloride coloration and in the t.l.c it showed two spots of very close Rf values indicating a mixture of two tautomeric forms-the diketone 154 A and diosphenol 154 B. The UV spectrum of the latter showed a maxima at  $269 \text{ m}\mu$  (  $\epsilon$  , 7532 ). The compound 154 B on acetylation with acetic anhydride - pyridine at room temperature gave the corresponding acetate 155, m.p.  $194 - 96^{\circ}$ ,  $(\alpha)_D + 9.43^{\circ}$  which showed a single spot on chromatoplate . UV spectrum showed a maxima at  $237 \text{ m}\mu$  (  $\epsilon$  , 9968 ). These physical data and chemical properties are in good agreement with the structures 154 B and 155 for diosphenol and diosphenol acetate respectively. Hydrogenation of diosphenol acetate 155 in presence of 10 % palladium - on - charcoal catalyst gave a product, m.p.  $223-25^{\circ}$ ,  $(\alpha)_D + 32.56^{\circ}$ . This compound has been assigned sturcture 156 on the basis of its chemical properties and in analogy to the previous work of Khastgir et al<sup>10a, 10b</sup>. The compound 156 was kept overnight on a basic column of alumina and after eluting



with benzene it afforded a new product, m.p.  $228-30^{\circ}$  which has been assigned structure 157, methyl-2-keto-3-acetoxy dihydrobetulinate, by analogy with previous work. This is formed by the migration of the acyl group from  $2\alpha$ - position to  $3\beta$ - position. This type of migration has been reported in literature both in the terpenoid and steroid fields<sup>13,17</sup>. This fact establishes the structure of 156 as  $2\alpha$ - acetoxy methyl dihydrobetulonate.  $2\alpha$ -acetoxy methyl dihydrobetulonate 156 on sodium borohydride reduction in methanol-dioxan solution at pH 8 to reduce isomerisation gave a crystalline solid 158, m.p.  $259-61^{\circ}$ ,  $(\alpha)_D - 7.14^{\circ}$ . The latter on a hydrolysis with a 15 % methanolic potassium hydroxide solution afforded a product which on chromatography gave a crystalline solid, 159, m.p.  $230-32^{\circ}$ . It did not show any depression in melting point when mixed with an authentic sample of methyl dihydroaliphitolate supplied by Prof. E. Ritchie. The infrared spectrum of the two compounds were also found to be identical throughout the entire range.

Recently, in our laboratories, Khastgir and co-workers<sup>10a,64</sup> have adduced additional evidence for the  $\alpha$ - equatorial configuration of the 2 - acetoxy group in the 2-acetoxy ketone 29 from O. R. D. studies. They have shown that the NMR spectrum of 29 showed a multiplet at  $\delta$  5.62<sup>39</sup> for proton at C - 2. This downfield signal for a proton with a clear quartet of lines



( sum of J. 20 Hz ) can be assumed for a methine proton  $\alpha$  both to an acetoxy group and a carbonyl group with the acetoxy group at C - 2 position. They argued that this type of NMR signals could explain equally both for 2 $\alpha$ -equatorial acetoxy group with the chair conformation of ring A and 2 $\beta$ -axial acetoxy group with the boat conformation of ring A. The 2 $\alpha$ -equatorial configuration of the acetoxy group has been strengthened by the O.R.D. studies of the acetoxy ketones. The octant rule<sup>62</sup> dictates that an  $\alpha$ -substituent will make a significant contribution to the ketone  $n \rightarrow \pi^*$  cotton effect only when in an axial orientation and the sign of the contribution will be determined by the sign of the octant in which the substituent falls. Since equatorial  $\alpha$ -substituents lie near a symmetry plane their contribution will be insignificant. The O.R.D. of  $\alpha$ -acetoxy ketones has been studied recently by Bull and Enslin<sup>63</sup> and also Klyne *et al*<sup>64</sup>. From their studies it emerged that in many cases ( though not in all ) the effect of an acetoxy group to the carbonyl chromophore is anti-octant i.e. the contribution of an acetoxy group to the carbonyl Cotton effect is opposite to that of an alkyl group in the same position.

In the parent ketone  $\beta$ -amyrene 26 the conformation of ring A is a flattened chair ( to relieve the diaxial interaction

between the  $10\beta$ -methyl and  $4\beta$ -methyl groups ) and this leads to a positive cotton effect. The same conformation is probable for the  $2\alpha$ -acetoxy derivative, but because of the flattening of ring A, the  $2\alpha$  (equatorial) acetoxy group does not lie in the nodal plane of the carbonyl group but protrudes into the back upper right octant. An alkyl group in that position would make a negative contribution to cotton effect, but the "anti-octant" acetoxy group makes a positive contribution. Thus compound 29, having a  $2\alpha$ -acetoxy configuration, would be expected to exhibit a more positive cotton effect, than the parent ketone  $\beta$ -amyrone 26. This has actually been found to be true in the present case. In the O. R. D. curve of  $2\alpha$ -acetoxy ketone 29 the amplitude - peak  $[\phi]_{307}$  - trough  $[\phi]_{269}$  has been found to be greater than that of the corresponding parent ketone ( $\beta$ -amyrone), peak  $[\phi]_{307}$  - trough  $[\phi]_{278}$ . On the basis of the above evidence Khastgir and colleagues observed that the compound 29 has the  $2\alpha$ -equatorial acetoxy configuration. They have also stated that the alternative  $2\beta$ -acetoxy configuration with the boat conformation of ring A would in all probability lead to a small negative cotton effect.

Thus our synthesis of methyl dihydroalphitolate and the configuration of the hydroxyl groups ( $2\alpha$ ,  $3\beta$ ) stand on

firm grounds - and we believe that this is the first unambiguous synthesis of methyl dihydroalphitolate having the same configuration and stereochemistry as the natural product.

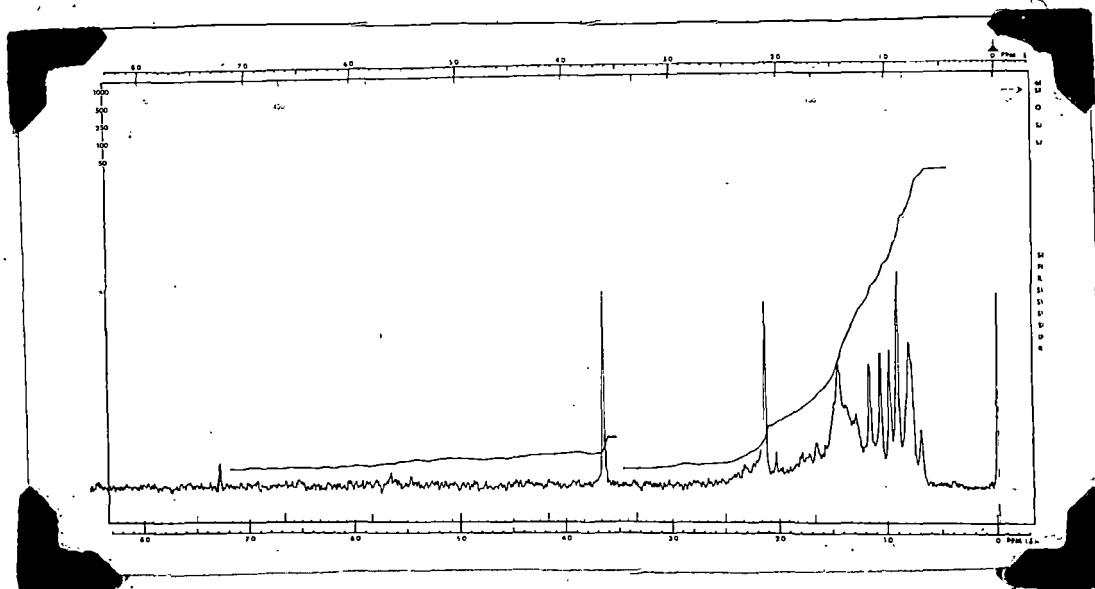


Fig. 1 : NMR spectrum of 2 $\alpha$ -acetoxy methyl dihydrobetulonate 156

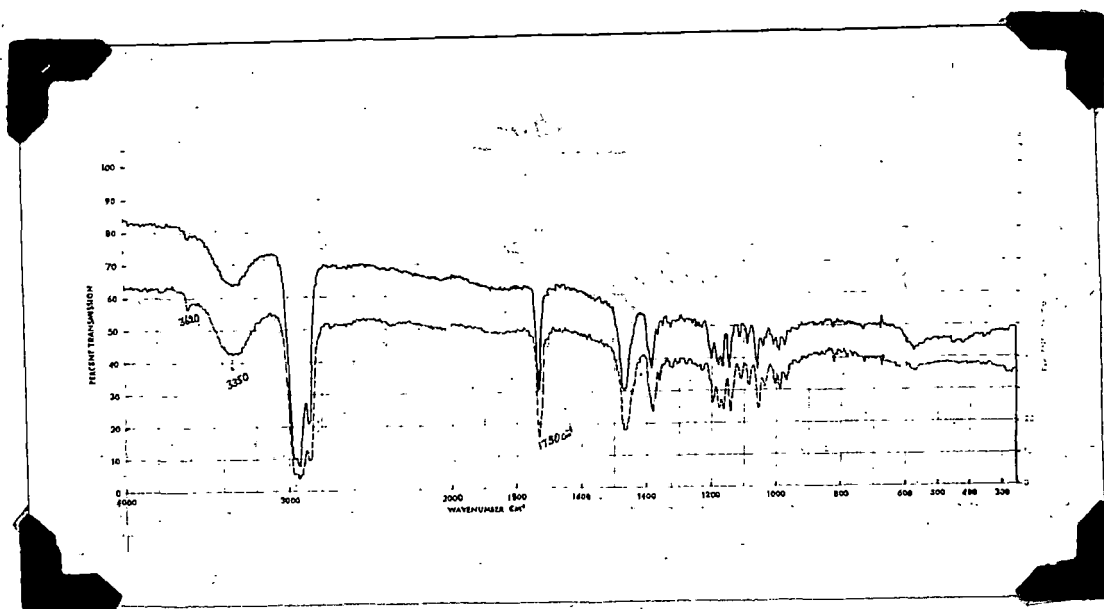


Fig. 2 : IR comparison of methyl dihydroalphitolate 159 (solid line) with an authentic specimen (dotted line)

### EXPERIMENTAL

Melting points are uncorrected. The petroleum used throughout the investigation had b.p.  $60 - 80^{\circ}$ . All optical rotations were determined in chloroform solution unless stated otherwise. NMR spectra were determined on Varian A - 60 and HA - 100 spectrophotometer using chloroform -  $d$  solution containing tetramethyl-silane as reference. The IR spectra were recorded in Perkin-Elmer 337 and 221 and Beckmann IR - 20 spectrophotometers in 95% ethanol solution unless stated otherwise. TLC was done on chromatoplate of silica gel G ( E. Merck ) and the spots were developed with  $H_2SO_4 - Ac_2O$  ( 9 : 1 ) mixture.

Extraction of Bischofia Javanica Blume ; Isolation of betulinic Acid 150 :

described on page 48 .

Esterification of betulinic acid 150 ; Preparation of methyl betulinate 151 :

Described on page 49

Hydrogenation of methyl betulinate 151 ; Preparation of methyl dihydrobetulinate 152 ;

Described on page 50

Jones oxidation of methyl dihydrobetulinate 152 ; Preparation of methyl dihydrobetulonate 153 ;

Described on page 51

Autoxidation of methyl dihydrobetulonate 153 ; Preparation of diosphenol 154 ;

Described on page 53 .

Acetylation of diosphenol 154 ; Preparation of diosphenol acetate 155 ;

Diosphenol 154 ( 1 gm ) was treated with acetic anhydride ( 10 ml ) and pyridine ( 10 ml ) and kept overnight at room temperature. After working up in the usual manner the crude acetate ( 0 .9 gm ) was obtained. This was chromatographed over a column of alumina ( 60 gm ) deactivated with 2.4 ml of 10%

aqueous acetic acid. The compound was dissolved in benzene ( 7 ml ) and the chromatogram was developed with petroleum. The following solvents were used as eluent ( Table - I )

Table - I

| Eluent                     | Fractions<br>50 ml each | Residue on<br>evaporation.        |
|----------------------------|-------------------------|-----------------------------------|
| Petroleum <del>ether</del> | 1 - 3                   | Nil                               |
| petroleum <del>ether</del> | 4 - 15                  | Solid (0.85 gm)<br>m.p. 187 - 90° |

Elution with more polar solvent did not afford ~~any~~ any solid material.

Fractions 4 - 15 ( 0.85 gm ) were combined and on crystallisation from methanol afforded needle shaped crystals of 155, m.p. 194 - 96°, ( $\alpha$ )<sub>D</sub> + 9.43°. It showed a single spot on a chromatoplate and did not respond to the colour-test with ferric chloride solution for diosphenol.

Found : C, 75.52; H, 9.73%

Calculated for  $C_{33}H_{50}O_5$  : C, 75.25; H, 9.57%

UV :  $\lambda_{\max}$  237  $m\mu$  ( $\epsilon$  . 9968 )

Hydrogenation of diosphenol acetate 155 :

Preparation of 2 $\alpha$ -acetoxy-methyl dihydrobetulonate 156 :

To diosphenol acetate 155 ( 1 gm ) dissolved in a mixture of ethyl acetate and ethanol ( 30 ml each ) was added 10% palladium-on-charcoal catalyst ( 100 mg ) and the mixture was shaken in an atmosphere of hydrogen till the absorption of hydrogen ceased. The solution was filtered and after removing the solvent from the filtrate a solid residue was obtained which after crystallisation from methanol afforded 156, m.p. 223-25 $^{\circ}$ ,  $(\alpha)_D + 32.56^{\circ}$

Found : C, 74.75 ; H, 9.97%

Calculated for  $C_{33}H_{52}O_5$  : C, 74.96 ; H, 9.91%

NMR (60Mc) :

Fig - 1

Sodium borohydride reduction of 2 $\alpha$ -acetoxy-methyl dihydrobetulonate 156 ; Preparation of 2 $\alpha$ -acetoxy-methyl dihydrobetulinate 158 :

The 2 $\alpha$ -acetoxy-methyl dihydrobetulonate 156 (200 mg)



dissolved in a mixture of dioxan and methanol ( 10 ml each ), was added, with cooling a slurry of sodium borohydride (200 mg) prepared in an  $\text{NH}_4\text{Cl} - \text{NH}_4\text{OH}$  buffer ( pH = 8, 3 ml ) and the mixture was stirred at room temperature for three hours. Most of the solvents were removed by distillation, cooled and acidified with dilute hydrochloric acid and then extracted with ether. The ethereal layer was washed with water till neutral, <sup>and</sup> dried (  $\text{MgSO}_4$  ). Removal of ether gave a solid residue <sup>the</sup> ( 150 mg ) which was chromatographed over a column of alumina ( 25 gm, deactivated with 1 ml of 10% aqueous acetic acid ). The residue was dissolved in benzene, poured on the column and was eluted with the following solvents ( Table - II ).

Table - II

| Fluent                           | Fractions<br>50 ml each | Residue on<br>evaporation        |
|----------------------------------|-------------------------|----------------------------------|
| Petroleum                        | 1 - 4                   | Oil ( 15 mg )                    |
| Petroleum ;<br>benzene ( 4 : 1 ) | 5 - 7                   | Nil                              |
| Petroleum ;<br>benzene ( 3 : 2 ) | 8 - 12                  | Solid (100 mg)<br>m.p. 254 - 57° |

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

Fractions 8 - 12 ( 100 mg ) were combined and on crystallisation from methanol furnished pure 2 $\alpha$ -acetoxy-methyl dihydrobetulinate 158, m.p. 259 - 61 $^{\circ}$ , ( $\alpha$ )<sub>D</sub> - 7.14 $^{\circ}$ .

Foundn; C, 74.43; H, 10.02%

Calculated for C<sub>33</sub>H<sub>54</sub>O<sub>5</sub> : C, 74.67; H, 10.25%

IR :

Hydrolysis of 2 $\alpha$ - acetoxy-methyl dihydrobetulinate 158

Preparation of methyl dihydroalphitolate 159 :

To 2 $\alpha$ - acetoxy-methyl dihydroalphitolate 158 ( 200 mg ) was added 15% methanolic potassium hydroxide ( 20 ml ) and the mixture was heated under reflux for three hours. The reaction mixture, after removal of solvent, was diluted with water and then extracted with ether. The ethereal layer was washed with water till neutral and dried ( Na<sub>2</sub>SO<sub>4</sub> ). The solvent was removed and a solid residue ( 180 mg ) was obtained. This residue dissolved in benzene was placed over a column of alumina ( 15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid ) developed with petroleum and was eluted with the following solvents ( Table - III )

Table - III

| Eluent                | Fractions<br>50 ml each | Residue on<br>evaporation       |
|-----------------------|-------------------------|---------------------------------|
| Petroleum             | 1 - 3                   | Oil (trace)                     |
| petroleum :           | 4 - 7                   | Nil                             |
| Benzene ( 4 : 1 )     |                         |                                 |
| Petroleum :           | 8 - 11                  | Solid ( 10 mg )                 |
| benzene ( 3 : 2 )     |                         | m.p. 259-61° (unreacted)        |
| Petroleum             | 12-14                   | Nil                             |
| benzene ( 1 : 4 )     |                         |                                 |
| Benzene               | 15-17                   | Nil                             |
| Benzene ; Ether (4:1) | 18-21                   | Solid ( 150 mg )                |
|                       |                         | <del>XXXXXXXXXXXXXXXXXXXX</del> |
|                       |                         | m.p. 228-30°.                   |

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

Fractions 18-21 ( 150 mg ) were combined and on crystallisation from methanol furnished pure methyl dihydroalphitolate 159, 230-32°. This compound showed no depression of melting point on

admixture with an authentic sample supplied by Prof. E. Ritchie. The infrared spectrum of the compound also found to be identical throughout the entire range.

Found : C, 76.32; H, 10.55%

Calculated for  $C_{31}H_{52}O_4$  : C, 76.18; H, 10.72%

IR : 3620, 3350, 1730  $cm^{-1}$

Isomerisation of 2 $\alpha$ -acetoxy methyl dihydrobetulonate 156 :

Preparation of 2 keto - acetyl-methyl dihydrobetulinate 157 :

2 $\alpha$ - acetoxy methyl dihydrobetulonate 156 ( 100 mg ) dissolved in benzene ( 3 ml ) was adsorbed on a column packed with basic alumina ( Brockman ) and left overnight. Next day it was eluted with benzene and the solid obtained was crystallised from methanol, m.p. 226-28°. This compound on admixture with the starting material melted at 196 - 98°.

Found : C, 74.69; H, 10.02%

Calculated for  $C_{33}H_{52}O_5$  : C, 74.96; H, 9.91%

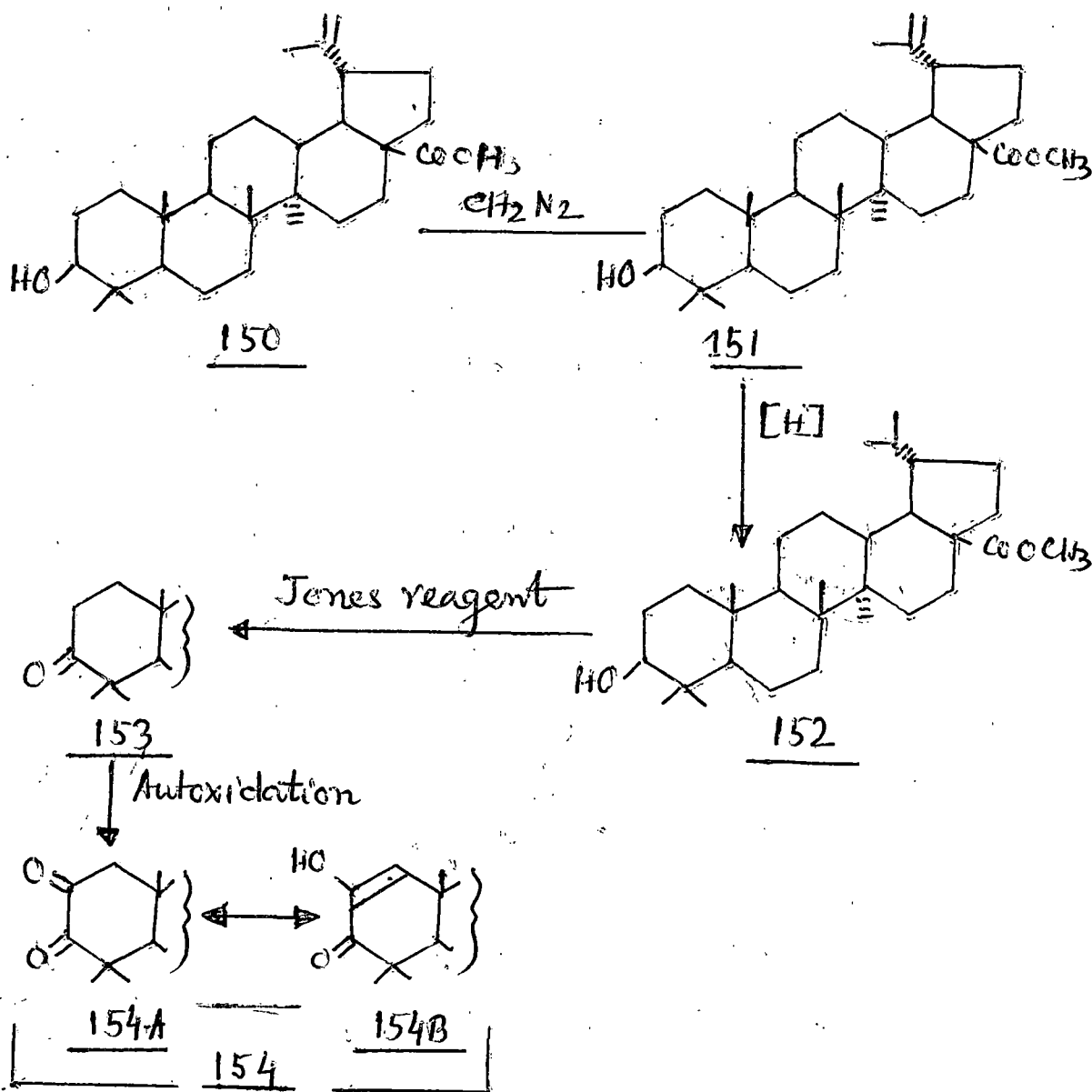
Section C : Synthesis of methyl 2 $\beta$ , 3 $\beta$ - dihydroxy betulinate from betulinic acid.

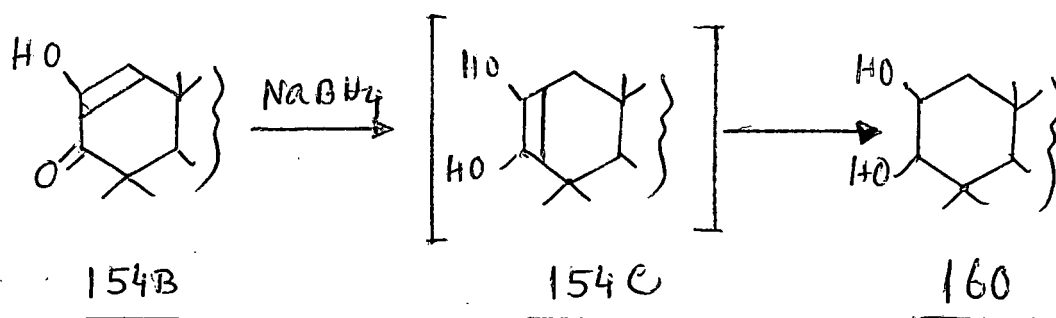
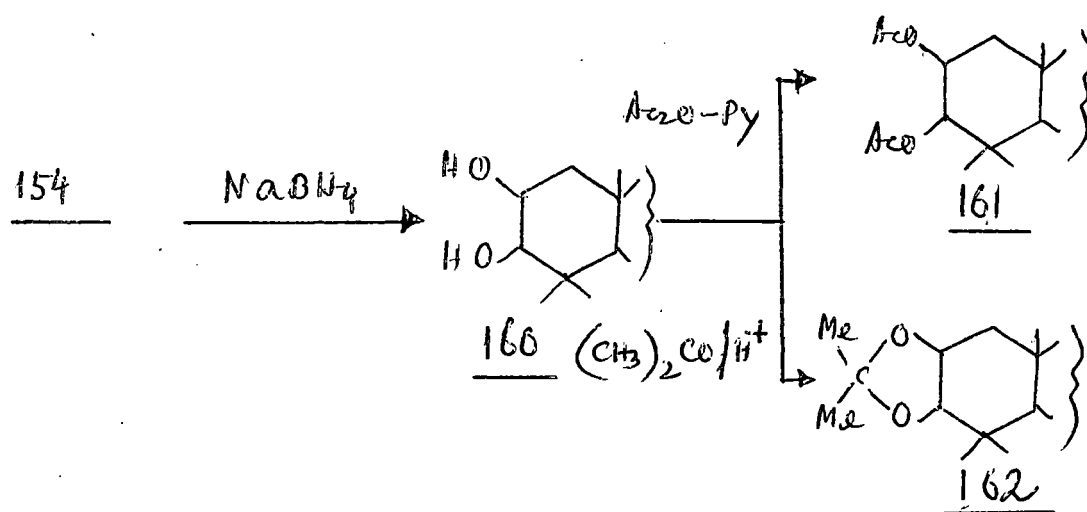
The preparation of the diosphenol 154 starting from betulinic acid 150 has already been discussed ( page 48-54). Diosphenol 154 on sodium borohydride reduction in methanol solution gave a compound 160, m.p. 261-63°, ( $\alpha$ )<sub>D</sub> 21.62°, no UV absorption in the region 220-300 $\mu$ ,  $\nu$  max 3525, 3360, 1720  $\text{cm}^{-1}$ . Treatment of 160 with pyridine and acetic anhydride gave the diacetate 161, m.p. 186-87°,  $\nu$  KBr max 1745, 1720, 1258  $\text{cm}^{-1}$ . Examination of the NMR spectrum of 160 showed a multiplet at 3.20 ppm assigned to C<sub>3</sub>- 3H and a broad unresolved multiplet at about 4.5 ppm ( C<sub>2</sub>- 2H ) which collapsed to a doublet ( J = 3.7 Hz ) and a multiplet ( $\leq J = 6\text{Hz}$ ) respectively upon exchange of hydroxyl proton with D<sub>2</sub>O. Thus the hydroxyl group at C - 3 is equatorial ( Ha ) and the one at C - 2 is axial ( He ). In the NMR spectrum of its diacetate 161, these signals were shifted to a downfield to 4.8 ppm (doublet, J = 4Hz) and at about 5.4 ppm ( broad multiplet ). The signals for the ester group and the acetate group appeared at 3.65 ppm ( singlet, 3H ) and at 2.06 ( singlet 6H ) ppm.

Sodium borohydride reduction would be expected to furnish a 2 $\alpha$ , 3 $\alpha$ -diol, or 2 $\beta$ , 3 $\beta$ -diol or a mixture of

$2\alpha$ ,  $3\beta$  and  $2\alpha$ ,  $3\alpha$  diols. However,  $2\beta$ ,  $3\beta$ - diol 160 could only be isolated which can be explained if it is assumed that the reduction proceeds via the intermediate 154 C

The diol 160 on treatment with acetone in presence of catalytic amount of p-toluene sulfonic acid gave an acetonide derivative 162, m.p.  $194-95^{\circ}$ ,





### EXPERIMENTAL

Melting points are uncorrected. The petroleum~~ether~~<sup>had</sup> used throughout the investigation, b.p. 60-80°. All optical rotations were determined in chloroform solution unless stated otherwise. NMR spectra were determined on Varian A - 60 and HA - 100 spectrophotometers using chloroform - d solution containing tetramethyl silane as reference. The IR spectra were recorded in Perkin - Elmer 337 and 221 and Beckmann I. R - 20 spectrophotometers. UV absorption spectra were taken in Ziess VSU - 1 and UV Beckmann DU - 2 spectrophotometers using 95% ethanol solution unless otherwise stated.

#### Sodium borohydride reduction of diosphenol 154 :

#### Preparation of methyl 2 $\beta$ ,3 $\beta$ - dihydroxy betulinate 160 :

To a solution of diosphenol 154 ( 200 mg ) in methanol ( 100 ml ) sodium borohydride ( 200 mg ) was added and the mixture was stirred for 4 - hours. The reaction



mixture was concentrated, diluted with water and then acidified with dilute hydrochloric acid ( 6 ml ) when a solid precipitated out . The solid ( 100 mg ) was dissolved in benzene and was poured on a column of alumina ( 10 gm , deactivated with 0.4 ml of 10% aqueous acetic acid ) developed with petroleum. The chromatogram was eluted with the following solvents ( Table - IV )

Table - IV

| Eluent            | Fractions<br>50 ml each | Residue on<br>evaporation        |
|-------------------|-------------------------|----------------------------------|
| Petroleum         | 1 - 2                   | Nil                              |
| Petroleum         | 3 - 4                   | Nil                              |
| benzene ( 3 : 1 ) | 5 - 6                   | Nil                              |
| Petroleum :       | 7 - 8                   | Nil                              |
| benzene ( 1 : 1 ) | 9 - 14                  | Solid ( 180 mg )<br>m.p. 256-58° |
| Petroleum         |                         |                                  |
| benzene ( 1 : 3 ) |                         |                                  |
| Benzene           |                         |                                  |

Elution with more polar solvent did not yield any solid material.

Fractions 9 - 14 ( 180 mg ) were collected and crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals of 160, m.p.  $261-63^{\circ}$ ,  $(\alpha)_D + 21.62^{\circ}$ .

Found : C, 76.49; H, 10.47%

Calculated for  $C_{31}H_{52}O_4$  : C, 76.18; H, 10.72%

UV : No absorption in the region 220-300  $m\mu$

IR :  $\nu_{\max}$  3525, 3360, 1729  $cm^{-1}$

NMR ( 60 Mc/S ) : Peaks at 3.15 ( multiplet ) and 4.4 ( multiplet ), 5.15 ppm.

Acetylation of methyl 2 $\beta$ , 3 $\beta$ - dihydroxy betulinate 160 :

Preparation of methyl 2 $\beta$ , 3 $\beta$ - diacetoxy betulinate 161 :

The diol 160 ( 200 mg ) was acetylated by heating with pyridine ( 4 ml ) and acetic anhydride ( 4 ml ) on a water bath for four hours. After working up in the usual manner it gave a solid residue ( 180 mg ). This residue dissolved in benzene was placed over a column of alumina ( 15 gm, deactivated with 0 . 6 ml of 10% aqueous acetic acid ) developed with petroleum and was eluted with the following solvents ( Table - V ).

Table - V

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation     |
|-----------|-------------------------|-------------------------------|
| Petroleum | 1 - 5                   | Solid (170 mg)<br>m.p. 181-4° |

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

Fractions 1 - 5 ( 170 mg ) were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless needles of 161, m.p. 186 - 87°

Found : C, 73.54; H, 9.71%

Calculated for  $C_{35}H_{56}O_6$  : C, 73.39; H, 9.85%

UV : No absorption in the region 220 - 300 m $\mu$

IR :  $\nu_{\max}$  1258, 1729, 1745 cm<sup>-1</sup>

NMR ( 60 Mc/s ) : Peaks at 3.65 ( singlet, COOMe )

2.06 ( singlet, 6 H ), 4.6, 5.4 ppm.

Preparation of acetone 162 of methyl 2 $\beta$ , 3 $\beta$ - dihydroxy  
betulinate 160 :

To 2 $\beta$ , 3 $\beta$ - diol 160 ( 100 mg ) dissolved in dry acetone ( 20 ml ) was added a few crystals of  $\beta$  - toluene sulfonic acid and the mixture was shaken for 10 - minutes and then kept overnight. After usual work up it gave a solid residue ( 80 mg ). This product dissolved in benzene ( 3 ml ) was placed over a alumina column ( 10gm, deactivated with 0.4 ml of 10% aqueous acetic acid ) developed with petroleum and was eluted with the following solvents ( Table - VI ).

Table - VI

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 3                   | Solid (70 mg)<br>m.p. 192 - 4° |

Elution with more polar solvent did not yield any solid material.

Fractions 1 - 3 ( 70 mg ) were combined and crystallisation from methanol afforded pure crystals of acetone derivative 162 m.p. 194 - 95°.

|                                                                 |                      |
|-----------------------------------------------------------------|----------------------|
| Found :                                                         | C, 77.45 ; H, 10.73% |
| Calculated for C <sub>34</sub> H <sub>56</sub> O <sub>4</sub> : | C, 77.22 ; H, 10.67% |

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PART - III

CHEMICAL INVESTIGATION ON THE RHIZOMES  
OF POLYPODIUM JUGLANDIFOLIUM DON

PART - III

CHAPTER - I

Morphological features of the fern of Polypodium juglandifolium Don (syn Arthromeris juglandifolia) :

The Polypodiaceae<sup>12-1b</sup> constitute the highest and largest family of true ferns. It is a family of 170 genera and 7,000 species, widely distributed over most of the land areas of the earth, especially abundant in forest and humid areas, but occurring in almost all floristic areas or zones from desert to rain forest and from tropics to arctic or antarctic. The following list includes most of the large genera : Pteris, Adiantum, Athyrium, Cheilanthes, Dryopteris, Polystichum, Asplenium, Blechnum, Elaphoglossum, and Polypodium.

Polypodium juglandifolium Don<sup>1c</sup> : Terrestrial and epiphytic ferns of moderate size; rhizome being creeping, long, stout, dictyostele and with sclerenchyma strands, clothed with lanceolate, attenuate, entire, brownish p/aleae affixed above the base; stipes remote, articulate, long; fronds uniform, imparipinnate, lateral, pinnae articulate to rachis, opposite, lanceolate, acuminate, naked or pubescent, subcoriaceous, margin contilaginous and entire, main veins conspicuous, minor venation not so,

areolae irregular, with simple or forked included veinlets running in any direction; sori solitary or plural between main veins, compital, round, superficial, without paraphyses, annulus of 14-16 cells, spores bilateral, brownish, obscurely tuberculate or spinulose.

Chapter-II

Section A : A short review on the chemical constituents of  
ferns of Polypodiaceae family.

The exceptional revival of interest in phytochemistry in recent years, which has accompanied the development of powerful modern techniques for structural investigations and has been stimulated by the potential usefulness of plants as a source of new therapeutic agents, has only touched in a marginal way the class of ferns (Pterophyta or Filicinae). Of the fourteen families and the many thousands of species into which this botanically very interesting class is subdivided, very few have been investigated by the phytochemist. With the exception of some genera of Polypodiaceae, only occasional and very incomplete attention has been paid to the various families, some of which, such as the Dipteridaceae and Salviniaceae, are completely unknown from the chemical point of view. However, recent results, such as the discovery of many new acylphloroglucinol derivatives in Dryopteridoideae and of several characteristic triterpenoids, and the availability of efficient methods for their analysis, indicate that the phytochemical investigation of ferns could be of great interest both to the taxonomist, as an aid in the very difficult task of their

classification, and to the natural products chemist in his search for novel structures.

We have undertaken, in this chapter, to give an up-to-date description of all organic compounds that have been isolated from ferns of Polypodiaceae family.

Polypodium vulgare L : The constituents of the fern Polypodium vulgare L. Growing in Europe, Asia and America were many times investigated. The outstanding sweet taste of the rhizomes of the plant in question in particular attracted the interest of chemists and pharmacists. Guignet<sup>2</sup> in 1885 reported that this property of rhizomes was caused by glycyrrhizine like in papilionaceous plant Glycyrrhiza glabra L. Fisher and Goodrich<sup>3</sup> agreed with Guignets<sup>2</sup> opinion, but shortly afterwards Fisher and Lynn<sup>4</sup> rejected this assertion and stated only that sweet taste of rhizomes was caused by relatively high content of sugars and that mainly by saccharose besides fructose and glucose. The named authors isolated besides less defined compounds the glycoside polydine, characterized by melting point 188-89°C. Volmer and Reebe<sup>5</sup> reported another substance of m.p. 150-51°C which they called polypodine. Freise<sup>6</sup> found in the rhizomes also a compound of a bitter taste which he named samambaine. The latter compound is a glycoside which on enzymatic hydrolysis

affords glucose, rhamnose, benzoic acid and resins. Later, Jermstad and co-workers<sup>7</sup> corroborated the absence of glycyrrhizine in P. vulgare and reported the presence of a series of compounds besides polydine, among others also caffeic acid chlorogenic acid. However, <sup>o</sup>through investigation of the rhizomes of P. vulgare was mainly due to Jizba and Herout<sup>8</sup>. These named authors mainly paid their attention on the hydrophilic part of the extract. The ethanolic extract of the rhizome<sup>s</sup> from which the lipophilic material was removed by extraction with light petroleum was chromatographed on polyamide powder.

Fraction which was eluted first contained relatively pure saccharose in a yield of about 2.5% on the weight of dry drug. The mother<sup>l</sup>liquors contained small amount of glucose and fructose by paper chromatography. Eluates which followed gave a <sup>substance,</sup> crystalline, m.p. 229-33°C in about 1.8% yield was found to be the mixture of two compounds. They separated the two constituents by chromatography on silica gel. One compound is polypodine crystallised as hydrate, m.p. 150-51°C. The second compound<sup>s</sup> they isolated, showed m.p. 244-46°C with the same molecular formula as the first one (Chart I) Jizba and Herout<sup>8</sup> named these compounds as polypodine A and polypodine B. Next eluates furnished a crystalline compound in about 0.6% <sup>yield</sup> which was identified as glucocaffeic acid. The following eluate they found, was a mixture of two compounds. These compounds were separated by partition chromatography, on solica gel.



The compound eluted first was not obtained crystalline, the other was polydine, m.p. 191-93°C,  $(\alpha)_D^{20} -121.6^\circ$  (C 0.244; MeOH). The mother-liquors after polydine contained a series of compounds of glycoside character. Their isolation was carried out by chromatography on polyamide powder. Aqueous methanol eluted "saponin I" m.p., 199-201°C, the aqueous solution of which formed a rich foam. This compound differed from "Saponin II" by the presence of methoxyl group in its molecule.

After separation of saponin I, they isolated the sweet principle, the interesting constituent, by partition chromatography on kieselguhr. The yield of the compound, m.p. 201-203°C (hydrated form) or 252-254°C (anhydrous form) was about 0.03% of the weight of the dry drug. These authors assumed that this compound was the true sweet principle of polypody rhizome. This compound affords unstable aglycone and rhamnose and glucose and they proposed the name osladine for the compound.

The eluates which yielded osladine on rechromatography on silicagel and elution by a mixture of chloroform containing 15% ethanol they isolated a small amount of crystalline substance of bitter taste. This compound, according to them, has a relation to samambaine already described by Fré<sup>6</sup> and also to the compound osladine by their preliminary results. These authors have also isolated a small amount of crystalline "saponin II", m.p. 213-14°C from the plant collected in another season of the year.

Chart - I

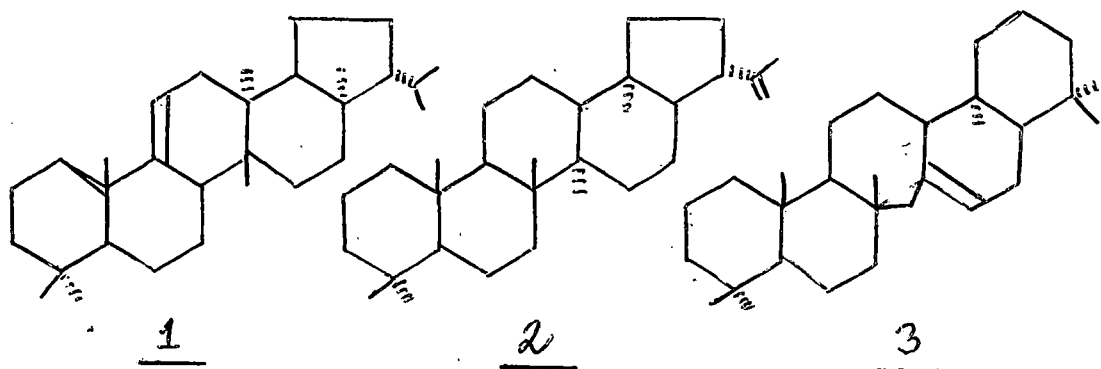
Compounds isolated from Polypodium vulgare L.

| Name              | M.P., °C | $\frac{20}{D}$ | Composition                              |
|-------------------|----------|----------------|------------------------------------------|
| Saccharose        | 184-85   | + 67.5         | $C_{12}H_{20}O_{11}$                     |
| Polypodine A      | 150-51   | + 61.8         | $C_{27}H_{44}O_7 \cdot 3H_2O$            |
| Polypodine B      | 244-46   | + 92.8         | $C_{27}H_{48}O_8 \cdot \frac{1}{2} H_2O$ |
| Glucocaffeic acid | 133-35   | - 80.4         | $C_{15}H_{18}O_9 \cdot H_2O$             |
| Polydine          | 191-93   | -121.6         | $C_{20}H_{22}O_{10} \cdot H_2O$          |
| Osladine          | 201-203  |                | a                                        |
| Saponin I         | 199-201  |                | a                                        |
| Saponin II        | 213-14   |                | a                                        |
| Samambaine        | 251-52   |                | a                                        |

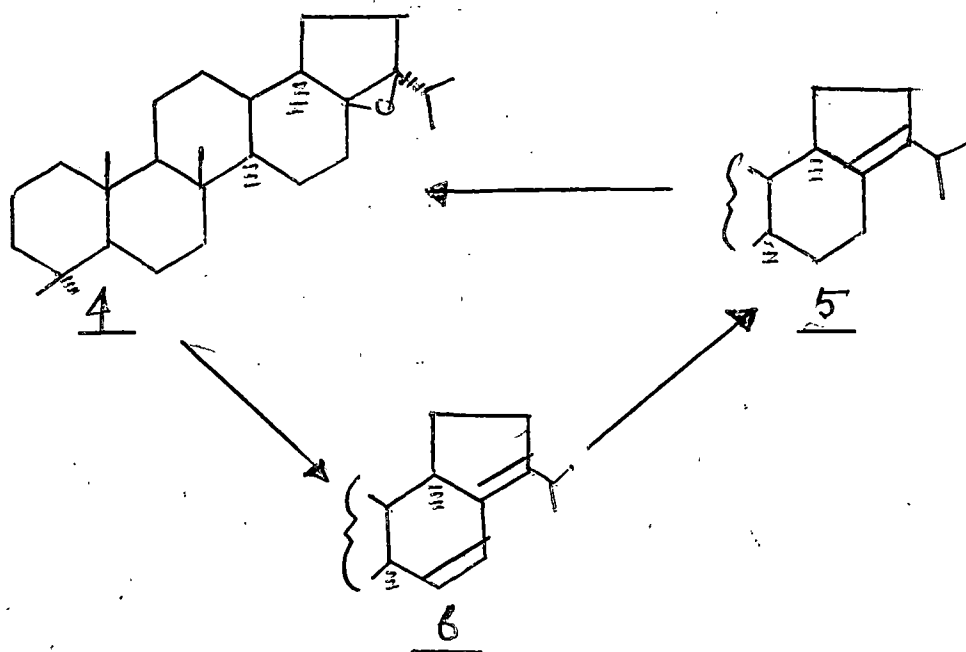
The authors have also reported the isolation of fernene from the lipophilic part of the extract.

Berti and co-workers<sup>9-11</sup> investigated the fern by means of modern methods and found the presence of several triterpenic hydrocarbons in the lipophilic part. The rhizomes of polypodium vulgare contain 0.4% of a mixture of triterpene hydrocarbons; gas liquid chromatographic analysis indicated the presence of three components - 9(11) fernene 1, m.p., 170-71° ( $\alpha$ )  $D_{20}^{25} - 16.5$ , 22(29) hopene 2, m.p., 211-12° ( $\alpha$ )  $D_{20}^{25} + 61$ ° and serratene 3, m.p. 237-39° ( $\alpha$ )  $D_{20}^{25} - 13.4$ °. The third component was isolated in a pure state by column

chromatography over silica gel impregnated with silver nitrate. This compound had been obtained before as the dehydroxylation product of serratene diol, a triterpenoid from Lyconodium serratum. 17,21- Epoxyhopane 4, m.p. 268-70°,  $(\alpha)_D^{28} +47^\circ (\text{CHCl}_3)$



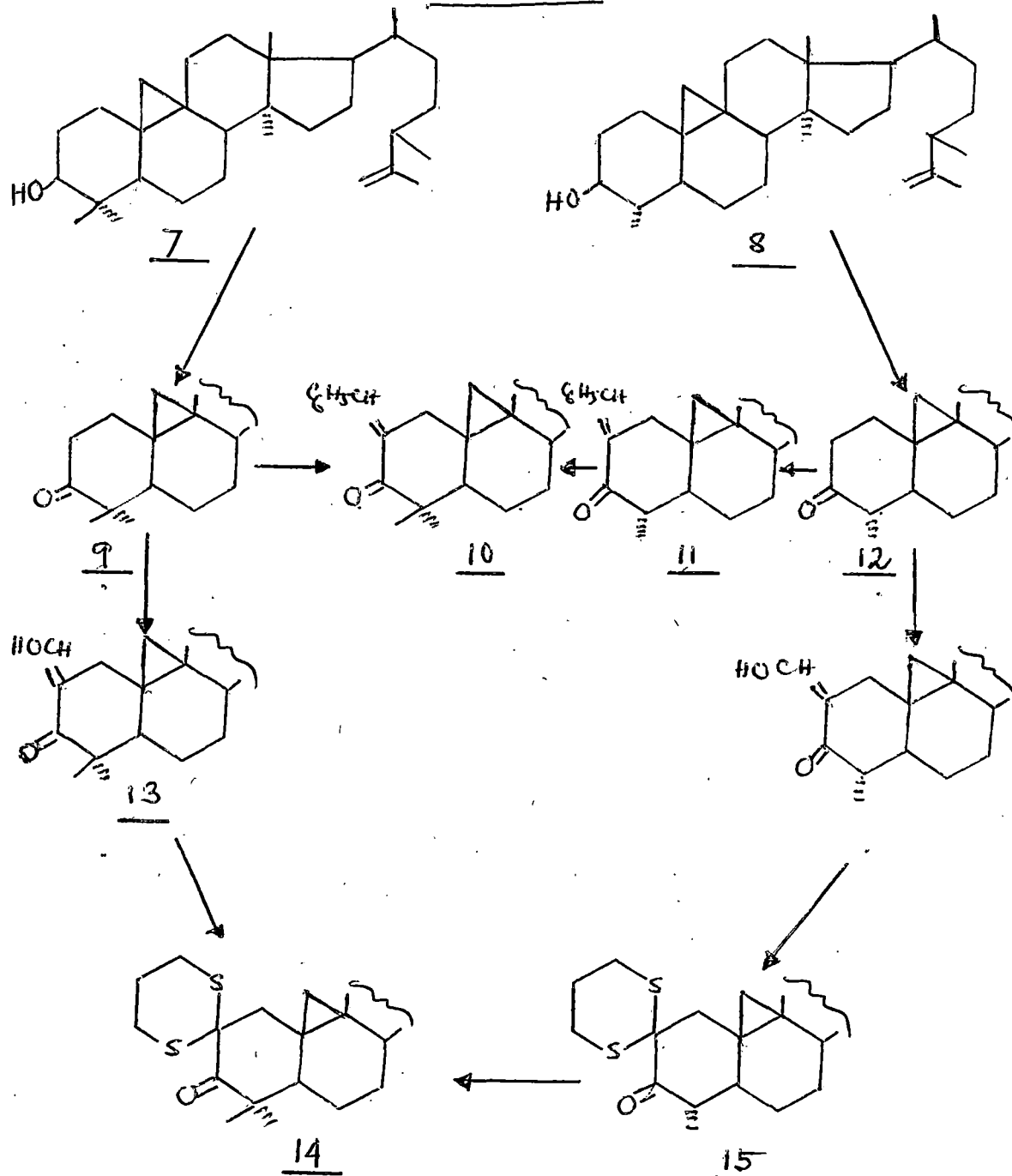
is one of the minor triterpenoids of Polypodium vulgare.<sup>10</sup> Treatment with hydrogen chloride in ethanol, or chromatography on alumina, converts it into the heterobannular diene 6 which is partially reduced to the known 17(21)-hopene (hopene-1) 5 by catalytic hydrogenation. The latter compound transformed back into the epoxide 4 with p-nitroperoxybenzoic acid; therefore, the stereochemistry of the epoxide ring of 4, although not rigorously proved, is very probably  $\beta$ , since peroxy acids usually attack double bonds from less hindered side.



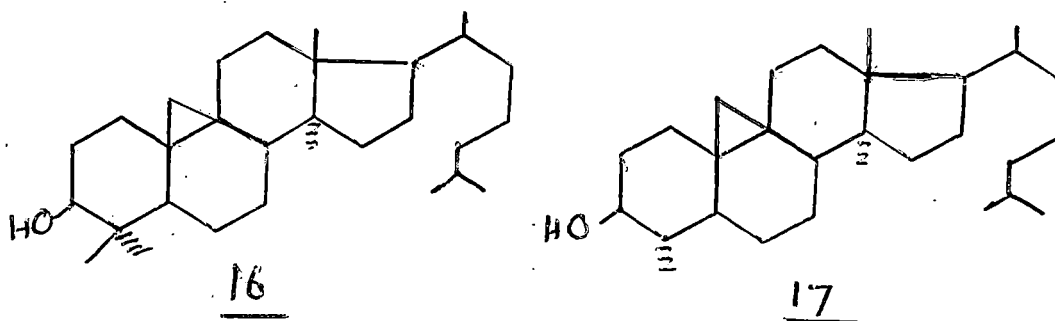
Triterpenoids of the 9,19- cyclolanostanol group has been reported from Polypodium vulgare. About 1.4% of the dry weight of P. Vulgare rhizomes consists of a mixture of alcohols, the main constituent of which is cyclolaudenol 7,<sup>9</sup> m.p. 123-25°, ( $\alpha$ )<sub>D</sub> + 45°, a compound that had previously been found in Papaver somniferum<sup>12</sup> and in Manihara bidentata.<sup>13</sup> A second constituent was identified as 31-norcyclolaudenol 8, m.p. 139-40°, ( $\alpha$ )<sub>D</sub> + 44°, on the basis of its i. r., n. m. r., and mass spectra<sup>9</sup>. The relation between 8 and 7 was established (scheme 1) by converting the corresponding ketone 12 into the benzal ~~XXXXXXXXXX~~ derivative 11 and methylating the latter to 10, which turned out to be identical with benzalcyclolaudenone. Another link ~~was~~

was established through the dithiane derivative 15, m.p.  $200^{\circ}$ , the methylation of which produced 14, m.p.  $162'$ , also prepared from 7 through 9 and 13.<sup>14</sup>

Scheme - 1



Two more 9,19 cyclolanostanol derivatives were <sup>0</sup>fund as minor constituents <sup>in</sup> Polypodium vulgare: cycloartanol 16, m.p. 101-2° ( $\alpha$ )<sub>D</sub> +51° and 31-norcycloartanol 17, m.p. 128-32° ( $\alpha$ )<sub>D</sub> +49°. The former had been tentatively identified as a trace constituent of rice brain oil<sup>15</sup>; the latter had never been found in nature but had been obtained as an intermediate in the structural elucidation of cycloeucaleenol<sup>16</sup>.

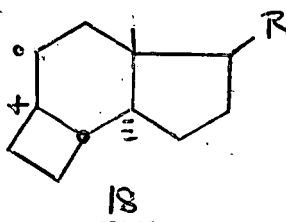


Compounds 7, 8, 16, and 17, were easily analysed by gas-liquid chromatography of their trimethylsilyl ethers on a neopentylglycol succinate column. The order of increasing retention times is : 17 < 16 < 8 < 7. The preparative separation is not satisfactory, since only an incomplete separation into mixtures of cycloartanol and 31-norcycloartanol, and of cycloartanol and 31-norcycloartanol is achieved. A much better method is based on the chromatography of the benzoates over

silver nitrate impregnated silica gel. Elution with petroleum-ether containing 2% benzene gives a mixture of the benzoates of cycloartanol and 31-norcycloartanol, while higher amounts of benzene are required for the elution of those of cyclolaudenol and 31-norcyclolaudenol. The 31-norderivatives cycloartanol and 31-norcycloartanol are then separated from the corresponding parent compounds cyclolaudenol and 31-norcyclolaudenol by conversion into the 3-ketones and chromatography over alumina; after separation, the ketones can be reconverted into the alcohols.

9,11 - cyclolanostanol derivatives are easily identified in the n.m.r. spectrum by an AB quartet ( $J=4$  cps) at very high field (between 9.40 and 9.80 $\tau$ ), which is due to the magnetically nonequivalent cyclopropane protons. The quartet for 4-monomethyl derivatives is at higher field than for 4,4-dimethyl derivatives<sup>9</sup>. Further evidence for the cyclolanostane structure is provided by the presence in the mass spectrum of the alcohols of a strong peak at  $M^+-C_9H_{16}O$  for compounds with two methyl groups in  $C_{(4)}$  such as cyclolaudenol and 31-norcycloartanol and at  $M^+-C_8H_{14}O$  for those with one such as 31-norcyclolaudenol and cycloartanol. This peak is due to the loss of ring A and  $C_{(19)}$ , and probably corresponds to the ion 18<sup>11,17</sup>. Mass spectra also provide useful information on the type of side chain, by the presence

of a peak due to fragmentation between  $C_{(17)}$  and  $C_{(20)}$ , with the charge remaining on the larger fragment. The methylene side chain of cyclolaudenol and 31-norcyclolaudenol has also been readily distinguished from the saturated one of cycloartanol and 31-norcycloartanol by the strong  $=CH_2$  i. r. bands at 6.10 and 11.22  $\mu$ .



9.19 - cycloartanol derivatives have recently been receiving much attention, because they could be more important than lanosterol as intermediates in the biosynthetic transformation of squalene into phytosterols<sup>18</sup>. It is a well known fact that cycloartenol is widely distributed in plants but it is becoming more and more evident that other compounds of this class, such as cycloeucalenol, 22-methylene cycloartanol, cyclolaudenol, etc. are also very common, even if usually present only in small amounts. The hypothesis has been put forward that squalene can cyclize directly to cycloartenol and this compound gives rise to sterols through a series of steps : elimination of the methyl groups  $C_{(30)}$ ,  $C_{(31)}$ , and  $C_{(32)}$ , opening of the cyclopropane ring, methylation and reduction of the side chain, etc.



The isolation from the same plant of cyclolaudenol, 31-norcyclolaudenol, artanol and 31-norcycloartanol may indicate that they are metabolic intermediates of two different pathways in the transformation of cycloartenol into sterols.

Fischer et al isolated a sterol, m.p. 132-133°, from P. vulgare<sup>4</sup> which was assumed to be  $\beta$ -sitosterol<sup>14</sup>. The presence in the same plant of another, unknown sterol, m.p. 162-163°, benzoate, m.p. 165-166° has been reported<sup>7</sup>.

Polypodium aureum L. Jizba et al<sup>19</sup>, in 1974, studied this fern, Polypodium aureum L. and in this connection they also investigated the fern Polypodium vulgare L.<sup>8</sup> The latter gave steroid compounds of cholestane type in form of Glycosides<sup>20, 21</sup>, phytoecdysones<sup>22, 23</sup> and polyphenolic compounds<sup>8, 24</sup>.

The ethanolic extract obtained from rhizomes and leaves of P. aureum L. was separated by partitioning between light petroleum and water. From the unpolar fraction triterpenic hydrocarbons fernene and vallichene were isolated. Both hydrocarbons were identified on the basis of their melting points and their identity was confirmed by IR spectra, mass spectra as well as by oxidation of fernene and vallichene with chromium trioxide to fernenone<sup>25</sup> and vallichienone<sup>26</sup> respectively. The more polar fractions gave  $\beta$ -glucoside of  $\beta$ -sitosterol, benzoic acid, saccharose, glucose, and three

phytoecdysones of which one was identical with ecdysterone and the other two remained unidentified.

Some other species of polypodiaceae family have also been investigated. Suzuki<sup>27</sup> reported the isolation of coumarin from Polypodium hastatum. In a recent<sup>28</sup> quantitative analysis of free sugars by gas-liquid chromatography on their trimethylsilyl ethers, fructose, <sup>a</sup>galactose, glucose and sucrose were detected from Polypodium polypodioides. 3-Desoxy-anthocyanidin glucosides, which at one time were erroneously identified as 6-hydroxypelargonidin and 6-hydroxycyanidin glucosides<sup>29</sup>, are present in Polypodium rhodoleuron.

### CHAPTER-III

Investigation on the neutral part of the benzene -  
extract of the fern Polypodium juglandifolium, and isolation  
of two new triterpenes - Polypodinol A,  $C_{30}H_{50}O$  and Polypodi-  
nol B,  $C_{30}H_{50}O$  along with fern-9(11)-ene, filicene, cyclolaudenol,  
nerifoliol and  $\beta$ -sitosterol and detection of a new  
triterpene  $C_{32}H_{54}O$  in the cyclolaudenol fraction from mass  
spectrometry :

Since no chemical examination on this plant has yet been reported in the literature, a detailed chemical examination was undertaken in this laboratory and the results are discussed below.

#### Section A : Extraction :

Dried and powdered <sup>rhizomes</sup> ~~root~~ of the fern Polypodium juglandifolium was extracted with benzene in a soxhlet apparatus for 20 hours. Benzene was distilled off and the residual gummy solid was taken up in ether. The ether layer was washed with 10% sodium hydroxide solution, and then with cold water till the washings were neutral, dried over anhydrous sodium sulphate and evaporated, when the neutral material was obtained as a gummy residue.

Section B : Chromatography of the neutral part :

Table - 1

| Fraction No | Eluent                       | Eluate          | Melting point<br>of the residue |
|-------------|------------------------------|-----------------|---------------------------------|
| 1.          | Petroleum                    | Solid with oil  | —                               |
| 2.          | Petroleum ;<br>benzene (4:1) | Solid (mixture) | —                               |
| 3.          | Petroleum ;<br>benzene (3:2) | Solid           | 240 - 43°                       |
| 4.          | Petroleum ;<br>benzene (2:3) | Solid           | 134 - 36°                       |

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

Section C : Examination of fractions 1 - 4 :

Fraction No. 1 : Isolation of filicene, fern-9(11)-ene :

The Fraction No. 1. on careful chromatography over deactivated alumina afforded a mixture of two compounds ( two spots in TLC) from the least polar component. The above mixture on crystallisation from a mixture of chloroform and acetone furnished a product, m.p. 231-32°, ( $\alpha$ )<sub>D</sub> 49.6° which was found to be identical with an authentic specimen of filicene<sup>30</sup> (m.m.p., co-TLC and IR). The mother-liquor from the crystallisation of

filicene on concentration yielded another product, m.p. 169-71°, ( $\alpha$ )<sub>D</sub> - 16.3°. This compound was identified as fern-9(11)-ene<sup>25</sup> by direct comparison with an authentic specimen (m.m.p and IR). The mass spectrum showed the characteristic peaks for fernene at m/e 257, m/e 243, m/e 231<sup>31</sup>.

Fraction No. 2 : Isolation of two new triterpenes - Polypodinol A, C<sub>30</sub>H<sub>50</sub>O and Polypodinol B, C<sub>30</sub>H<sub>50</sub>O and detection of a new cyclolaudenol homologue, C<sub>32</sub>H<sub>54</sub>O in the cyclolaudenol fraction from mass spectrometry :

The fraction no. 2 was rechromatographed over an active column of alumina. The first four fractions of the petroleum - benzene (4:1) eluate gave a solid, m.p. 218-20°. This product on crystallisation from a mixture of chloroform and methanol furnished needle-shaped crystals, m.p. 223-25°, ( $\alpha$ )<sub>D</sub> - 89.13°. This compound has been characterized as a new triterpene alcohol, C<sub>30</sub>H<sub>50</sub>O, named as Polypodinol A. The chemistry and structure-elucidation of this compound has been described in chapter - IV (page-192).

Elution with petroleum-benzene (3:2) gave a compound, m.p. 148-50°, C<sub>30</sub>H<sub>50</sub>O in a very poor yield. Further work is in progress to isolate it in quantity to enable us to investigate its structure. Elution with the same solvent mixture gave another product, m.p. 162-64°. This compound on crystallisation from a mixture of chloroform and methanol afforded colourless

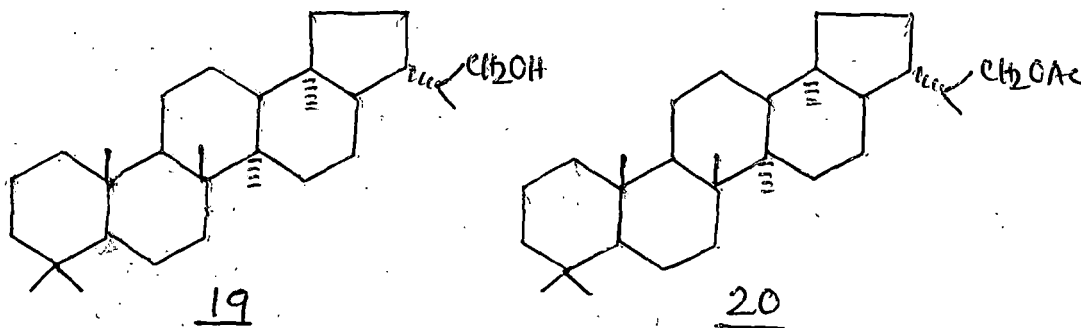
needles,  $C_{30}H_{50}O$ , m.p.  $165-66^{\circ}$ ,  $(\alpha)_D$   $28.57^{\circ}$ . The compound is a new triterpene and has been named Polypodinol B and its partial structure has been assigned from physical and chemical evidence. The chemistry and its structure elucidation has been described in Chapter - V (page -218).

Further elution with the same solvent mixture furnished a solid, m.p.  $121-22^{\circ}$  which was seen to be a mixture of two compounds by TLC. This mixture could not be separated either by chromatography or by fractional crystallisation. However, the separation of one of the compounds was realised by careful chromatography of the benzoate derivative of the mixture followed by fractional crystallisation. The first crop obtained was again found to be a mixture but careful fractionation of the mother-liquor afforded a benzoate, m.p.  $176-78^{\circ}$  identical with an authentic specimen of cyclolaudenol benzoate supplied by Prof. Berti, (m.m.p. and IR). Hydrolysis of the latter afforded a solid, m.p.  $121-22^{\circ}$ , which on crystallisation from methanol had m.p.  $122-23^{\circ}$ . It showed no depression in melting point on admixture with an authentic sample of cyclolaudenol (identical IR). The second component in the mixture has been identified as a cyclolaudenol homologue from mass-spectrometric studies. The details of the work has been described in chapter - VI (page -232).

Fraction No. 3 : Isolation and identification of nerifoliol :

Rechromatography of the fraction no. 3 over a column of active alumina and elution with petroleum - benzene (2:3) gave an alcohol, m.p.  $244-46^{\circ}$ ,  $(\alpha)_D 35.5^{\circ}$ ,  $(M^+ 428$ ; Fig. 1A)  $\nu_{\text{max}}^{\text{nujol}} 3340 \text{ cm}^{-1}$  (OH; Fig-1B). NMR of the compound showed broad signal at  $\tau 6.4$  corresponding to two protons -  $\text{CH}_2\text{OH}$  and this signal was further resolved to AB quartets ( $J=11$  cps) indicating that the carbon atom to which  $\text{CH}_2\text{OH}$  is bonded is asymmetric.

On acetylation, the alcohol furnished an acetate - 20. m.p.  $195-96^{\circ}$ ,  $(\alpha)_D 21.3^{\circ}$ ,  $(M^+ 470$ ; Fig. 2A),  $\nu_{\text{max}}^{\text{nujol}} 1725$ ,  $1230 \text{ cm}^{-1}$  (Fig. 2B), (NMR : Fig. 2C). The physical data of the alcohol and the acetate are in quite good agreement with those recorded for nerifoliol by Mitra et al.<sup>26</sup> who isolated this compound from Oleandra nerifolia and established structure as shown in 19.



Some reactions on nerifoliol have been studied and the results are described in chapter - VII (page - 250).

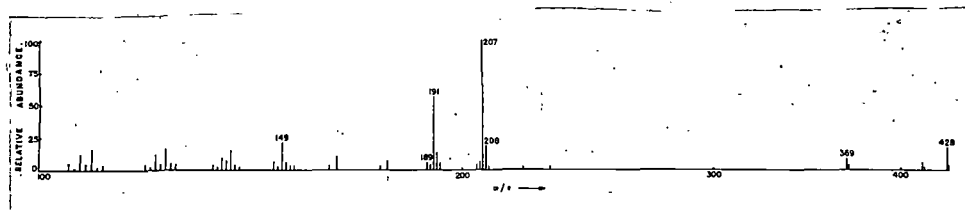


**Fig. 1 A :** Mass spectrum of nerifoliol 19

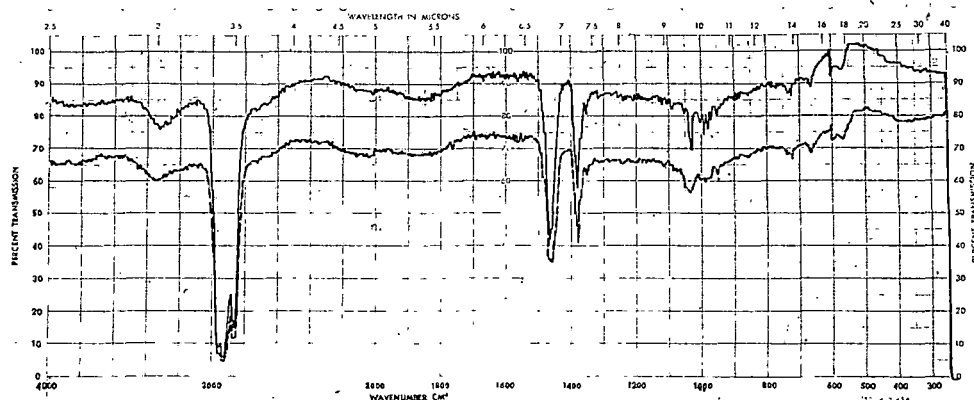


**Fig. 1B :** IR comparison of nerifoliol 19 (solid line) with an authentic sample (dotted line)



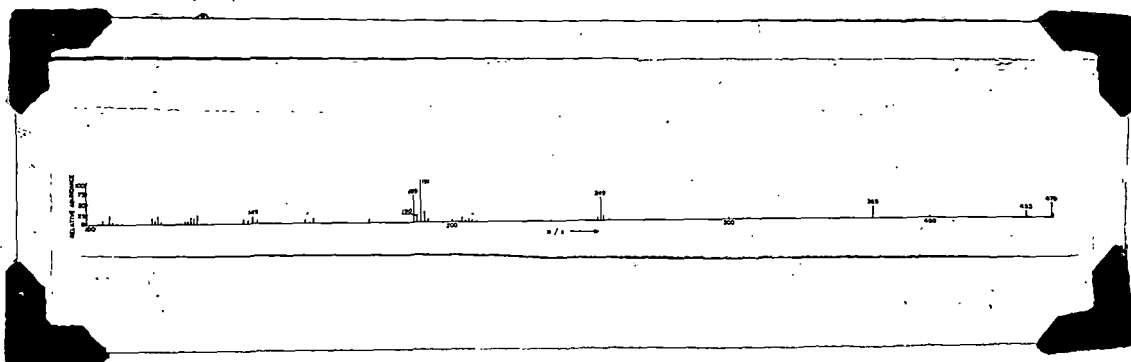


Fig. 2A : Mass spectrum of nerifolici acetate 20

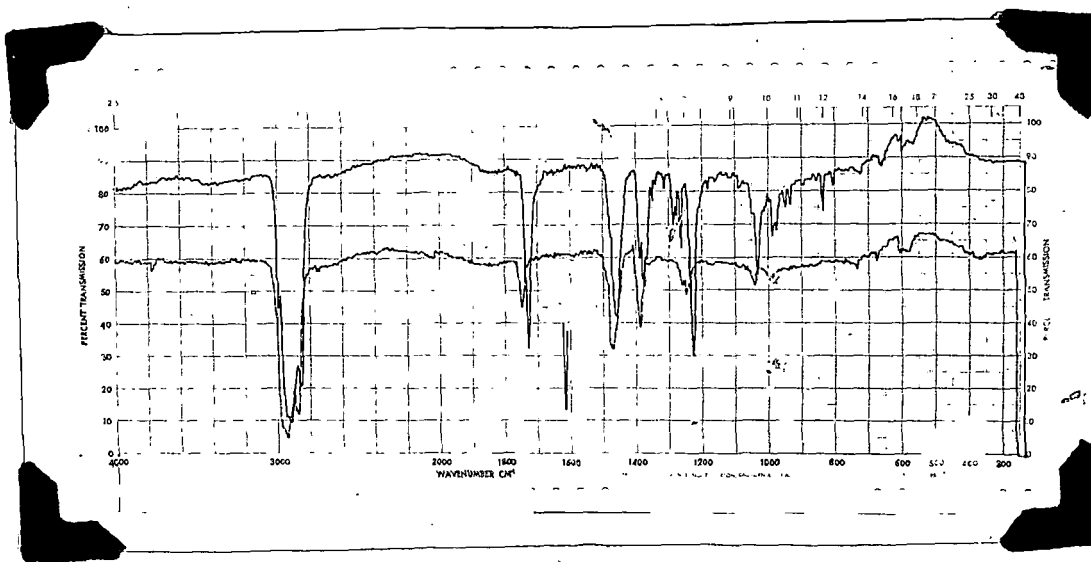


Fig. 2B : IR comparison of nerifolici acetate 20  
(solid line) with an authentic specimen  
(dotted line)

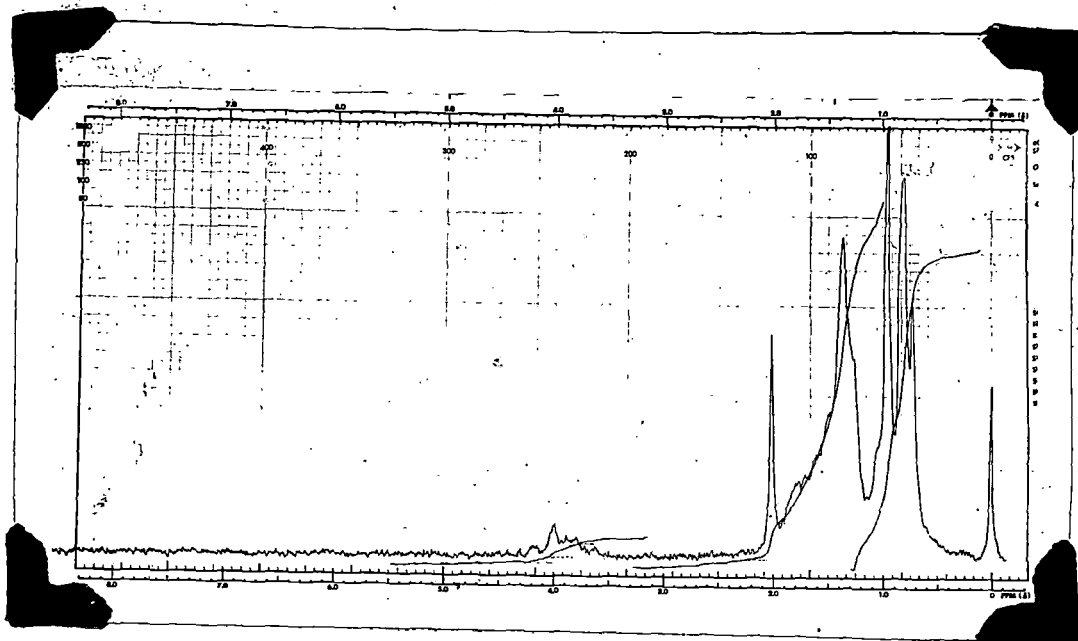


Fig.2C : NMR spectrum of nerifoliol acetate 20

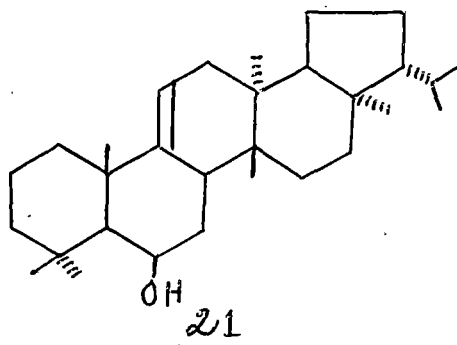
Fraction No. 4 : Identification of  $\beta$  - sitosterol :

Fraction No. 4 on rechromatography over active alumina with petroleum - benzene (1:4) yielded a solid which on crystallisation from a mixture of chloroform and methanol furnished fine needle - shaped crystals, m.p.  $136-37^{\circ}$ ,  $(\alpha)_D - 32^{\circ}$ . Elemental analysis established the molecular formula as  $C_{29}H_{50}O$ . It gave an acetate,  $C_{31}H_{52}O_2$ , m.p.  $127-29^{\circ}$ ,  $(\alpha)_D - 40^{\circ}$ . The alcohol and its acetate were identified as  $\beta$ -sitosterol and  $\beta$ -sitosterol acetate respectively by direct comparison with their respective authentic specimen.

CHAPTER-IV

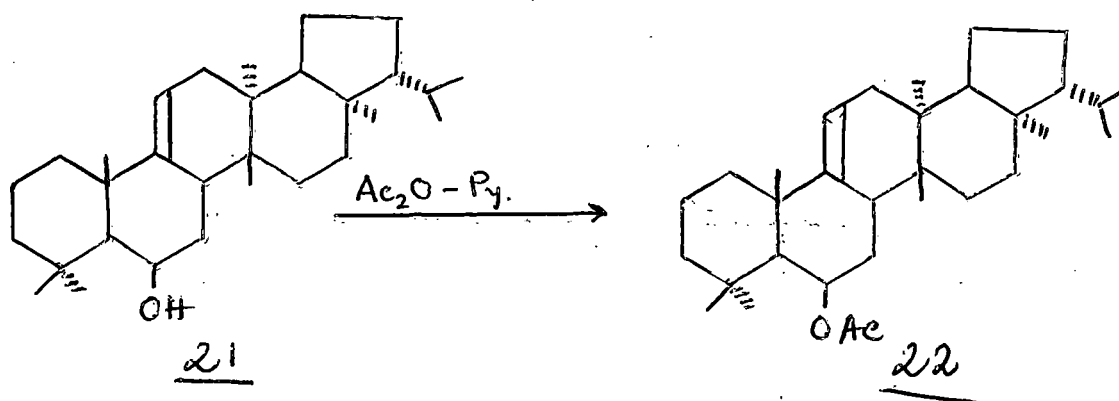
Isolation of a new triterpene alcohol, Polypodinol-A,  $C_{30}H_{50}O$ ,  
 $(\alpha)_D - 89.13^\circ$  and investigation on its structure ;

Fraction No. 2 (Chapter-III, page -187, Table - I) on rechromatography and crystallisation of the fractions 6-9 (Chapter-IV, page -204, Table - II) from a mixture of chloroform and methanol furnished needle-shaped crystals of 21 having m.p.  $223-25^\circ$ ,  $(\alpha)_D - 89.13^\circ$ . It gave a positive Liebermann-Burchard test and a reddish-brown colour with tetranitromethane indicating that it is a triterpene having a double bond. Elemental analysis and mass-spectrometric determination closely corresponded to the molecular formula  $C_{30}H_{50}O$  ( $M^+$  426). IR spectrum of the compound showed peaks at  $3605\text{ cm}^{-1}$  (OH, Fig - 3) and its NMR spectrum (Fig - 4) showed signals for eight methyl groups between  $\delta$  0.8 to 1.28, a multiplet centered at  $\delta$  5.40 (1H, trisubstituted double bond) and a broad diffused multiplet centered at  $\delta$  4.40 attributed to the proton attached to the carbon containing the hydroxyl group. The coupling pattern indicates that this proton is attached to a carbon atom in the system -  $\overset{|}{\text{CH}}-\text{CHOH}-\text{CH}_2-$



Section A : Nature of the oxygen function :

On treatment with acetic anhydride - pyridine, the compound afforded a monoacetate 22, m.p.  $203-4^{\circ}$ ,  $(\alpha)_D -83.72^{\circ}$ . Elemental analysis and mass-spectrometric determination suggested the molecular formula as  $C_{32}H_{52}O_2$  ( $M^+$  468, Fig-5). The acetate had IR peaks (nujol) at  $1725$  and  $1245\text{ cm}^{-1}$  (Fig-6) but no hydroxyl peak in the region  $3100 - 3650\text{ cm}^{-1}$ . It did not show any UV absorption in the region between  $200-300\text{ m}\mu$ . NMR spectrum (60Mc, Fig - 7) of the compound showed the presence of eight methyl groups between  $\delta 0.8$  to  $1.16$ , a sharp peak at  $\delta 2.00$  ( $3H$ ,  $-O-COCH_3$ ), a multiplet at  $\delta 5.40$  ( $1H$ , vinyl proton) and an unresolved multiplet centered at  $\delta 5.20$  [ $1H$ ,  $-CH-CH_2(OAc)-$ ]. Hence the oxygen function is present as a hydroxyl group which is acetylatable. The acetate also did not show any UV absorption in the region  $220-300\text{ m}\mu$ .

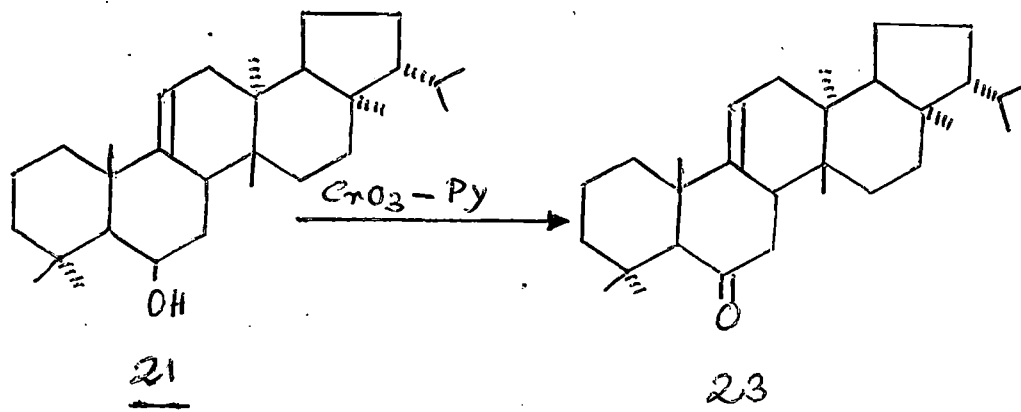


Hence from the IR and UV spectra of the alcohol and its acetate, the absence of any ketonic carbonyl function in the molecule was deduced.

section B : Nature of the carbon skeleton :

The nature of the carbon skeleton was revealed by the chemical transformations and physical evidence described below.

Oxidation of 21 with chromium trioxide - pyridine complex gave a compound 23, m.p. 221-23°, ( $\alpha$ )<sub>D</sub> 16.66°. Elemental analysis and mass - spectrometric determination suggested the



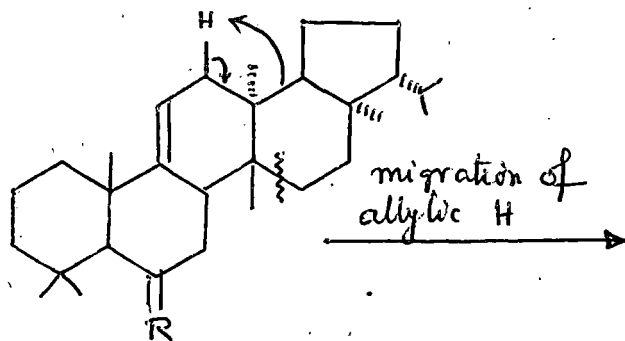
molecular formula as  $\text{C}_{30}\text{H}_{48}\text{O}$  ( $\text{M}^+$  424, Fig - 3). The ketone 23 had IR peak (nujol) at  $1720\text{ cm}^{-1}$  (six membered ring ketone Fig - 9). Its NMR spectrum (Figs - 10A and 10B) showed <sup>signals</sup> ~~singls~~ at  $\delta$  5.40 (IH, vinyl proton) and peaks between  $\delta$  0.8 to 1.04 for eight methyl groups. Wolff - Kishner reduction of the ketone 23 under anhydrous condition furnished a hydrocarbon 24, m.p. 168-69°, ( $\alpha$ )<sub>D</sub> 16.6°. Elemental analysis and mass-

spectrometric determination suggested the molecular formula as  $C_{30}H_{50}$  ( $M^+$  410, Fig - 11). This compound was found to be identical with an authentic sample of fern-9(11)-ene (m.m.p. no depression, superimposable IR and identical mass fragmentation pattern) supplied by Prof. Berti. Hence the skeleton is of fernene type with a double bond in 9(11)-position - indicating that the original compound is a  $\Delta^9(11)$  fernene derivative.

Section C : Position of the hydroxyl function :

The position of the hydroxyl group was established from the following observations. From the foregoing discussion it seems that the position of the oxygen function may be at C-3, C-6, C-7, C-15 and C-16. Of these possibilities the first one is eliminated, since the ketone 23 did not respond to Zimmerman's colour test for 3-keto group nor did it react with any carbonyl reagent. Moreover, it was not identical with 3-keto-fern-9(11)-ene. The study of the mass spectra of the acetate 22 and the ketone 23 indicated that the oxygen function was probably present in ring B, either at C-6 or at C-7. The compounds (Chart - II) 22 and 23 exhibited a mass peak (A) at 257 for 23 and 241 for 22 [ formed by the loss of acetic acid from the ion (A) ]. In addition to species (A) and its further decomposition products, the spectra of 22 and 23 showed a very abundant fragment (B) at 271 for 23 and at 255 for 22.

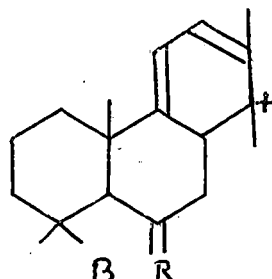
Chart - II



21 , R = OH ( $\beta$ ), H

22 , R = OAc ( $\beta$ ), H

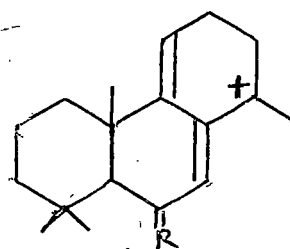
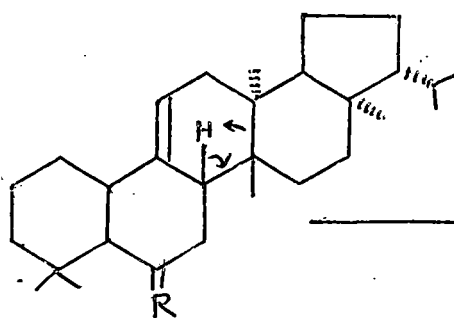
23 , R = O



m/e 271 ( $R=O$ )

m/e 255 ( $B-AcOH$ )

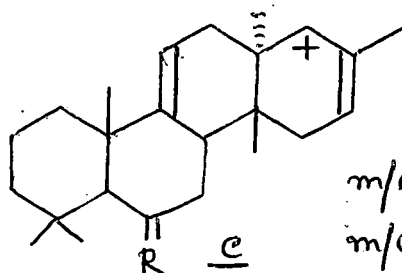
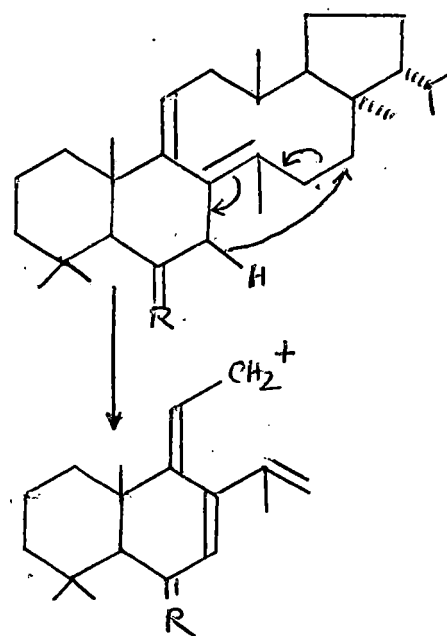
R = OAc, (H)



m/e 257 ( $R=O$ )

m/e 241 ( $A-AcOH$ )

R = OAc, (H)



m/e 339 ( $R=O$ )

m/e 323 ( $C-AcOH$ )

R = OAc, (H)

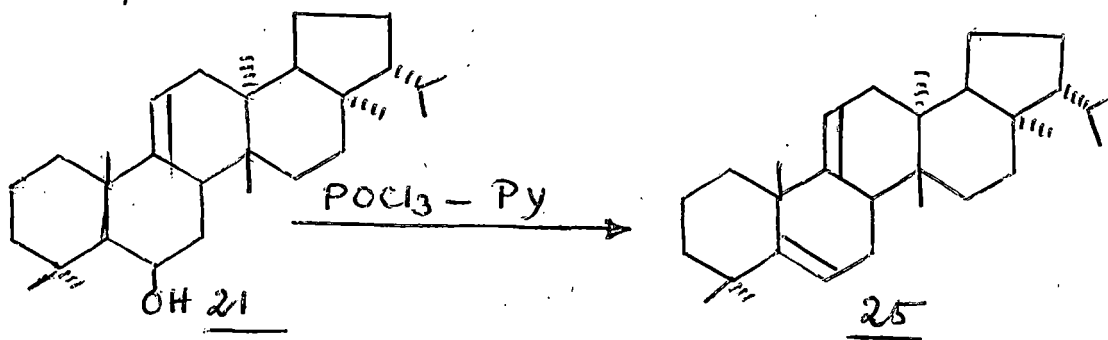


The acetate 22 and the ketone 23 also show a small peak (C) at 323 and at 339 respectively. This type of fragmentation pattern and other evidence discussed earlier can be explained by assuming the presence of hydroxylic function at ring B either at C-6 or at C-7. A careful examination of circular dichroism curve (Jouan Dichrograph - 185) of 23 (Fig - 12) established the presence of oxygen function at C-6. The compound gave a strong positive Cotton effect  $\Delta \epsilon + 3.39$  at 296 nm. Inspection of Drieding models suggest that the C-6 ketone would adopt a twist conformation with a positive cotton effect. But if the carbonyl group is situated at C-7 position then the chirality of the  $\beta$ - $\gamma$  unsaturated keto chromophore <sup>328-320</sup> would most probably give rise to a negative Cotton effect.

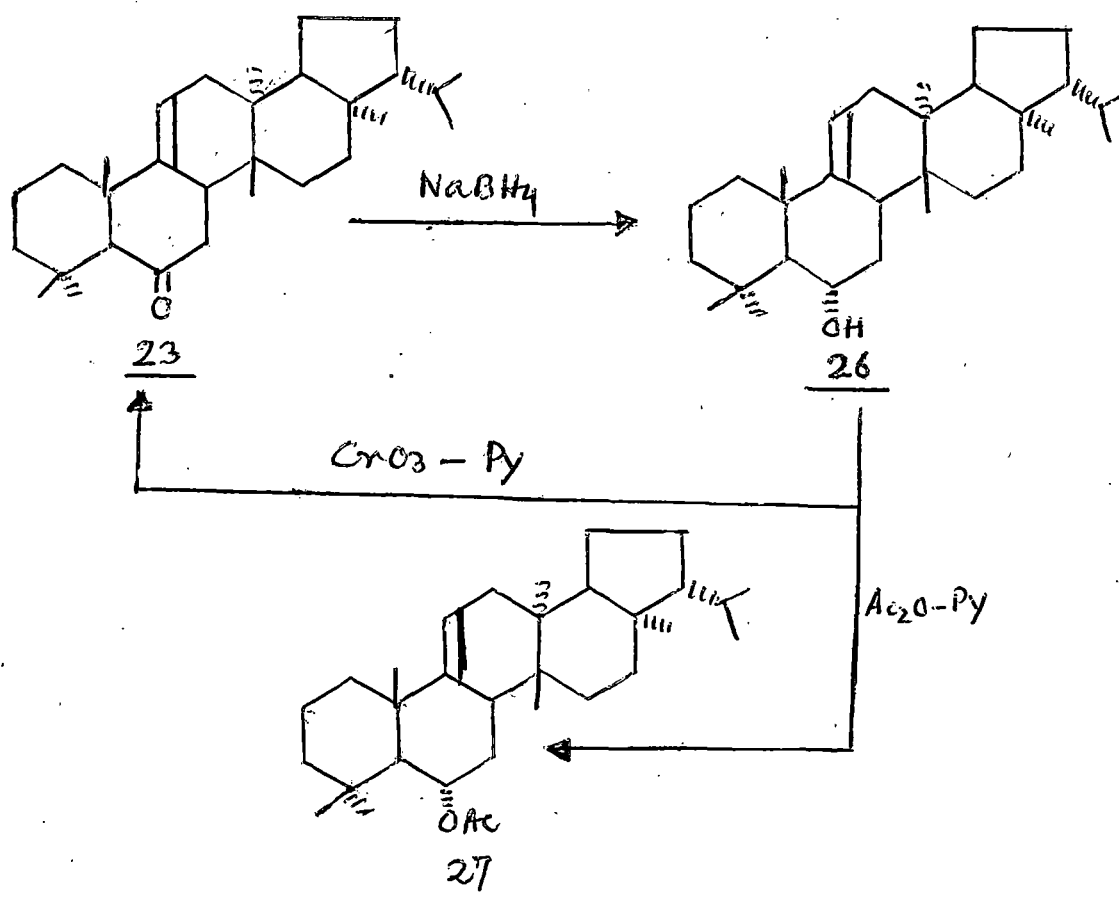
Thus from the foregoing physical evidence it may be inferred that in compound 21 the hydroxyl group is present at position C-6.

#### Section D: Stereochemistry of the hydroxyl function:

Dehydration of 21 with  $\text{POCl}_3$ -pyridine afforded a single product 25 (TLC single spot), m.p.  $157-58^\circ$ , no UV absorption above 220  $\text{m}\mu$ , and no-OH absorption in IR spectrum (Fig - 13).

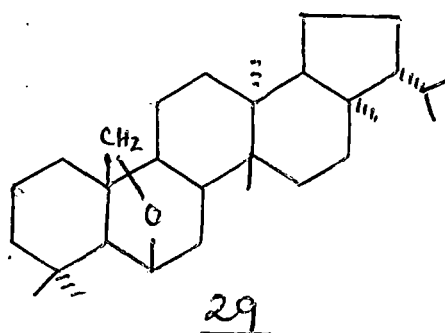
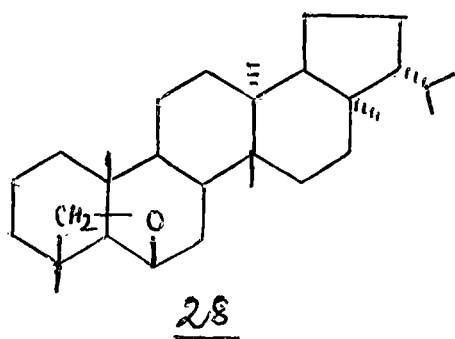


NMR spectrum of this compound (Fig - 14) showed the presence of two vinyl protons (two multiplets, one centered at  $\delta$  5.28 and the other at  $\delta$  5.44). Compound 25 on acid isomerisation afforded an oily compound which did not show any UV absorption above 220 m $\mu$ . The formation of a single product during dehydration indicated the  $\beta$  - axial orientation of the hydroxylic function at C-6. Sodium borohydride reduction of the ketone 23 gave the epimeric compound 26, m.p. 200-201 $^{\circ}$  (NMR : Fig - 15); monoacetate 27, m.p. 177-79 $^{\circ}$ . Oxidation of 26 gave back the original ketone. This also established that during the reduction process 6 - ketogroup was converted to the 6 $\alpha$  - equatorial hydroxy compound 26.



The NMR spectral data also indicated the axial nature of hydroxyl group in 21<sup>32</sup>. The signal for C<sub>10</sub>-CH<sub>3</sub> group in 21 appeared at considerably low field ( $\delta$  1.28) and the corresponding signal in the acetate 22 was shifted to  $\delta$  1.16. The observation is indicative of 1,3 - diaxial relationship between C<sub>10</sub>-CH<sub>3</sub> and the hydroxylic group at C-6.

With a view to adducing further evidence for the axial nature of the 6-OH group and also the position of the hydroxy group at C-6 we attempted to prepare the five membered ring ethers 28 and/or 29 by stirring the compound 21 in dry CCl<sub>4</sub> with finely divided HgO and iodine in presence of high pressure mercury lamp according to the method of Barton *et al*<sup>33</sup>. But we found that in different runs of the same experiment we



could isolate only the dehydration product 25, m.p. 157-58°. This experiment, however, again indirectly suggests the axial nature of the hydroxyl group at C-6, which readily undergoes elimination.

The acetate 22 was treated with selenium dioxide in acetic acid but no product having a diene dienone system could be achieved which is in conformity with work of Talapatra et al <sup>34</sup> and is reminiscent of the similar behaviour exhibited by  $\Delta^{9(11)}$  pentacyclic triterpenes like arborenyl <sup>35</sup> and fernemyl acetates <sup>36</sup>.

Discussions on the 300 MHz NMR spectrum of the ketone 23 :

We have obtained the 300 MHz NMR spectrum of compound as shown in Figs. 10A and 10B. The reference is TMS and the full sweep range is 2500 Hz.

The compound displays a peak at about  $\delta$  5.3 which is assigned to the olefinic proton on C-11. This proton appears to be coupled rather weakly to the neighbouring protons on C-12 and also the protons on C-8. These coupling constants are 2-3 Hz or less.

The compound (Fig. 10A), displays several signals labelled A-E about which the following comments appear reasonable and is in conformity with the structure assigned for it. Addition of shift reagent stepwise to 23 resulted in peaks A, B, and C moving downfield (left) about  $\delta$  0.25 before they began to broaden, while peaks D and E were essentially unaffected. The peaks D and E could be assigned to the  $\alpha$ - and  $\beta$ - protons on C-12, and the peak C could be reasonably assigned to the proton on C-5, since it would be a singlet, and should be affected by

shift reagent [in this case Europium in  $\text{Eu}(\text{FOD})_3$ ]. However some discussions are necessary <sup>g</sup> regarding peaks A and B. It appears that, both ~~both~~ A and B cannot arise from C-7, since these two protons are strongly spincoupled together, being in the same carbon, and the signals A and B show no evidence of mutual coupling (Portions of the spectra have been expanded to show the multiplets more clearly). Multiplet B moves faster than A and eventually superimposes on it with addition of shift reagent, so B is assigned tentatively to the  $7\beta$ -proton and a splitting of about 8-10 Hz due to diaxial coupling to the proton on C-8. The assignment of multiplet A is, however, uncertain, and would probably arise from a proton near the paramagnetic ion, in this case Europium in  $\text{Eu}(\text{FOD})_3$ . Although, all the foregoing assignments can reasonably explain the assigned structure 23, we believe that to arrive at a <sup>firm</sup> ~~four~~ conclusion from NMR studies a considerable experimental work  $V_2$  decoupling, changing solvents would be required. Moreover, observations after complete deuteration might offer additional clues ~~to~~ for identification of different protons in the complex between  $\delta$  1.0 to  $\delta$  2.0.

There is, however, one <sup>pi</sup> striking point which seems to rule out the 7-keto structure and thus provide an indirect proof for the  $\delta$ -keto structure 21. If it were the 7-keto compound, where the  $8\alpha$ -proton is flanked by the 7-keto compound and the

$\Delta^{9-11}$  group, the chemical shift value should have been discernible at a much lower field beyond the region of  $\delta 2.00$  complex. Moreover, a larger shifting effect would be predicted towards the left, (larger  $\delta$ ) due to the influence of the 7-keto group and the  $\Delta^{9-11}$  group.

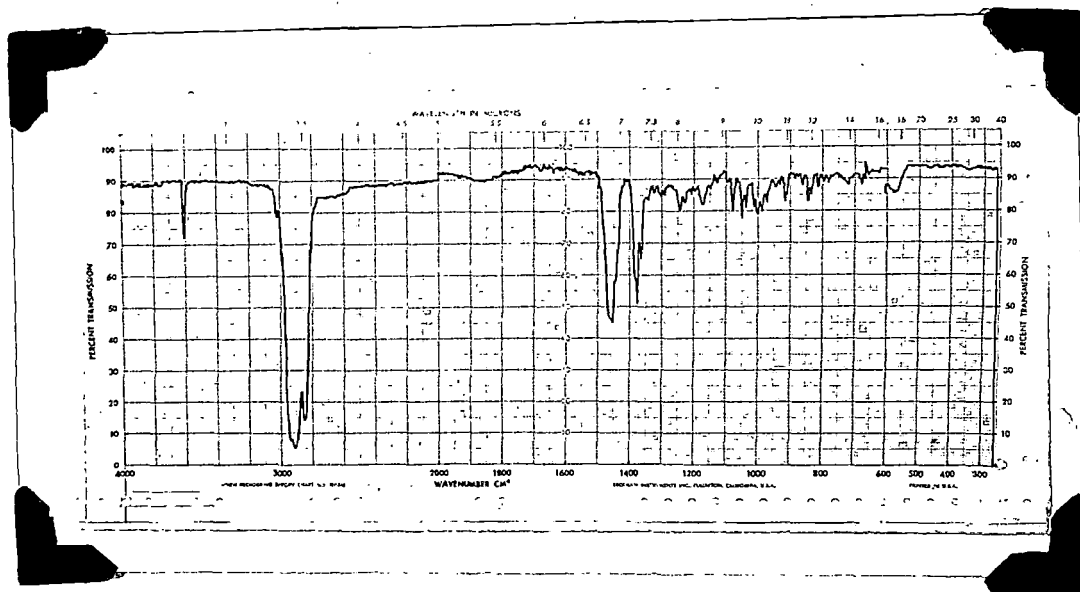


Fig. 3 : IR spectrum of Polypodinol A 21

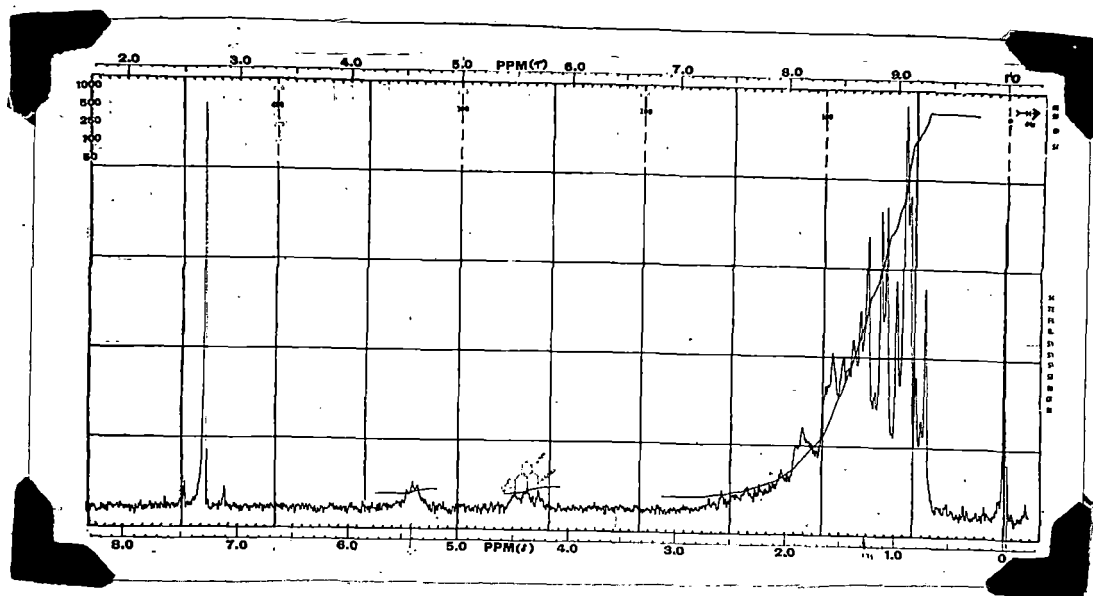


Fig. 4 : NMR spectrum of Polypodinol A 21

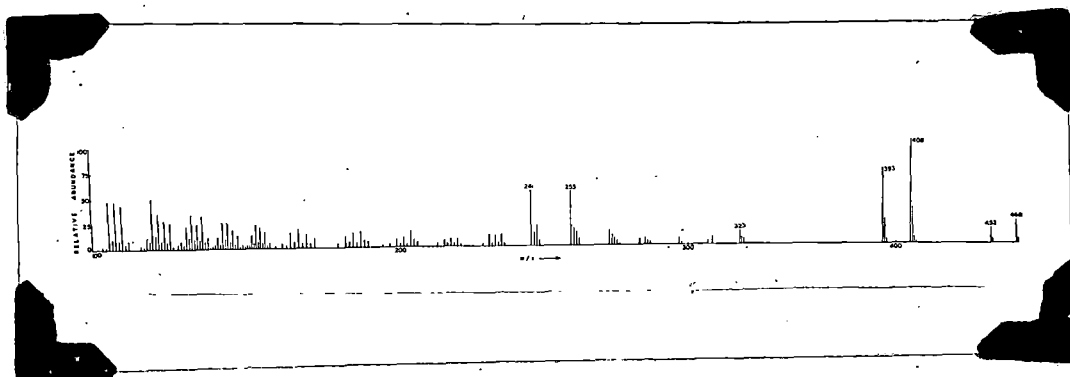


Fig. 5 : Mass spectrum of the acetate 22

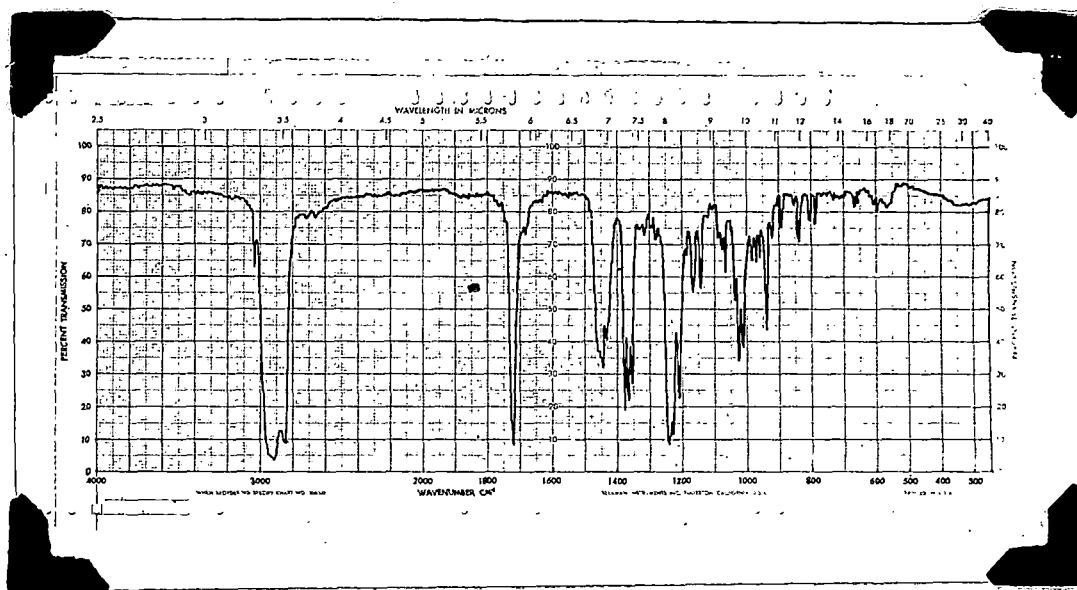


Fig. 6 : IR spectrum of the acetate 22



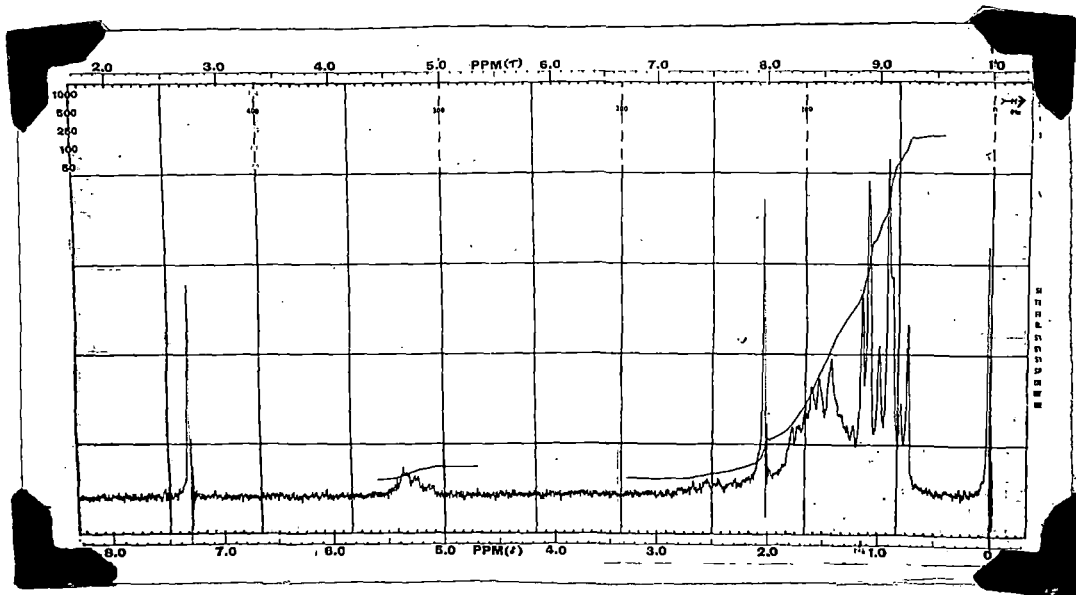


Fig. 7 : NMR spectrum of the acetate 22

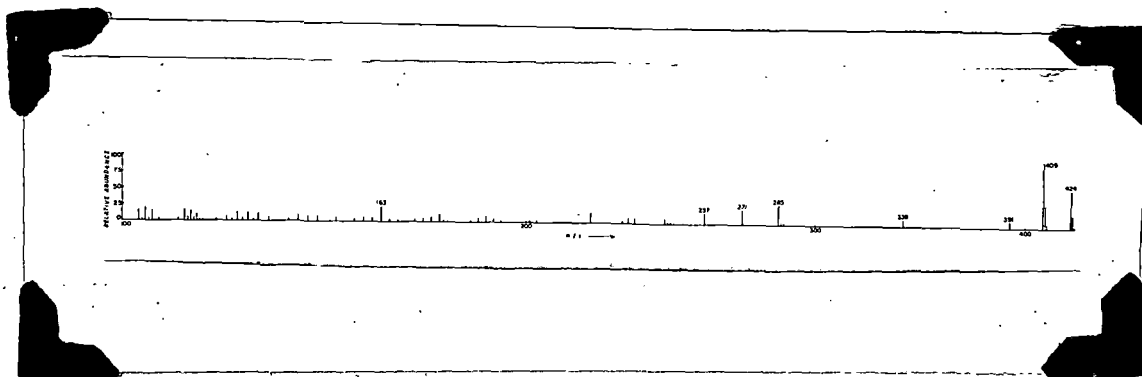


Fig. 8 : Mass spectrum of the ketone 23

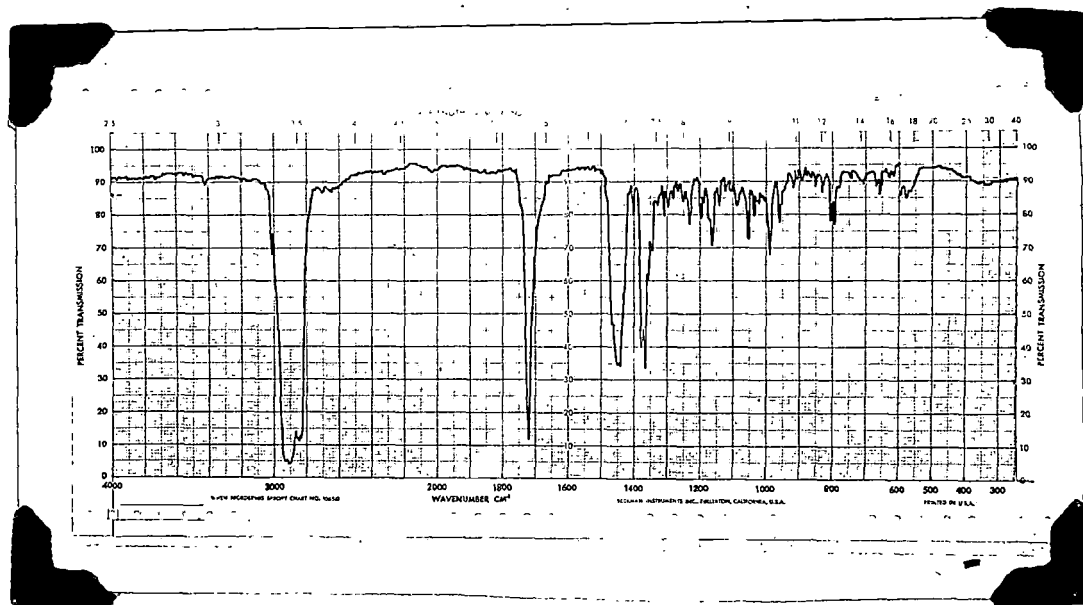


**Fig. 9 :** IR spectrum of the Ketone **23**

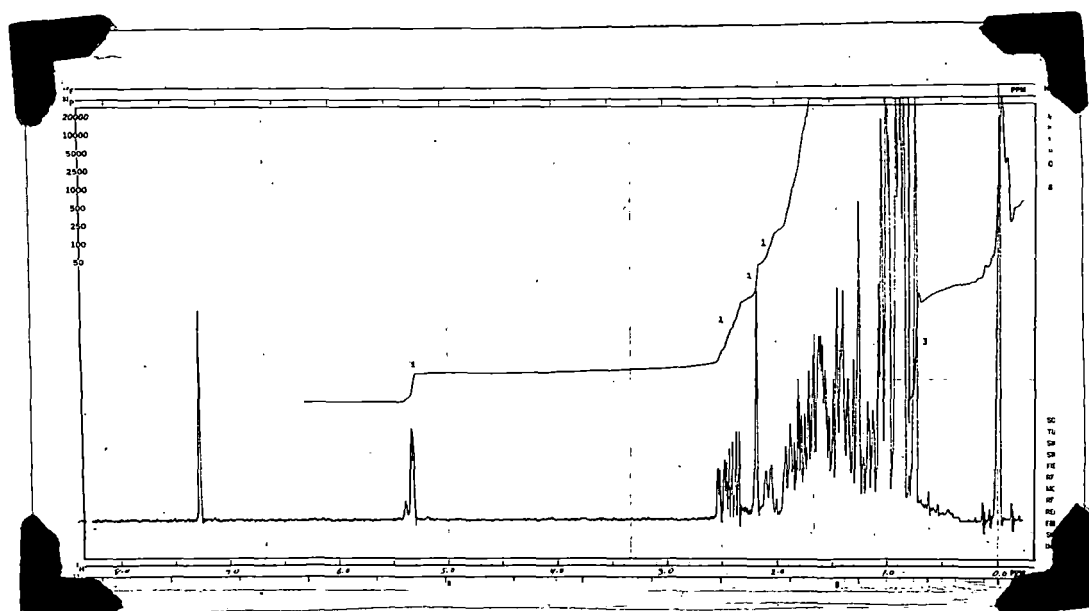


**Fig. 10A :** NMR spectrum of the Ketone **23** ( 300 MHz)

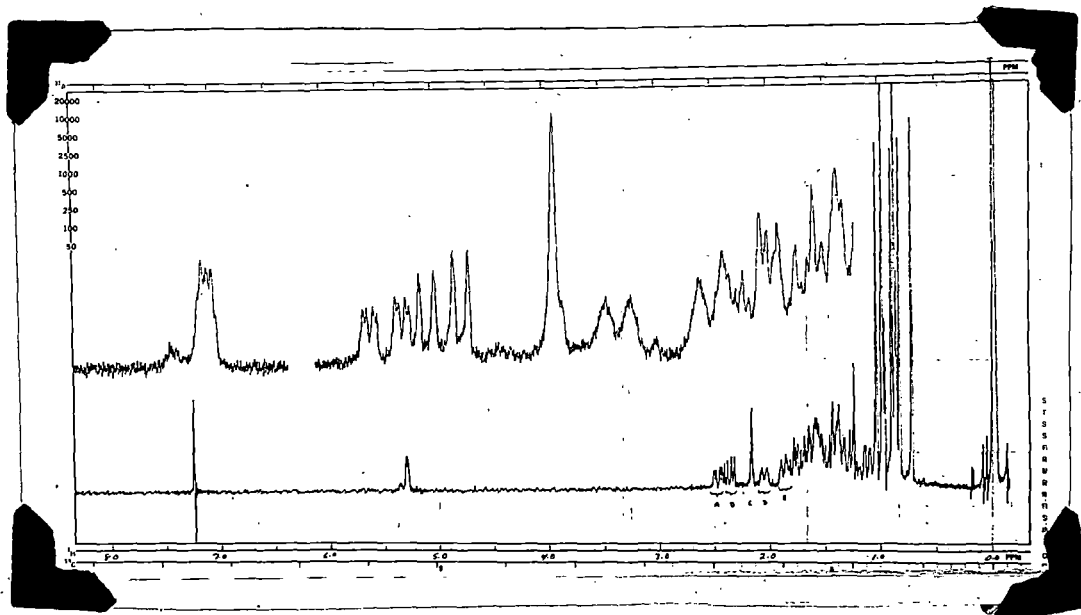


**Fig. 10B :** NMR spectrum of the ketone 23 (300 MHz)

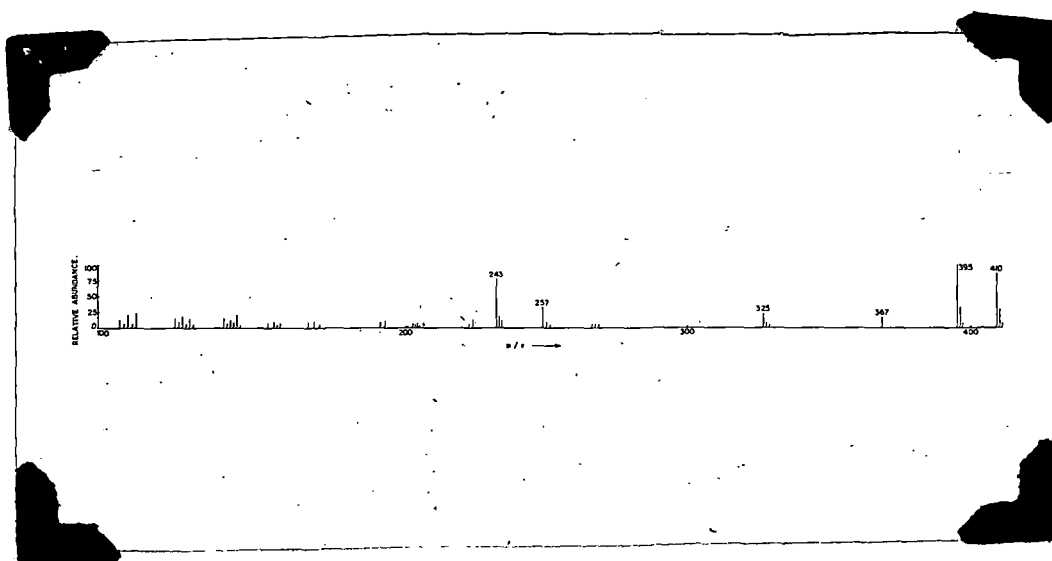


**Fig. 11 :** Mass spectrum of fern-9(11)-ene 24

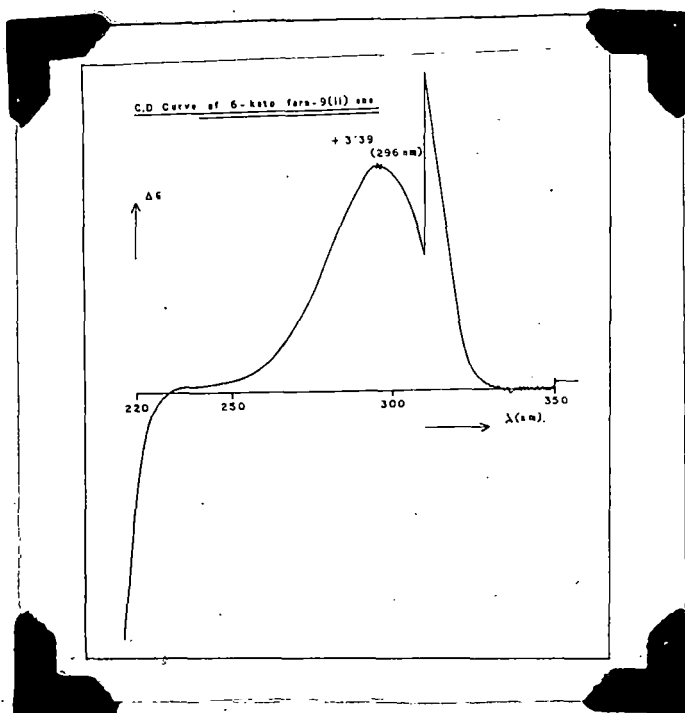


**Fig. 12 :** C. D. of the Ketone 23

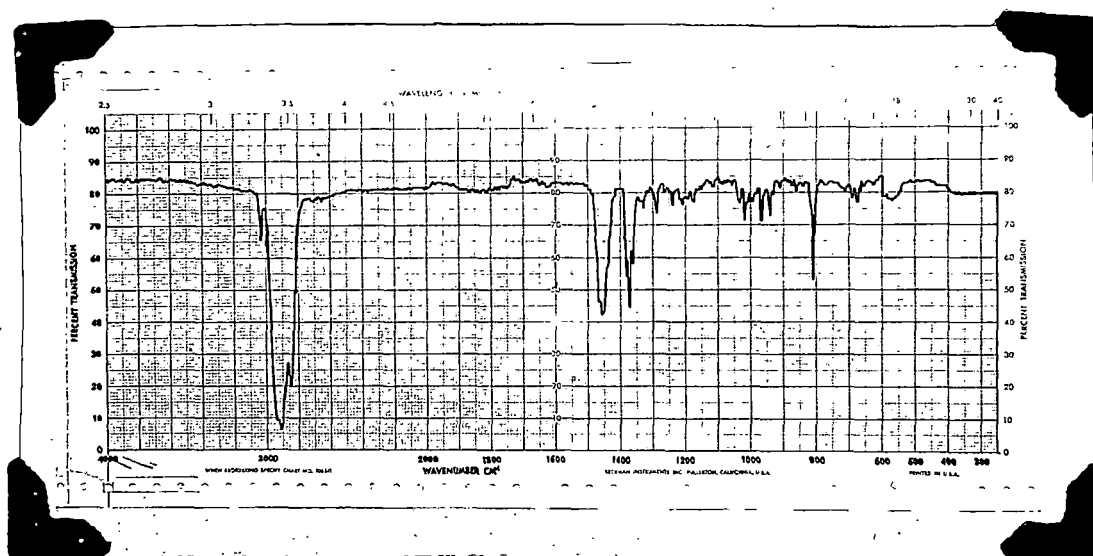


**Fig. 13 :** IR spectrum of the dehydrated product 25

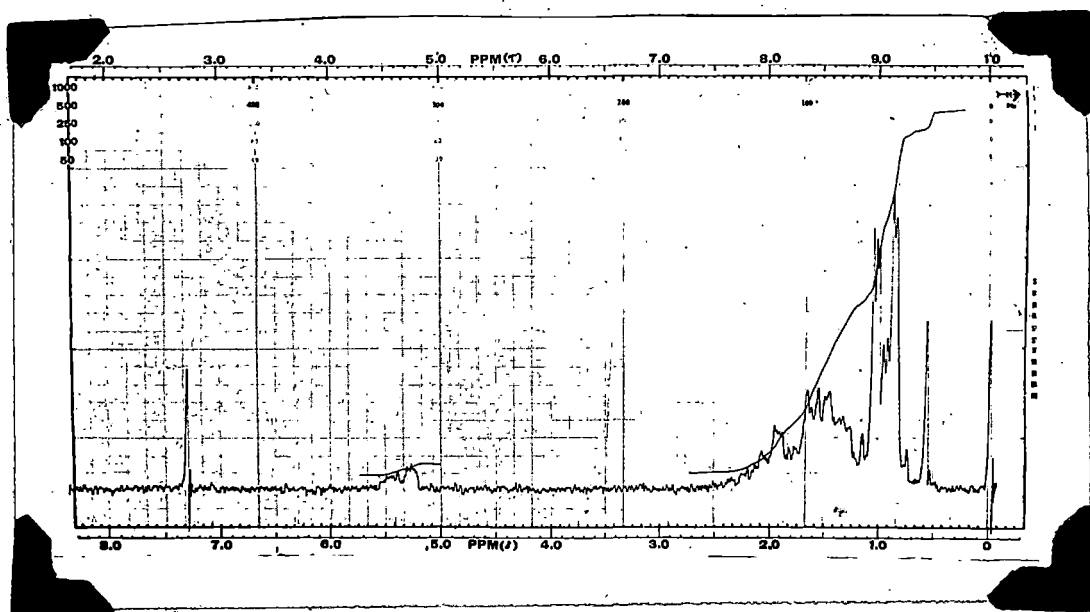


**Fig. 14 :** NMR spectrum of the dehydrated product **25**

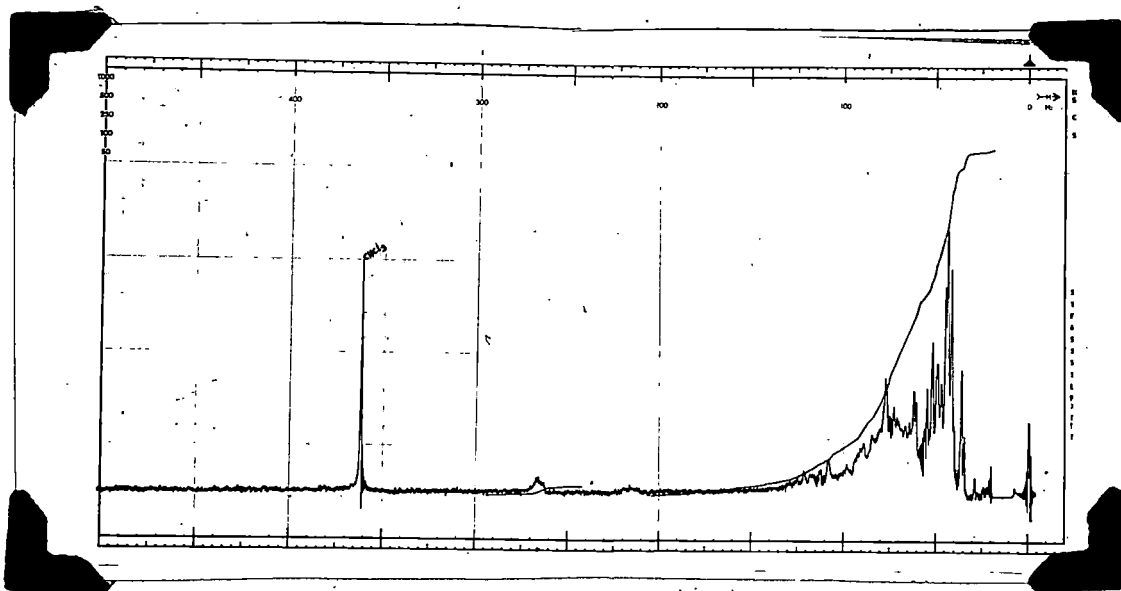


**Fig. 15 :** NMR spectrum of the epimeric alcohol **26**

### EXPERIMENTAL

Melting points are uncorrected. The petroleum used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution unless stated otherwise. NMR spectra were determined on varian HR-300, varian A-60 and HA - 100 spectrophotometers using chloroform-d solution containing tetramethyl-silane as reference. The IR spectra were recorded in Perkin-Elmer 337 and 221 and Beckmann IR-20 spectrophotometers, UV absorption spectra were taken in Zeiss VSU-1 and UV Beckman DU-2 spectrophotometers. The mass spectra were determined with an MS-9 mass spectrophotometer, using direct sample introduction into the ion source.

#### Rechromatography of fraction no. 2 (Chapter-III, ~~Table-I~~ Page -187, Table - I):

The gummy solid (5 gm) obtained in fraction no. 2 (Chapter-III, Page -187, Table-I) was placed on a column of active alumina (300 gm). The chromatogram was developed with petroleum and eluted with the following solvents (Table - II).

Table - II

| Eluent        | Fractions<br>50 ml each | Residue on<br>evaporation | Melting<br>point |
|---------------|-------------------------|---------------------------|------------------|
| Petroleum     | 1 - 3                   | Oil                       | -                |
| Petroleum ;   | 4 - 5                   | Oil                       | -                |
| benzene (4:1) | 6 - 9                   | Solid (2gm)               | 218-20°          |
|               | 10 - 11                 | Oil                       |                  |
| Petroleum ;   | 12 - 14                 | Solid (0.5gm)             | 148-50°          |
| benzene (3:2) | 15-17                   | Solid (0.8gm)             | 162-64°          |
|               | 18-20                   | Solid (0.8gm)             | 115-20°          |

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

Fractions 6-9 were combined and on crystallisation from a mixture of chloroform and methanol furnished fine needle-shaped crystals of 21, m.p. 223-25°, ( $\alpha$ )<sub>D</sub> - 89.13°

Found : C, 84.41; H, 11.85%

Calculated for  $C_{30}H_{50}O$  : C, 84.44; H, 11.81%

IR :  $3605\text{ cm}^{-1}$  (OH) Fig - 3

UV : no absorption in the region 200-300  $m\mu$

Mass spectrum :  $M^+$  426

NMR spectrum :  $\delta$  0.8-1.28 ( 8 methyl groups )

$\delta$  5.40 (multiplet, 1H, trisubstituted double bond)

$\delta$  4.40 (multiplet, -  $\overset{|}{\text{CH}}\text{-CH}(\text{OH})\text{-CH}_2\text{-}$ )

Fig - 4

Preparation of the acetate 22 of Polypodinol A 21 :

The compound 21 (200 mg) was treated with pyridine (2 ml) and acetic anhydride (2 ml) and heated on a water bath for 3-hours. After working up in the usual way it gave a solid residue (180 mg). The residue dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - III).

Table - III

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation       |
|-----------|-------------------------|---------------------------------|
| Petroleum | 1 - 3                   | Solid (170 mg)<br>m.p. 198-200° |

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



Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless needles of 22, m.p.  $203-4^{\circ}$ ,  $(\alpha)_D - 83.72^{\circ}$ .

Found : C, 81.93; H, 11.21%

Calculated for  $C_{32}H_{52}O_2$ : C, 81.99; H, 11.18%

IR :  $1725, 1245\text{ cm}^{-1}$  Fig - 6

Mass spectrum :  $M^+$  468 Fig - 5

NMR spectrum (60 Mc):  $\delta$  0.8-1.16 (8 methyl peaks)

$\delta$  2.00 (3H,  $-O-CO-CH_3$ )

$\delta$  5.40 (multiplet, 1 H, vinyl proton)

$\delta$  5.20 (multiplet, 1H,  $-CH-CH(oAc)-CH_2-$ )

Fig - 7

Preparation of the ketone 23 with chromium trioxide-pyridine complex :

A solution of the Polypodinol A 21 (200 mg) in pyridine (2 ml) was added to chromium trioxide-pyridine complex prepared from pyridine (2 ml) and chromium trioxide (200 mg) and the mixture was kept at room temperature for 14 hours. The product (180 mg) obtained after working up in the usual manner was dissolved in benzene (3 ml) and placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents ( Table - IV ).

Table - IV

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation.     |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 3                   | Solid (145 mg)<br>m.p. 219-21° |

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

~~and~~

Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished fine needle-shaped crystals of ketone 23, m.p. 221-23°, ( $\alpha$ )<sub>D</sub> 16.66°.

Found : C, 84.89; H, 11.35%

Calculated for C<sub>30</sub>H<sub>48</sub>O : C, 84.84; H, 11.39%

IR : 1720 cm<sup>-1</sup> Fig - 9

Mass spectrum : M<sup>+</sup> 424 Fig - 8

NMR spectrum (300 MHz) :  $\delta$  0.8-1.04 ( 8 methyl groups )  
 $\delta$  5.40 ( 1H, vinyl proton )

Figs - 10A and 10B

Wolff-Kishner reduction<sup>37</sup> of the ketone 23: Preparation of  
fern -9(11)-ene 24 :

The ketone 23 (200 mg) in diethylene glycol (30 ml) was refluxed with hydrazine hydrate (2.3 ml) for 30 minutes. After addition of KOH (200 mg) the mixture was further refluxed for one hour. The condenser was removed and the mixture was heated to 190°. After refluxing for another 2½ hours the reaction mixture was cooled, diluted with water when a solid separated out. The solid (185 mg) dissolved in petroleum was poured over a column of active alumina (15 gm) developed with petroleum and eluted with following solvents (Table V).

Table - V

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation        |
|-----------|-------------------------|----------------------------------|
| Petroleum | 1 - 3                   | Solid (150 mg)<br>m.p. 165 - 68° |

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

Fractions 1-3 were <sup>combined</sup>~~crystallised~~ and on crystallisation from a mixture of chloroform and methanol furnished colourless needles of 24 which was found to be identical with fern-9(11)-ene (no, m.m.p. depression, superimposable IR and identical mass fragmentation pattern) supplied by Prof. Berti.

Found : C, 88.01; H, 12.15%

Calculated for  $C_{30}H_{50}$  : C, 87.73; H, 12.27%

IR :

Mass spectrum :  $M^+$  410

Fig - 11

Preparation of the dehydration product 25 with  $POCl_3$ - pyridine :

To Polypodinol A 21 (200 mg) in pyridine (2 ml) was added a solution of phosphorous oxychloride (2 ml) in pyridine (4 ml) and the mixture was kept at room temperature for 24-hours. The mixture was diluted with water, extracted with ether, the ether layer washed successively with saturated sodium bicarbonate solution, dil. hydrochloric acid and water (till neutral) and then dried (anhyd.  $Na_2SO_4$ ). The ether layer on evaporation gave a residue (150 mg) which was chromatographed over a column of active alumina (10 gm) developed with petroleum using the following solvents ( Table - VI ).

Table - VI

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 3                   | Solid (130 mg)<br>m.p. 155-57° |

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

Fractions 1-3 were combined and on crystallisation from chloroform-methanol mixture yielded fine crystals of 25, m.p. 157 - 58°

Found : C, 88.32; H, 11.81%

Calculated for  $C_{30}H_{48}$  : C, 88.16; H, 11.84%

IR : Fig - 13

NMR spectrum (60 Mc) :  $\delta$  5.28 (multiplet), Fig - 14  
 $\delta$  5.44 (multiplet)

Attempted acid isomerisation of the dehydrated product 25:

To the dehydrated product 25 (200 mg) in glacial acetic acid (20 ml) was added conc. hydrochloric acid (1 ml) and heated on a water bath for 30 minutes. The reaction mixture

was diluted with water and after usual working up gave a oily residue (185 mg). This product, dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of a 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - VII).

Table - VII

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation |
|-----------|-------------------------|---------------------------|
| Petroleum | 1 - 3                   | Oil                       |

Further elution with more polar solvent did not yield any oil or solid material.

Fractions 1- 3 were combined and this could not be induced to crystallisation.

UV : Transparent in the region 220-300 m $\mu$ .

Sodium borohydride reduction of the ketone 23 : Preparation of the 6-epimer 26 of Polypodinol-A 21 :

To a solution of the ketone 23 (200 mg) in methanol (100 ml), sodium borohydride (200 mg) was added and the mixture was stirred

for 3 hours. The reaction mixture was concentrated, diluted with water and the solid which separated out was filtered, washed and dried. This product (190 mg), dissolved in benzene (4 ml) was poured on a column of alumina (15 gm deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - VIII).

Table - VIII

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation       |
|-----------|-------------------------|---------------------------------|
| Petroleum | 1 - 4                   | Solid (170 mg)<br>m.p. 196-98°. |

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

Fractions 1 - 4 were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless needles 26, m.p. 200-1°.

Found : C, 84.51; H, 11.85%

Calculated for  $C_{30}H_{50}O$  : C, 84.44; H, 11.81%

NMR spectrum (100 MHz) : Fig - 15

Acetylation of the above compound 26 :

The compound 26 (100 mg) was acetylated with pyridine (1 ml) and acetic anhydride (1 ml) in the usual way. After working up in the usual manner it gave a solid residue (90 mg). This product dissolved in benzene (2 ml) was placed over a column of alumina (10 gm, deactivated with 0.4 ml of 10% aqueous acetic acid). developed with petroleum and eluted with the following solvents (Table - IX)

Table - IX

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 3                   | Solid (80 mg)<br>m.p. 174-77°. |

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

Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless crystals of 27, m.p. 177-79°.

Found : C, 81.91; H, 11.23%

Calculated for  $C_{32}H_{52}O_2$  : C, 81.99; H, 11.18%



Oxidation of the epimeric alcohol 26 with chromium trioxide - pyridine complex :

A solution of the epimeric alcohol 26 (200 mg) in pyridine (2 ml) was added to chromium trioxide - pyridine complex prepared from pyridine (2 ml) and chromium trioxide (200 mg) and the mixture was kept at room temperature for 12-hours. The product (170 mg) obtained after working up in the usual manner was dissolved in benzene (3ml) and placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - X).

Table - X

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 3                   | Solid (148 mg)<br>m.p. 218-20° |

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

Fractions 1-3 were combined and on crystallisation from chloroform-methanol mixture gave crystals, m.p. 221-23° identical with the ketone 23 (m.m.p.)

HgO - iodine treatment of Polypodinol A 21 in CCl<sub>4</sub> in presence of high pressure mercury lamp <sup>33</sup>:

Polypodinol A 21 (500 mg) in dry CCl<sub>4</sub> (100 ml) was stirred for 1 hour with finely divided HgO (900 mg) and iodine (1.07 <sup>mm</sup>g) while being irradiated with a high pressure mercury lamp in the usual way <sup>33b-33d</sup>. The mixture was filtered, washed with aq. sodium thiosulphate and the solvent removed in vacuo. The product (460 mg) obtained was dissolved in petroleum (5 ml) and placed over a column of alumina (30 gm, deactivated with 1.2 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XI).

Table - XI

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation                    |
|-----------|-------------------------|----------------------------------------------|
| Petroleum | 1 - 5                   | Solid (425 <del>mg</del> mg)<br>m.p. 155-57° |

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

Fractions 1-5 were combined and on crystallisation from a mixture of chloroform and methanol yielded colourless needles; m.p. 157-58° which was found to be identical with the dehydration product 25 (m.m.p. and IR).

Found : C, 88.23; H, 11.78%

Calculated for  $C_{30}H_{48}$  : C, 88.16; H, 11.84%

Selenium dioxide oxidation of the acetate 22 :

To the acetate 22 (200 mg) in glacial acetic acid (50 ml) was added  $SeO_2$  (200 mg) in 96% acetic acid (5.2 ml) and heated under reflux for 24 hours. The reaction mixture was filtered ~~and the filtrate~~ and the filtrate diluted with water. The aqueous solution was extracted with ether, washed with sodium bicarbonate and then with water till neutral. Evaporation of the solvent gave a solid residue (180 mg). This product dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XII).

Table - XII

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 3                   | Solid (160 mg)<br>m.p. 154-57° |

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

Fractions 1 - 3 were combined and crystallisation from a mixture of chloroform and methanol yielded colourless needles, m.p.  $157-58^{\circ}$  which was found to be identical with the dehydration product 25 (m.m.p. and IR).

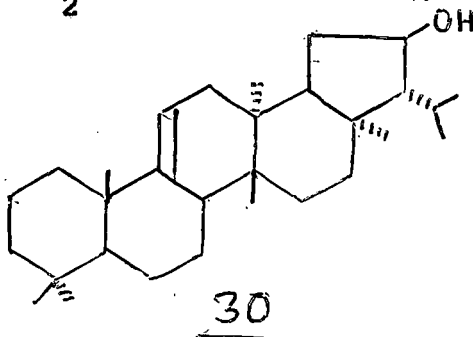
Found : C, 88.31; H, 11.89%

Calculated for  $C_{30}H_{48}$  : C, 88.16; H, 11.84%

CHAPTER - V

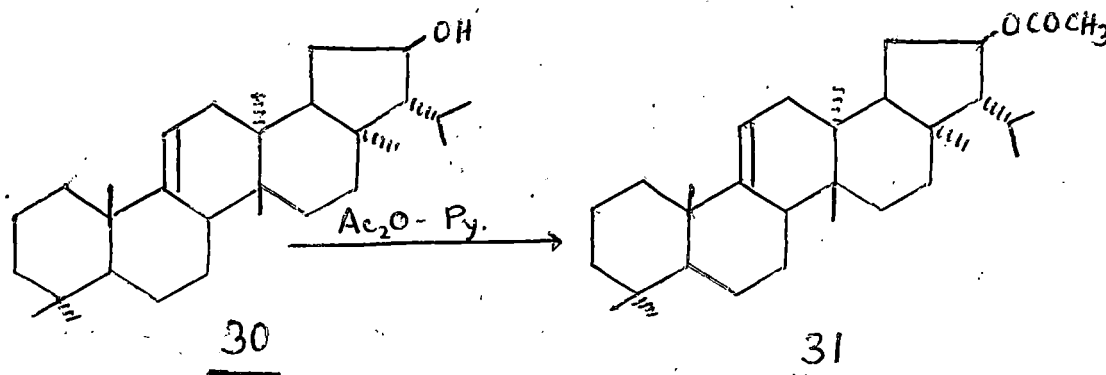
Isolation of a new triterpene alcohol, Polypodinol B,  
 $C_{30}H_{50}O$ ,  $(\alpha)_D$  28.57° and investigation on its structure :

Fraction No. 2 ( Chapter-III, Page-187, Table - I )  
 on rechromatography ( Chapter-IV, Page-204, Table-II) and  
 crystallisation of the fractions 15-17 from a mixture of  
 chloroform and methanol furnished needle-shaped crystals  
 having m.p. 165-66°,  $(\alpha)_D$  28.57°. This new triterpene 30  
 gave positive Libermann Burchard test and a yellow colour  
 with tetranitromethane. Elemental analysis and mass-spectro-  
 metric determination closely corresponded to the molecular  
 formula  $C_{30}H_{50}O$  ( $M^+$ 426). IR spectrum of 30 (Fig-16) showed  
 bands at 3590, 3510  $cm^{-1}$  (OH) and its NMR spectrum (60 Mc,  
 Fig-17) showed signals for eight methyl groups between  
 $\delta$  0.8 to 1.28, a multiplet centered at  $\delta$  5.40 (IH, trisubs-  
 tituted double bond) and a broad diffused multiplet  
 centered at  $\delta$  4.36 attributed to the proton attached to the  
 carbon containing - OH group. The coupling pattern again  
 indicates that this proton is attached to a carbon atom in  
 the system -  $\overset{|}{CH}-\underset{|}{CH}OH-CH_2$



Section A : Nature of the oxygen function :

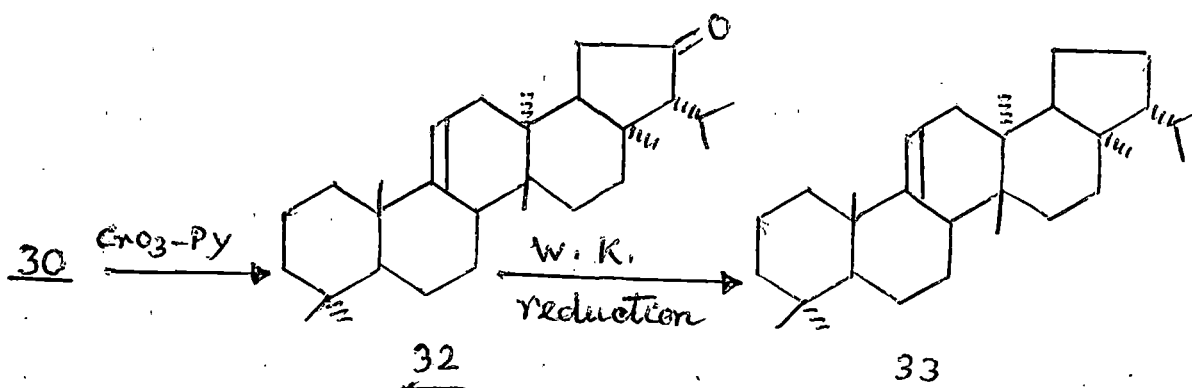
On treatment with acetic anhydride - pyridine, the compound 30 furnished a monoacetate 31,  $C_{32}H_{52}O_2$  ( $M^+$  468, Fig - 18), m.p.  $207-8^\circ$ ,  $(\alpha)_D$   $38.00^\circ$ ,  $\gamma_{\text{max}}^{\text{nujol}}$  1720 and  $1240\text{ cm}^{-1}$  (Fig - 19). Its NMR spectrum (60 Mc, Fig-20) showed the presence of eight methyl groups between  $\delta 0.8$  to 1.16, a sharp peak at  $\delta 2.08$  (3H,  $-O-CO-CH_3$ ), an unresolved multiplet centered at  $\delta 5.16$  attributable to one proton  $[1H, -CH-CH(OAc)-CH_2-]$  and a multiplet centered at  $\delta 5.45$  (1H, vinyl proton). Hence the oxygen function is present as a hydroxyl group which is acetytable. The acetate 31 did not show any UV absorption in the region 200-300  $m\mu$ . Hence it is evident from the above data that the compound does not contain any carbonyl function.



Section B : Nature of the carbon skeleton :

The nature of the carbon <sup>skeleton</sup> ~~skeleton~~ of the new triterpene was deduced from the following physical and chemical evidence

described below. Oxidation of 30 with  $\text{CrO}_3$ -pyridine complex gave a compound 32,  $\text{C}_{30}\text{H}_{48}\text{O}$  ( $M^+$  424, Fig-21), m.p.  $174-75^\circ$ ,  $\nu_{\text{max}}^{\text{nujol}}$  1725 (five membered ring ketone, Fig-22), NMR signals (Figs-23A and 23B) at  $\delta$  5.38 (1H, vinyl proton) and peaks between  $\delta$  0.8 to 1.04 for eight methyl groups. Wolff-Kishner reduction<sup>37</sup> of the ketone 32 furnished a hydrocarbon 33 ( $M^+$  410, Fig-24) m.p.  $169-71^\circ$ ,  $(\alpha)_D - 13.04^\circ$  which was found to be identical with an authentic sample of fern-9(11)-ene<sup>25</sup> (m.m.p. no depression, superimposable IR and identical mass fragmentation pattern) supplied by Prof. Berti of University of Pisa, Italy.



### Section C : Position of the hydroxyl function :

The position of the oxygen function could not be ascertained but a tentative proposal has been advanced on the basis of available chemical and physical evidence which are described below.

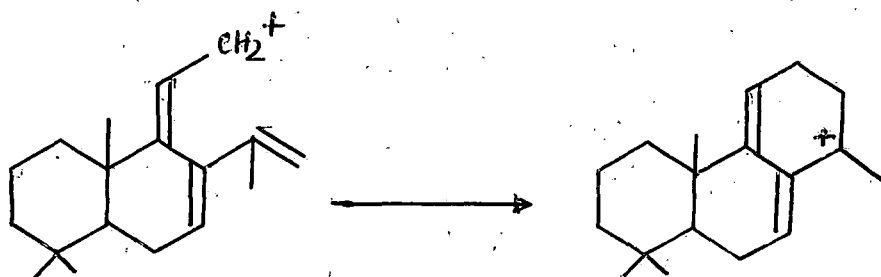
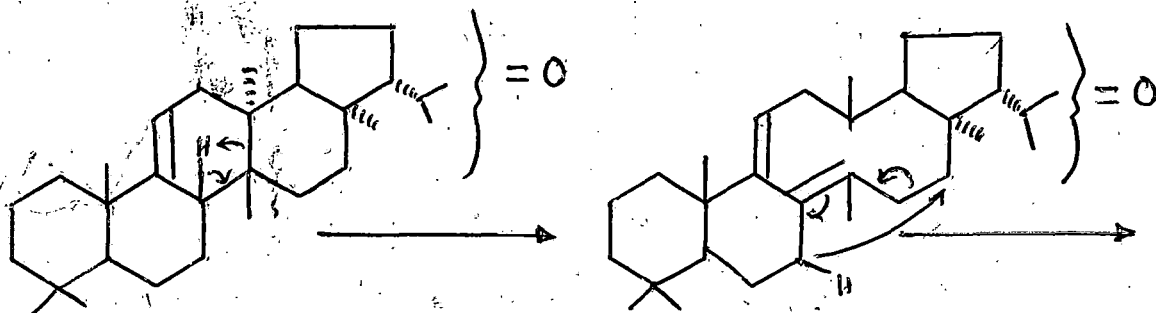
The ketone 32 did not respond to Zimmerman's colour test for 3-keto group<sup>38</sup> and did not react with any carbonyl reagent. The ketone was also found to be different from fernenone which is 3-keto-fern-9(11)-ene. Therefore, the oxygen function is not present at C-3.

Mass spectrum (Fig-21) of the ketone showed the molecular ion peak at  $M^+$  424 in addition to peaks at  $m/e$  409 ( $M^+ - 15$ ),  $m/e$  243 and  $m/e$  231 characteristic of a  $\Delta^{9(11)}$ -fernene system. The mass spectrum (Fig - 18) of the monoacetate 31 was more informative and exhibited a mass fragmentation pattern having the following peaks :  $M^+$  468,  $m/e$  453 ( $M - 15$ ),  $m/e$  393 ( $M - 15-HOAc$ ) in addition to peaks at  $m/e$  231 and  $m/e$  243. The fragmentation pattern is shown in chart III and again reveals that it is similar to that of  $\Delta^{9(11)}$ -fernene system. The presence of base peak at  $m/e$  243 in both the ketone 32 and the acetate 31 indicates that there is no substitution in rings A and B.

The other possible alternative positions for the (OH) group are at C-15, C-16 in ring D or some other position in ring E. At this stage of the work we thought it worthwhile to take the circular dichroism curve (Jouan Dichrograph -185) of the ketone 32. A careful examination of the CD curve (Fig-25) of 32 gave some clue about the position of the carbonyl group. We have carried out a systematic study of

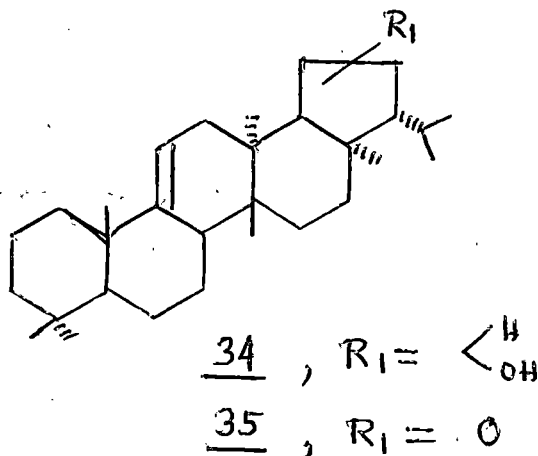


Chart - III



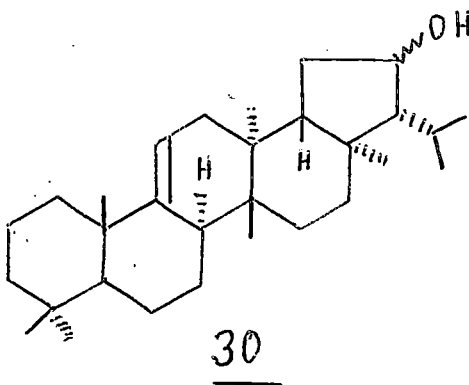
m/e 243

Drieding models with C-15, and C-16 ketone in the fem-9(11)-ene nucleus separately and inspection of the respective models suggest that the compound with the ketone at C-15 would have a very small Cotton effect - probably positive - whereas the compound with C - 16 carbonyl group would show a strong positive Cotton effect. Since a negative Cotton effect is actually observed for 32, positions C-15 and C-16 are ruled out. Therefore, we believe that the carbonyl group is most probably situated at some position in ring E. We can therefore put forward the following partial structures <sup>34 and 35</sup> for the new triterpene and the corresponding ketone obtained from its oxidation.



300 MHz NMR spectrum of 32 was taken as shown in Figs. -23A and 23B. The spectrum displays two pairs of doublets, Labelled A and C and a strong line labelled B.

Spectrum (Fig-23B) shows the integral which suggests that B represents three protons. The assignment of A and C could be attributed to the  $12\alpha$ - and  $12\beta$ - protons. Addition of shift reagent to this sample resulted in a shift of peak B and as more shift reagent is added, a splitting of peak B into two signals is discernible. It appears that one of the protons of peak B move over to the vicinity of peak A, while the other two protons move beyond peak A to the left, eventually moving about 0.25 ppm. This suggests that if the keto group is placed at C-20 (structure 32) then the peak B could arise from the superposition of  $21\beta$  and  $19\alpha$ - and  $19\beta$ - protons. Thus we propose a tentative structure 30 for Polypodinol B.



It is worth mentioning here that the above assignment 30 is tentative and would require considerable experimental work to confirm. Unfortunately, this was not possible in view of the lack of proper NMR facilities. However, further work is in progress to finally confirm its structure.

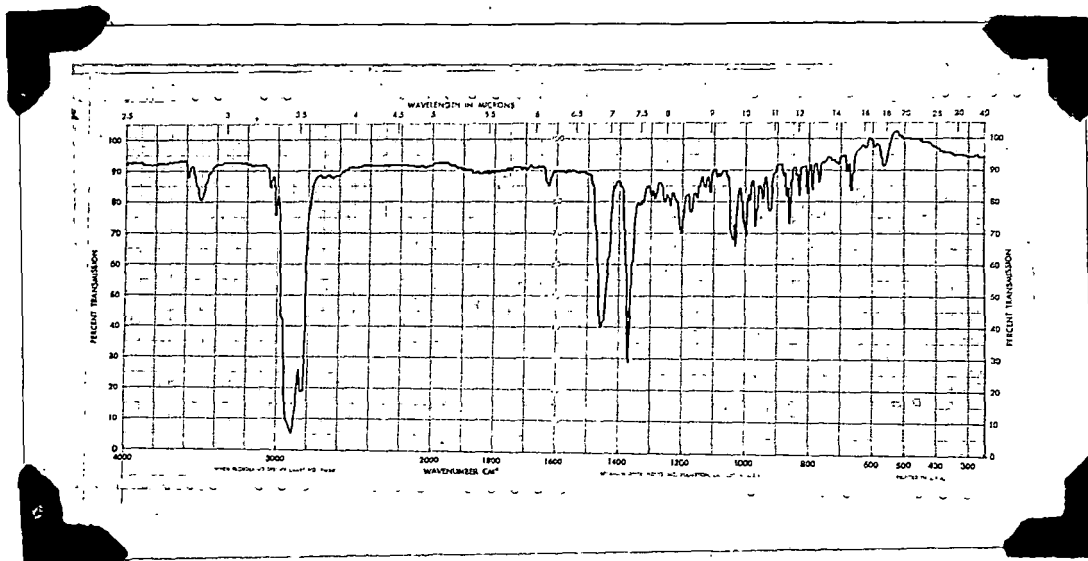


Fig. 16 : IR spectrum of Polypodinol B 30

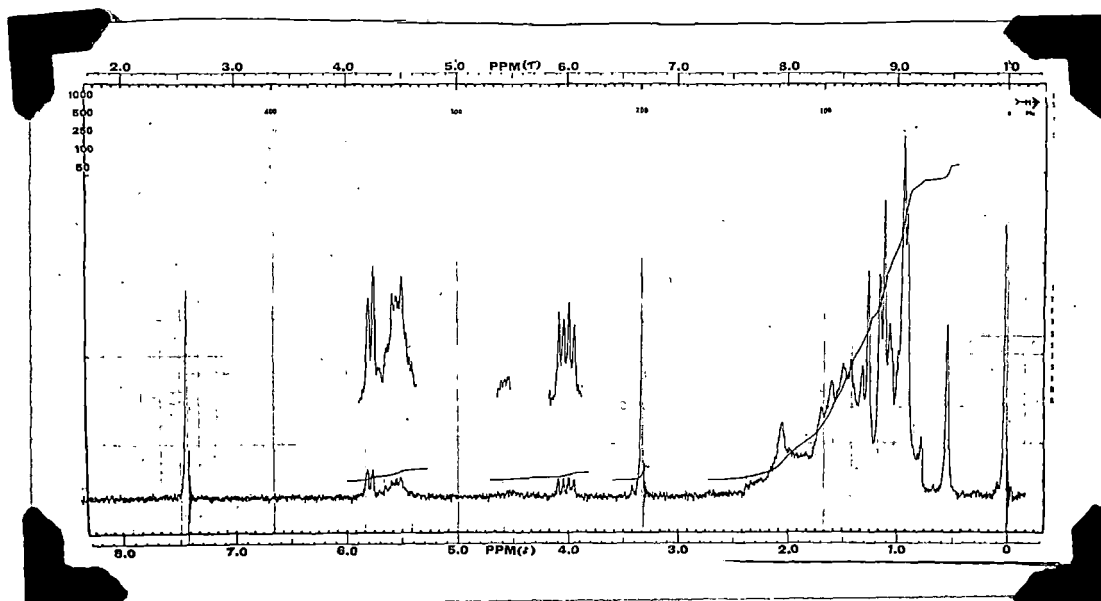
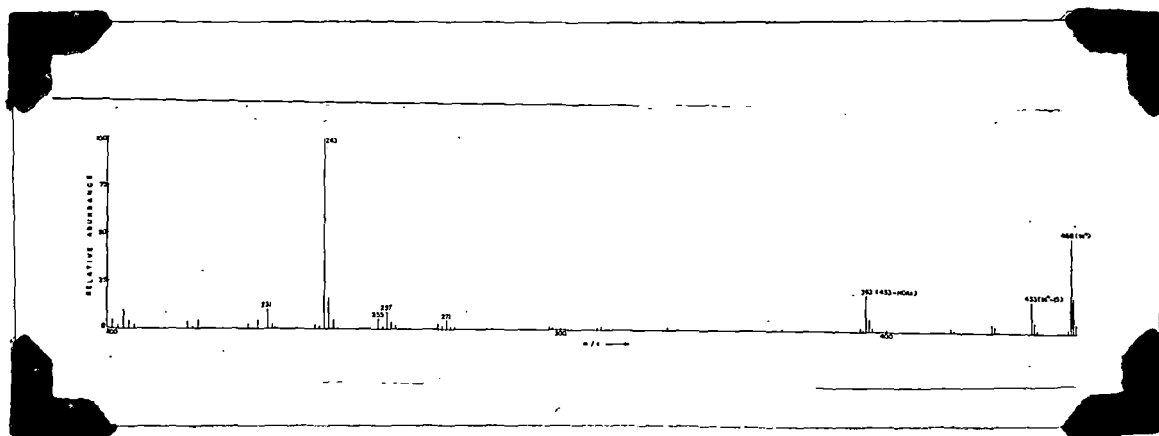


Fig. 17 : NMR spectrum of Polypodinol B 30



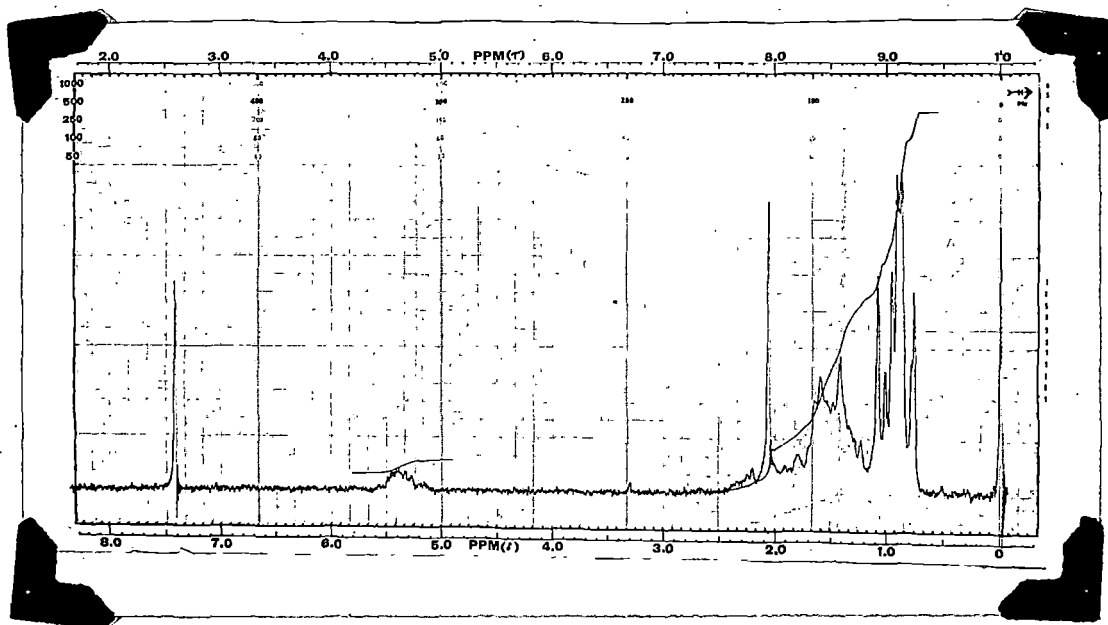


Fig. 20 : NMR spectrum of the acetate 31

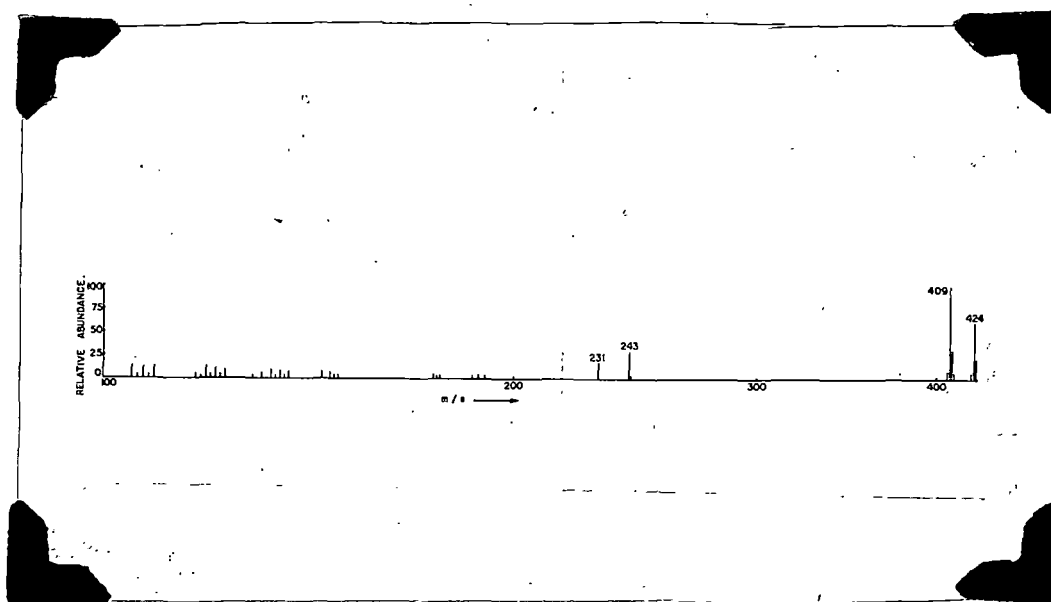


Fig. 21 : Mass spectrum of the Ketone 32

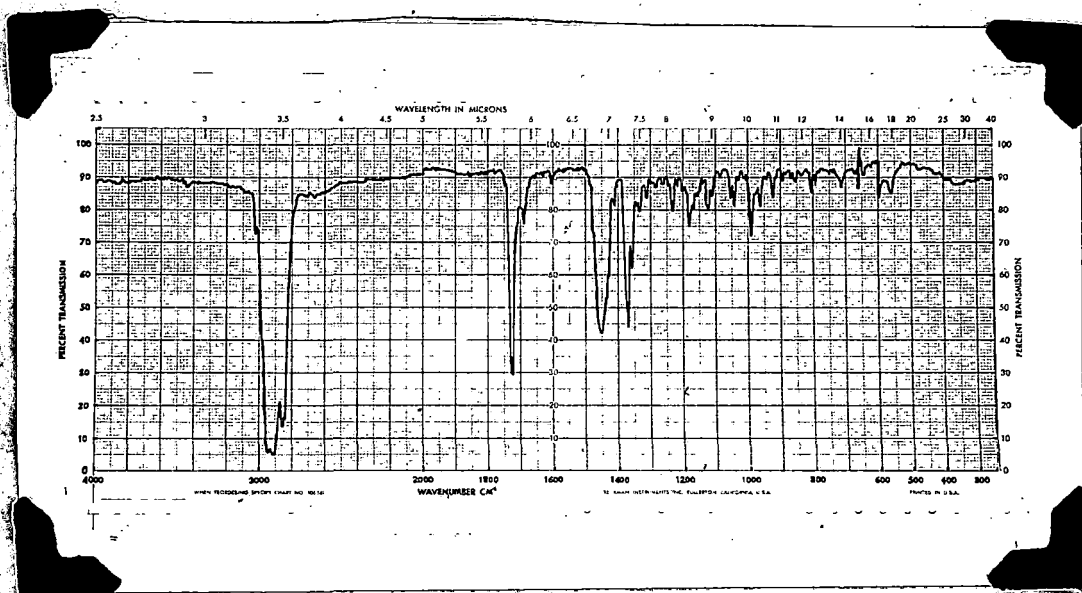


**Fig. 22 :** IR spectrum of the Ketone 32

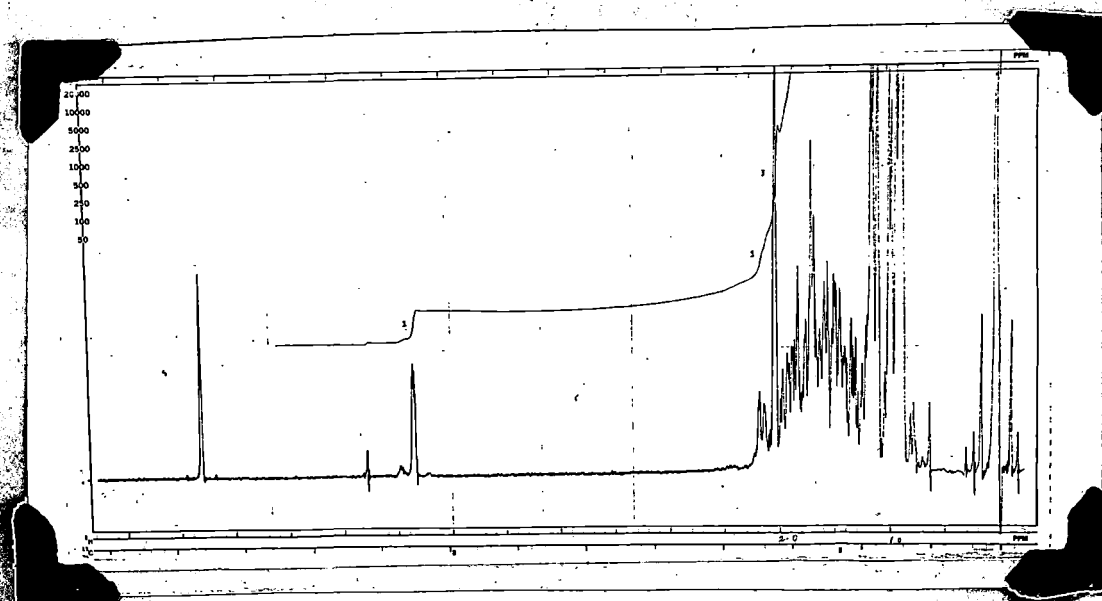


**Fig. 23A :** NMR spectrum of the Ketone 32 ( 300 MHz )

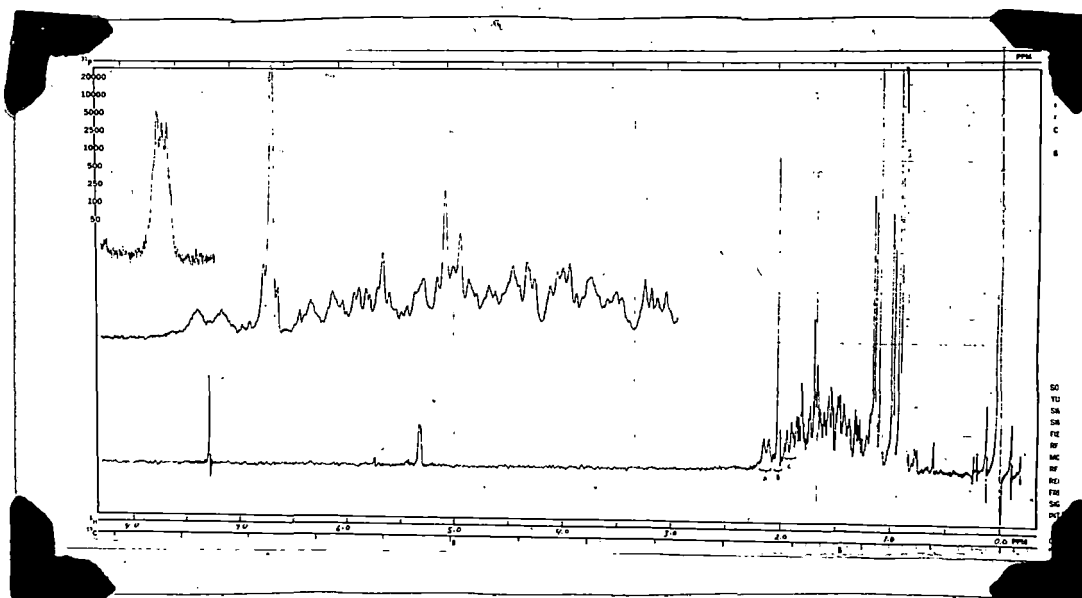


**Fig. 23B :** NMR spectrum of the Ketone 32 (300 MHz)

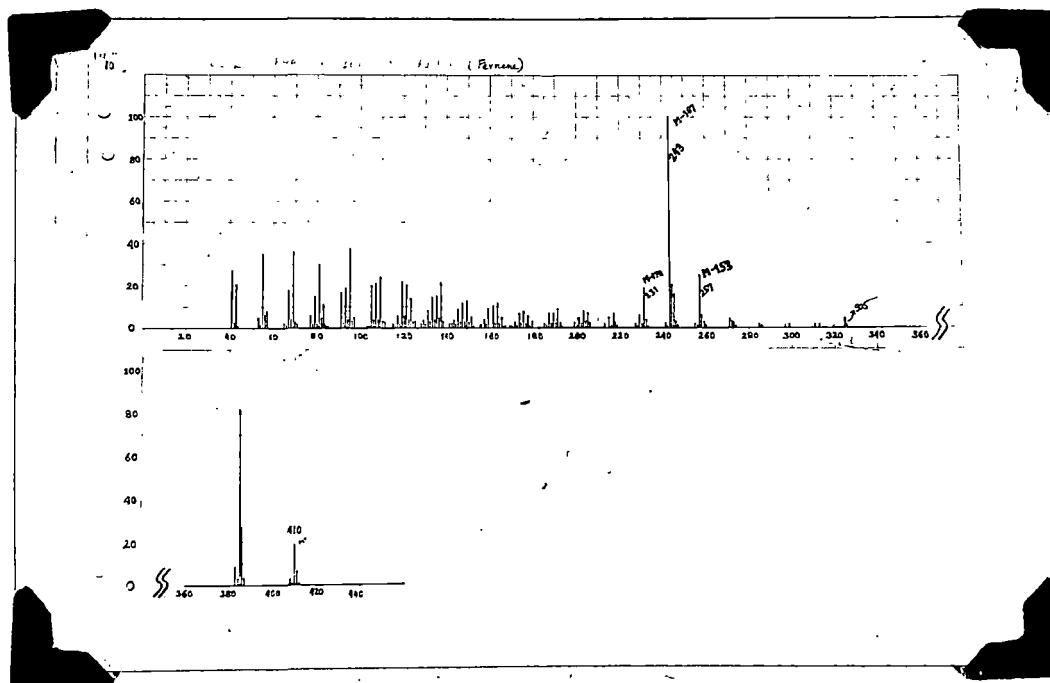


**Fig. 24 :** Mass spectrum of fern-9(11)-ene 33



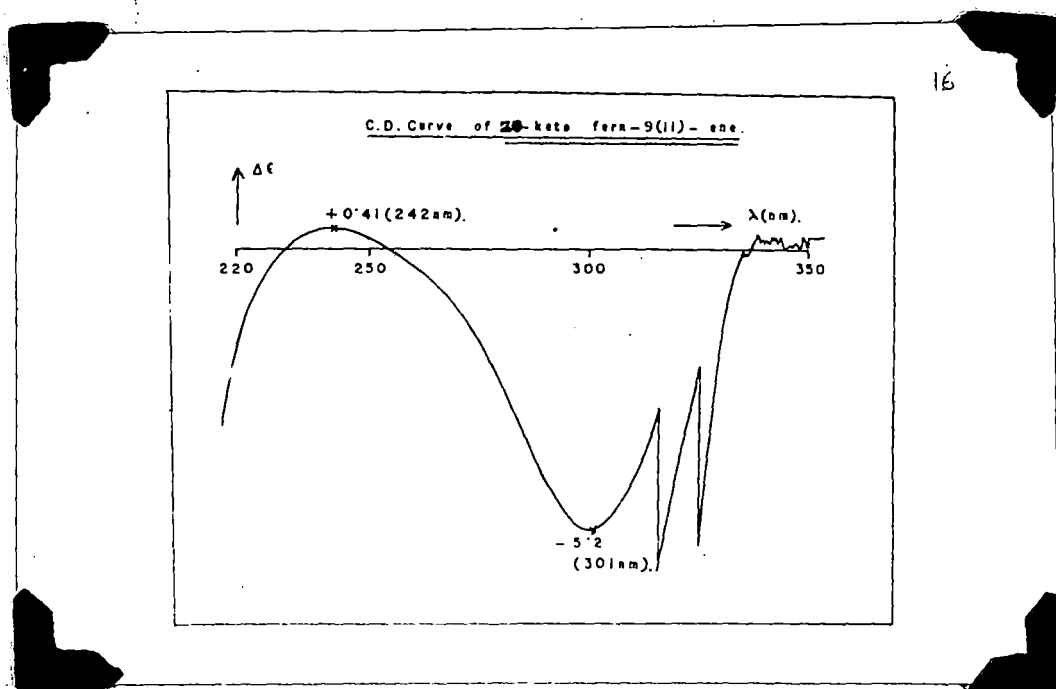


Fig. 25 : C. D. of the Ketone 32

EXPERIMENTAL

Rechromatography of fraction no. 2 (Chapter-III, Page - 187,

Table - I) :

Rechromatography of fraction no. 2 has been described (Chapter - IV, Page -204, Table - II). Fractions 15-17 (Chapter- IV, Page -204, Table - II) were combined and on crystallisation from a mixture of chloroform and methanol furnished fine colourless crystals of 30, m.p. 165-66°, ( $\alpha$ )<sub>D</sub> 28.57°.

Found :

C, 84.47; H = 11.80%

Calculated for C<sub>30</sub>H<sub>50</sub>O : C, 84.44; H = 11.81%

IR : 3590, 3510 cm<sup>-1</sup> Fig. - 16

Mass spectrum : M<sup>+</sup> 426

NMR spectrum (60 Me) :  $\delta$  0.8-1.28 (8 methyl groups)

$\delta$  5.40 (multiplet, 1H, trisubstituted double bond)

$\delta$  4.36 (multiplet,  $\text{-CH-CH(OH)-CH}_2$ )

Fig. - 17

Preparation of the acetate 31 of Polypodinol B 30 :

The compound 30 (200 mg) was dissolved in pyridine (2ml) and acetylated with acetic anhydride (2ml) by heating on a water bath for 3-hours. After working up in the usual manner

it gave a solid residue (185 mg). The residue dissolved in benzene (3 ml) was placed over a column of alunina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XIII).

Table - XIII

---

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation.     |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1-3                     | Solid (170 mg)<br>m.p. 204-6°. |

---

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

---

Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless crystals of 31, m.p. 207-8°, ( $\alpha$ )<sub>D</sub> 38.00°.

Found : C, 82.11; H, 11.07%

Calculated for  $C_{32}H_{52}O_2$  : C, 81.99; H, 11.18%

IR : 1720, 1240  $cm^{-1}$  Fig - 19

Mass spectrum :  $M^+$  468 Fig - 18

NMR (60 Mc):  $\delta$  0.8 - 1.16 (8 methyl groups)

$\delta$  2.08 (3H,  $-O-CO-CH_3$ )

$\delta$  5.16 [multiplet,  $-CH-CH(OAc)-CH_2-$ ]

$\delta$  5.45 (multiplet, 1H, vinyl proton)

Fig - 20

Preparation of the ketone 32 of Polypodinol B 30 with chromium trioxide - pyridine complex :

A solution of the Polypodinol B 30 (200 mg) in pyridine (2 ml) was added to chromium trioxide - pyridine complex prepared from pyridine (2 ml) and chromium trioxide (200 mg) and the mixture was kept at room temperature for 12 hours. The product (170 mg) obtained after working up in the usual manner was dissolved in benzene (3 ml) and placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XIV).

Table - XIV

Table - XIV

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation       |
|-----------|-------------------------|---------------------------------|
| Petroleum | 1 - 3                   | Solid (130 mg)<br>m.p. 171-73°. |

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

Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless crystals of 32, m.p. 174-75°.

Found ; C, 84.81; H, 11.42%

Calculated for  $C_{30}H_{48}O$  : C, 84.84; H, 11.39%

IR :  $1725\text{ cm}^{-1}$  (five membered ring ketone) Fig -22

Mass spectrum :  $M^+$  424 Fig -21

NMR spectrum :  $\delta$  0.8 - 1.04 (8 methyl groups)

(300 MHz)

$\delta$  5.38 (1H, vinyl proton)

Figs-23A and 23B

Wolff - Kishner reduction<sup>37</sup> of the ketone 32: Preparation of  
fern -9(11)-ene 33:

The ketone 32 (200 mg) in diethylene glycol (30 ml) was refluxed with hydrazine hydrate (2.3 ml) for 30 minutes. After addition of KOH (200 mg) the mixture was further refluxed for one hour. The condenser was removed and the mixture was heated to 190°. After refluxing for another 2½ hours the reaction mixture was cooled, diluted with water when a solid separated out. The solid (180 mg) dissolved in petroleum was placed over a column of active alumina (15 gm) developed with petroleum and eluted with the following solvents (Table - XV).

Table - XV

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation       |
|-----------|-------------------------|---------------------------------|
| Petroleum | 1-3                     | Solid (145 mg)<br>m.p. 164-68°. |

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

Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless crystals of 33 which was found to be identical with fem-9(11)-ene (no m.m.p. depression, superimposable IR and identical mass fragmentation pattern, Fig-24) ~~Fig-25~~ supplied by Prof. Berti.

Found : C, 87.68; H, 12.31%

Calculated for  $C_{30}H_{50}$  : C, 87.73; H, 12.27%

Mass spectrum :  $M^+$  410 Fig - 24

Attempted acid-isomerisation of the ketone 32 :

To the ketone 32 (25 mg) in glacial acetic acid (3 ml) was added 6% hydrochloric acid (0.5 ml) and heated on reflux for 5-hours. The reaction mixture was diluted with water and after usual working up gave a crystalline solid (17 mg) which on crystallisation from a mixture of chloroform yielded needle-shaped crystals, m.p. 174-75°. This compound was identical with the starting ketone 32 (m.m.p. and IR)

Attempted selenium dioxide oxidation of the acetate 31 :

To the acetate 31 (200 mg) in glacial acetic acid (50 ml) was added  $SeO_2$  (200 mg) in 96% acetic acid (5.2 ml) and heated under reflux for 24 hours. The reaction mixture was filtered and the filtrate diluted with water. The aqueous solution was extracted

with ether, washed with sodium bicarbonate and then with water till neutral. Evaporation of the solvent gave a solid residue (175 mg). This compound on crystallisation from a mixture of chloroform and methan<sup>ol</sup><sub>A</sub> gave crystals, m.p. 207-8° which was found to be identical with the starting compound (m.m.p. and IR)



CHAPTER - VI

Detection and structure determination of a new triterpene -  
a cyclolaudenol homologue,  $C_{32}H_{54}O$  by mass - spectrometry.

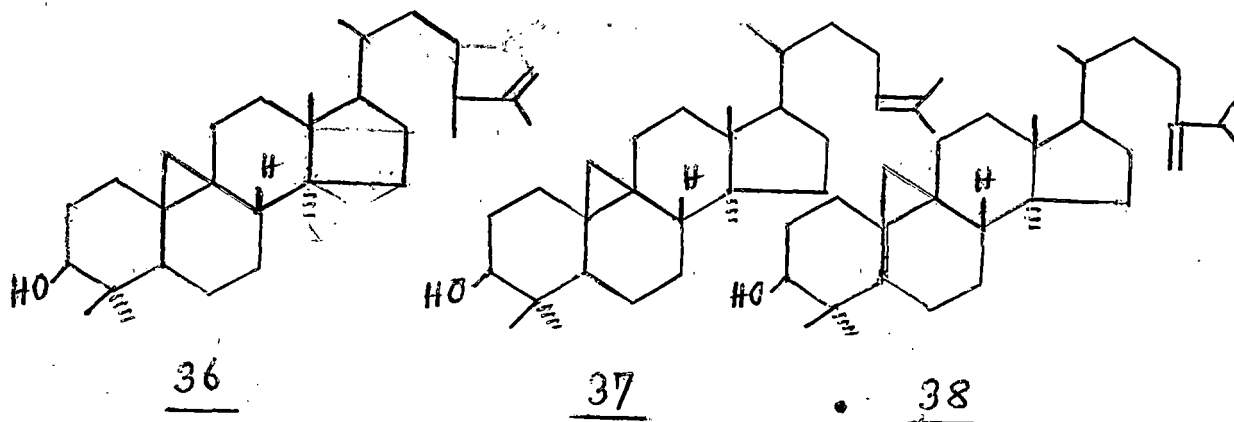
Fraction No. 2 (Chapter - III, Page -187, Table I) was rechromatographed and the separation of Polypodinol A, Polypodinol B, a compound m.p.  $148-50^{\circ}$  has been described in Chapter - IV, Page -204, Table - II. The next fraction from the petroleum - benzene (3:2) eluate was found to be a ~~poly~~mixture of cyclolaudenol and cyclolaudenol homologue (A). Isolation of ~~trixnonol~~ cyclolaudenol and its homologue has also been described in Chapter - IV (Page -204, Table -II, fractions 18-20).

(IR: Fig-26)

The above mixture (A) was acetylated with acetic anhydride and pyridine when an acetate (B), m.p.  $107-8^{\circ}$ ,  $(\alpha)_D$   $55.17^{\circ}$  was obtained. Hydrogenation of the original mixture in presence of Adam's catalyst in alcohol gave a compound (C), m.p.  $132-33^{\circ}$ , which on acetylation gave an acetate (D), m.p.  $127-28^{\circ}$ . NMR spectrum of the original mixture (A, Fig - 27) and its acetate (B, Fig - 28) both showed distinct peaks at  $\delta 4.65$  ( $=CH_2$ ) and  $\delta 4.65$  (3H,  $=CH_2$  and  $-CH-OAc$ ) respectively but in the spectrum of the hydrogenated product (C, Fig-29) the peak due to vinylidene group was no longer present indicating thereby that both the

compounds in the mixture probably contained a  $=CH_2$  group. Oxidation of the original mixture (A) with Jones's reagent gave a mixture of ketones (E), m.p.  $117-18^\circ$ . We have measured the mass spectra of the original mixture (A, Fig-30) its acetate (B, Fig-31), the dihydrocompound (C, Fig-32) and its acetate (D, Fig-33) and it was observed that along with cyclolaudenol  $M^+ 440$ , a second component  $M^+ 454$ , that is a new C-32 triterpene alcohol,  $C_{32}H_{54}O$ , was present in the original mixture.

The mass spectrum of this new compound was found to correspond very closely to that of cyclolaudenol 36. The

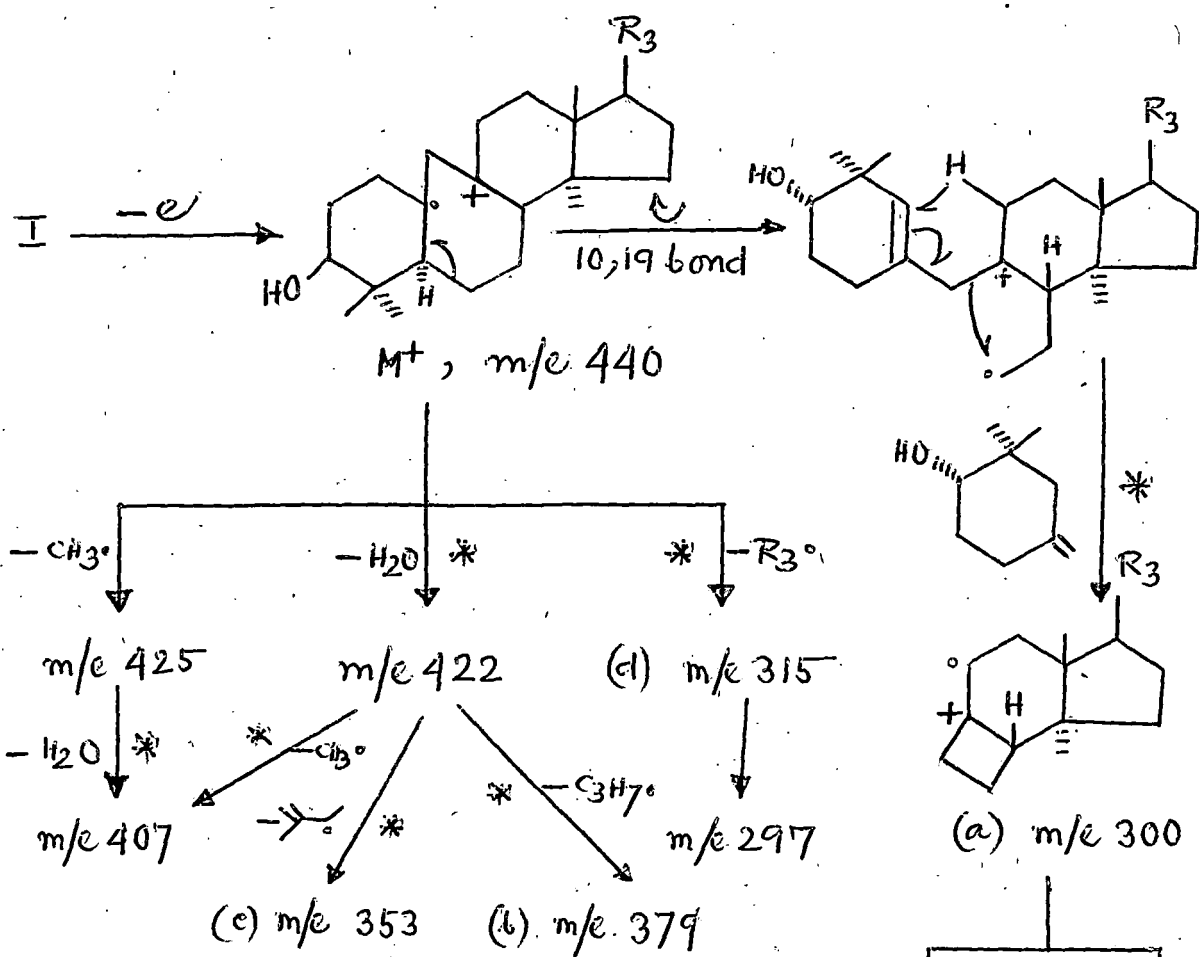
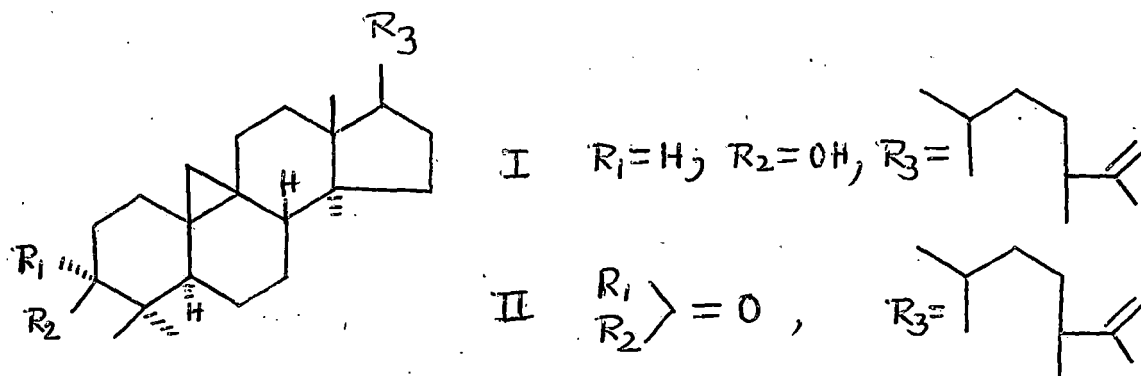


mass spectra of 9,19-cycloartenol 37 and methylene - 24 -

cycloartenol 38 derivatives has been discussed by Benveniste, Hirth and Ourisson<sup>39</sup> who have mentioned that the compounds showed strong peaks at  $m/e$  286 and  $m/e$  300 respectively corresponding to loss of ring A. Audier, Bengelmans and Das<sup>40</sup> have also discussed the mass spectra of some 9,19 - cyclolanostane derivatives and indicated four types of fragmentation peaks. Aplin and coworker<sup>41</sup> have also described in detail about application of mass spectrometry to the structural investigation of 9, 19 - cyclosterols and triterpenes and have recorded detailed mass spectral fragmentation of various triterpenes containing 9,19 - cyclofunction including cyclolaudenol. It will be evident from the discussions which follow that our data are in agreement with the earlier findings<sup>39-41</sup>.

The unusual feature of the spectrum of cyclolaudenol I<sup>41</sup> (Chart - IV) is the intense fragment (a) at  $m/e$  300 and have the composition  $C_{22}H_{36}$  and corresponds to loss of ring A plus one hydrogen atom, since it remains at  $m/e$  300 in the spectrum of the corresponding ketone II. This is formed from the molecular ion  $m/e$  440 by a one step process. The other fragments, besides the loss of a methyl group ( $m/e$  425), water ( $m/e$  422) and methyl-plus-water ( $m/e$  407), arise from M-18 fragment and involve cleavage of the side chain - fragment (b)  $m/e$  379 (M-18- $C_3H_7$ .) and (c)  $m/e$  353 (M-18- $C_5H_9$ ). A further fragment (e)  $m/e$  175, common to all spectra,

CHART - IV



\* Metastable peak observed

corresponds to the loss of both ring A and the side chain. Another significant peak (d) at  $m/e$  315 ( $M-R_3\cdot$ ) is common to all its side chain derivatives which arises by elimination of  $R_3$  group from M. The most plausible mechanism<sup>42</sup> for the formation of these fragments is depicted in Chart - IV where the initial ionisation of 9-10 bond relieves the strain imposed on ring B, fission of the activated 5-6 bond followed by transfer of one of the  $C_{11}$  hydrogen via a "Mc Lafferty" type of rearrangement.

Aplin et al<sup>41</sup> have also stated in their paper that in the spectra of the corresponding ketone II the intensity of the fragment (a)  $m/e$  300 is greatly reduced, presumably owing to competitive ionisation at the carbonyl group. However, fragments corresponding to the well established preferential fragmentation in the vicinity of the carbonyl groups<sup>43a</sup> were not apparent.

Discussions on the mass spectrum of the original mixture  
(Chart - V) :

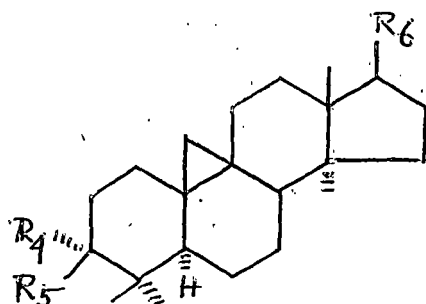
The mass spectrum of the original mixture (III A + III B ; Chart - V) was measured and it has been observed that it contained all the peaks characteristic of cyclolaudenol and a cyclolaudenol homologue,  $C_{32}H_{54}O$  having the molecular ion peak at  $M^+$  454. It will be observed from Chart - ~~V~~ that in addition to the peak at  $m/e$  300 corresponding to fragment (a)

in Chart -IV for cyclolaudenol there is an intense peak at ~~m/e~~ m/e 314 (III B, fragment a') indicating the presence of an extra carbon atom in the side chain of the new triterpene. Moreover, the prominent peak (d) at m/e 315 for fragment M-R<sub>6</sub> also indicated the presence of an extra carbon atom in the side chain. A further single peak at m/e 175 due to fragment(e) is also indicative of the presence of the extra carbon atom in the side chain of the cyclolaudenol nucleus. The other major fragment (b), besides the loss of methyl radical at m/e 439 (III B) water at M/e 436 (III B) and methyl-plus-water at m/e 421 (III B) is from the M-18 fragment (m/e 436) and appears at m/e 393 (III B) and involve cleavage of the side chain (M-18-C<sub>3</sub>H<sub>7</sub>.) The appearance of all these mass peaks can be explained by the same mechanism as that for cyclolaudenol as is given in Chart - IV. We, therefore, conclude from the above studies that the mixture contains a new triterpene having the same cyclolaudenol nucleus but having a extra -CH<sub>2</sub>- in the cyclolaudenol side chain.

Similarly, the mass fragmentation of the acetate ( IV A + IV B ; Chart - V) also leads us to the same conclusion that the new cyclolaudenol homologue contains an extra - CH<sub>2</sub>- in the side chain.

Examination of the mass spectra of the dihydroalcohol mixture ( V A + V B ; Chart - VI ) also indicates the presence

CHART - V



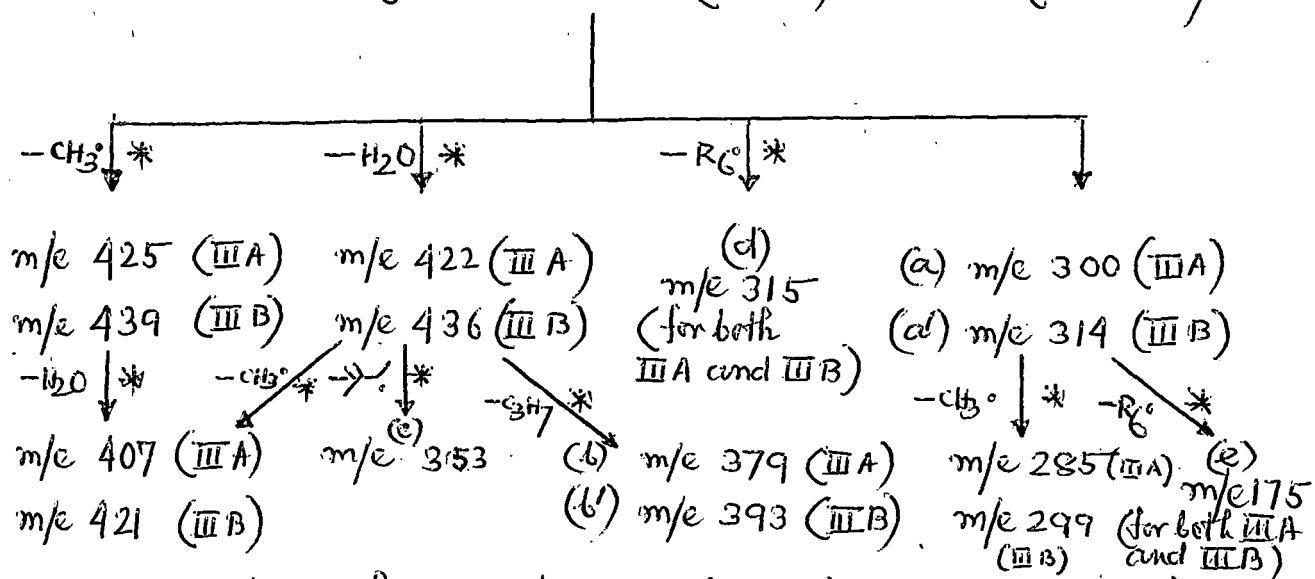
III A  $R_4=H, R_5=OH, R_6=C_9H_{17}$

III B  $R_4=H, R_5=OH, R_6=C_{10}H_{19}$

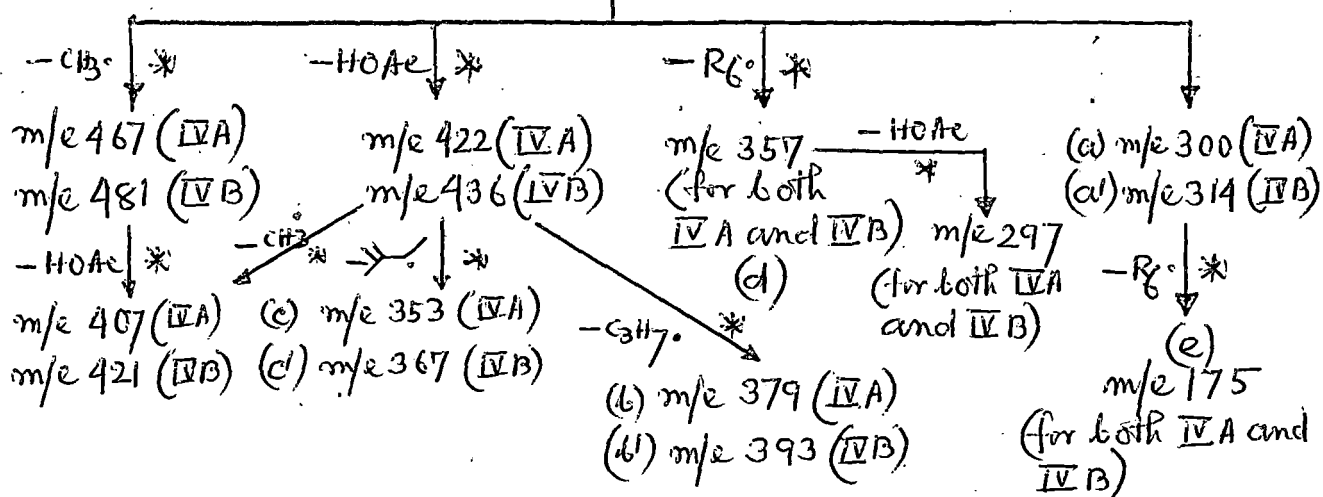
IV A  $R_4=H, R_5=OAc, R_6=C_9H_{17}$

IV B  $R_4=H, R_5=OAc, R_6=C_{10}H_{19}$

Mixture of alcohols III A ( $M^+ 440$ ) and III B ( $M^+ 454$ )



Mixture of acetates IV A ( $M^+ 482$ ) and IV B ( $M^+ 496$ )



of the extra carbon atom in the side chain cyclolaudenol nucleus. The presence of the fragment (a') at m/e 316 (VB) and the fragment (d) (M-R<sub>9</sub>) at m/e 315 (single peak) of VB again established beyond doubt that the extra carbon atom is present in the side chain R<sub>9</sub> of dihydrocyclo-laudenol homologue. The other fragments depicted in the Chart - VI also bear strong evidence for the presence of a extra - CH<sub>2</sub> - in the side chain of the dihydrocyclo-laudenol nucleus.

Similar examination on mass fragmentation pattern of the dihydroacetate mixture (VI A + VI B; Chart - VI) also corroborates the presence of the extra - CH<sub>2</sub> - in the side chain of cyclo-laudenol nucleus.

The position of the double bond (= CH<sub>2</sub>, NMR : Fig-27) in the side chain at 25(26) position may be tentatively assigned from the mass spectra of (III A + III B), (IV A + IV B), (VA + VB) and (VI A + VIB) as the M-84 fragment observed in the "McLafferty" rearrangement involving <sup>176, 436</sup>24-28 double bond and the C-20 hydrogen has been found to be absent in their spectra.

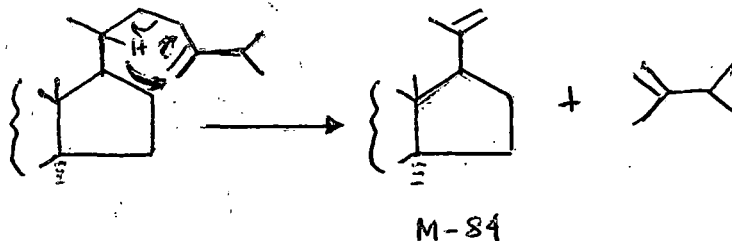
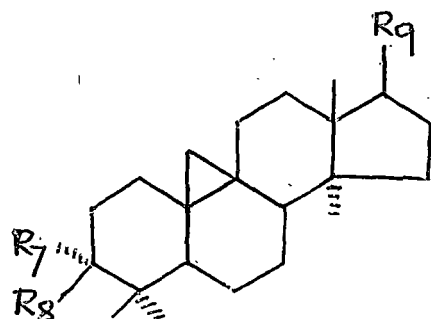




CHART - VI



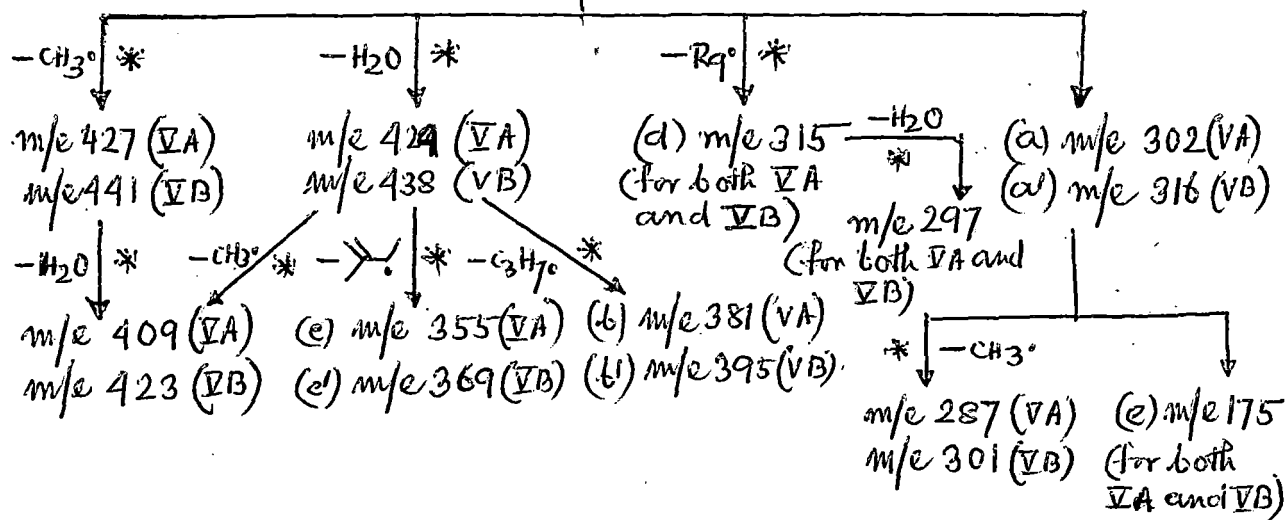
V A  $R_7=H, R_8=OH, R_9=C_9H_{19}$

V B  $R_7=H, R_8=OH, R_9=C_{10}H_{21}$

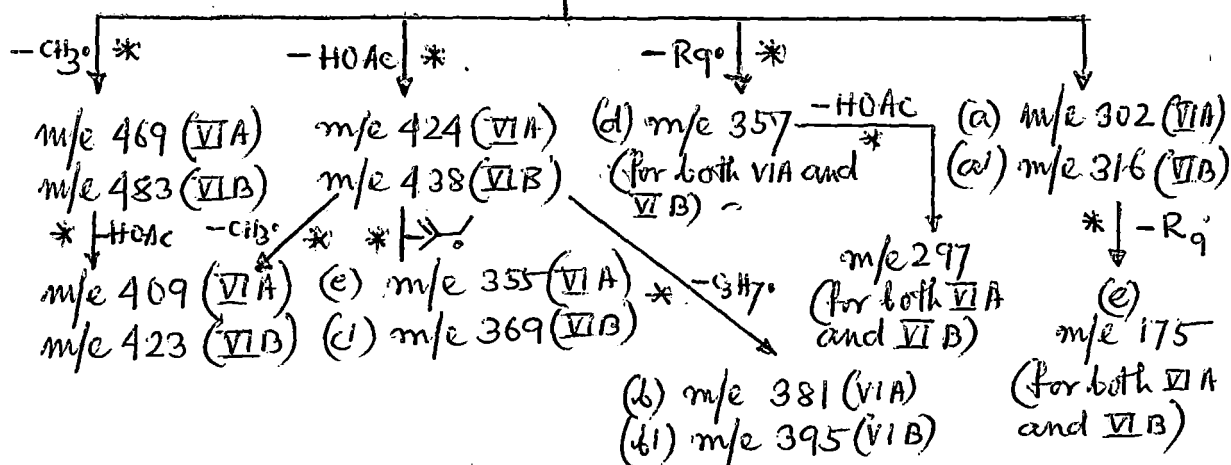
VIA  $R_7=H, R_8=OAc, R_9=C_9H_{19}$

VIB  $R_7=H, R_8=OAc, R_9=C_{10}H_{21}$

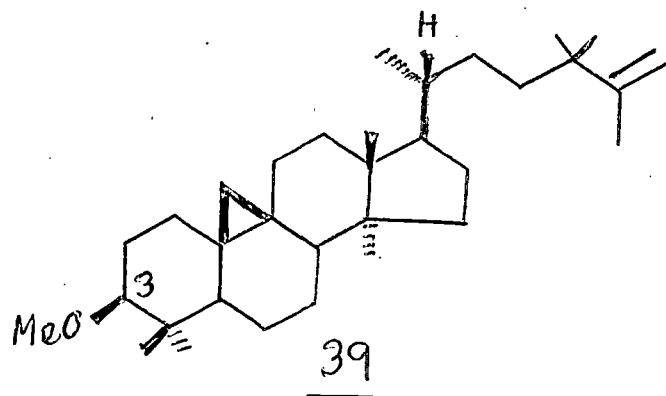
Mixture of Dihydroalcohols V A ( $M^+ 442$ ) and V B ( $M^+ 456$ )



Mixture of the corresponding acetates V I A ( $M^+ 484$ ) and V I B ( $M^+ 498$ )



From the foregoing evidence, it may be reasonably concluded that the new triterpene is a cyclolaudenol derivative with a extra carbon ( $-\text{CH}_2-$ ) in the side chain and a probable structure can be arrived at by merely placing the extra carbon atom in the side chain of cyclolaudenol. In the known triterpenes any additional carbon atom or atoms are always attached to C-24 as either methyl, methylene, ethyl or ethylidene groups<sup>44</sup>. Recently, Ritchie and coworkers<sup>45</sup> have isolated a new triterpene,  $\text{C}_{33}\text{H}_{56}\text{O}$ , cycloneolitsin from Neolitsea dealbata R.Br. Merr. for which a 24,24 - dimethyl structure 39 has been assigned on the basis of chemical and physical evidence. This is, also the first example of 24,24 - dimethylation on the



side chain of known triterpenes. Accordingly, two probable structures 40 and 41 can be assigned to the new cyclolaudenol derivative.

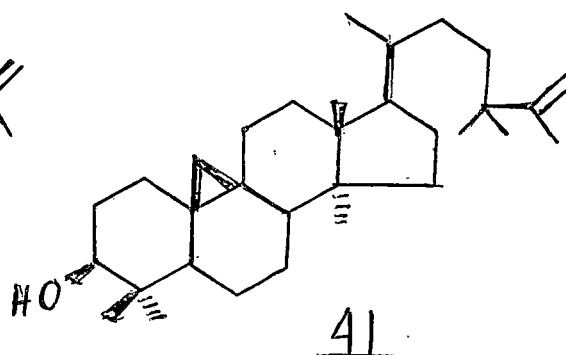
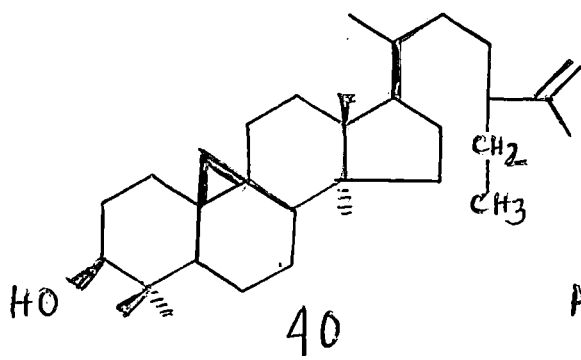
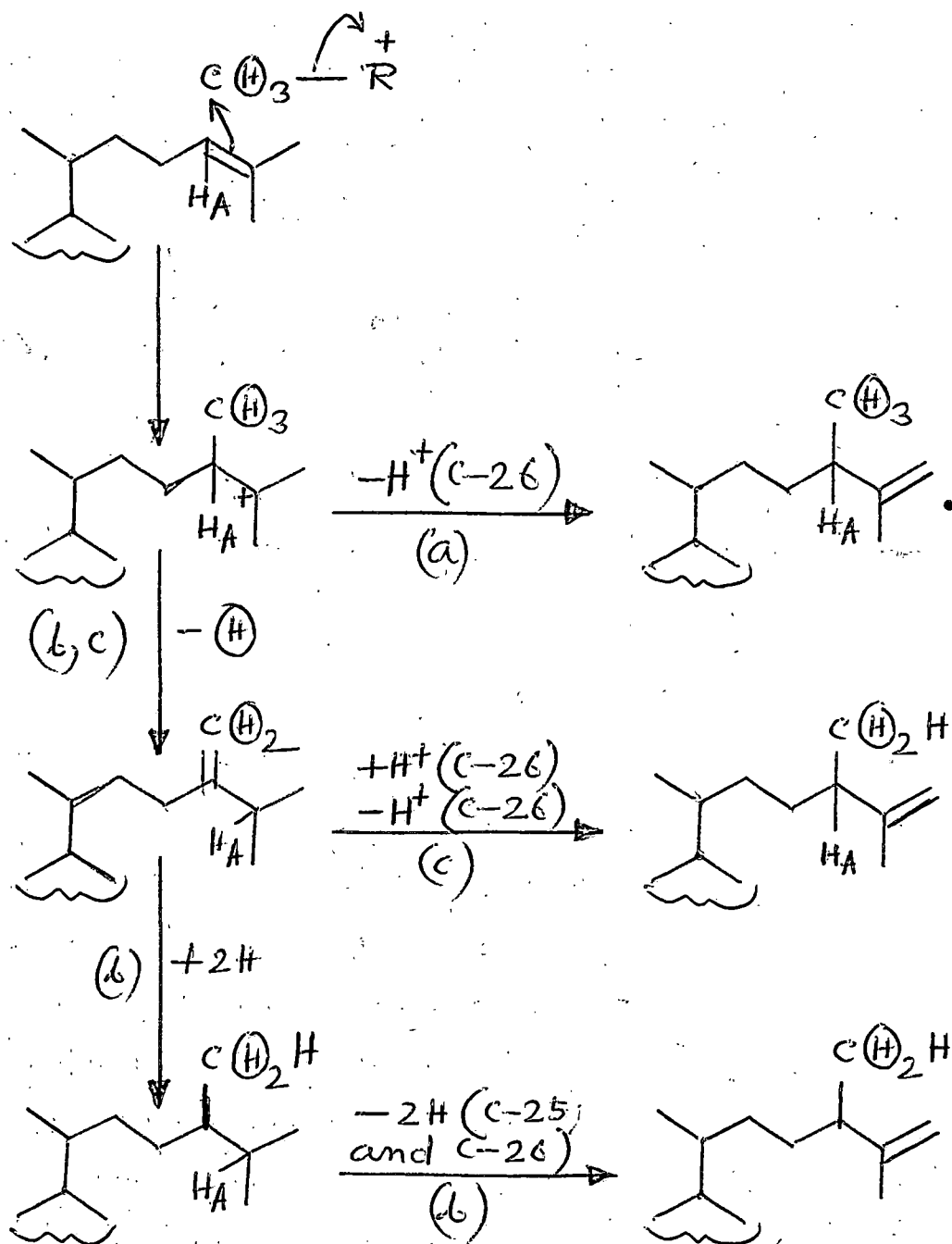


Chart - VII



Possible mechanism of methylation to produce cyclolaudenol. The circled H is derived from the methyl group of methionine, and  $\text{H}_A$  arises from the 4-pro-R hydrogen of mevalonic acid.

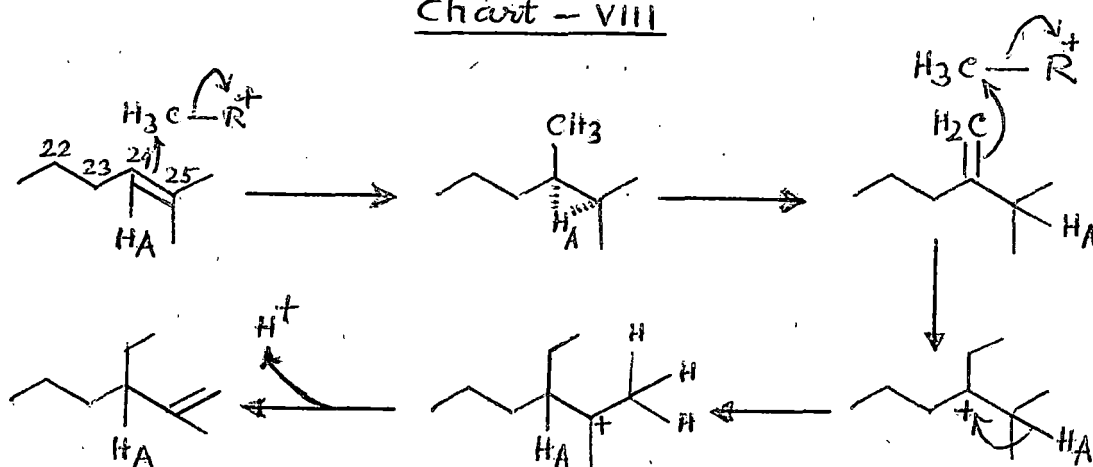
The outlines of the biosynthetic pathways involved in the formation of C-24 alkylated derivatives of tetracyclic triterpenes are now established<sup>45</sup>. The alkylation mechanism linked with the formation of  $\Delta^{25-26}$  bond, as in the formation of cyclolaudenol I and 24S-24-ethylcholesta- 5,22,25 - trien-3 $\beta$ -ol is now well known. Three possibilities exist for the methylation and  $\Delta^{25-26}$  double bond formation in cyclolaudenol (Chart - VII). Route (a) involves elimination of a proton from C-26 from the intermediate cation indicated, which would give the cyclolaudenol side chain directly with retention of all three hydrogens of the C-24 methyl group and hydrogen ( $H_A$ ) of a precursor (e.g. cycloartenol) remaining at C-24. Routes (b) and (c) both involve a C-24 methylene intermediate and thus the retention of only two of the hydrogens of the incoming methyl group; however, route (c) would result in retention of  $H_A$  at C-24 and route (b) would result in its elimination. Experiments with  $[2-^{14}\text{C}-(4R)-4^3\text{H}_1]$  MVA showed that cyclolaudenol formed by rhizomes of *Polypodium vulgare* retained  $H_A$  at C-24 and that the methylene group at C-25 arose stereospecifically from C-2 of MVA. Thus route (c) was eliminated; evidence which favours route (a) was obtained by comparing the  $^3\text{H} : ^{14}\text{C}$  ratio in cyclolaudenol and 24 - methylene cycloartanol synthesized from  $[^{14}\text{C} : ^3\text{H}_3]$  methionine by *P. Vulgare* in the

same experiment. The ratio in cyclolaudenol (17.4:1) was very close to that of starting methionine (16.5:1) whereas in 24-methylene cycloartanol was significantly lower<sup>47</sup> (14.1 :1). However, this result needs confirmation by use of  $[C^2H_3]$  methionine, in order to eliminate any possible isotopic effect.

Since biosynthesis of triterpenes and sterols having ethyl group at C-24 is known and has been established, we prefer, tentatively for "biogenetic prejudice" the structure 40 for the new triterpene - cyclolaudenol homologue,  $C_{32}H_{54}O$ . The mechanism for its biosynthesis in *Polypodium juglandifolium* is indicated in Chart - VIII and is probably operating in producing a simultaneous alkylation and desaturation at C-24<sup>48</sup>.

However, the final structure determination of this new triterpene must await its isolation in pure form from its mixture with cyclolaudenol. Further work in this direction is in progress in our laboratories.

Chart - VIII



Mechanism of alkylation to produce 24-ethyl-cyclolaudenol

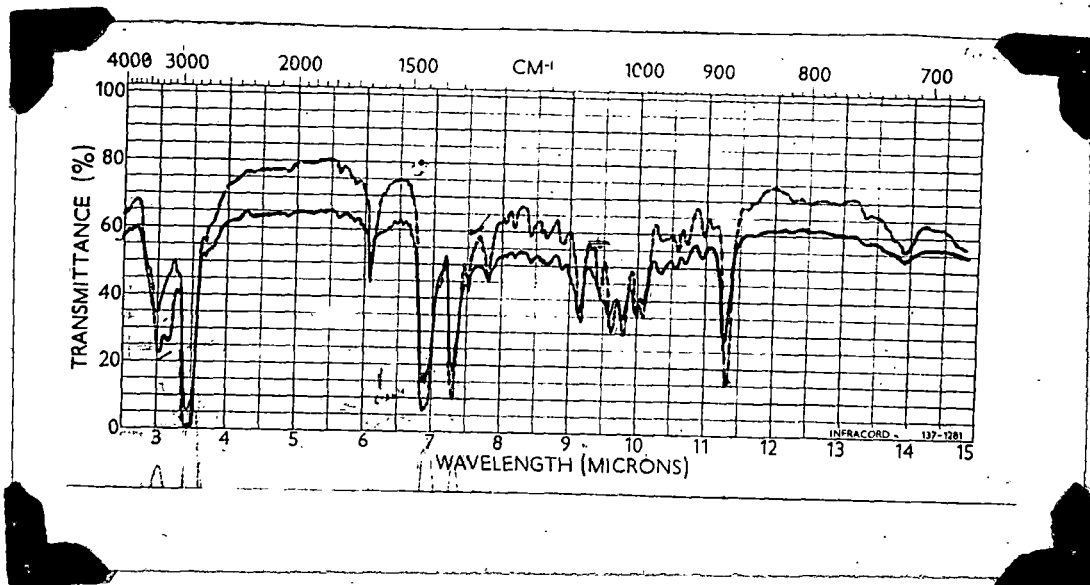


**Fig. 26 :** IR spectrum of the cyclolaudenol-cyclodaudenol homologue mixture A (solid line) and authentic cyclolaudenol (Dotted line)

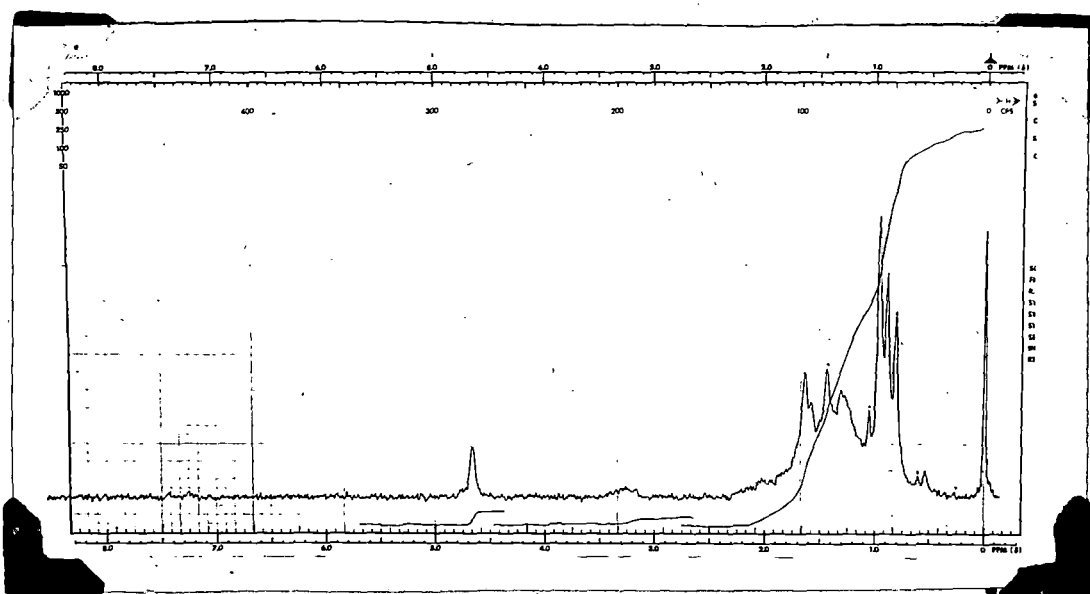


**Fig. 27 :** NMR spectrum of the mixture of cyclolaudenol and its homologue A

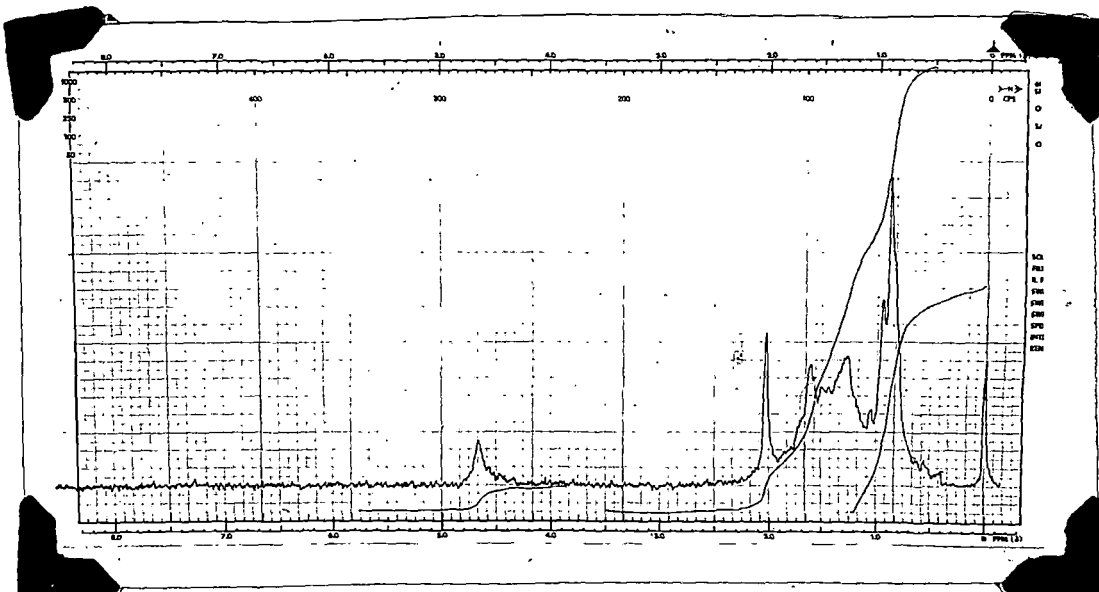


**Fig. 28 :** NMR spectrum of the mixture of acetates of cyclolaudenol and its homologue B

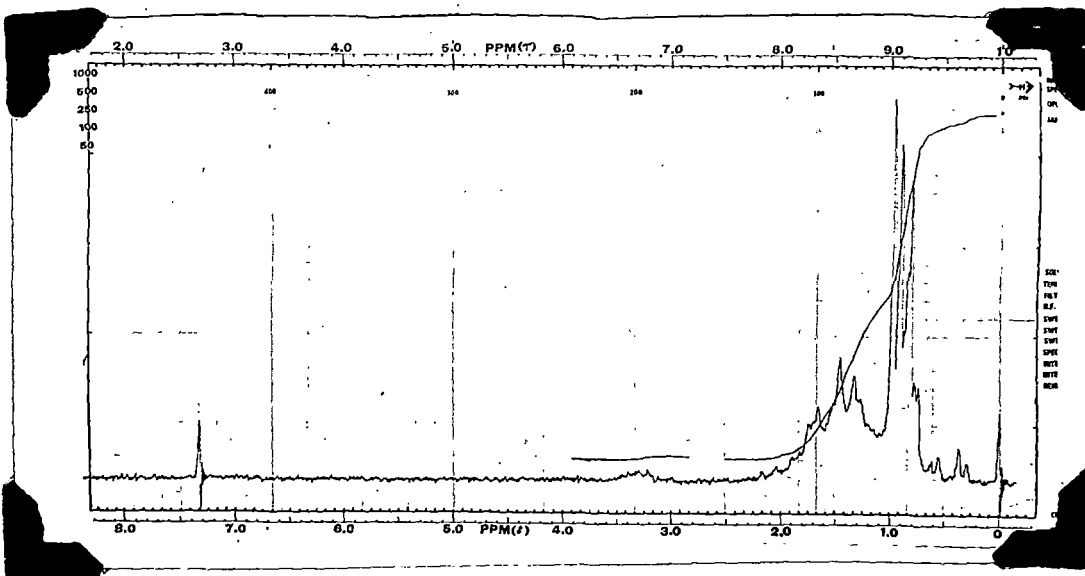


**Fig. 29 :** NMR spectrum of the mixture of dihydro-derivatives of cyclolaudenol and its homologue C

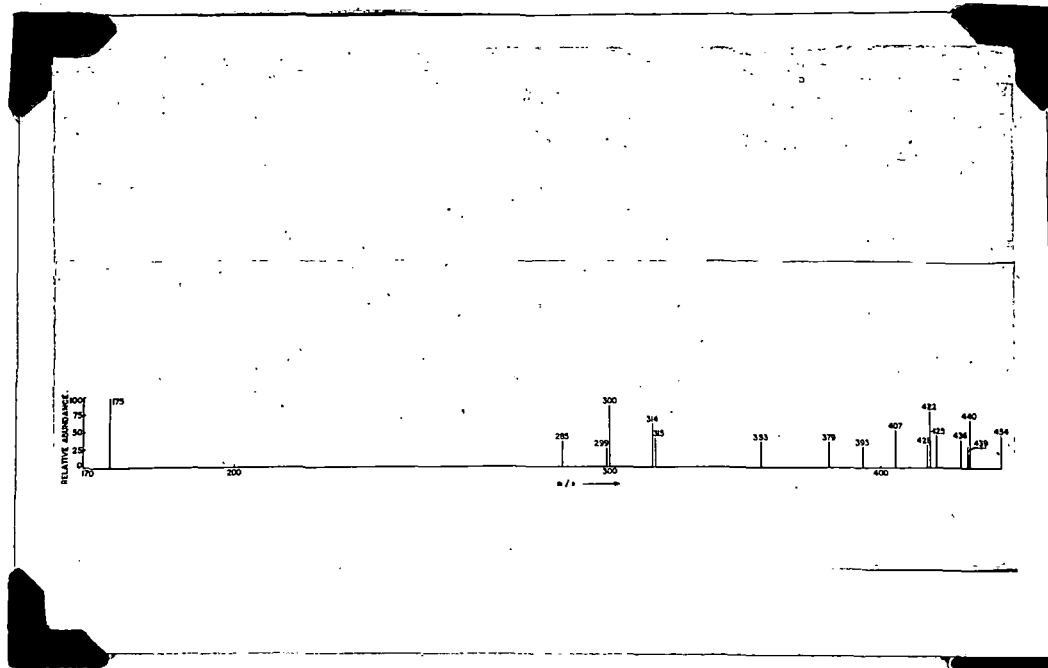


**Fig. 30 :** Mass spectrum of the mixture of cyclolaudenol and its homologue A

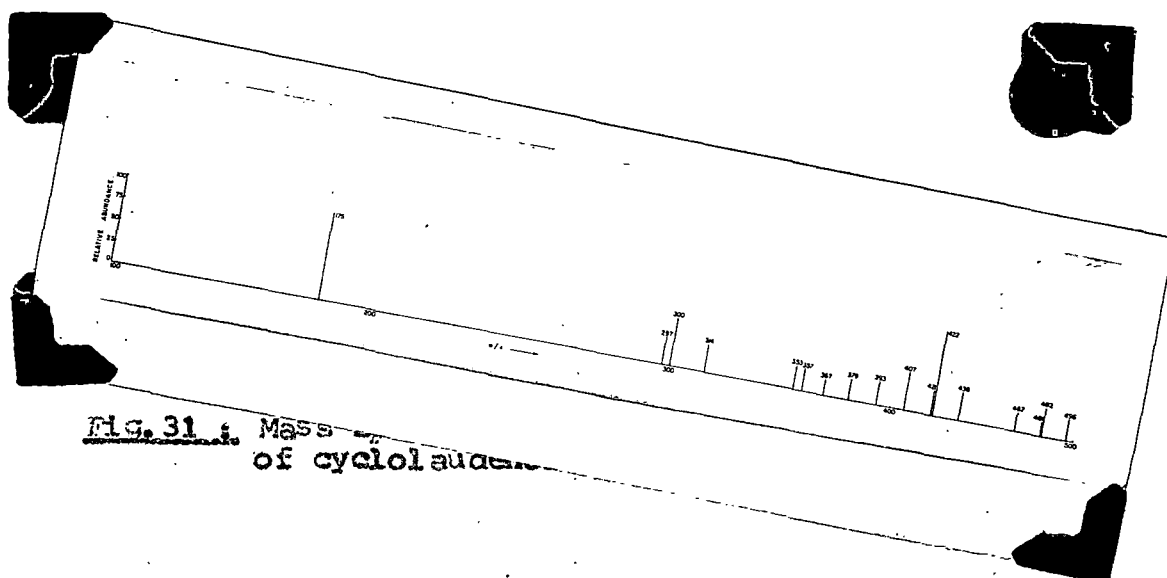


**Fig. 31 :** Mass spectrum of cyclolaudenol



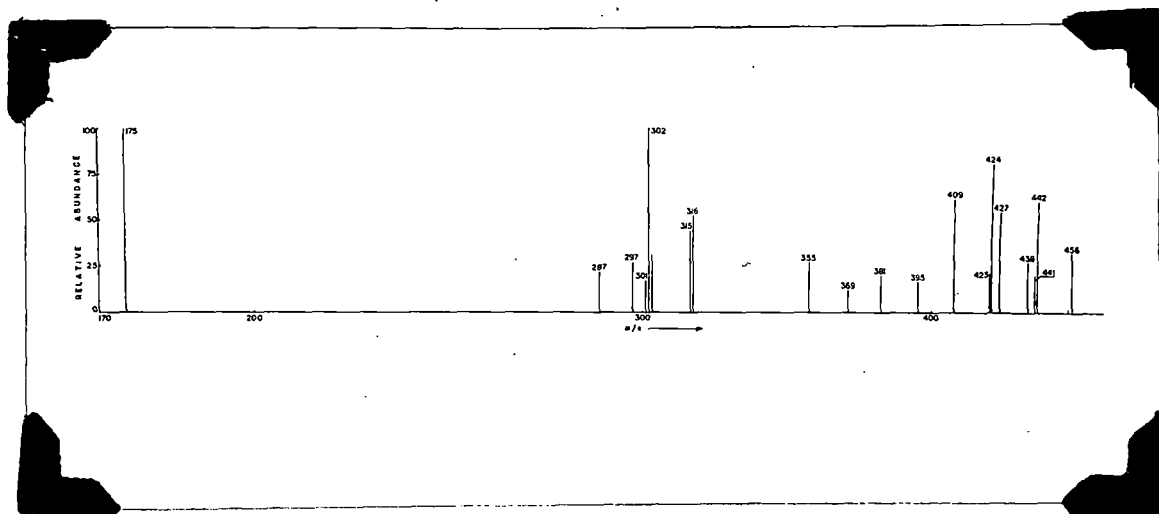


**Fig. 32 :** Mass spectrum of the mixture of dihydro-derivatives of cyclolaudenol and its homologue C

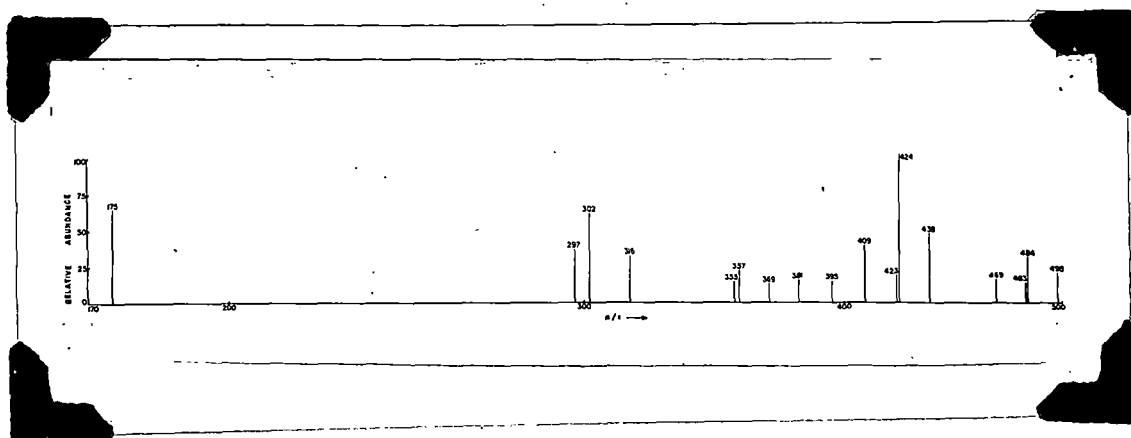


**Fig. 33 :** Mass spectrum of the mixture of acetates of dihydro-derivatives of cyclolaudenol and its homologue D

EXPERIMENTAL

Rechromatography of fraction no. 2 (Chapter - III, Page -187,

Table - I) :

Rechromatography of fraction no. 2 (Chapter - III, Page -187, Table - I) has been described (Chapter - IV, Page -204, Table - II). Fractions 18-20 were combined and on crystallisation from methanol gave a solid (A), m.p. 121-22°. This product (A) was found to be a mixture of two compounds (Two spots in TLC and mass fragmentation pattern)

IR :  $3340\text{ cm}^{-1}$  (OH) Fig - 26

Mass spectrum :  $M^+$  440 and  $M^+$  454 Fig - 30

NMR spectrum (60 Mc) :  $\delta$  4.65 ( $=\text{CH}_2$ ) Fig - 27

Acetylation of the above mixture (A) : Preparation of the mixture of acetates (B) :

The above alcohol mixture (A, 200 mg) was dissolved in pyridine (2 ml) and acetylated with acetic anhydride (2 ml) by heating on a water bath for 5-hours. After working up in the usual manner it gave a solid residue (185 mg). The residue dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XVI).

Table - XVI

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 3                   | Solid, m.p. 105-7°<br>(170 mg) |

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

Fractions 1-3 were combined and on crystallisation from a mixture of methanol and acetone furnished crystals (B), m.p. 107-8°, ( $\alpha$ )<sub>D</sub> 55.17° which was found to be a mixture of two compounds (TLC and mass fragmentation pattern)

NMR spectrum (60 Mc) :  $\delta$  4.65 (= CH<sub>2</sub>)

Fig-28

Mass spectrum : M<sup>+</sup> 482 and M<sup>+</sup> 496

Fig-31

Hydrogenation of the original mixture (A) :

Preparation of the mixture of dihydrocompounds (C) :

The original mixture (A, 500 mg) dissolved in alcohol (50 ml) was shaken in an atmosphere of hydrogen in presence of PtO<sub>2</sub> catalyst (50 mg) until absorption of hydrogen ceased.

The solid obtained (450 mg) after usual working up was subjected to chromatography over alumina (30 gm, deactivated with 1.2 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table -XVII).

Table - XVII

| Eluent                       | Fractions<br>50 ml each | Residue on<br>evaporation       |
|------------------------------|-------------------------|---------------------------------|
| Petroleum                    | 1 - 3                   | Nil                             |
| Petroleum ;<br>Benzene (4:1) | 4 - 7                   | Solid (400 mg)<br>m.p. 128-30°. |

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

Fractions 4-7 were combined and on crystallisation from acetone gave (C), m.p. 132-33°. The product (C) was found to be a mixture of two compounds (TLC and mass fragmentation pattern)

NMR spectrum (60 Mc) :

Fig-29

Mass spectrum :  $M^+$  442,  $M^+$  456

Fig-32

Acetylation of the dihydro mixture (C) : Preparation of the corresponding acetate mixture (D) :

The dihydro mixture (C, 200 mg) was acetylated with pyridine (2ml) and acetic anhydride ( 2 ml ) by heating on a water bath for 5-hours. After working up in the usual manner it gave a solid residue (180 mg). This product dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XVIII).

Table - XVIII

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation       |
|-----------|-------------------------|---------------------------------|
| Petroleum | 1 - 3                   | Solid (160 mg)<br>m.p. 125-27°. |

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

Fractions 1 - 3 were combined and on crystallisation from a mixture of chloroform and methanol gave a solid (D), m.p. 127-28°. This product (D) was found to be a mixture of two compounds (TLC and mass fragmentation pattern)

Mass spectrum :  $M^+$  484 and  $M^+$  498

Fig-33

Oxidation of the original mixture (A) with Jones's reagent :

Preparation of the mixture of ketones (E) :

To a solution of the original mixture (A, 200 mg) in pure acetone (100 ml) was added Jones's reagent dropwise until a faint orange colour persisted. The mixture was kept at room temperature for 10 minutes, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried ( $Na_2SO_4$ ) and the ether evaporated. The residue (180 mg) dissolved in benzene (3 ml) was chromatographed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table - XIX).

Table - XIX

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation       |
|-----------|-------------------------|---------------------------------|
| Petroleum | 1 - 3                   | Solid (160 mg)<br>m.p. 115-18°. |

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

Fractions 1-3 were combined and on crystallisation from a mixture of chloroform and methanol furnished shining crystals (E), m.p. 117-18°. This compound again, was found to be a mixture of two compounds (TLC).

-2501-

CHAPTER - VII

Some new observations in the (i)  $\text{CrO}_3$ -Pyridine oxidation of nerifoliol 19, and (ii) in the attempted preparation of the tosyl derivative of nerifoliol:

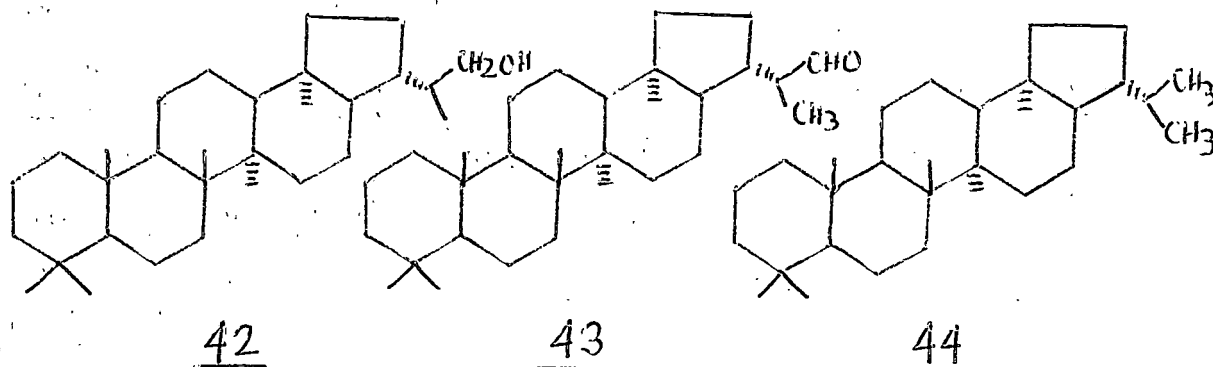
Section A :  $\text{CrO}_3$  - Pyridine oxidation of nerifoliol :

Mitra et al<sup>26</sup> converted nerifoliol 19 to the corresponding aldehyde nerifolial by sarret oxidation and recorded its m.p. as  $76^\circ$ . They reduced this aldehyde by Wolff-Kishner reduction and obtained a hydrocarbon, m.p.  $190-92^\circ$ ,<sup>( $d_4$ ) + 36°</sup> which they reported was same as hopane but did not establish its identity by comparison<sup>(m.m.p.)</sup> with an authentic sample. We, therefore, attempted to repeat their above reaction in order to obtain hopane and compare it with an authentic specimen of hopane.

Sarret oxidation of nerifoliol with hydrated  $\text{CrO}_3$  - pyridine complex was performed and the product obtained was found by TLC to consist of two compounds. Careful chromatography of this mixture over alumina followed by crystallisation from a mixture of chloroform and methanol resulted in the isolation of a compound, m.p.  $228-32^\circ$ . TLC of the compound was carried out and the Rf value (0.46; Benzene - Petrol 9:11) was found to be identical with that of an authentic sample of

isoadiantone. Mixed melting point of the compound with an authentic sample supplied by Prof. Berti also showed no depression. The residue from the mother liquor of the above compound was again chromatographed over alumina. The solid eluted from different fractions on TLC examination showed the presence of adiantone and isoadiantone having same melting point and  $R_f$  values with their respective authentic specimen. In this reaction we failed to obtain any aldehyde nerifolial, having, m.p.  $76^\circ$  described by Mitra et al<sup>26</sup>. Moffatt<sup>49</sup> oxidation on nerifolial was attempted several times but we failed to isolate the desired aldehyde, the original alcohol being recovered each time. We next tried  $\text{CrO}_3$  - Pyridine oxidation with anhydrous  $\text{CrO}_3$  - Pyridine complex according to the method described by Ratcliffe and Rodehorst<sup>50</sup>. The product from this oxidation was a single compound (TLC) which on chromatography followed by crystallisation afforded crystals m.p.  $205-6^\circ$ ,  $\mu_{\text{max}}^{\text{nujol}}$  2720,  $1730 \text{ cm}^{-1}$  (Fig - 34),  $M^+$  426 (Fig - 35). NMR spectrum of the compound (Fig - 36) showed a peak characteristic of the aldehydic proton at  $\delta 9.65$ . Elemental analysis corresponded to the formula  $\text{C}_{30}\text{H}_{50}\text{O}$ . It is significant to note here that all these data are consistent with the structure of nerifolial 43. Wolff-Kishner reduction of the aldehyde gave hopane 44 m.p.  $218-19^\circ$  identical with an authentic sample (IR - Fig - 37).

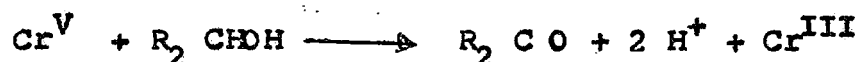
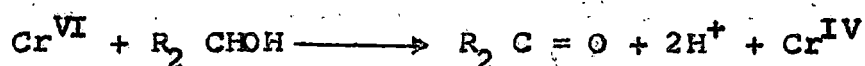




The above observations concerning the products of the chromic acid reaction under different conditions suggest that the reaction proceeds by different mechanisms. In the Sarret oxidation with the hydrated complex obviously cleavage and oxidation takes place, but in the absence of kinetic and other data it is not possible to put forward any mechanism at this stage. This reaction is, however being studied in these laboratories in more detail. The product of oxidation by anhydrous  $\text{CrO}_3$  - Pyridine - methylene chloride being an aldehyde only, it appears that, in this case normal oxidation<sup>51</sup> takes place.

Studies of the mechanisms of oxidations with chromium compounds<sup>51</sup> have been complicated by the fact that each stage in the oxidation of most organic compounds is accompanied by the net transfer of two electrons although the oxidising agents normally accept a total of three or five electrons. It is

therefore evident that intermediate valence states of chromium are important in the overall process. The problem is well illustrated by the oxidation of a secondary alcohol with a hexavalent chromium compound, one of the many possible reaction schemes for which is presented in the accompanying equations



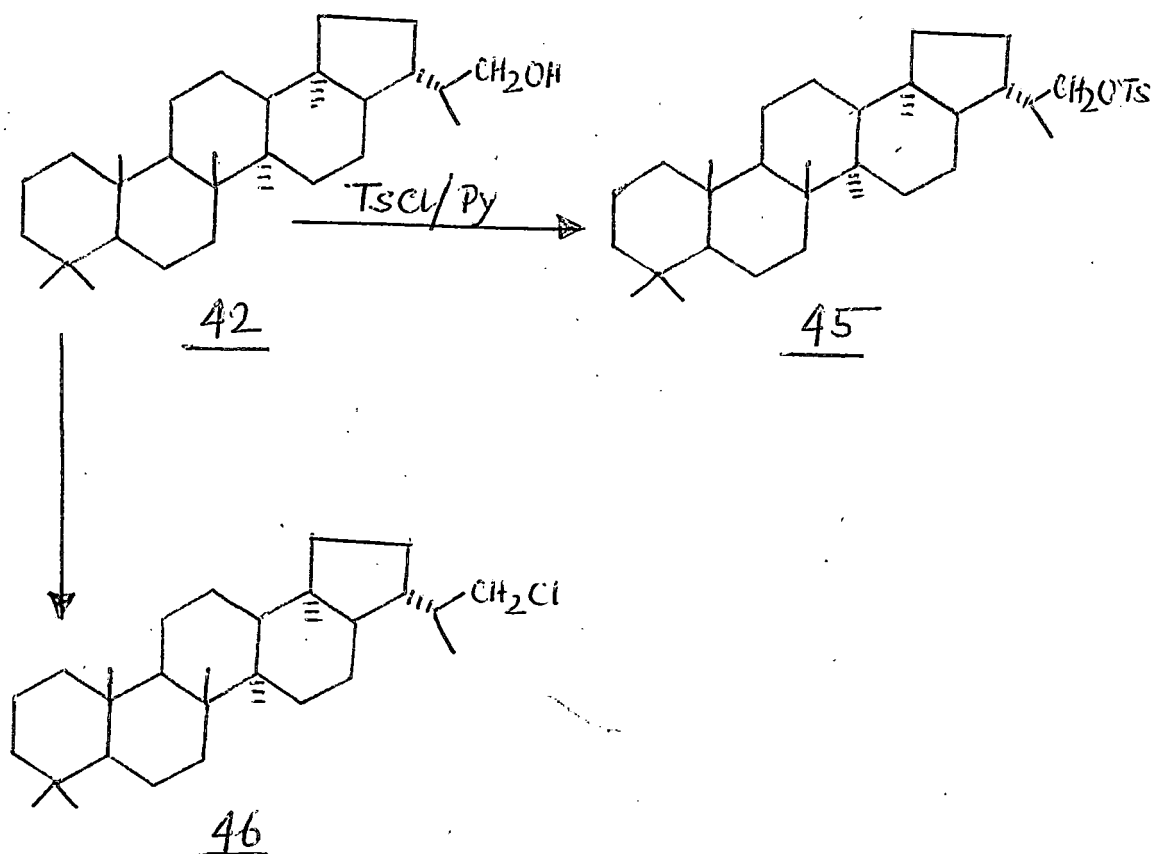
Both the  $\text{Cr}^{\text{V}}$  and the  $\text{Cr}^{\text{IV}}$  species appear to be more powerful oxidising agents<sup>51</sup> than  $\text{Cr}^{\text{VI}}$ . It is evident from the equations that these intermediate valence states may be responsible for as much as two-thirds of the total oxidation and in some cases may lead to unwanted side reactions such as carbon-carbon bond cleavage<sup>52</sup>.

#### Section B : Attempted preparation of Nerifoliol tosylate :

##### Isolation of Nerifoliol chloride :

We had also planned to prepare nerifoliol tosylate 45 by the usual procedure with purified p-toluene sulfonyl chloride and pyridine at room temperature and then convert the tosylate to hopane by LAH reduction. But in our attempt to prepare the tosylate 45 by the usual procedure with highly purified

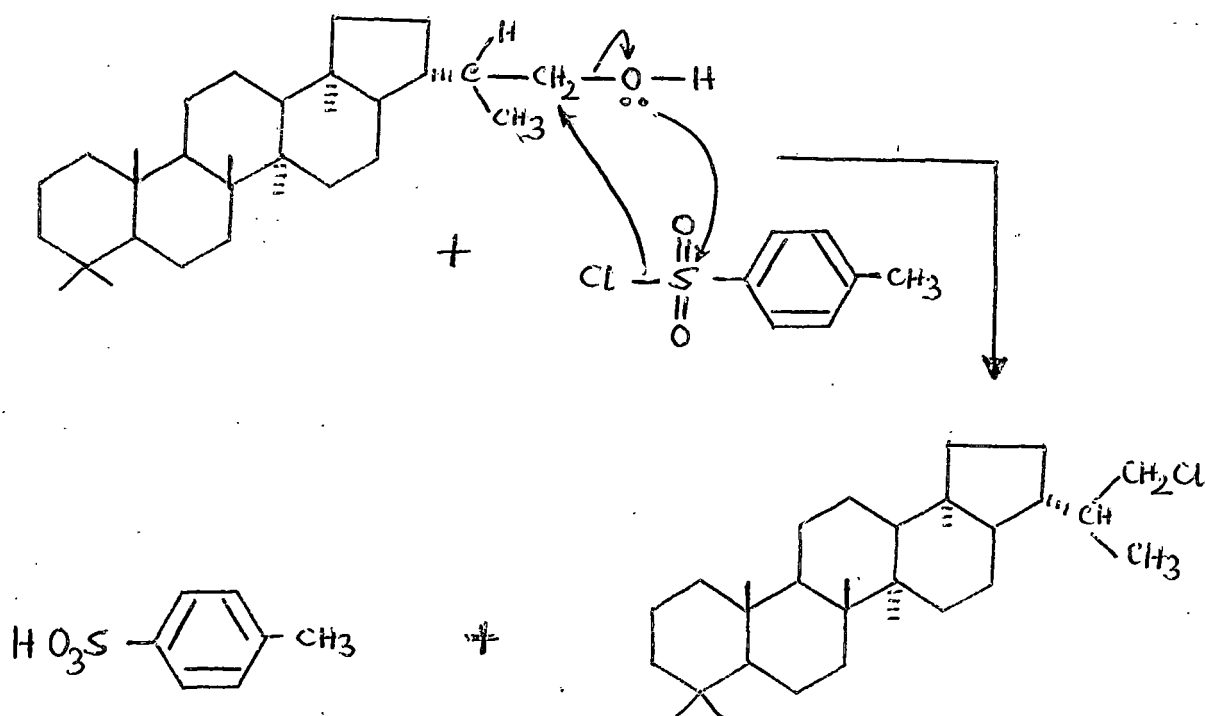
p - toluene sulfonyl chloride and pyridine we failed to isolate the desired tosylate but instead obtained a compound, m.p. 221-22° which gave a positive test for halogen. Elemental analysis



corresponded to the molecular formula giving correct analysis for the presence of one chlorine atom (see experimental). NMR spectrum (Fig-38) of the compound showed peak at  $\delta$  3.55.

which are closely in conformity with the structure 46. The mass spectrum (Fig-39) of the compound showed peaks at  $m/e$  446 ( $M^+$ ), 431, 369, 227, 226, 225, 191, 149 which again confirms the assigned structure.

In the above reactions we see that the normal reaction is retarded and instead a different reaction takes place giving a product by substitution with a chloride ion. The most probable mechanism for the reaction may be depicted as shown below.



The reason for this retardation of the reaction may be due to steric factors. Construction of the model for Nerefoliol shows

that in the formation of the tosyl derivative severe steric interactions result - which in all probability diverts the reaction to a substitution reaction by frontal attack as shown in the mechanism above. We are, however studying the reaction with different substrates of similar nature and the results will be reported in a future communication. At the present moment, no definite conclusions can be drawn for this reaction behaviour. The mechanism, shown above, however, seems reasonable.

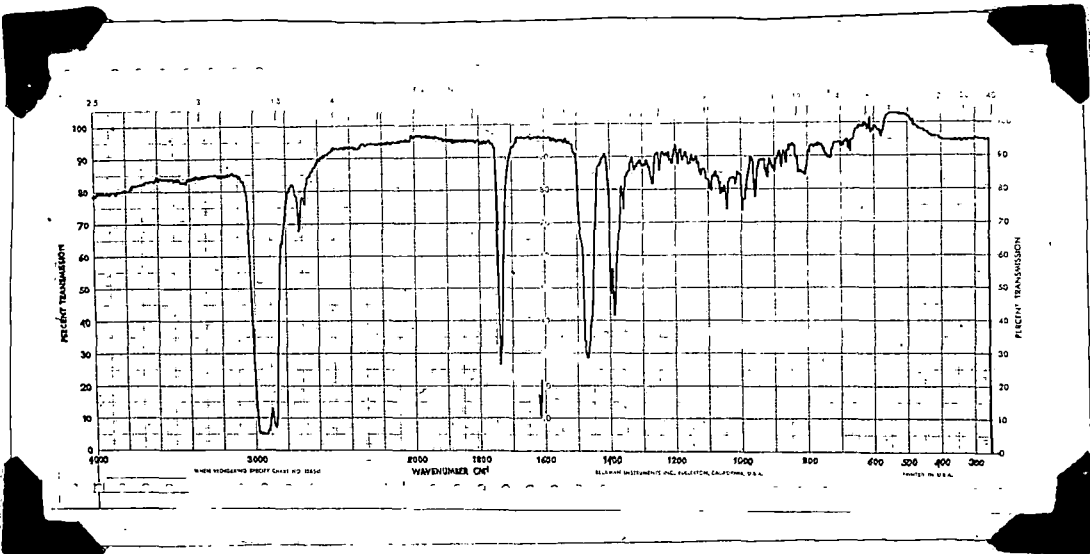


Fig. 34 : IR spectrum of nerifolial 43

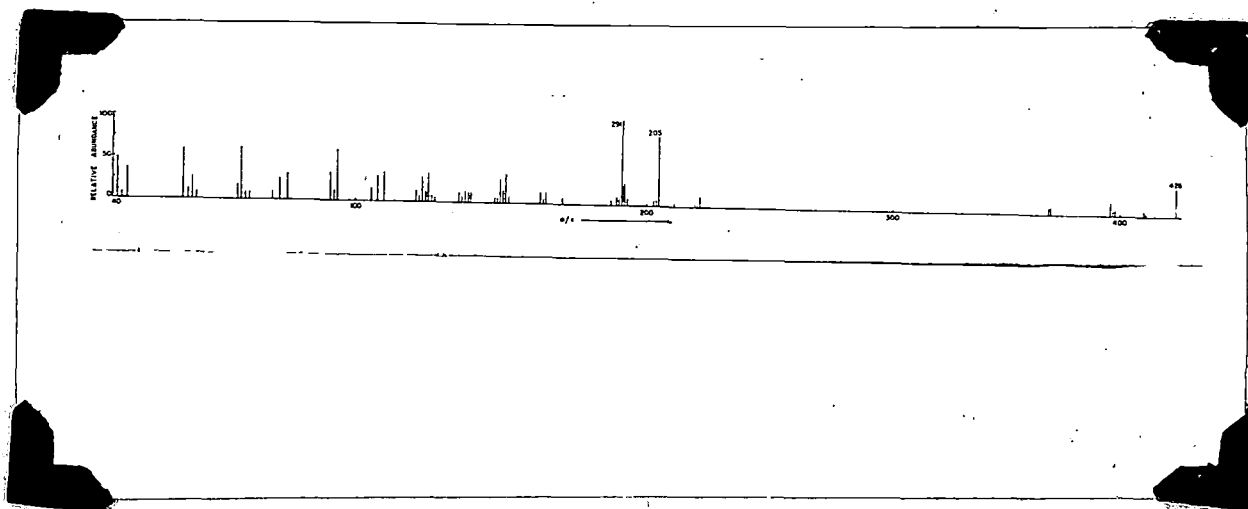


Fig. 35 : Mass spectrum of nerifolial 43

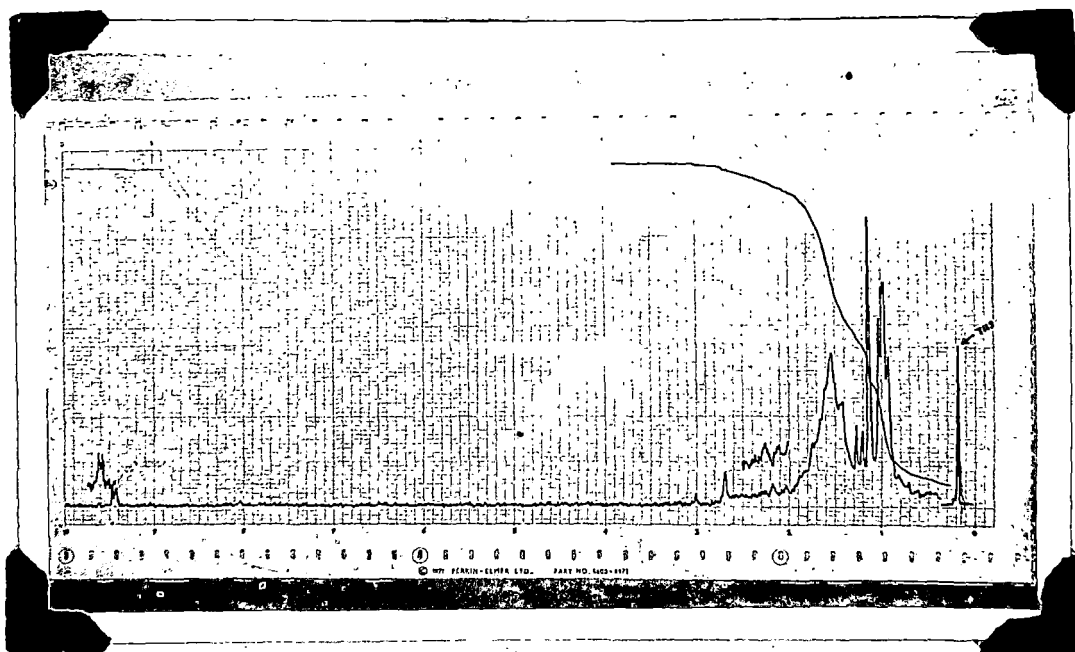


Fig. 36 : NMR spectrum of nerifolial 43

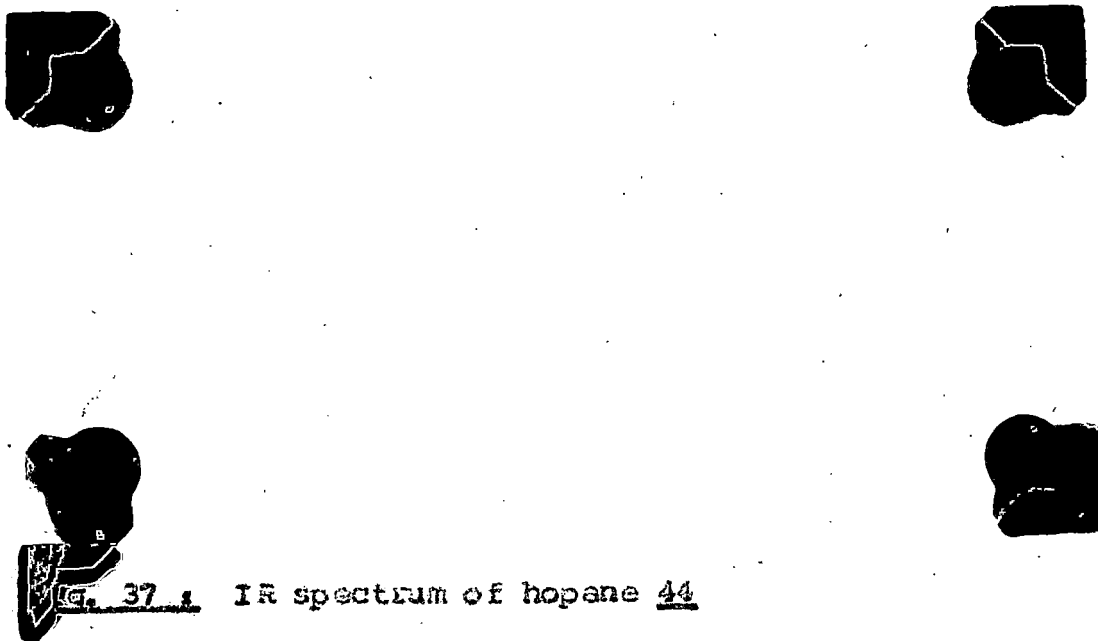


Fig. 37 : IR spectrum of hopane 44

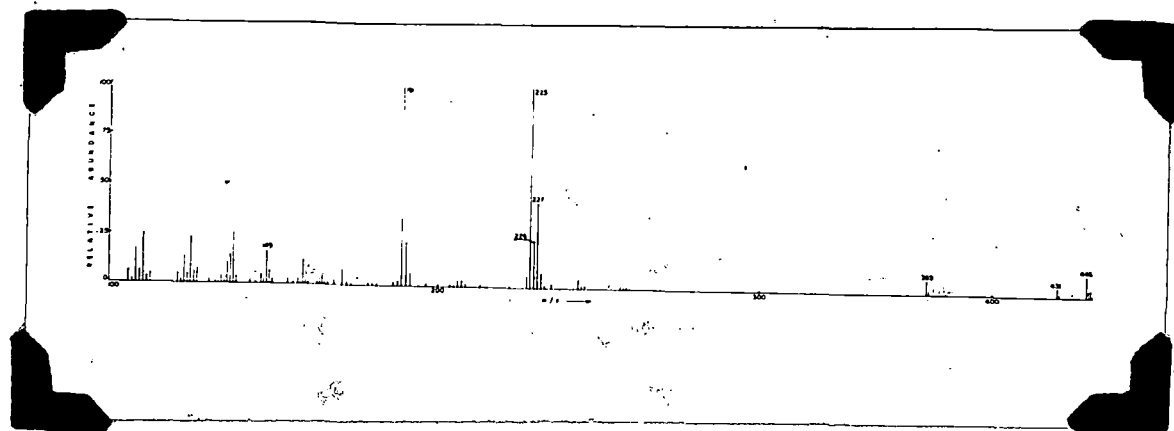
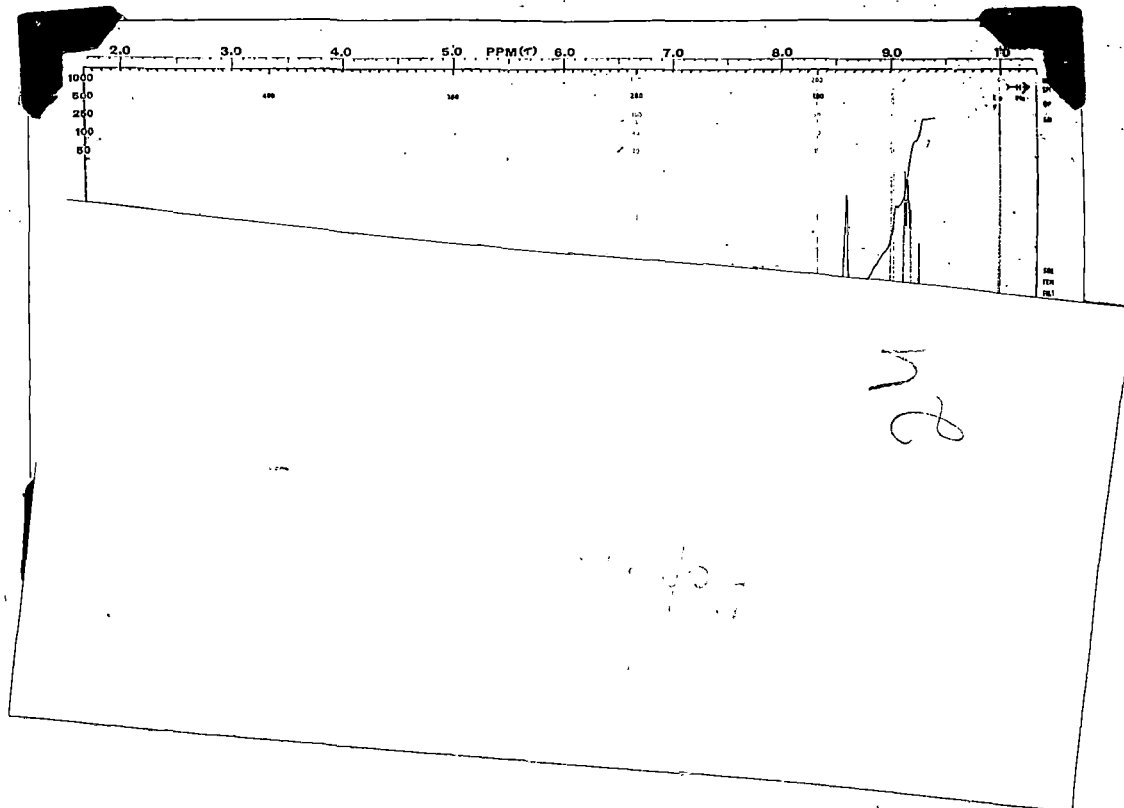


Fig 39 : Mass spectrum of the chloride 46



EXPERIMENTAL

Acetylation of nerifoliol 19 : Preparation of nerifoliol acetate 20 :

The alcohol 19 (200 mg) was treated with acetic anhydride (4ml) and pyridine (4ml) and kept overnight at room temperature. After working up in the usual manner the crude acetate (190 mg) was obtained. This product dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - XX).

Table - XX

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-----------|-------------------------|--------------------------------|
| Petroleum | 1 - 5                   | Solid (180 mg)<br>m.p. 192-4°. |

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

Fractions 1 - 5 (180 mg) were combined and on crystallisation from a mixture of chloroform and methanol furnished nerifoliol acetate 20, m.p. 195-96°.

Found : C, 81.69; H, 11.50%

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

IR : Fig - 2

Chromic acid - pyridine oxidation of nerifoliol 19 :

Preparation of adiantone and iso-adiantone :

Nerifoliol 19 (200 mg) was oxidised with  $CrO_3$  - Py complex prepared from pyridine (2 ml) and  $CrO_3$  (200 mg) at 15°C. Excess  $CrO_3$  was destroyed by adding 15 ml methanol, diluted with ethyl acetate and filtered. Ethyl acetate was removed, the concentrate was taken up in ether. The ether solution was washed with 5% hydrochloric acid solution, then with water until neutral and dried ( $Na_2SO_4$ ). Removal of ether gave a gummy residue. It showed two spots in TLC examination. The residue (160 mg) dissolved in benzene (3 ml) was placed over a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents. (Table XXI).

Table - XXI

| Eluent      | Fractions<br>50 ml each | Residue on<br>evaporation      |
|-------------|-------------------------|--------------------------------|
| Petroleum   | 1 - 2                   | Solid, m.p. 192-94°<br>(trace) |
| Petroleum : | 3 - 4                   | Solid, m.p. 225-30°            |

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

Fractions 1-2 were combined and on crystallisation from a mixture of chloroform and methanol furnished ~~some~~ crystals of m.p. 193-95°. This could not be investigated further for want of sufficient material.

Fractions 3 - 4 were combined and on crystallisation gave crystals, m.p. 230-32°. This compound was identified as isoadiantone (IR and co - TLC). The mother liquor from isoadiantone on rechromatography and subsequent TLC examination showed the presence of adiantone.

Attempted oxidation of nerifoliol 19 according to the method of Moffatt et al.<sup>49</sup>:

Nerifoliol 19 (300 mg) was added to DMSO (2.5 ml) and benzene (2.5 ml) containing DCC (0.5 gm), pyridine (0.6 ml) and the solution was kept overnight. Benzene (50 ml) was added and

filtered. The benzene solution was extracted with water, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. The residue (27 mg) on chromatography over silicagel (20 gm) and subsequent crystallisation of the solid (eluate -  $\text{C}_6\text{H}_6$ ) from a mixture of chloroform and methanol furnished fine needle-shaped crystals, m.p.  $244-46^\circ$ . This compound was found to be identical with the starting material (m. m.p.).

Oxidation of nerifolial <sup>0</sup>19 in anhydrous condition ;

Preparation of nerifolial 43 :

Dried chromium trioxide powder (0.6 gm) was added to a magnetically stirred solution of pyridine (0.95 gm) and anhydrous methylene chloride (15 ml). The flask was stoppered with a drying tube containing drierite, and the deep burgandy solution was stirred for 15 minute at room temperature. At the end of this period, a solution of the alcohol (0.426 gm, 1 mmol) in a small volume of methylene chloride was added in one portion. A tarry, black deposit separated immediately. After stirring an additional 15 minute at room temperature, the solution was decanted from the residue, which was washed with 100 ml of ether. The combined organic solution was washed with three 50 ml portions of 5% aqueous sodium hydroxide solution, 50 ml of 5% aqueous HCl, 50 ml of 5% aq. sodium bicarbonate solution, 50ml of saturated aq. sodium chloride solution, and was dried over anhydrous sodium sulphate. Evaporation of the solvent at reduced pressure afforded a gummy residue (390 mg)

This product dissolved in benzene (5 ml) was placed over a column of alumina (25 gm, deactivated with 1 ml of a 10% aqueous acetic acid) and was eluted with the following solvents (Table - XXII)

Table - XXII

| Eluent                       | Fractions<br>50 ml each | Residue on<br>evaporation |
|------------------------------|-------------------------|---------------------------|
| Petroleum                    | 1 - 3                   | Nil                       |
| Petroleum ;<br>benzene (4:1) | 4 - 11                  | Solid, m.p. 202-4°.       |

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

Fractions 4-11 were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless needles of aldehyde - nerifolial 43, m.p. 205-6°.

Found : C, 84.41; H, 11.85%

Calculated for  $C_{30}H_{50}O$  : C, 84.44; H, 11.81%

IR : 2720, 1730  $cm^{-1}$  Fig - 34

NMR spectrum (90 MHz) : 9.65 (doublet, - C H O) Fig - 36

Mass spectrum :  $M^+$  426 Fig - 35

Wolff - Kishner reduction<sup>37</sup> of nerifolial 43 :

Preparation of hopane 44 :

Nerifolial 43 (200 mg) in diethylene glycol (30 ml) was refluxed with hydrazine hydrate (2.3 ml) for 30 minutes. After addition of KOH (200 mg) the mixture was further refluxed for one hour. The condenser was removed and the mixture was heated to 190°. After refluxing for another 2½ hours the reaction mixture was cooled, diluted with water when a solid separated out. The aqueous solution after usual working up gave a solid residue (180 mg). This product dissolved in petroleum was placed over a column of active alumina (15 gm) developed with petroleum and eluted with the following solvents (Table - XXIII).

Table - XXIII

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation  |
|-----------|-------------------------|----------------------------|
| Petroleum | 1 - 3                   | Solid, 216 - 18°<br>160 mg |

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

Fractions 1 - 3 were combined and on crystallisation from a mixture of chloroform and methanol furnished fine needle-shaped crystals 44, m.p. 218-19°. This compound was found to be identical with an authentic sample of hopane (m.m.p.) and identical IR).

Found : C, 87.43; H, 12.67%.

Calculated for  $C_{30}H_{52}$  : C, 87.30; H, 12.70%

IR :

Fig - 37

Attempted tosylation of nerifoliol 42 :

Formation of Nerifoliol chloride 46 :

To nerifoliol 19 (200 mg) dissolved in pyridine (10 ml) was added p-toluene sulfonyl chloride (200 mg; purified by crystallisation) and heated on a water bath for 3-hours. The reaction mixture after usual ~~XXXX~~ working up and subsequent crystallisation from a mixture of chloroform and methanol furnished fine crystals 46, m.p. 221-22°. Elemental analysis corresponds to the presence of one chlorine atom. This compound gave positive test for halogen.

Found : C, 80.70; H, 11.44%

Calculated for  $C_{30}H_{51}Cl$  : C, 80.62; H, 11.42%

NMR spectrum :  $\delta$  3.55 Fig - 38

Mass spectrum :  $M^+$  446 Fig - 39

Attempted dehydration of nerifoliol 19 :

To nerifoliol 19 (200 mg) dissolved in pyridine (10 ml) was added  $POCl_3$  (5 ml). The reaction mixture was heated on water bath for 5 minutes and kept overnight at room temperature. This mixture after usual working up gave a solid residue (180 mg) which was subjected to chromatography over alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum and eluted with the following solvents (Table-XXIV)

Table - XXIV

| Eluent    | Fractions<br>50 ml each | Residue on<br>evaporation              |
|-----------|-------------------------|----------------------------------------|
| Petroleum | 1 - 3                   | Solid, m.p. $220-22^\circ$<br>(175 mg) |

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



Fractions 1 - 3 were combined and on crystallisation from a mixture of chloroform and methanol gave crystals, m.p.  $221-22^{\circ}$  which was found to be identical with the chloride 46 (m.m.p) IR, NMR and mass spectrum).

Attempted hydrogenolysis of the chloride 46 :

The chloride 46 (200 mg) dissolved in ethyl acetate (25ml) was shaken in an atmosphere of hydrogen in presence of Adam's catalyst. The reaction mixture after usual working up gave a solid residue (180 mg) which on subsequent crystallisation from a mixture of chloroform and methanol furnished colourless crystals, m.p.  $221-22^{\circ}$ , which was again found to be identical with the starting material (m.m.p.)

Attempted dehydrohalogenation of the chloride 46 with potassium hydroxide.

The chloride 46 (200 mg) was added to 10% methanolic KOH (10 ml) and refluxed for 7 hours. The reaction mixture after usual work up gave a solid residue (185 mg) which on crystallisation from a mixture of chloroform and methanol furnished crystals, m.p.  $221-22^{\circ}$ . This compound was found to be identical with the starting material (m.m.p.)

Attempted conversion of the chloride 46 to the corresponding iodide :

The chloride 46 (200 mg) dissolved in acetone (20 ml) was treated with NaI (200 mg) and refluxed on a water bath for 7 - hours. The reaction mixture after usual working up gave a solid residue (180 mg) which on crystallisation from a mixture of chloroform and methanol furnished crystals, m.p. 221-22<sup>o</sup>, identical with the starting material (m.m.p.).

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PART - IV

CHEMICAL INVESTIGATION ON  
INDIAN PLANTS

PART - IV

Chemical investigation on some Indian Plants :

Chemical investigation on the following Plants was undertaken and the results are shown in the attached REPRINT NO.1 and REPRINT NO.2.

REPRINT NO. 1 :

1. *Cyathes spinulosa* Wall (Filices)
2. *Antidesma diandrum* Roth (Euphorbiaceae)
3. *Euphorbia jacquemontii* Boiss (Euphorbiaceae)
4. *Gelonium bifarium* Roxb (Euphorbiaceae)
5. *Soyamida febrifuga* A. Juss (Meliaceae)

REPRINT NO. 2 :

1. *Fluggea microcarpa* Blume (Euphorbiaceae)
2. *Sapium baccatum* Roxb (Euphorbiaceae)
3. *Skimmia wallichii* (Rutaceae)

REPRINT NO. 1

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identity in absolute configuration was established by CD comparison— $\Delta\epsilon_{196} +7.2$  from either source. The NMR spectrum of the isolated calamenene showed it to be the *cis*-isomer<sup>8</sup> essentially free of diastereomeric material. The negative  $^1L_b$  band ( $\Delta\epsilon_{278} = -0.25$ ) suggests structure II.<sup>8</sup>

*Acknowledgement*—The work at U.W. was supported by NIH Grant GM-18143.

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## FILICES, etc.

### PHYTOSTEROLS IN PLANTS

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**Key Word Index**—*Cyathea spinulosa*; Filices; Fern; lupeol; *Antidesma diandra*; *Euphorbia jacquemontii*; *Gelonium bifarium*; Euphorbiaceae; Angiospermae taraxerone; sitosterol; epimultiflorenol; multiflorenol; bauerenol; *Soyimida febrifuga*; Meliaceae; methyl angolensate; sitosterol.

*Plant. Cyathea spinulosa* Wall<sup>1</sup> (Syn. *Hemitelia decipines* J. Scott). Filices. *Occurrence*. Middle and upper hill forest, rarely found near Kalimpong and Darjeeling, India. *Previous work*. None.

*Isolation and identification*. The powdered whole plant was extracted with  $C_6H_6$  and the neutral fraction gave lupeol,<sup>2</sup> m.p. 214–215°,  $[\alpha]_D +26.4^\circ$ , its acetate m.p. 216–217°,  $[\alpha]_D +47.5^\circ$  confirmed by m.m.p., IR and co-TLC with an authentic sample. The second solid has been identified as sitosterol.

*Plant. Antidesma diandrum* Roth.<sup>3</sup> Euphorbiaceae. *Occurrence*. Tropical Himalaya, from Garwhal eastwards and southwards to Travancore, India.

*Isolation and identification*. The powdered trunk was extracted with benzene and the neutral part afforded sitosterol.

*Plant. Euphorbia jacquemontii* Boiss; Euphorbiaceae.<sup>4</sup> *Occurrence*. Throughout the Western Himalayan region of India. *Previous work*. None.

*Isolation and identification*. The powdered trunk bark was extracted with  $C_6H_6$  and the neutral part on chromatography first gave taraxerone,  $C_{30}H_{50}O$ ,\* m.p. 238–240°,  $[\alpha]_D 10.8^{05}$  confirmed by IR, NMR and co-TLC with authentic specimen and by conversion to

\* Satisfactory analysis,  $[\alpha]_D$  in  $CHCl_3$ , 60 Mc NMR in  $CDCl_3$  with TMS as internal standard.

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<sup>4</sup> HOOKER, J. D. (1954) *Flora of British India*, p. 238, Reeve, London.

<sup>5</sup> POLLOCK, J. R. A. and STEVENS, R. (1965) *Dictionary of Organic Compounds*, 4th Ed., Vol. 5, p. 2943, Eyre & Spottiswoode, London and references cited therein.

taraxerol, m.p. 278–280°,  $[\alpha]_D + 3.7^\circ$ , acetate, m.p. 295–297°,  $[\alpha]_D + 9.16^\circ$ . The second solid was an alcohol,  $C_{23}H_{48}O$ , m.p. 87–88°,  $[\alpha]_D - 22.42^\circ$  (IR: 3275  $cm^{-1}$ ); acetate,  $C_{25}H_{50}O_2$ , m.p. 70–71° (IR: 1725 and 1245  $cm^{-1}$ ) but could not be identified for want of sufficient material. The last solid *sitosterol*,  $C_{29}H_{50}O$ , m.p. 137–138°,  $[\alpha]_D - 36^\circ$  confirmed by m.m.p., IR and co-TLC with authentic specimen. Acetate, m.p. 127°,  $[\alpha]_D - 4^\circ$ .

*Plant. Gelonium bifarium* Roxb;<sup>6</sup> Euphorbiaceae. *Occurrence.* Andaman islands, Malay islands. *Previous work.* Other sister species.<sup>7,8</sup>

*Isolation and identification.* The powdered trunk bark was extracted with  $C_6H_6$ . The neutral part gave a complex mixture of crystalline substances which on acetylation afforded a mixture of at least 3 acetates (TLC). On fractional crystallization it first afforded *bauerenol* acetate,  $C_{32}H_{52}O_2$ , m.p. 282–284°,  $[\alpha]_D 0^\circ$ , hydrolysis of which gave *bauerenol*,  $C_{30}H_{50}O$ , m.p. 208–209°,  $[\alpha]_D - 20^\circ$  confirmed by m.m.p., IR and co-TLC with authentic specimen. From the mother liquor two other solids were separated by fractional crystallization. The first solid multiflorenol acetate  $C_{32}H_{52}O_2$ , m.p. 220–222°,  $[\alpha]_D 0^\circ$  and its corresponding alcohol, *multiflorenol*,  $C_{30}H_{50}O$ , m.p. 188–190°,  $[\alpha]_D - 30^\circ$  confirmed by m.m.p. and IR comparison with authentic specimen. The third solid isolated from the mother liquor has been identified as epimultiflorenol acetate,  $C_{32}H_{52}O_2$ , m.p. 220–222°,  $[\alpha]_D 0^\circ$  and its corresponding alcohol *epimultiflorenol*, m.p. 206–208°,  $[\alpha]_D 0^\circ$  and its identity has been confirmed by preparing the alcohol from multiflorenone by the method of Paton *et al.*<sup>9</sup> The last solid m.p. 135–137° has been identified as *sitosterol*.

*Plant. Soyimida febrifuga* A. Juss.<sup>10</sup> Meliaceae. *Occurrence.* Dry forests of Western Peninsula, extending northwards to Marwara, the Mirzapur Hill and Chot Nagpur of India. *Medicinal use.* Bark, astringent, bitter tonic, febrifuge, used in general debility, intermittent fevers, diarrhoea and dysentery. *Previous work.* bitter substances from bark.<sup>11</sup>

*Isolation and identification.* The powdered trunk bark was extracted with  $C_6H_6$  and the chromatography of the neutral part over alumina first afforded *sitosterol*, m.p. 137–138°,  $[\alpha]_D - 36^\circ$  confirmed by m.m.p. and IR comparison with authentic specimen. The second solid, m.p. 202–204°,  $[\alpha]_D - 42^\circ$  coming out in  $C_6H_6$ –petrol. (4:1) showed in the NMR spectrum the presence of two  $\alpha$  ( $\delta$  7.37) and one  $\beta$ -furanic protons ( $\delta$  6.35), the H-17 proton ( $\delta$  5.62)  $\alpha$ -to the furan ring, two vinyl proton singlet  $\sim$   $\delta$  5.00, characteristic of the vinylidene group exocyclic to a cyclohexane ring, two doublets ( $J$  14 Hz) each one proton, assignable to the isolated geminal protons at C-15 and five three proton singlet assignable to one methyl ester and for quaternary methyls. All the above spectral data are in accord with *methyl angolensate*<sup>12</sup> and the compound has been found to be identical with the same confirmed by m.m.p. IR, co-TLC and NMR spectra with an authentic specimen.

*Acknowledgements*—The authors thanks are due to Professor T. G. Halsall, Dyson Perrins Laboratory, U.K. for an authentic sample of methyl angolensate. T.K.R. and D.B.N. are thankful to East India Pharmaceutical Works Ltd., Calcutta and University of North Bengal respectively for grant of research fellowships.

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# PHYTOCHEMICAL REPORTS

## PHYTOSTEROLS IN EUPHORBIACEAE AND RUTACEAE\*

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( Received 19 March 1974 )

Key Word Index - *Fluggea microcarpa*; Euphorbiaceae; hexacosane; friedelin; friedelanol; sitosterol; *Sapium baccatum*; Euphorbiaceae; acetoxy aleuritolic acid; *Skimdia wallichii*; Rutaceae; taraxerone; 3-epitaraxerol; taraxerol; sitosterol.

Plant. *Fluggea microcarpa* Blume [2, 3] (Syn *Flueggea microcarpa* Blume); Euphorbiaceae. Occurrence. Throughout India; from Kashmir ascending the Himalaya to 5000 ft. to Bhutan, and Assam and southwards to Malacca and Travancore. Uses. Medicinal [4]. Previous work [5]. Bergenin and isocoumarin in leaves.

Isolation and identification. The powdered trunk bark was extracted with  $C_6H_6$  and the neutral fraction on chromatography over deactivated alumina afforded several crystalline solids; hexacosane, mp  $58-59^\circ$ ; friedelin mp  $259-261^\circ$ ,  $[\alpha]_D - 32^\circ$ ,  $\nu_{max}$   $1708\text{ cm}^{-1}$  (six-membered ring ketone), oxime, mp  $293-295^\circ$ ,  $[\alpha]_D + 54.6^\circ$ , friedelanol, mp  $296-298^\circ$ ,  $[\alpha]_D + 15.5^\circ$ , acetate, mp  $314-316^\circ$ , and sitosterol, mp  $136-137^\circ$ ,  $[\alpha]_D - 34^\circ$ , acetate, mp  $127-129^\circ$ ,  $[\alpha]_D - 39^\circ$ . The identity of the above compounds was confirmed by mmp IR and co - TLC with authentic samples.

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\* Part II of a series on Plant Phytosterols; for Part I see Ref. / 1 /.

Plant. *Sapium-baceatum* Roxb; [6] Euphorbiaceae. Occurrence. Assam, Sylhet and Khasia Mountains, India. Previous work. [7-10] Isolation and characterization of taraxerone, taraxerol, sitosterol, 1-hexacosanol, 3-3'-di-O-methyl ellagic acid [11].

Isolation and identification. The acidic fraction from the  $C_6H_6$  extract of the stem and trunk bark of the plant on esterification with  $CH_2H_2$  followed by chromatography furnished a crystalline solid, mp 241-243°,  $[\alpha]_D + 21.8^\circ$ , no UV absorption above 220 nm,  $\nu_{max}^{CHCl_3}$  1738  $cm^{-1}$  (broad, -OCOMe and COOMe), 1245  $cm^{-1}$  (-OCOMe), NMR signals at  $\delta$  5.45 (1H, vinyl proton, trisubstituted double bond),  $\delta$  4.42 (1H, H-C-O-COOMe),  $\delta$  2.05 (3H, -OCOMe),  $\delta$  3.54 (3H, -COOMe) and several sharp signals between  $\delta$  0.8 to 1.55 (21H, seven methyl groups). Hydrolysis of the ester with 5% methanolic KOH yielded an alcohol, mp 208-210°,  $[\alpha]_D + 15.6^\circ$ ,  $\nu_{max}^{CHCl_3}$  3490  $cm^{-1}$  (-OH), 1738  $cm^{-1}$  (-COOMe). Oxidation of alcohol by  $CrO_3-C_6H_5N$  complex furnished a ketone, mp 174-176°,  $[\alpha]_D + 14.2^\circ$ ,  $\nu_{max}^{KBr}$  1708  $cm^{-1}$  (C=O), 1738  $cm^{-1}$  (-COOMe). The acid obtained from the plant is therefore acetoxyleuritic acid, the physical data on the ester, alcohol and ketone being strikingly similar to those obtained for the known compound [12].

Plant. *Skimmia wallichii* Hk [13]; Rutaceae. Occurrence. A small procumbent shrub about 4ft. high, fairly common in Singalila Range, from 9000-11000 ft. Previous work. None. Isolation and identification. The powdered bark of the plant was extracted with  $C_6H_6$  and the neutral fraction on chromatography over deactivated alumina first gave four crystalline compounds; the first mp 238-239°,  $[\alpha]_D + 11^\circ$ ,  $\nu_{max}$  1710  $cm^{-1}$  (six membered ring ketone) was identical with taraxerone

(mp and IR); 3-epitaraxerol [14] mp 261-263°,  $[\alpha]_D - 25.6^\circ$ ,  $\nu_{\max} 3420 \text{ cm}^{-1}$  (-OH) and  $825 \text{ cm}^{-1}$  (trisubstituted double bond), acetate, mp 160-162°,  $[\alpha]_D - 43^\circ$ . Oxidation of the alcohol by  $\text{CrO}_3 - \text{C}_6\text{H}_5\text{N}$  complex-furnished taraxerone. The alcohol, 3-epitaraxerol and its oxidised product, taraxerone were identified by mp, IR and co-TLC; taraxerol, mp 272-274°,  $[\alpha]_D + 5.5^\circ$ , acetate, mp 295-297°,  $[\alpha]_D + 10^\circ$  confirmed by mp, IR and co-TLC with an authentic sample; and sitosterol (mp and IR).

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For Convention of Chemists 1974 : Section : Organic Chemistry.

TRITERPENES FROM POLYPODIUM JUGLANDIFOLIUM

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ABSTRACT

The neutral portion of the benzene extract of the rhizomes of Polypodium juglandifolium on chromatography afforded, from the least polar-fraction, two hydrocarbons- Fernene,  $C_{30}H_{50}$ , m.p.  $169-70^{\circ}$ ,  $(\alpha)_D - 16.3^{\circ}$  and Filixicene,  $C_{30}H_{50}$ , m.p.  $231-32^{\circ}$  (lit.  $288.5-229.5^{\circ}$ , Ref : H. Ageta, K. Iwata and S. Natori, Tetrahedron Letters, No. 46, pp 3413-3418, 1964),  $(\alpha)_D + 49.6^{\circ}$  identical with the authentic specimens (m.m.p and IR). The next fraction obtained from the chromatography m.p.  $121-2^{\circ}$  has been found to be a mixture of cyclolaudenol,  $C_{31}H_{52}O$ , m.p.  $122-23^{\circ}$  and a new C-32 triterpene alcohol  $C_{32}H_{54}O$ . The new triterpene alcohol  $C_{32}H_{54}O$  has established as having cyclolaudenol nucleus with the extra carbon in the side chain from mass spectrometric studies of the original mixture, its acetate and the dihydroderivative and the acetate of the dihydro compound. The position of the double bond in the side chain has been tentatively assigned at 25 (26) also from mass spectrometric fragmentation pattern. Further work is in progress to locate the exact position of the extra carbon atom.

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Partial Synthesis of Dimethyl Dihydroceanothate from  
Methyl Betulonate

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The diosphenol (2b) obtained by the autoxidation of methyl dihydrobetulonate, on  $H_2O_2$  oxidation followed by esterification affords the trimethyl ester (3b) of the seco-acid (3a). Dieckmann condensation of (3b) furnishes after purification methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-A(1)-norlupan-28-oate (1e) which on  $NaBH_4$  reduction in methanol-dioxane gives the desired dimethyl dihydroceanothate (1d), identical with an authentic sample.

Ceanothic acid (1a)<sup>1, 2, 3</sup> was originally isolated by Julian et al from *Ceanothus americanus* and has since been isolated from a number of Australian Rhamnaceae species<sup>5, 6</sup>. Huneck<sup>7</sup> in an attempt towards the partial synthesis of ceanothic acid developed a method for the synthesis of both epimeric A(1)-norlup-20(29)-en-3-carboxylic acids (1b) and (1c) by photochemical Wolff rearrangement of the diazoketone (2a). Kundu et al<sup>8</sup> also reported the synthesis of (1b).

We report here a successful synthesis of dimethyl dihydroceanothate (1b). Autoxidation of methyl dihydrobetulonate by passing a stream of oxygen in presence of K-tert.butoxide in tert.butanol gave the diosphenol (2b), m.p. 131-33°, ( $\alpha$ )<sub>D</sub> - 1.96°. The diosphenol on oxidation with 30% hydrogen peroxide gave the seco-acid (3a), m.p. 175-77°,  $\nu_{max}$  1690, 1710 (COOH)  $cm^{-1}$ . Treatment of (3a) with diazomethane

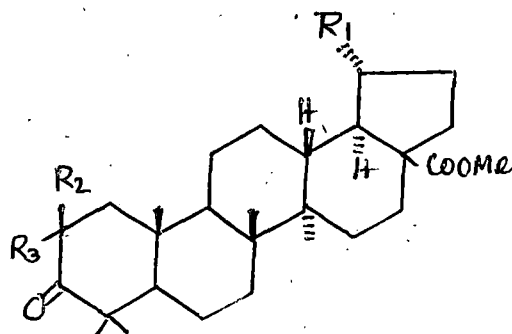
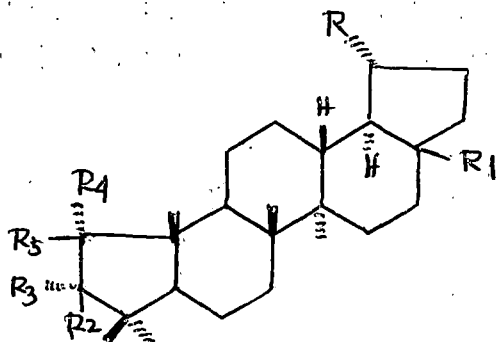
afforded the trimethyl ester (3b)<sup>1,6</sup>, M.P. 146-47°,  $\gamma$  <sub>max</sub> nuzol  
1745, 1725 cm<sup>-1</sup> (CO<sub>2</sub>Me). Dieckmann condensation of (3b)  
in the presence of potassium tert-butoxide in benzene under  
nitrogen blanket<sup>9a,9b</sup> gave a gummy product which on chromato-  
graphy over alumina gave methyl 2 $\alpha$ -methoxycarbonyl-3-oxo-  
A(1)-norlupan-28-oate(1e) m.p. 191-93°, ( $\alpha$ )<sub>D</sub> + 89°,  $\gamma$  <sub>max</sub> nujol  
1750, 1720 cm<sup>-1</sup>, and its epimer (1f) 175-77°, ( $\alpha$ )<sub>D</sub> + 42°,  
 $\gamma$  <sub>max</sub> nujol 1750, 1730 cm<sup>-1</sup> in the ratio of 17 : 1. The high  
yield of the  $\beta$ -keto ester (1e) with the desired stereochemis-  
try in this reaction is significant and is at variance with  
the observation of Eade et al.<sup>3</sup> who stated that methyl 2 $\alpha$ -  
methoxycarbonyl-3-oxo-A(1)-norlup-20(29)-en-28-oate (dimethyl  
dehydrocanothate) was rapidly epimerized by alkali to an  
equilibrium mixture containing 40% of the starting material  
and 60% of the isomer epimeric at C-2.

The  $\beta$ -keto ester (1e) on reduction with sodium  
borohydride in methanol-dioxane gave a mixture (two spots in  
TLC) which on chromatography over alumina first eluted (benzene-  
pet ether, 3:2) a solid, m.p. 261-62°, ( $\alpha$ )<sub>D</sub> + 22°,  $\gamma$  <sub>max</sub> nujol  
3540, 1730, 1710 cm<sup>-1</sup>, identical with an authentic specimen of  
dimethyl dihydroceanothate (1d) supplied by Prof. de Mayo. Further  
elution with the same solvent gave another solid, m.p. 140-42°,  
 $\gamma$  <sub>max</sub> nujol 3560, 1745, 1705 cm<sup>-1</sup>. The yield of the material was  
not sufficient for NMR studies but by analogy with previous  
work<sup>3</sup> it is believed to be the C-3 epimer (1g). Further work is  
in progress to elucidate its stereochemistry.

Investigation was also initiated to prepare the  $\beta$ -keto  
ester (1e) by carbomethoxylation of the A-nor ketone (4b) at C-2.  
The diosphenol (2b) on treatment with 10% ethanolic sodium  
hydroxide solution underwent benzilic acid rearrangement to

furnish the  $\alpha$ -hydroxy acid (4a), m.p. 255-56°,  $\gamma$  nujol 3440, max 3340, 1720  $\text{cm}^{-1}$ . The latter on treatment with lead dioxide<sup>10a, 10b</sup> in glacial acetic acid under mild refluxing conditions gave the nor-ketone (4b)<sup>1, 11</sup>, m.p. 179-80° ( $\alpha$ )<sub>D</sub> + 75.4°.

Attempts on carbomethoxylation of this ketone using dimethylacarbonate in the presence of sodium methoxide, potassium-tert butoxide or sodium hydride in various solvents proved unsuccessful.



1a,  $R_1=R_4=\text{COOH}$ ,  $R_2=\text{OH}$ ,  $R=\text{CH}_2$ ,  $R_3=R_5=\text{H}$

1b,  $R_1=\text{CH}_3$ ,  $R_3=\text{COOH}$ ,  $R_2=R_4=R_5=\text{H}$ ,  $R=\text{CH}_2$

1c,  $R_1=\text{CH}_3$ ,  $R_2=\text{COOH}$ ,  $R_3=R_4=R_5=\text{H}$ ,  $R=\text{CH}_2$

1d,  $R_1=R_4=\text{COOMe}$ ,  $R_2=\text{OH}$ ,  $R_3=R_5=\text{H}$ ,  $R=\text{CH}_2$

1e,  $R_1=R_4=\text{COOMe}$ ,  $R_2=\text{O}$ ,  $R_5=\text{H}$ ,  $R=\text{CH}_2$

1f,  $R_1=R_5=\text{COOMe}$ ,  $R_2=\text{O}$ ,  $R_4=\text{H}$ ,  $R=\text{CH}_2$

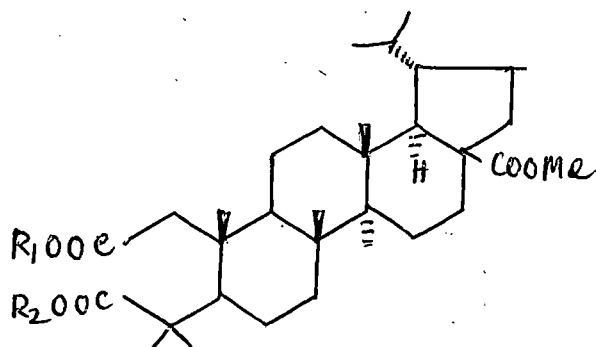
1g,  $R_1=R_4=\text{COOMe}$ ,  $R_3=\text{OH}$ ,  $R_2=R_5=\text{H}$ ,  $R=\text{CH}_2$

2a,  $R_2=R_3=\text{N}_2$ ,  $R=\text{CH}_2$

$R=\text{CH}_2$

2b,  $R_2=R_3=\text{O}$ ,  $R_1=\text{CH}_2$

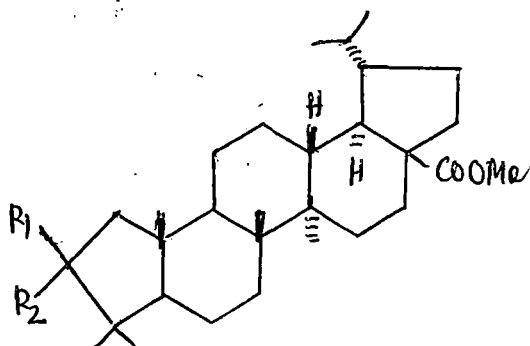
$R_1=\text{CH}_2$



3a,  $R_1 = R_2 = H$

3b,  $R_1 = R_2 = CH_3$

References :



4a,  $R_1 = OH, R_2 = COOH$

4b,  $R_1 = R_2 = O$

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