SUMMARY

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

A. PART-I :

Chapter-I: describes the morphology of the fern *Polypodium Juglandifolium Don.*

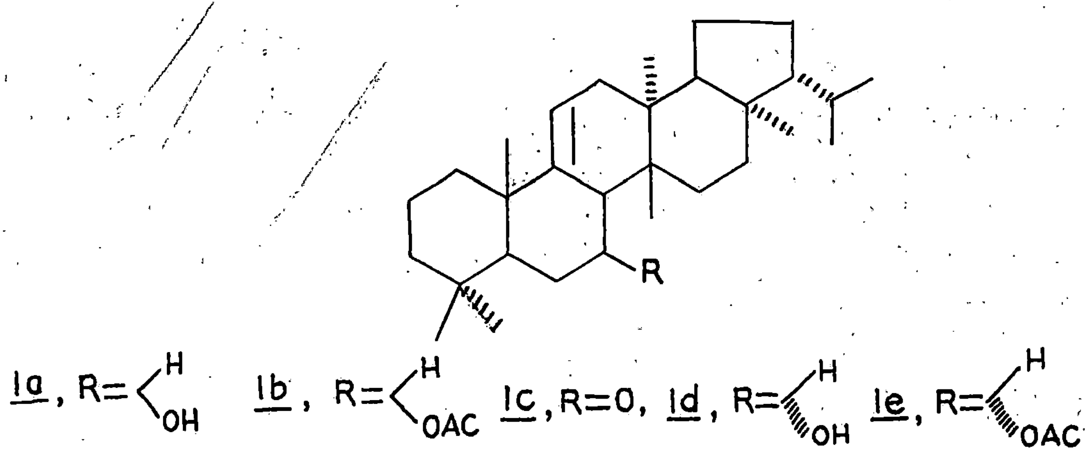
Chapter-II: gives a short review on the chemical constituents of fern of Polypodiaceae family.

Chapter-III: deals on the investigation on the neutral part of the benzene extract of the fern *Polypodium-Juglandifolium* and isolation of two new triterpene- Polypodinol B, $C_{30}H_{50}O$ and Polypodinol C, $C_{30}H_{50}O$, along with fern-9(11)-ene, filicene, Polypodinol A $C_{30}H_{50}O$, Cyclolaudenol, dryocrassol $C_{30}H_{52}O$, β-sitosterol and detection of a cyclolaudenol homologue, 'Cycloneolitsol' $C_{32}H_{54}O$, in the Cyclolaudenol fraction from mass-spectrometry.

Chapter-IV: describes the investigation on the structure elucidation of Polypodinol B, $C_{30}H_{50}O$:

Polypodinol B Ia $C_{30}H_{50}O$ (M^{+} 426), m.p. 165-66°, $(\alpha)_D$ 23.57°, gave a positive Libermann-Burchard test and a yellow colour with TNM. IR spectrum of Ia showed bands at 3390, 3510 cm^{-1} (-OH) and its NMR spectrum showed signals at δ 0.8 to 1.25, a multiplet centered at δ 5.40 (1H, trisubstituted double bond) and a broad diffused multiplet centered at δ 4.36 attributed to the proton attached to the carbon containing -OH group. The coupling pattern indicates that this proton attached to a carbon atom in the system $-CH-\underline{CH}OH-CH_2-$. On treatment with acetic anhydride-pyridine it furnished a monoacetate <u>Ib</u> $C_{32}H_{52}O_2$ (M^+ 468), m.p. 207-8°, $(\alpha)_D$ 38°, ν_{max}^{nujol} 1720, 1240 ($-O.CO.CH_3$) cm^{-1}. Its NMR spectrum showed the presence of eight methyl groups between δ 0.8 to 1.16, a sharp peak at δ 2.08 (3H, $-O.CO.CH_3$), a multiplet at δ 5.45 (1H, vinyl proton) and an unresolved multiplet centered at δ 5.16 [1H, $-CH-\underline{CH}-(OAc)-CH_2-$].

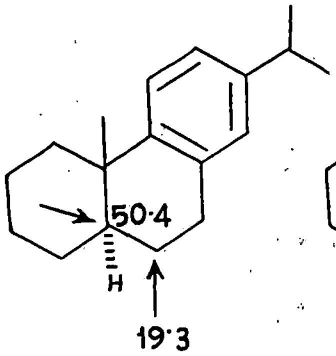
Oxidation of <u>Ia</u> with CrO_3-Pyridine complex gave a compound <u>Ic</u>, $C_{30}H_{48}O$ (M^+ 424), m.p. 174-75°, ν_{max}^{nujol} 1720 cm^{-1} (six membered ring ketone), NMR signals at δ 5.33 (1H, vinyl proton) and peaks between δ 0.8 to 1.04 for eight methyl groups. Wolff Kichner reduction of the ketone <u>Ic</u> under anhydrous condition furnished a hydrocarbon, $C_{30}H_{50}$ (M^+ 410) m.p. 169-71°, $(\alpha)_D$ -13.04° and this was found to be identical with an authentic sample of fern-9(11)-ene (m.m.p, IR comparison and mass comparison). This experiment established that the triterpene was a hydroxy derivative of $\Delta^{9(11)}$-fernene.



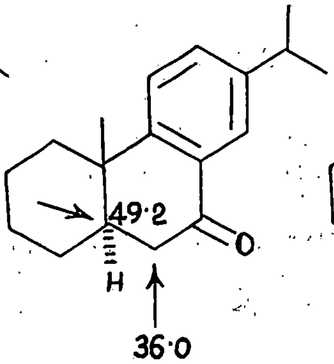
The position of the oxygen function was established from the following observation. The ketone Ic was different from 3-keto-fern-9(11)-ene. C.D. curve of the ketone Ic showed a strong negative cotton effect. Examination of the Drieding models with C-15 and C-16 ketone in the fernene nucleus separately suggest that the compound with C-15 ketone would have a very small Cotton effect probably positive, whereas the compound with C-16 carbonyl group would show a strong positive Cotton effect. Examination of the Drieding models also suggest that if the carbonyl group is situated at C-7 position then the chirality of the βγ-unsaturated keto chromophore would most probably give rise to a negative Cotton effect. Since a negative Cotton effect was observed we believe that the carbonyl group is probably situated at C-7 as in Ic and Polypodinol B is Ic. This is further confirmed from CMR of the ketone Ic (Table-1) by comparison with model compounds A_1, B_1, C_1, D_1 and calculating the deshielding effects due to 4-gemdimethyl group, Δ^{9-11} double bond and an α-keto group.

Table-1

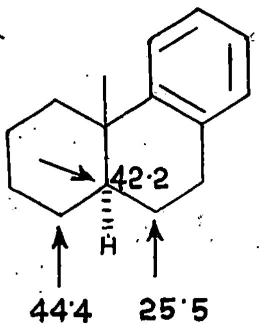
		Predicted with (models A_1, B_1, C_1, D_1)	Predicted (with $\Delta^{9(11)}$-fernene as models	Found
7-keto compound:	$CH(C_3)$	67	72.7	69.4
	$CH(C_5)$	49	52.1	46.2
	$CH_2(C_6)$	36		35.0



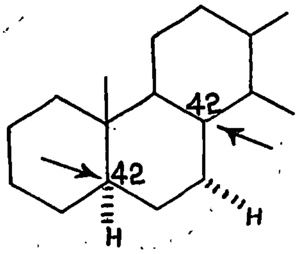
A1



B1



C1



D1 *(Estimate based on model-C1)*

Chapter-V: deals with investigation on the structure of the new triterpene Polypodinol C, $C_{30}H_{50}O$:

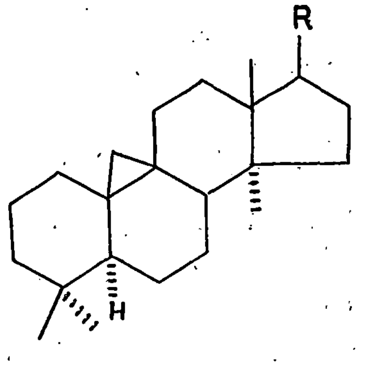
Polypodinol C Id, $C_{30}H_{50}O$ (M^+ 426), m.p. 149-51°, $(\alpha)_D$ -3.23° ν_{max}^{nujol} 3430 cm^{-1}(-OH), NMR ($CDCl_3$): δ 0.8-1.2 (8-tert-CH_3 groups), δ 5.35 (1H, vinyl proton), δ 4.4 ($-\overset{OH}{CH}-CH_2-$), gave a monoacetate Ie, $C_{32}H_{52}O_2$ (M^+ 468), m.p. 193-95°, $(\alpha)_D$ 30°, ν_{max}^{nujol} 1720, 1245 cm^{-1} (-O-CO-CH_3), no UV absorption between 220-300 mμ. Chromiumtrioxide and pyridine oxidation of Id gave a ketone m.p. 169-71°, ν_{max}^{nujol} 1720 cm^{-1}. Wolff Kishner reduction of the ketone gave fern-9(11)-ene indicating that Id is a hydroxy derivative of fern-9(11)-ene.

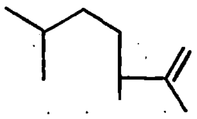
C.D. spectrum of the ketone showed a strong negative Cotton effect for the carbonyl group, $\Delta\epsilon$ −7.58 at 301 nm in methanol solution. The ketone m.p. 169-71° was found to be identical with 7-keto-fern-9(11)-ene Ic in all respects (m.m.p, IR, NMR, CMR), showing thereby that the Polypodinol C Id was an epimer of Polypodinol B Ia. When Ic (derived from Ia) was reduced with sodium borohydride in methanol afforded an alcohol m.p. 151-52° identical with Polypodinol C, Id. Thus Polypodinol C Id is most probably the equatorial isomer and Polypodinol B Ia is the axial isomer. We have also run the CMR of a sample of Δ^{9-11} Fernene where C_5 is assigned at 52.1 ppm and C_8 is assigned at 59.7 ppm instead of at 50 and 55 as estimated from models of compound A_1, B_1, C_1, D_1. If 13 ppm is added for the effect of an α-carbonyl we find that the fit is much better for the 7 keto compound Ic ~~respectively~~ (Table-1).

Chapter-II: describes the detection and structure determination of a cyclolaudenol Homologue $C_{32}H_{54}O$ by mass spectrometry; and preparative thinlayer chromatography:

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 component was isolated as cyclolaudenol benzoate.

The original mixture (A) on acetylation gave an acetate(B) m.p. 107-8°, $(\alpha)_D$ 55.17°. Hydrogenation of the mixture gave a compound (C) m.p. 132-33°, which on acetylation gave an acetate (D) m.p. 127-28°. Oxidation of the original mixture gave a mixture of ketones (E), m.p. 107-8°. Mass spectra of the original mixture (A), its acetate (B), the dihydrocompound (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 cyclolaudenol homologue has been found to correspond very closely to that of cyclolaudenol <u>IIa</u> and are in agreement with earlier findings (P.Benveniste, L.Hirth and G.Ourisson, <u>Phyto Chem.</u>, <u>5</u>, 31, 1966, M.E.Audier, R.Beugelmans and B.C.Das, <u>Tetrahedron Letters</u>, 4341, 1966, R.T.Aplin and G.M.Hornby, <u>J.Chem.Soc</u> (B), 1077, 1966).



IIa, R = 

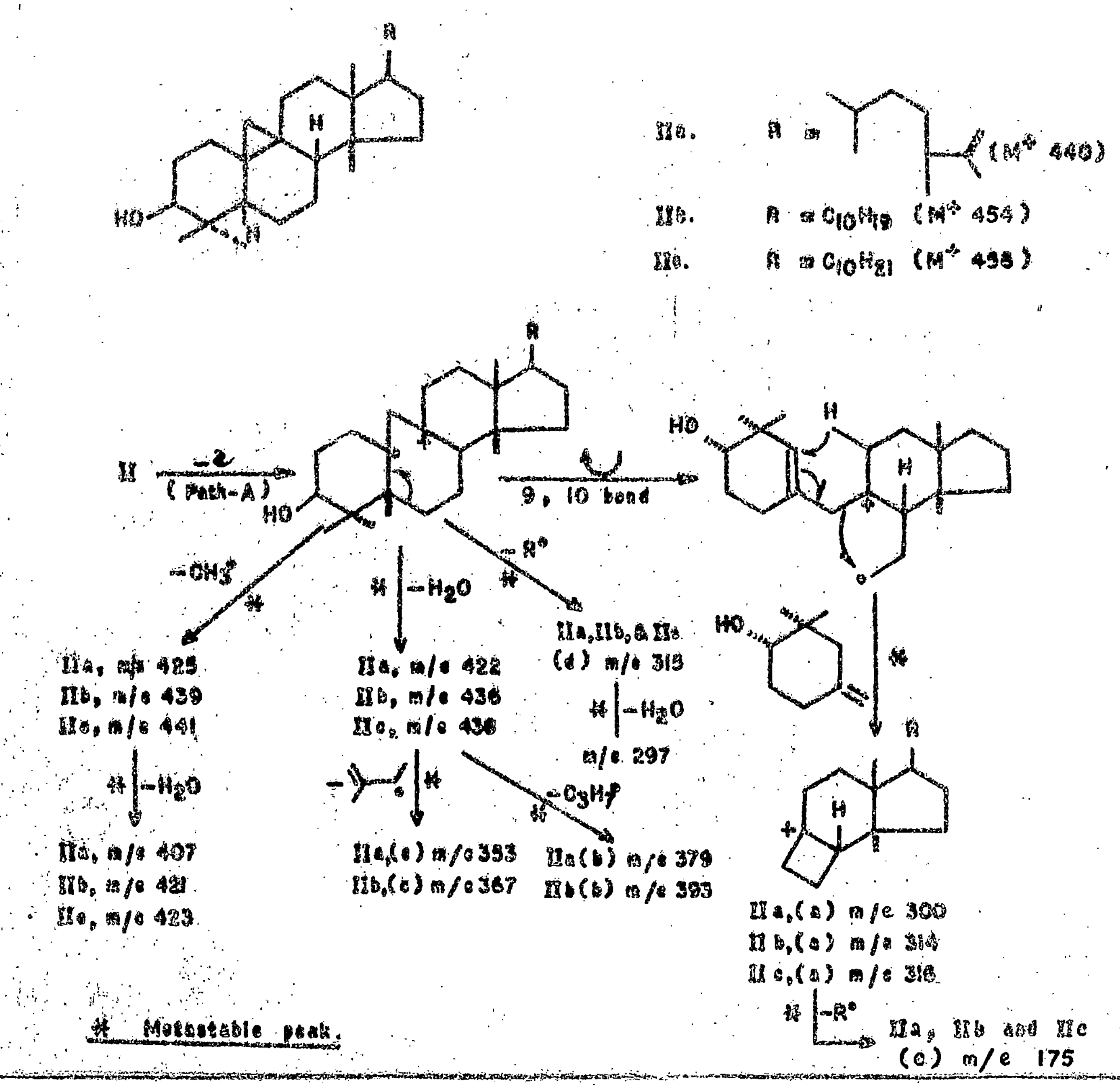
IIb, R = $C_{10}H_{19}$

IIc, R = $C_{10}H_{21}$

The unusual feature of the spectrum (Chart-A) of cyclolaudenol *IIa* 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 ($M-18-C_3H_7^{\cdot}$), (c) m/e 353 ($M-18-C_5H_9^{\cdot}$). 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($M-R^{\cdot}$) is common to cyclolaudenol *IIa*, its homologue *IIb* and its dihydroderivative *IIc* 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 in Chart -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 "McLafferty" type of rearrangement.

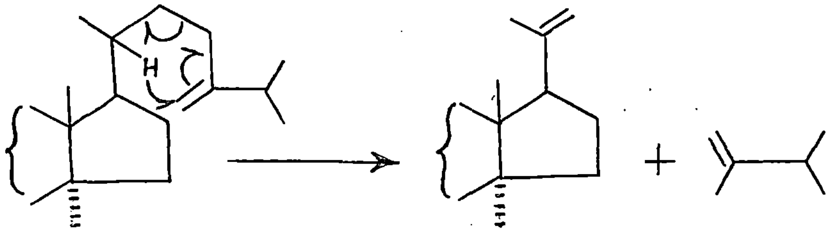
The mass spectra of the C-32 triterpene *IIb* ($\overset{+}{M}$ 454) and its dihydro compound *IIc* has been found to contain all the peaks (Chart-A) characteristic of cyclolaudenol but in this case the peak

CHART-A

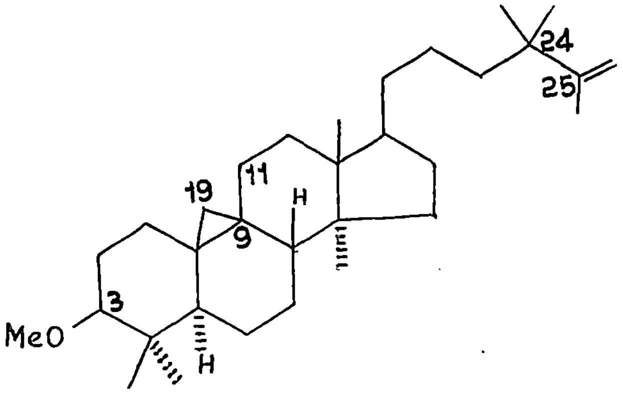

R
H
HO
H
IIa. R = (M+ 440)
IIb. R = C10H19 (M+ 454)
IIc. R = C10H21 (M+ 456)
II
−2
(Path-A)
HO
9, 10 bond
HO
H
H
−CH3•
#
−H2O
−R•
#
IIa, m/e 425
IIb, m/e 439
IIc, m/e 441
IIa, m/e 422
IIb, m/e 436
IIc, m/e 438
IIa, IIb, & IIc
(d) m/e 315
−H2O
m/e 297
HO
#
R
H
−H2O
IIa, m/e 407
IIb, m/e 421
IIc, m/e 423
#
−C3H7•
#
IIa,(e) m/e 353
IIb,(e) m/e 367
IIa(b) m/e 379
IIb(b) m/e 393
IIa,(a) m/e 300
IIb,(a) m/e 314
IIc,(a) m/e 316
−R•
IIa, IIb and IIc
(c) m/e 175
Metastable peak.

found by loss of side chain $\overset{+}{M}$-R for compounds IIb and IIc was at 315 indicating the presence of an extra carbon atom in the side chain. The intense fragment at m/e 314 for IIb have the composition $C_{23}H_{38}$ 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 dihydro compound IIc. 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 ($\overset{+}{M}$-R) for IIb and IIc and at m/e 357 for the acetates of IIb and IIc again established the presence of the extra carbon atom in the side chain (R) of the cyclolaudenol nucleous. 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 ($M-18-C_3H_7^{\circ}$) and m/e 367 ($M-18-C_5H_9^{\circ}$). 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 IIb and its acetate as the M-84 fragment observed in "McLafferty"[a]- rearrangement involving 24-28 double bond and the C-20 hydrogen has been found to be absent in the spectra of IIb and its acetate (J.Bergman, B.O.Lindgren and C.M.Svahn, <u>Acta.Chem. Scand.</u>, <u>19</u>, 1661, 1965).

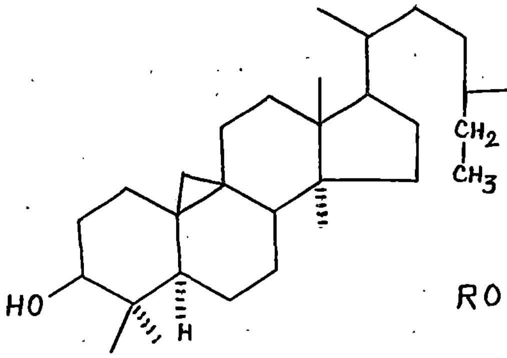

M-84

All these facts has been discussed in detail in <u>Chapter-VI</u> and established beyond doubt that the C-33 compound contains a cyclolaudenol nucle~~o~~us with the extra carbon atom in the side chain. In the known triterpenes any additional carbon atom or atoms are always attached to C-24 as either methyl, methylene, ethyl or ethylidene group. Recently Ritchie and Coworkers have isolated a new triterpene, $C_{33}H_{56}O$ 'cycloneolitsin' from <u>Neolitsea dealbata-R. Br. Merr.</u> for which a 24-24 dimethyl structure <u>IIIa</u> has been assigned on the basis of chemical and physical evidence.

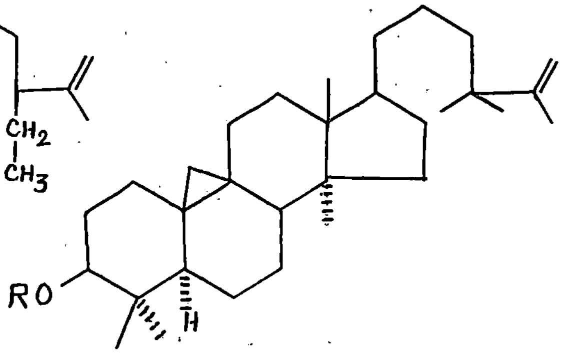


<u>III a</u>

This is also the first example of 24, 24-dimethylation on the side chain of known triterpenes. Accordingly two probable structures IIIb and IIIc can be assigned to the C-32 cyclolaudenol derivative.



IIIb.



IIIc, R=H
IIId, R=AC

After extensive PLC of the original mixture of alcohols(A), cyclolaudenol IIa and cycloneolitsol IIIc, m.p. 178-80° (6 mg) could be isolated (see experimental). The acetate of the latter IIId m.p. 165-67°, was found to be identical with an authentic sample of 'cycloneolitsol acetate' supplied by Prof. G.Ourisson, Laboratoire Associe au CNRS, Institut-de-Chimie, Strasbourg, France.

B. PART-II:

Chapter-I: describes the morphology of the fern Polypodium Amoenum Wall.

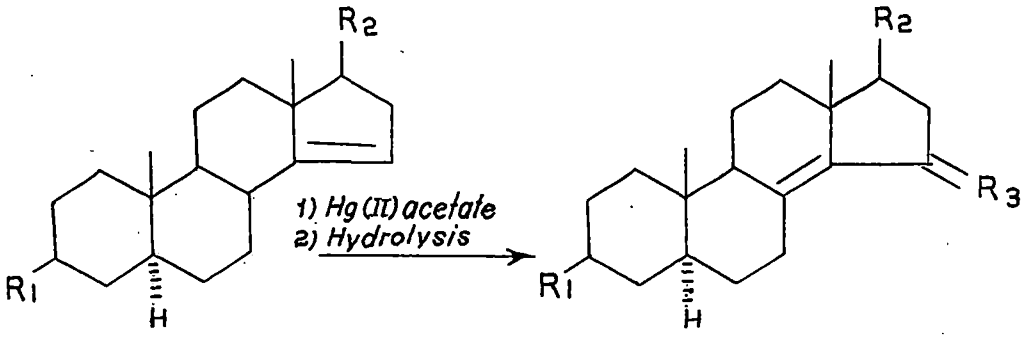
Chapter-II: deals on the investigation on the neutral part of the benzene extract of the fern Polypodium Amoenum wall and isolation of three triterpenes, $\Delta^{22(29)}$-Hopene (Diploptene), m.p. 210-11°, $(\alpha)_D$ 61°, $C_{30}H_{50}$ (M^+ 410), $\Delta^{9(11)}$-Fernene, m.p. 166-68°, $(\alpha)_D$ -16.1°, $C_{30}H_{50}$ (M^+ 410) and β-sitosterol.

C. PART-III: Chapter-I : Section A: describes Treibs reaction on olefins with Hg(II) acetate and discussions on the mechanism of the reactions:

Chapter-I: Section B: describes reaction of Hg(II) Acetate with di-and tri-substituted double bond of steroids: (E.C.Blossey and Phillip Kucinski, Chem.Commun., 2, 86, 1973).

Blossey and Kucinski have shown that Hg(II) Acetate oxidation with di-and tri-substituted double bond of certain steroids give αβ-unsaturated ketones. The following are the examples:

(XIII)

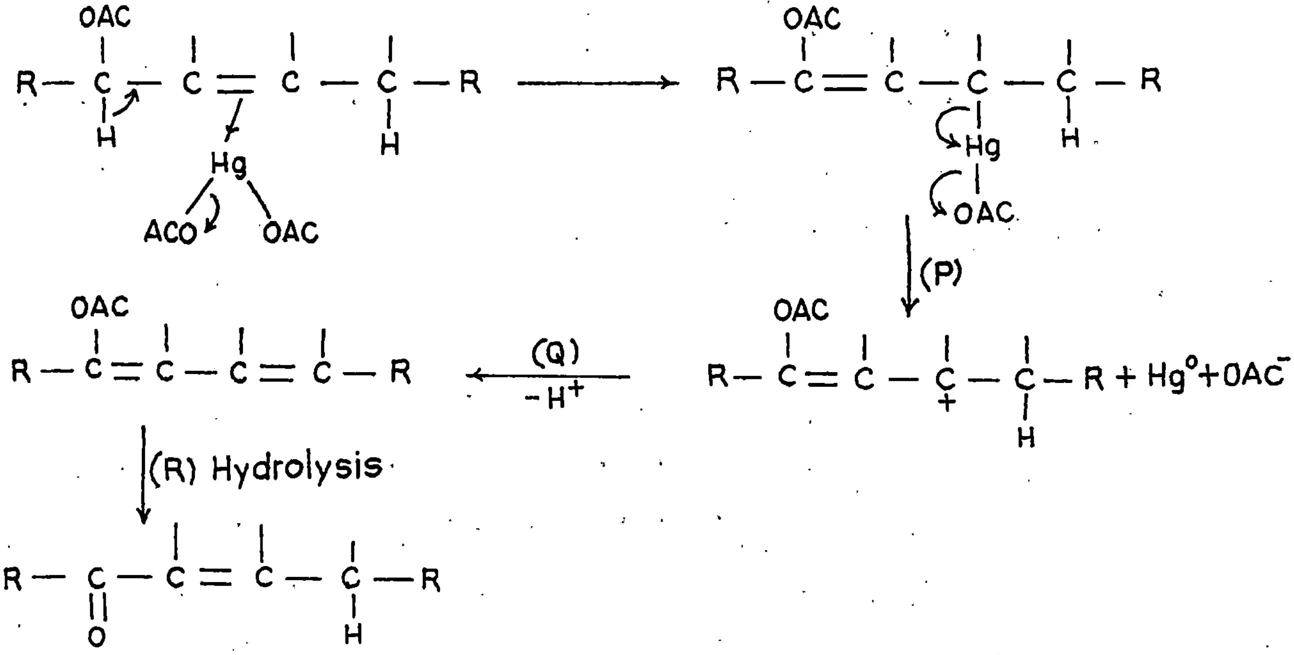


1 2

1	R1	R2
(a)	H	C_9H_{19}
(b)	$-O\cdot CO\cdot Me$	C_9H_{19}
(c)	$-O\cdot CO\cdot Me$	C_8H_{17}

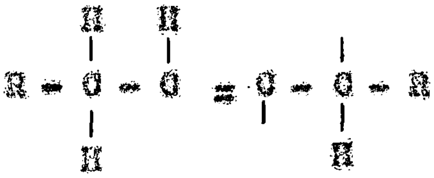
2	R1	R2	R3
(a)	H	C_9H_{19}	0
(b)	$-O\cdot CO\cdot Me$	C_9H_{19}	0
(c)	$-O\cdot CO\cdot Me$	C_8H_{17}	0

The Mechanism suggested by Blossey and Kucinski:



Chapter-II: describes the aims and object of the present work:

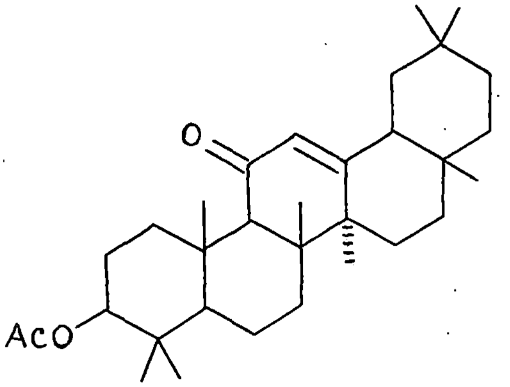
Encouraged by this report of unusual $Hg(OAc)_2$ oxidation, we planned to apply this method on triterpenes, viz. β-amyrin acetate 3, α-amyrin acetate 5 and Taraxeryl-acetate 7. Both β-and α-amyrin acetates contains the system (A), which is the same as that required, if the reactions were to take the path shown by the mechanism of Blossey and Kucinski (Chapter-I, Section-B, Page-XIII).



$$\begin{array}{ccccccccccc} & & H & & H & & & & & & \\ & & | & & | & & | & & | & & \\ R & - & C & - & C & = & C & - & C & - & R \\ & & | & & & & | & & | & & \\ & & H & & & & & & H & & \end{array}$$

(A)

If the reaction follows this path then one would expect to obtain 11-keto-β-amyrin acetate 4 and 11-keto-α-amyrin-acetate 6 as the products. (Mechanism, Part-III, Chapter-II, Page 110-111).



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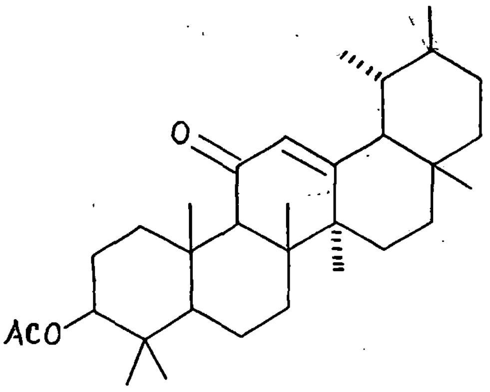

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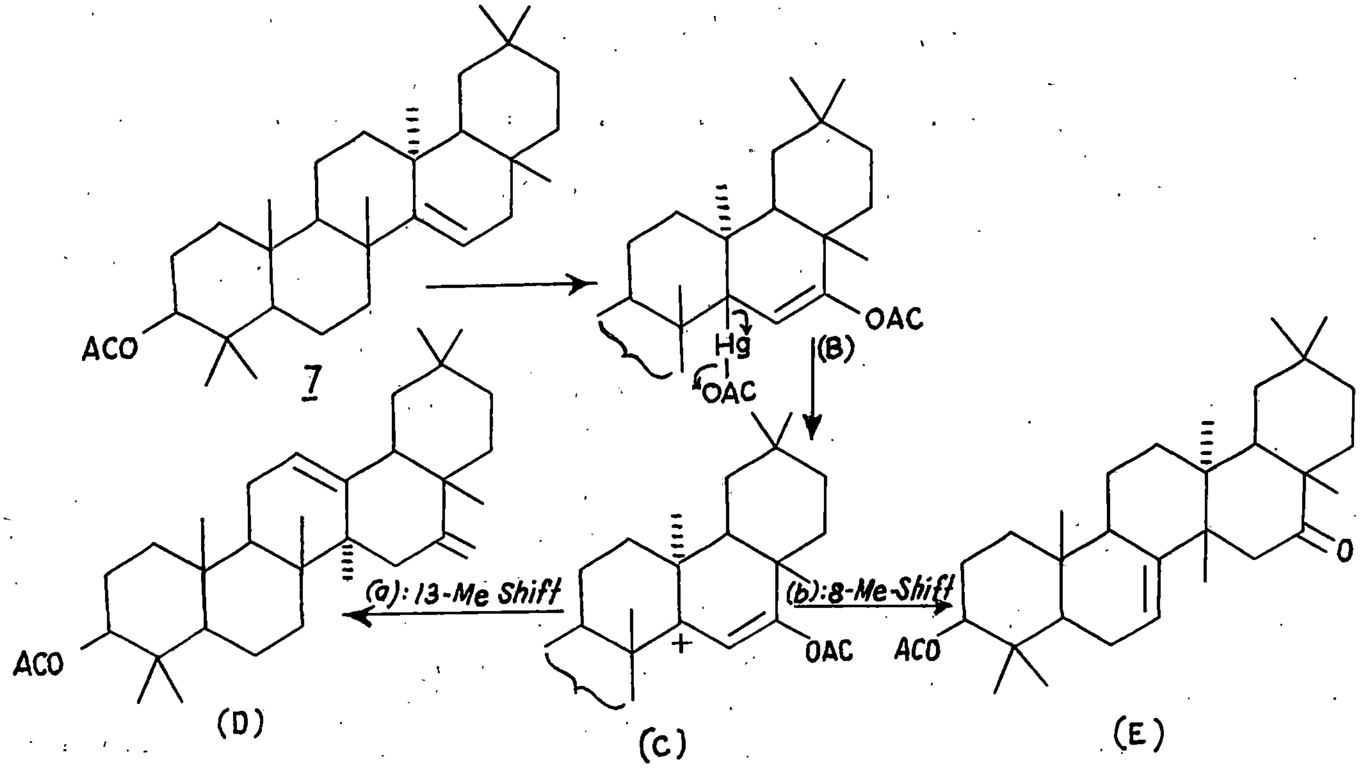
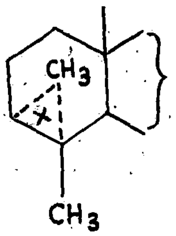
However, we thought that with Taraxeryl acetate 7, which does not contain the system (A), Blossey and Kucinski's scheme(Chapter-I, Section-B, Page- XIII)would not apply and the reaction might take a different course. There are two possibilities, conceivable for this reaction. The mechanism shown below, indicate the two paths by which the reaction may proceed. The organomercurial (B) if it is formed, through the intermediacy of the carbocation (C), may take path (a) with methyl shift from C_{13} to C_{14} to furnish a 16-keto-β-amyrin derivative (D) after hydrolysis or it may proceed by path (b) with methyl shift from C_8 to C_{14} to give a 16-keto-multiflorenol derivative (E) after hydrolysis.



<u>Chapter-III</u>: describes the results of Hg(ic)Acetate oxidation of β-amyrin acetate <u>3</u>, α-amyrin acetate <u>5</u>, Taraxeryl acetate <u>7</u> and structure elucidation of the crystalline product <u>8</u>, m.p. 193°, $(\alpha)_D$ 18.18° obtained from the reaction of $Hg(OAc)_2$ with Taraxeryl acetate <u>7</u>:

Hg(ic)Acetate oxidation of β-amyrin acetate <u>3</u>, and α-amyrin acetate <u>5</u>, in $CHCl_3$-CH_3COOH solution afforded β-amyrenonyl acetate <u>4</u> and α-amyrenonyl acetate <u>6</u> in about 15% yield after working up of the reaction, hydrolysis and reacetylation. Starting materials β-amyrin acetate <u>3</u> and α-amyrin acetate <u>5</u> were recovered from the reaction in about 70% yield. The formation of the αβ unsaturated ketones most probably follows the mechanistic path depicted by Blossey and Kucinski (Part-III, Chapter-II, Page 110-111).

However, when the same reaction was carried out on taraxeryl acetate <u>7</u>, a new hydrocarbon <u>8</u>, m.p. 193°, $(\alpha)_D$ 18.18° was isolated in 18% yield along with recovery of taraxeryl acetate <u>7</u> in about 70% yield. Elemental analysis and mass spectrometric determination established its molecular formula as $C_{30}H_{48}$ (M^+ 408). IR spectrum of the compound <u>8</u> showed the absence of bands due to hydroxyl or acetoxy group indicating that it was a hydrocarbon. The compound <u>8</u> showed no UV absorption between 220-300 nm. The molecular formula and IR spectrum clearly indicated that the acetoxy group in taraxeryl acetate 7 was eliminated as a molecule of acetic acid in the reaction with $Hg(OAc)_2$. Clearly taraxeryl acetate 7 had undergone some rearrangement via formation of the non classical carbonium ions either 9 or 10.



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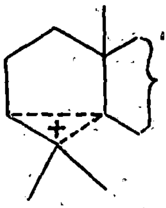

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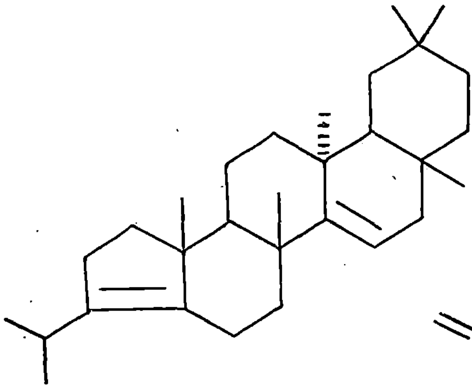
NMR spectrum (60 MHz, Fig-5, Part-III, Chapter-III) of this new hydrocarbon 8 showed signals for methyl groups between δ 0.9 -1.23 accounting for seven methyl groups (six tert.methyl groups and one secondary methyl group). The presence of one vinyl proton was indicated by the signal at δ 5.55 (multiplet). It exhibited a peak at δ 1.65 which integrated approximately for one methyl group and a multiplet at δ 2.65 which could not be integrated. Thus from the above physical evidence the following alternative structures 8, 11, 12 and 13 which may arise by rearrangement via the cations 9 and 10, may be put forward.



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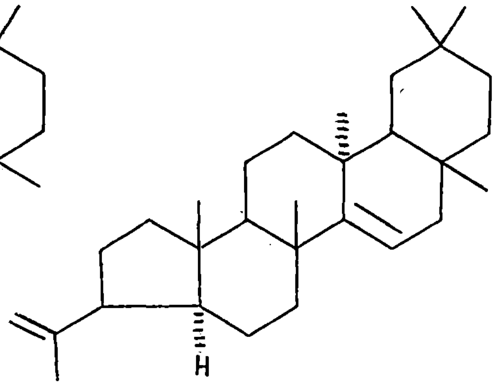

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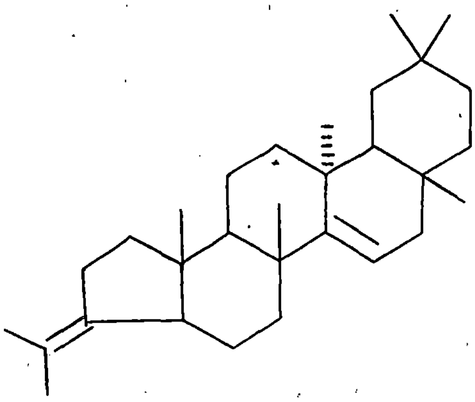

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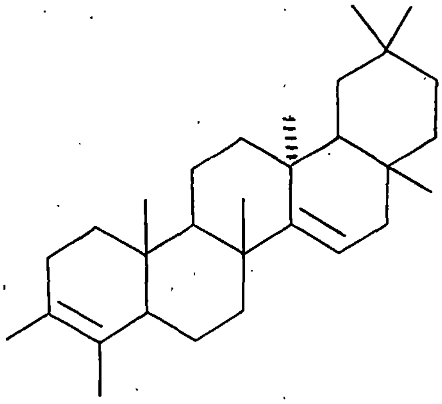

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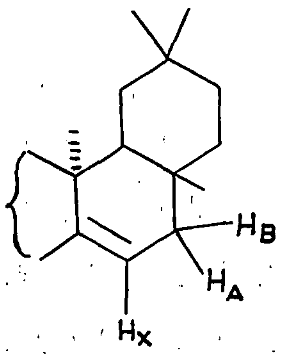
^{1}H spectrum of the compound 8 at 60 MHz, showed the presence of only one olefinic proton, presumably from the D-ring, in which it is coupled to two non equivalent protons on the adjacent carbon to give a typical X pattern of ABX group. Thus structure 11 is ruled out. Both structures 12 and 13 would be expected to show two methyl groups between δ 1.60 and δ 2.0 whereas the signal at δ 1.65 integrated to just one methyl. Thus structures 12 and 13 may also be ruled out. The multiplet centred at δ 2.65 appeared to be due to CH of an isopropyl group. Thus the above observations support structure 8. In order to obtain convincing proof we ran the spectrum at 300 MHz (Fig. 6, Part-III, Chapter-III) which showed the improved resolution at the higher field, the -CH of the isopropyl centred at δ 2.66 and the ABX protons of the D-ring . Decoupling in the methyl region gave the expected collapse of the CH- multiplet shown in Fig. 6A, (Part-III, Chapter-III), which also clearly shows the 7-line nature of the multiplet, confirming the isopropyl function. From ABX coupled spectrum and X-decoupled spectrum (Fig. 6B and Fig. 6C, Part-III, Chapter-III) NMR firmly establishes the location of the double bond in ring-D as shown in 14.



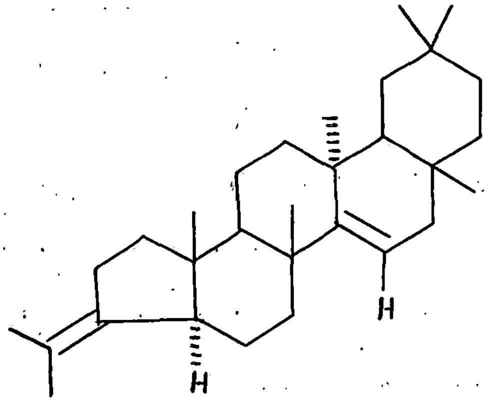
14

Thus structure 8 deduced from the 80 MHz spectrum, receives convincing support from 300 MHz spectral evidence and is the correct one.

^{13}C NMR spectrum of the compound 8 is shown in Fig-7 (Part-III, Chapter-III). The spectrum confirms the presence of the second-double bond since four olefinic carbons are seen in the down field region. It also shows that it is tetrasubstituted, since three of the lines are narrower and weaker than the fourth one which is due to the one protonated olefinic carbon in the D-ring. A plot expansion of the high field part of the spectrum shows a well resolved multitude of lines (Fig-7A, Part-III, Chapter-III) which could be integrated (Fig-7B, Part-III, Chapter-III) to give a total carbon count. Including the four olefinic carbons (down field) the total is 30, as required by the formula. Fig-7C (Part-III, Chapter-III) gives the total proton count as 48, which again firmly establishes the molecular formula as $C_{30}H_{48}$. The mass spectrum of 8 (Part-III, Chapter-III, Chart-II, Page 125-126) is also consistent with the structure proposed.

Chemical evidence in support of structure 8 assigned to the $Hg(OAc)_2$ rearrangement product:

Treatment of Taraxerol m.p. 279-81°, $(\alpha)_D$ 3.7° with PCl_5 gave a hydrocarbon 13 $C_{30}H_{48}$ m.p. 164-65°, $(\alpha)_D$ 25.8° to which we assign structure 12 (Page-XVIII, Chapter-III) from IR, NMR and mass spectral (Part-III, Chapter-III, Chart-III, Page 127-128) studies when the hydrocarbon <u>15</u> m.p. 184-85°, $(\alpha)_D$ 23.8° was



<u>15</u>

treated with Lithium-ethylenediamine at 120° under N_2 atmosphere for five hours a product was obtained, m.p. 190-91°, identical with the hydrocarbon <u>8</u> (m.m.p and Co-IR).

A probable mechanism for the formation of the hydrocarbon <u>8</u> m.p. 193°, $(\alpha)_D$ 18.18° in the reaction of Taraxeryl acetate <u>7</u> with $Hg(OAc)_2$ has been suggested as shown below:

(XXII)

$$Hg(OAC)_2 \rightleftharpoons ACO\,Hg^+ + OAC^-$$