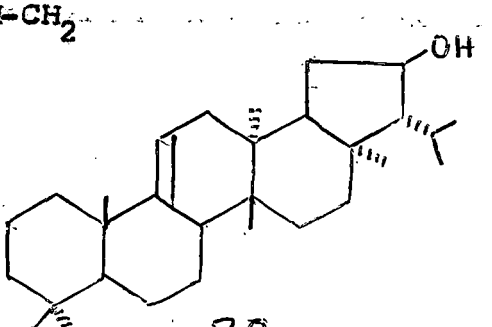
CHAPTER - V

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-spectrometric 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 δ 0.8 to 1.28, 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 again indicates that this proton is attached to a carbon atom in the system - $\overset{|}{C}H-C\underline{H}OH-CH_2$



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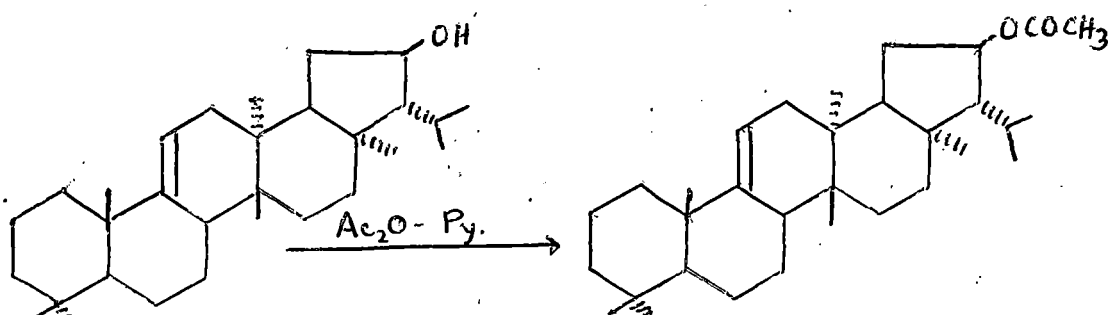
Section A : Nature of the oxygen function :

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°, $(\alpha)_D$ 38.00°, ν_{max}^{nujol} 1720 and 1240 cm^{-1} (Fig - 19). Its NMR spectrum (60 Mc, Fig-20) showed the presence of eight methyl groups between δ0.8 to 1.16, a sharp peak at δ 2.08 (3H, -O-CO-CH_3), an unresolved multiplet centered at δ 5.16 attributable to one proton [IH, -C<u>H</u>-CH(OAc)-CH_2-] and a multiplet centered at δ5.45 (IH, 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 mμ. Hence it is evident from the above data that the compound does not contain any carbonyl function.

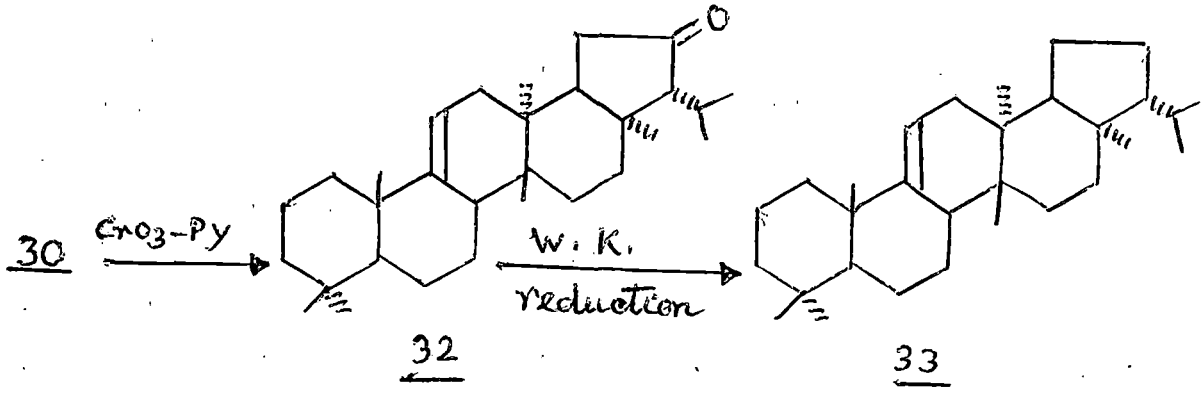


30 31

Section B : Nature of the carbon skeleton :

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

described below. Oxidation of 30 with CrO_3-pyridine complex gave a compound 32, $C_{30}H_{48}O$ (M^+ 424, Fig-21), m.p. 174-75°, ν_{max}^{nujol} 1725 (five membered ring ketone, Fig-22), NMR signals (Figs-23A and 23B) at δ 5.38 (1H, vinyl proton) and peaks between δ 0.8 to 1.04 for eight methyl groups. Wolff-Kishner reduction[37] of the ketone 32 furnished a hydrocarbon 33 (M^+ 410, Fig-24) m.p. 169-71°, $(\alpha)_D$ - 13.04° which was found to be identical with an authentic sample of fern-9(11)-ene[25] (m.m.p. no depression, superimposable IR and identical mass fragmentation pattern) supplied by Prof. Berti of University of Pisa, Italy.



32 33

Section C : Position of the hydroxyl function :

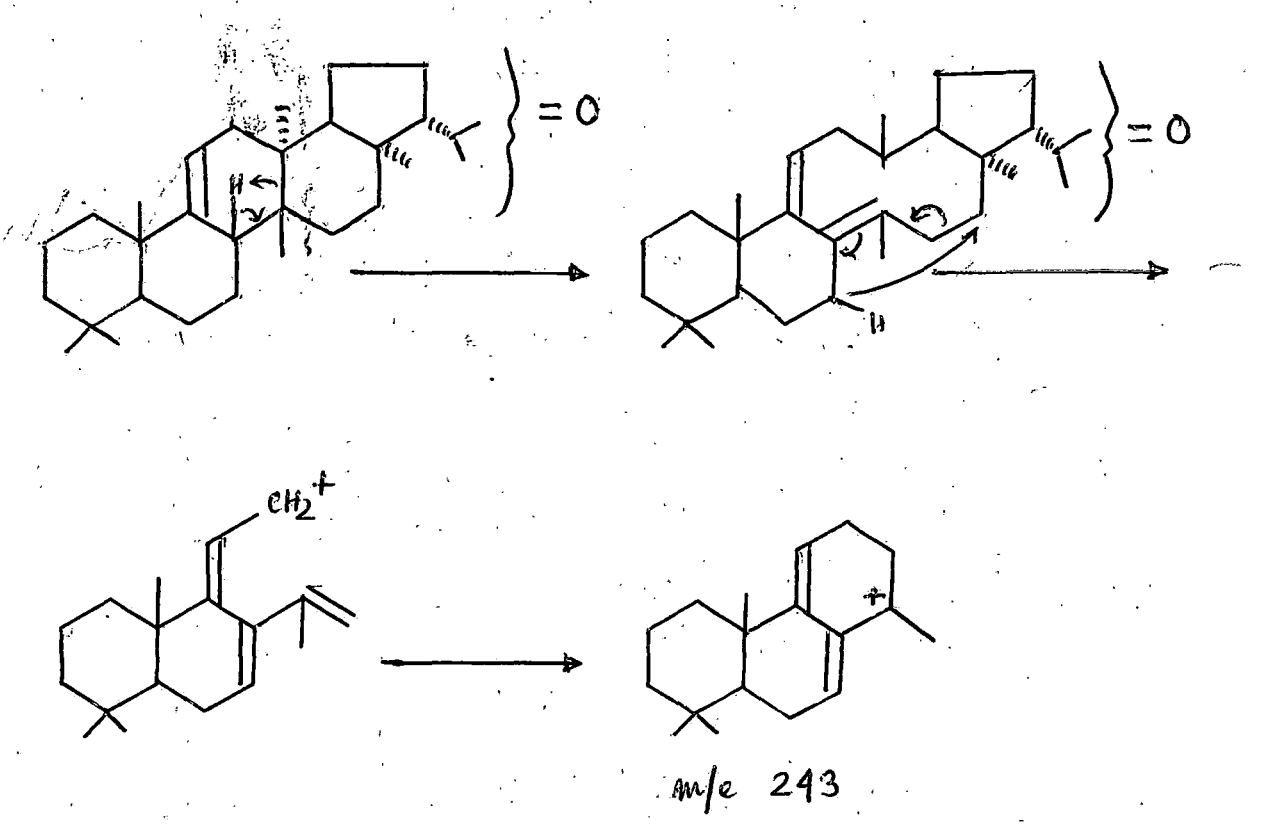
The position of the oxygen function could not be ascertained but a tentative proposal has been advanced on the basis of available chemical and physical evidence which are described below.

The ketone 32 did not respond to Zimmerman's colour test for 3-keto group[38] 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.

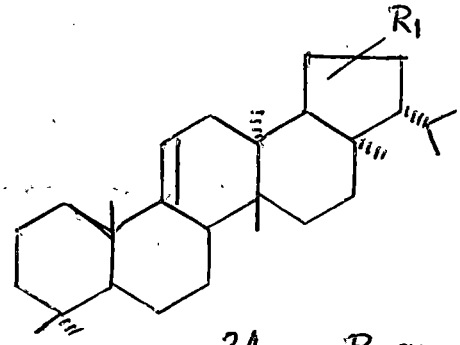
Mass spectrum (Fig-21) of the ketone showed the molecular ion peak at M^+ 424 in addition to peaks at m/e 409 ($M^+ - 15$), m/e 243 and m/e 231 characteristic of a $\Delta^{9(11)}$- fernene system. The mass spectrum (Fig - 18) of the monoacetate 31 was more informative and exhibited a mass fragmentation 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



Drieding models with C-15, and C-16 ketone in the fern-9(11)-ene nucleus sęeparately 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 34 and 35 for the new triterpene and the corresponding ketone obtained from its oxidation.

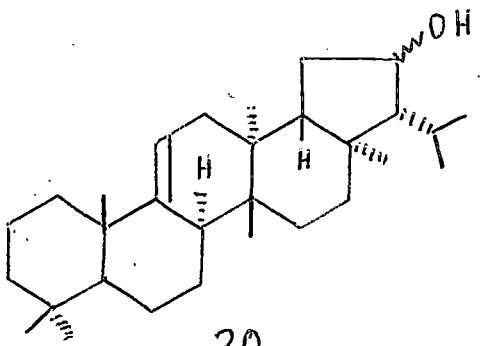


34 , $R_1 = \langle^{H}_{OH}$

35 , $R_1 = O$

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 12α- and 12β- 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 21β and 19α- and 19β- protons. Thus we propose a tentative structure 30 for Polypodinol B.



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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 facilitites. However, further work is in progress to finally confirm its structure.

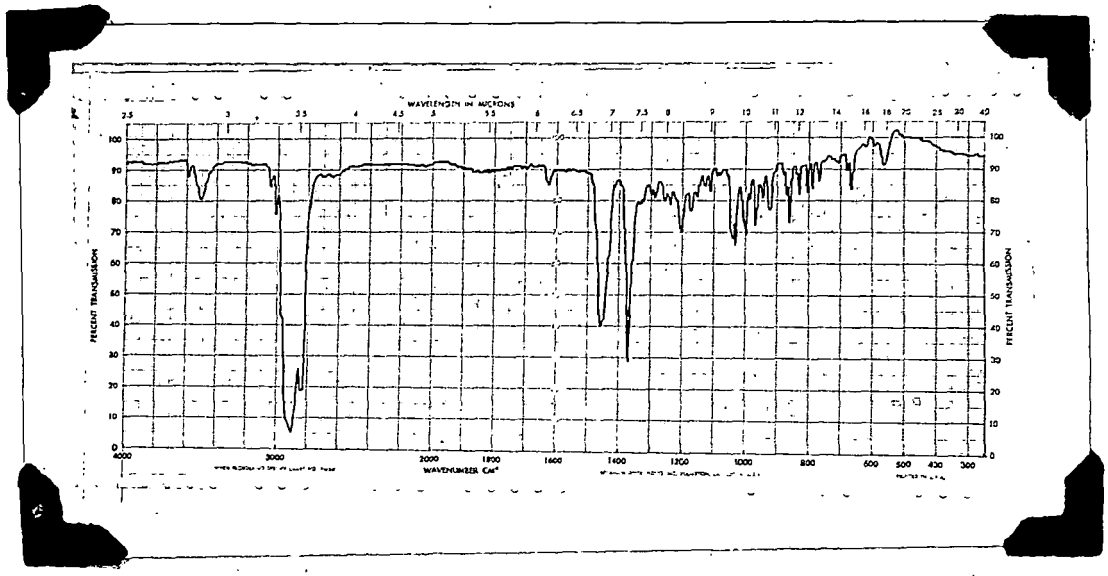

Fig. 16 : IR spectrum of Polypodinol B 30

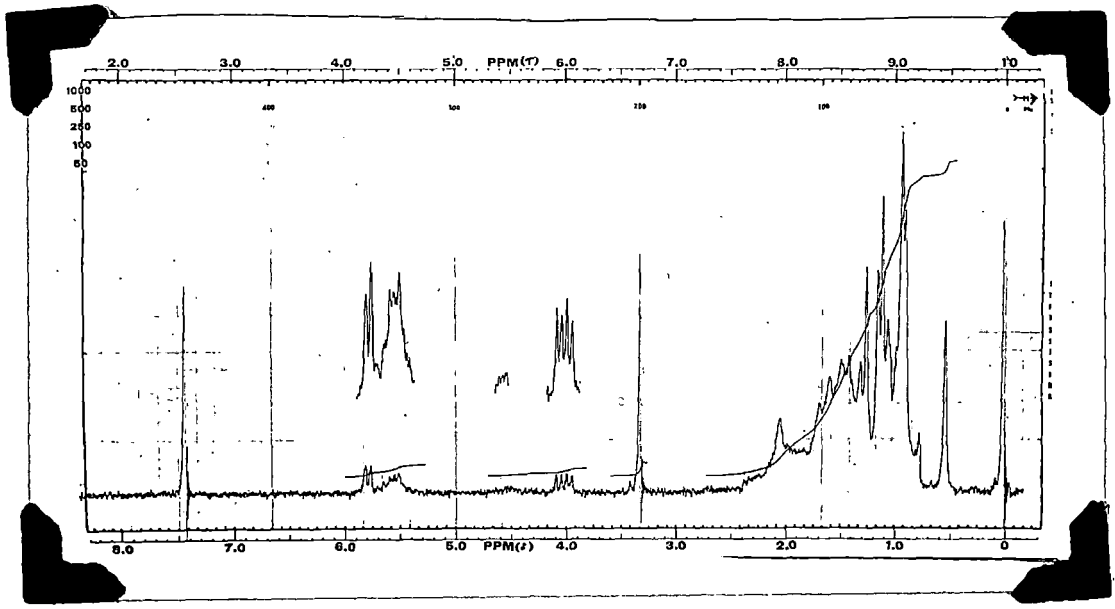

Fig. 17 : NMR spectrum of Polypodinol B 30

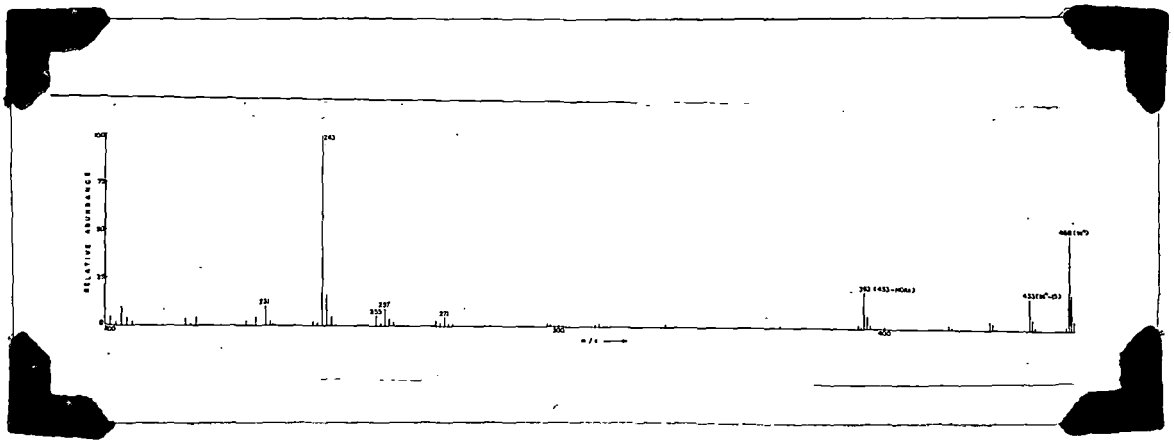

Fig. 18 : Mass spectrum of the acetate 31

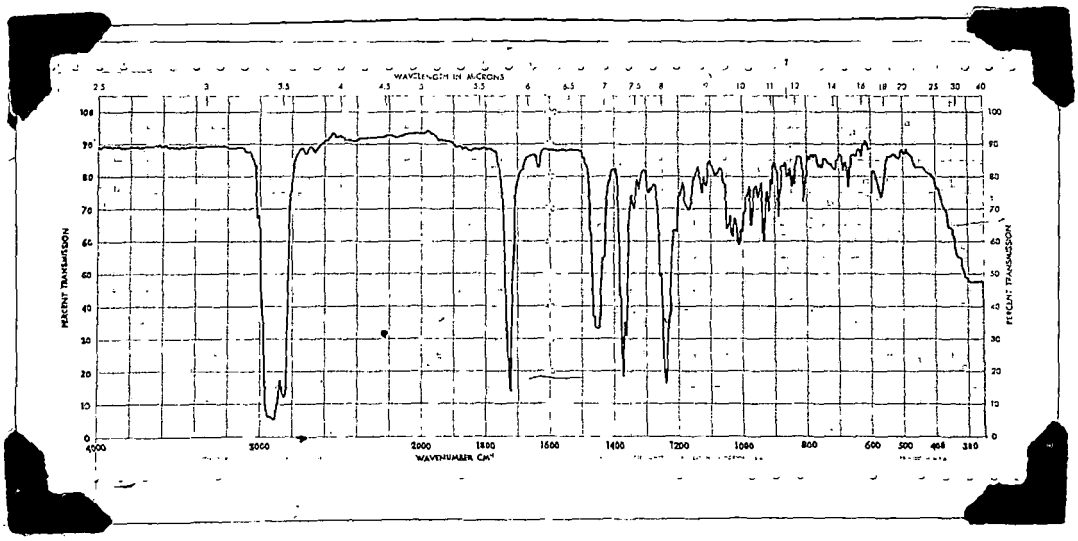

Fig. 19 : IR spectrum of the acetate 31

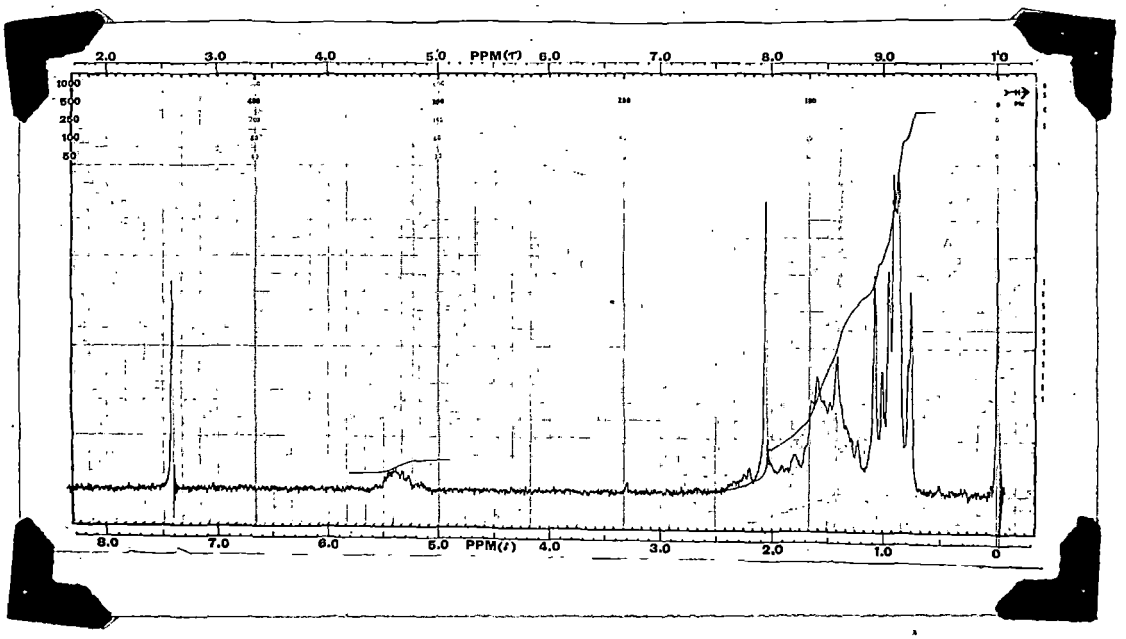

Fig. 20 : NMR spectrum of the acetate 31

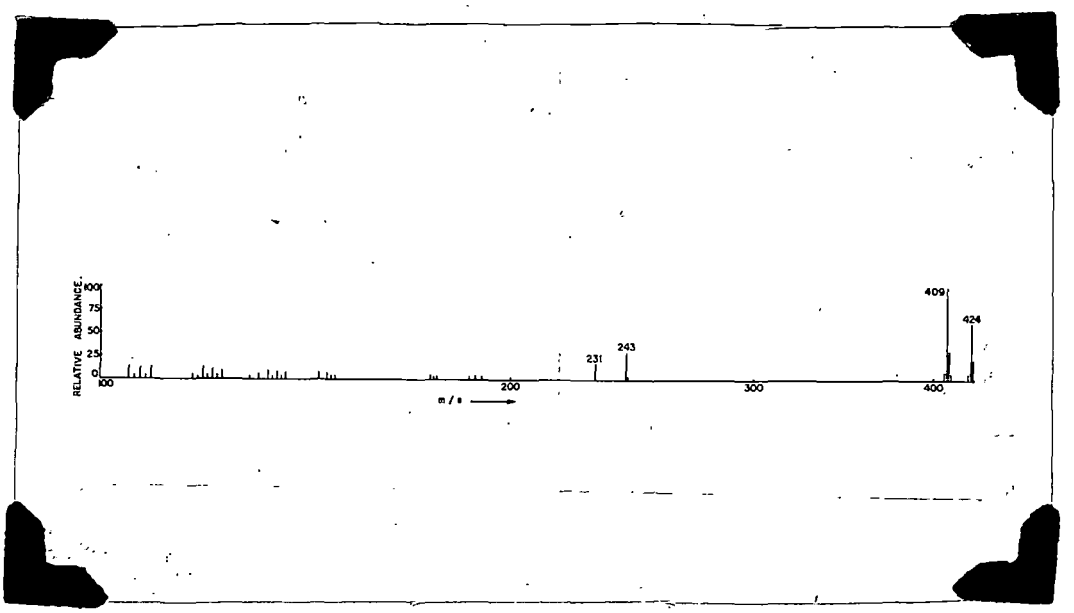

Fig. 21 : Mass spectrum of the Ketone 32

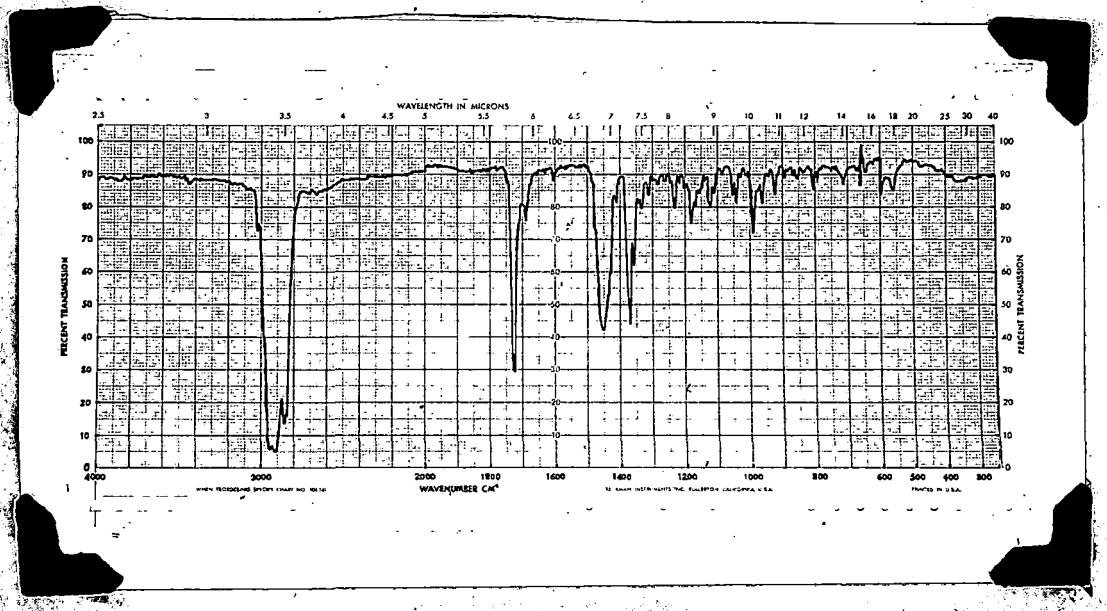

Fig. 22 : IR spectrum of the Ketone 32

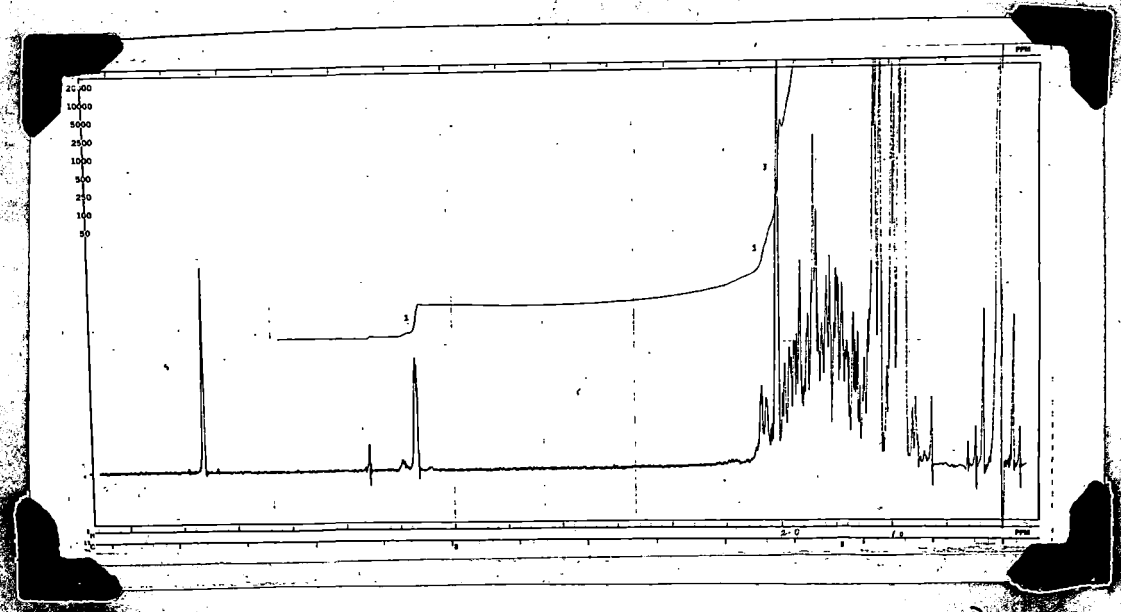

Fig. 23A : NMR spectrum of the Ketone 32 (300 MHz)

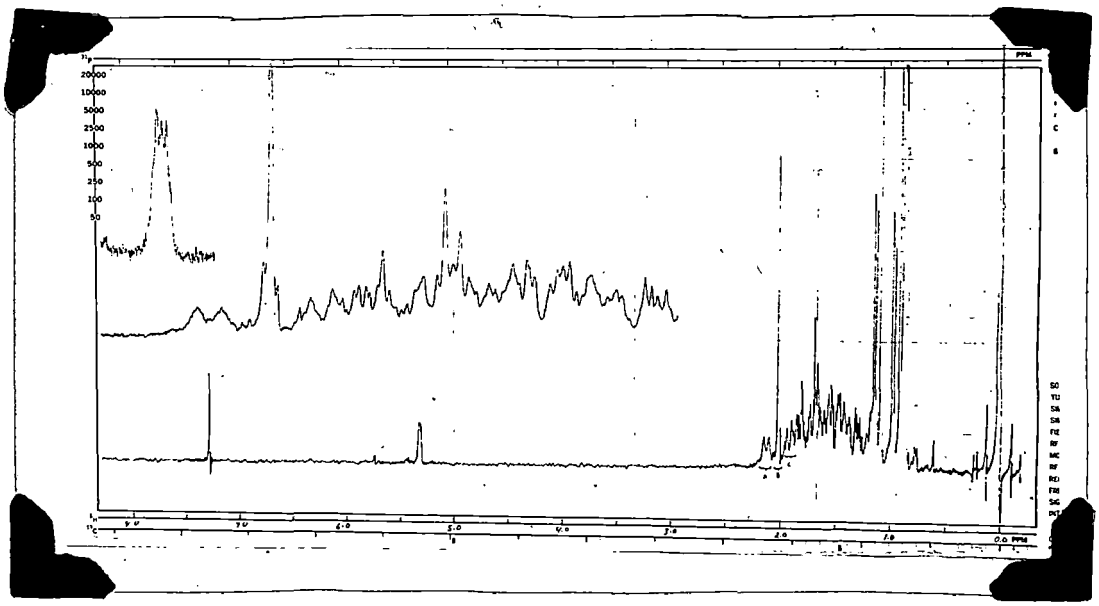

Fig. 23B : NMR spectrum of the Ketone 32 (300 MHz)

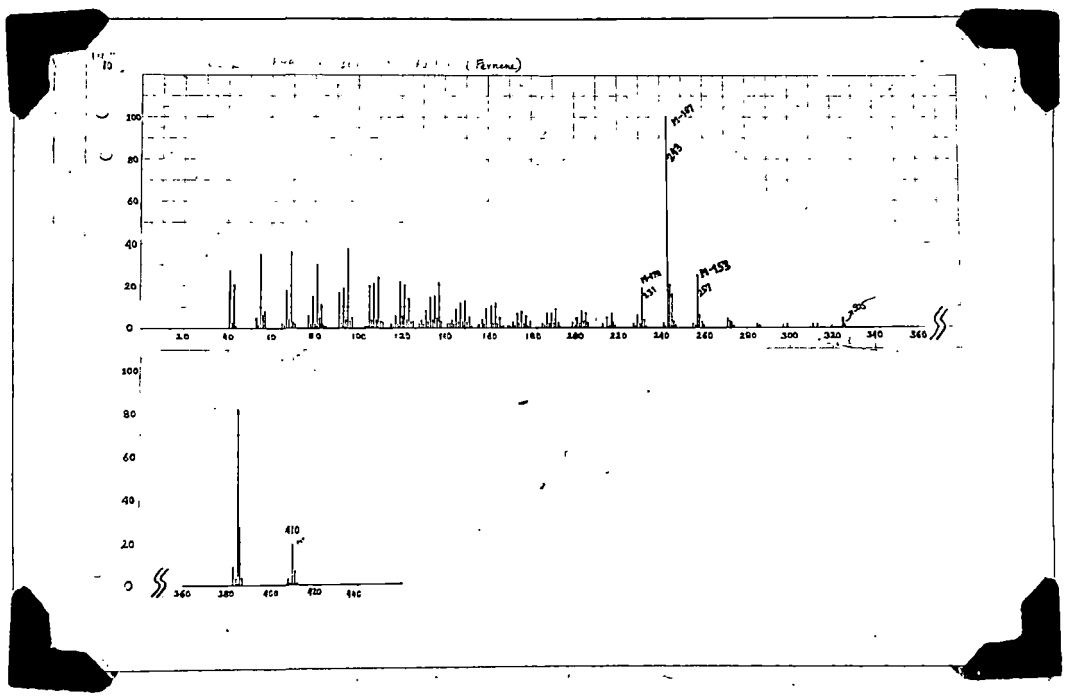

Fig. 24 : Mass spectrum of fern-9(11)-ene 33

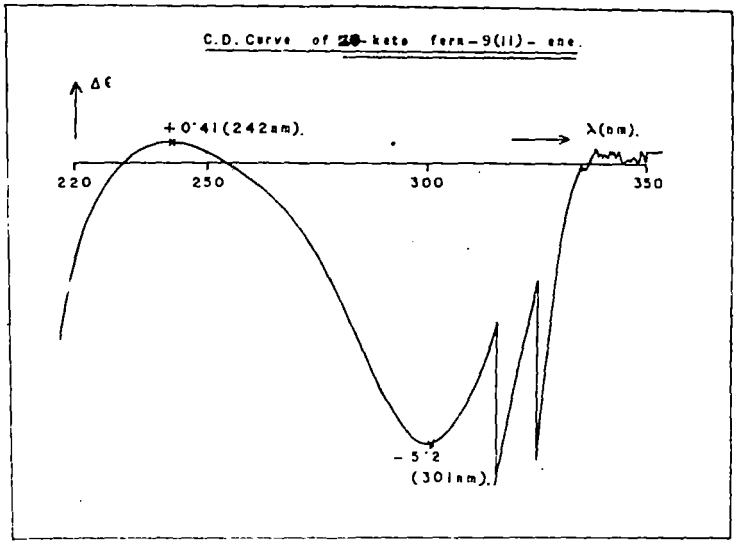

Fig. 25 : C. D. of the Ketone 32

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°, $(\alpha)_D$ 28.57°.

Found : C, 84,47; H = 11.80%

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

IR : 3590, 3510 cm^{-1} Fig - 16

Mass spectrum : M^+ 426

NMR spectrum (60 Me) : δ 0.8-1.28 (8 methyl groups)

δ 5.40 (multiplet, IH, trisubstituted double bond)

δ 4.36 (multiplet, $-\overset{|}{C}H-C\underline{H}OH-CH_2$)

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 50 ml each	Residue on evaporation.
Petroleum	1-3	Solid (170 mg) 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°, $(\alpha)_D$ 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) : δ 0.8 - 1.16 (8 methyl groups)

δ 2.08 (3H, -O-CO-C<u>H</u>$_3$)

δ 5.16 [multiplet, -CH-C<u>H</u>(OAc)-CH_2-]

δ 5.45 (multiplet, IH, 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 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (130 mg) 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 cm^{-1} (five membered ring ketone) Fig -22

Mass spectrum : M^+ 424 Fig -21

NMR spectrum : δ 0.8 - 1.04 (8 methyl groups)

(300 MHz)

δ 5.38 (1H, vinyl proton) Figs-23A and 23B

Wolff - Kishner reduction[37] 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 50 ml each	Residue on evaporation
Petroleum	1-3	Solid (145 mg) 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 fern-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 refulx 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 methanol gave crystals, m.p. 207-8° which was found to be identical with the starting compound (m.m.p. and IR)