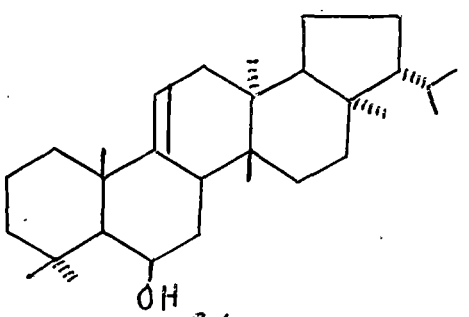
CHAPTER-IV

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

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°, $(\alpha)_D$ - 89.13°. 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 cm^{-1} (OH, Fig - 3) and its NMR spectrum (Fig - 4) 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 4.40 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 - $\overset{|}{C}H-C\underline{H}OH-CH_2-$



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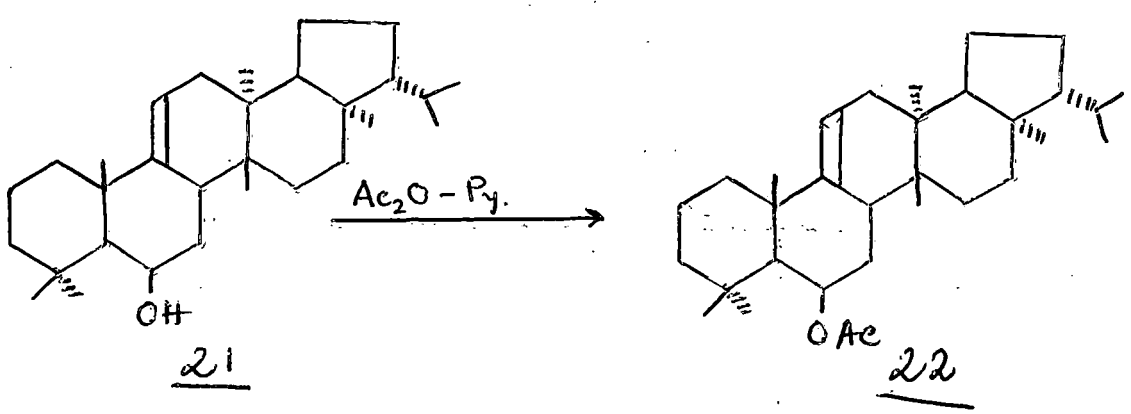
Section A : Nature of the oxygen function :

On treatment with acetic anhydride - pyridine, the compound afforded a monoacetate 22, m.p. 203-4°, $(\alpha)_D$ -83.72°. Elemental analysis and mass-spectrometic 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 cm^{-1} (Fig-6) but no hydroxyl peak in the region 3100 - 3650 cm^{-1}. It did not show any UV absorption in the region between 200-300 mμ. NMR spectrum (60Mc, Fig - 7) of the compound showed the presence of eight methyl groups between δ 0.8 to 1.16, a sharp peak at δ 2.00 (3H, - O-CO$\underline{CH}_3$), a multiplet at δ 5.40 (1H, vinyl proton) and an unresolved multiplet centered at δ 5.20 [1H, - C$\underline{H}$ (OAc)-CH_2-]. Hence the oxygen function is present as a hydroxyl group which is acetylable. The acetate also did not show any UV absorption in the region 220-300 mμ.



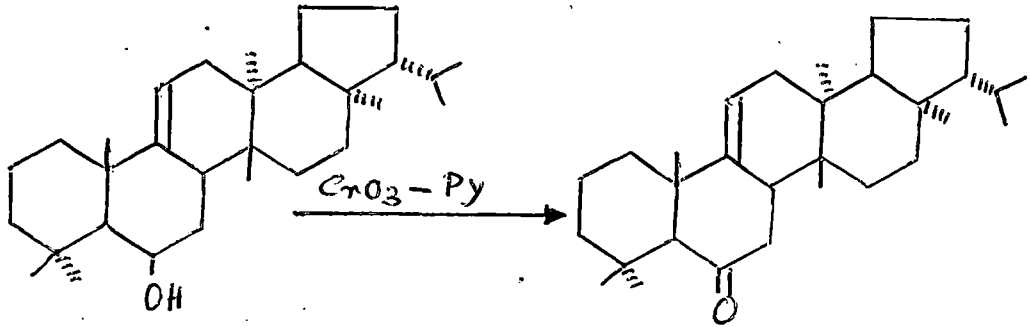
21 22

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°, $(\alpha)_D$ 16.66°. Elemental analysis and mass - spectrometric determination suggested the



21 23

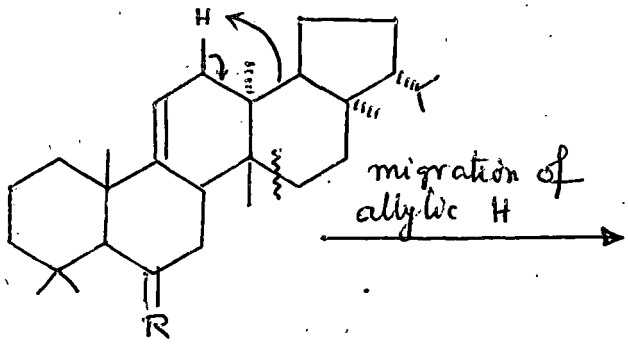
molecular formula as $C_{30}H_{48}O$ (M^+ 424, Fig - 8). The ketone 23 had IR peak (nujol) at 1720 cm^{-1} (six membered ring ketone Fig - 9). Its NMR spectrum (Figs - 10A and 10B) showed signals at δ 5.40 (1H, vinyl proton) and peaks between δ 0.8 to 1.04 for eight methyl groups. Wolff - Kishner reduction of the ketone 23 under anhydrous condition furnished a hydrocarbon 24, m.p. 168-69°, $(\alpha)_D$ - 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 I.R 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 Δ9(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.

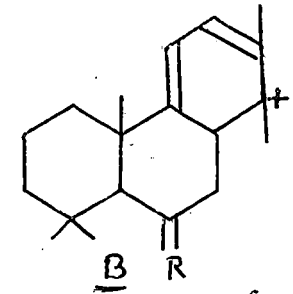
Chart - II



21 , R = OH (β) ; H

22 , R = OAc (β) ; H

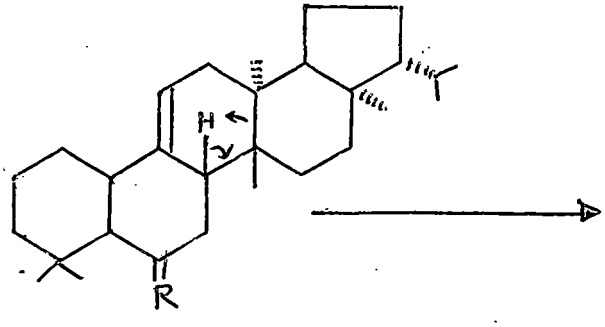
23 , R = O

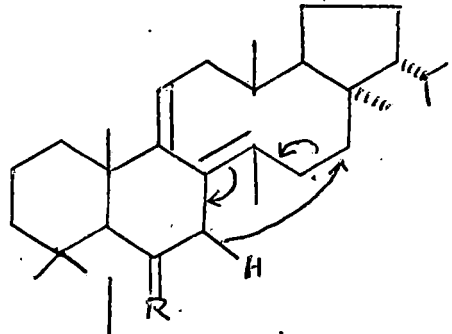


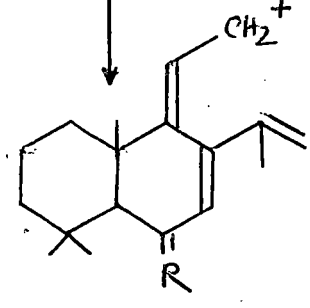
m/e 271 B (R=O)

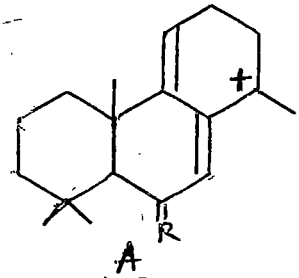
m/e 255 B (B - AcOH)

R = OAc, (H)







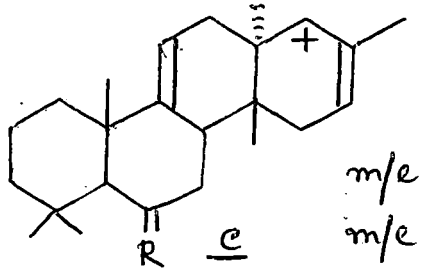


A

m/e 257 (R=O)

m/e 241 (A - AcOH)

R = OAc, (H)



C

m/e 339 (R=O)

m/e 323 (C - AcOH)

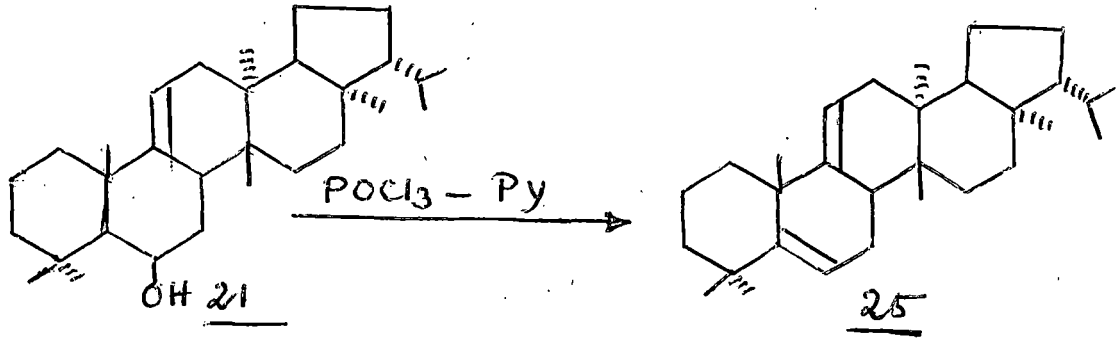
R = OAc, (H)

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 $\Delta E + 3.39$ 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 β-γ unsaturated keto chromophore[32a-32e] would most probably give rise to a negative Cotton effect.

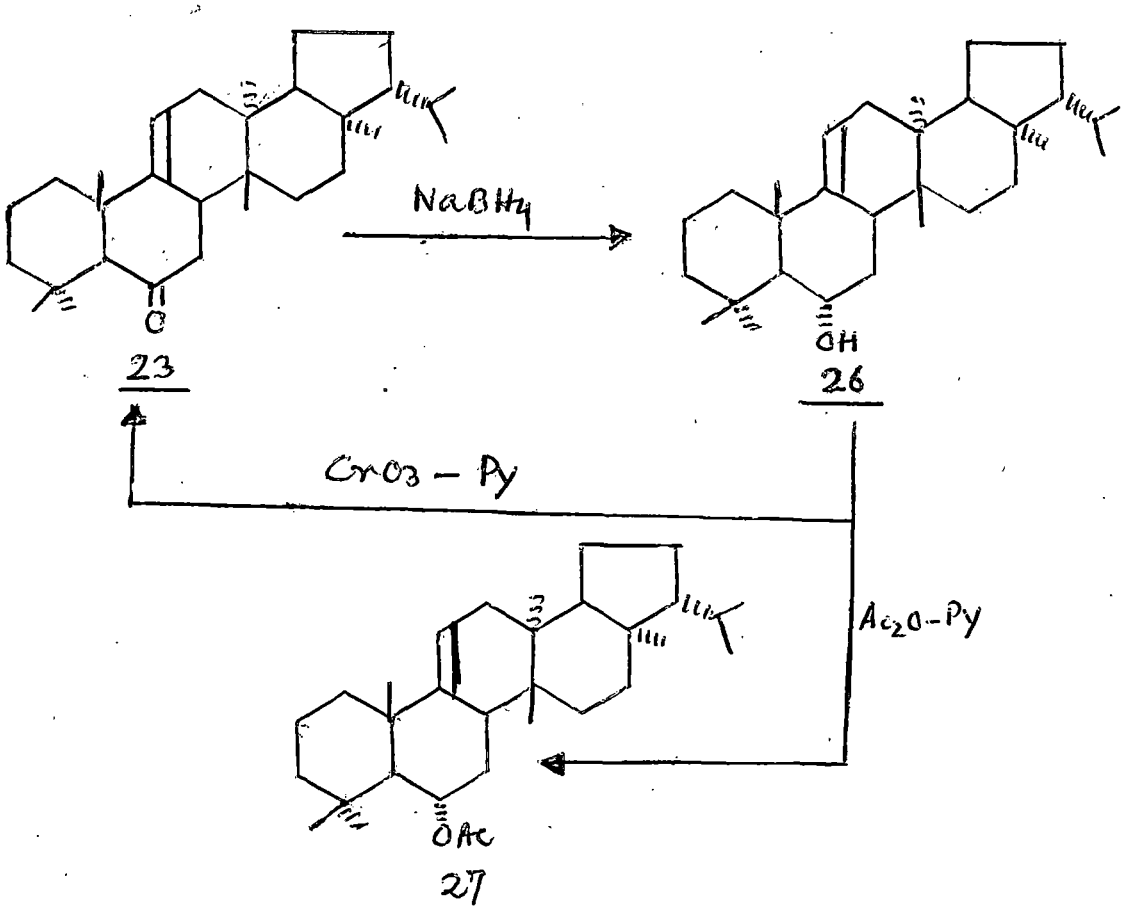
Thus from the foregoing physical evidence it may be inferred that in compound 21 the hydroxyl group is present at position C-6.

Section D : Stereochemistry of the hydroxyl function :

Dehydration of 21 with $POCl_3$- pyridine afforded a single product 25 (TLC single spot), m.p. 157-58°, no UV absorption above 220 mμ. and no-OH absorption in IR spectrum (Fig - 13).

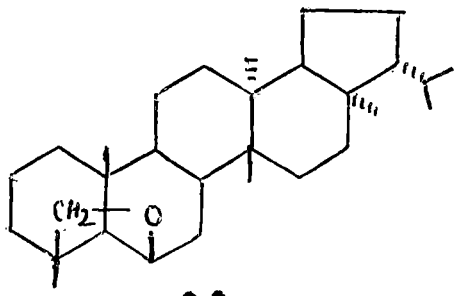


NMR spectrum of this compound (Fig - 14) showed the presence of two vinyl protons (two multiplets, one centered at δ 5.28 and the other at δ 5.44). Compound 25 on acid isomerisation afforded an oily compound which did not show any UV absorption above 220 mμ. The formation of a single product during dehydration indicated the β - 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° (NMR : Fig - 15); monoacetate 27, m.p. 177-79°. Oxidation of 26 gave back the original ketone. This also established that during the reduction process 6 - ketogroup was converted to the 6α - equatoral hydroxy compound 26.



The NMR spectral data also indicated the axial nature of hydroxyl group in 21[32]. The signal for C_{10} - CH_3 group in 21 appeared at considerably low field (δ 1.28) and the corresponding signal in the acetate 22 was shifted to δ 1.16. The observation is indicative of 1,3 - diaxial relationship between C_{10}- CH_3 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_4 with finely divided HgO and iodine in presence of high pressure mercury lamp according to the method of Barton et al[33]. But we found that in different runs of the same experiment we



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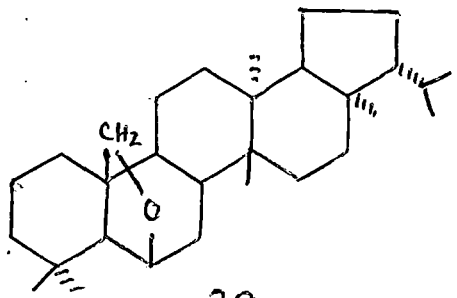

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could isolate only the dehydration product 25, m.p. 157-58°. This experiment, however, again indirectly suggests the axial nature of the hydroxyl group at C-6, which readily undergoes elimination.

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[34] and is reminiscent of the similar behavour exhibited by $\Delta^{9(11)}$ pentacyclic triterpenes like arborenyl[35] and fernenyl acetates[36].

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 δ 5.3 which is assigned to the olefenic 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), desplays 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 δ 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 α- and β- 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 Eu (FoD)$_3$] . However some discussions are necessary regarding peaks A and B. It appears that, 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 7β- 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 Eu (FoD)$_3$. Although, all the foregoing assignments can reasonably explain the assigned structure 23, we believe that to arive at a firm conclusion from NMR studies a considerable experimental work viz decoupling, changing solvents would be required. Moreover, observations after complete deuteration might offer additional clues for identification of different protons in the complex between δ 1.0 to δ 2.0.

There is, however, one striking point which seems to rule out the 7-keto structure and thus provide an indirect proof for the 6-keto structure 21. If it were the 7-keto compound, where the 8α-proton is flanked by the 7-keto compound and the

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

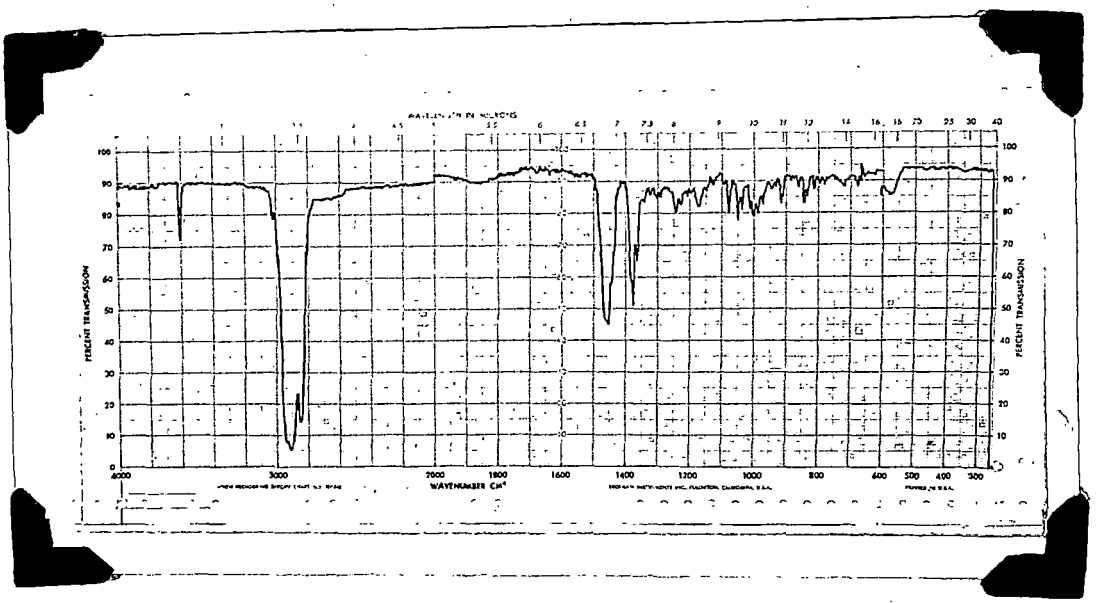

Fig. 3 : IR spectrum of Polypodinol A 21

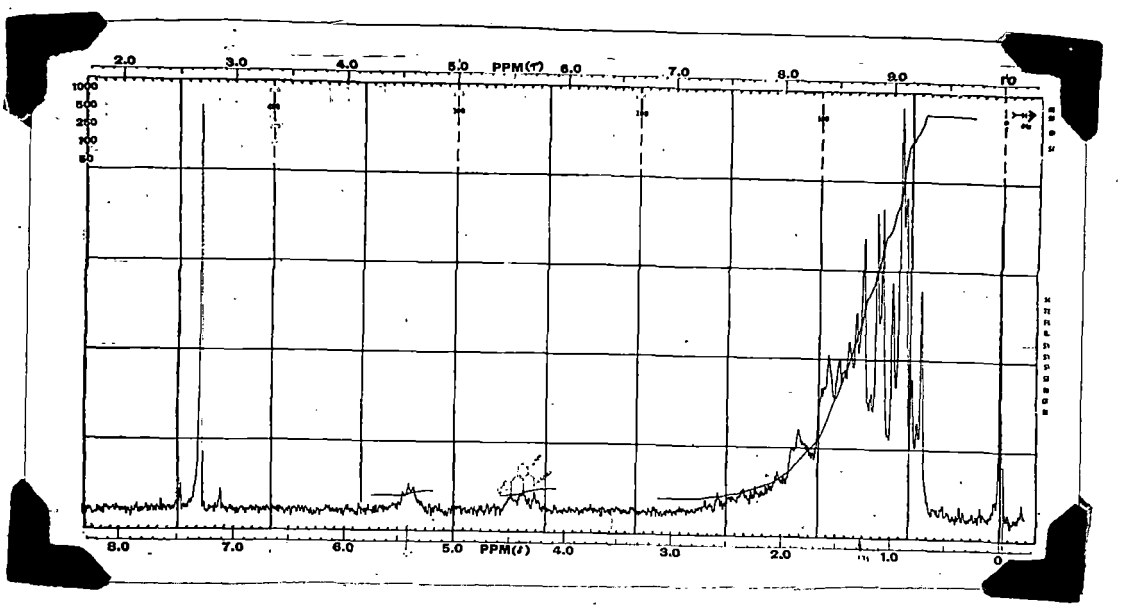

Fig. 4 : NMR spectrum of Polypodinol A 21

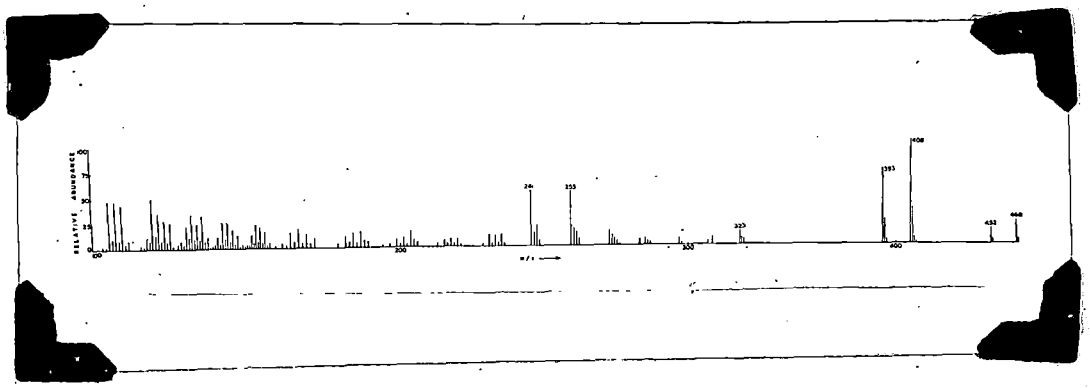

Fig. 5 : Mass spectrum of the acetate 22

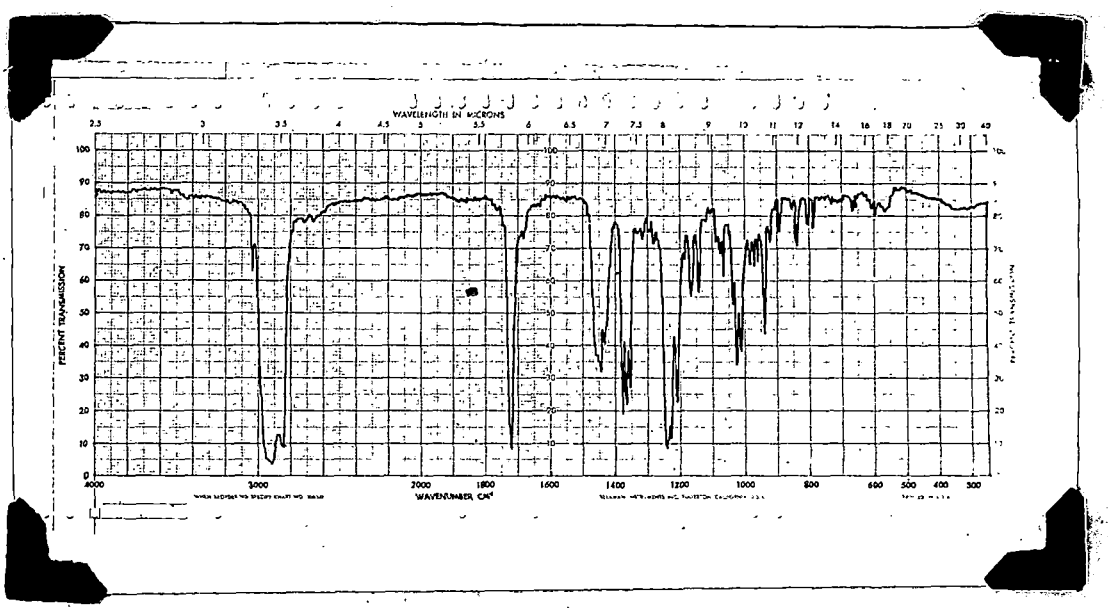

Fig. 6 : IR spectrum of the acetate 22

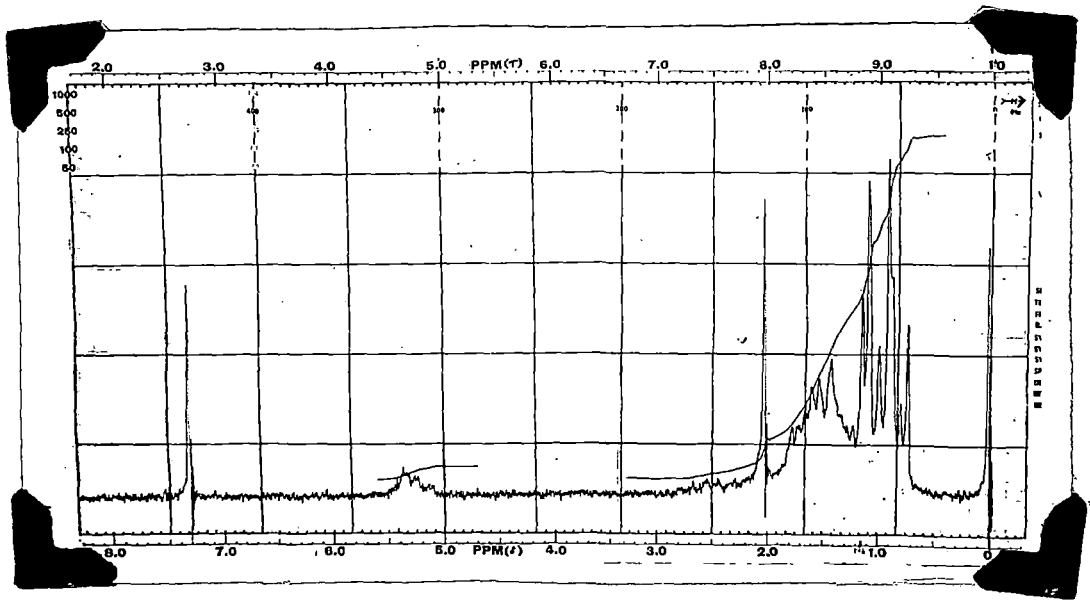

Fig. 7 : NMR spectrum of the acetate 22

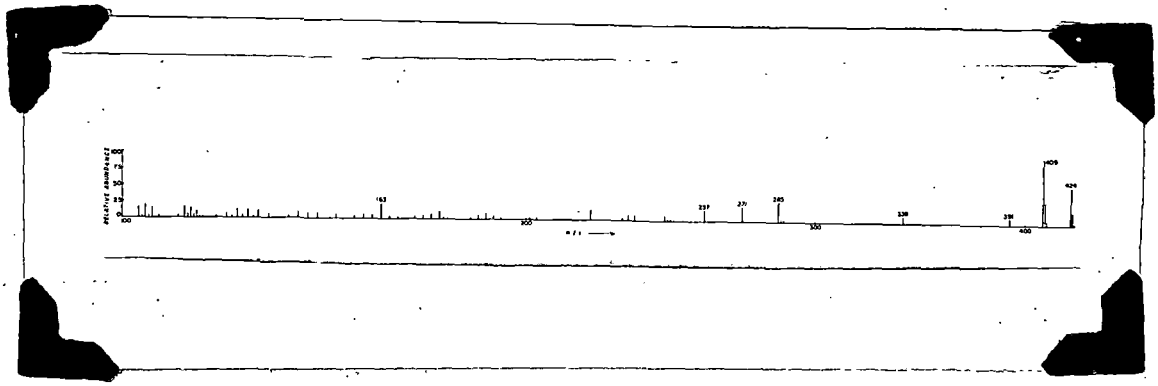

Fig. 8 : Mass spectrum of the ketone 23

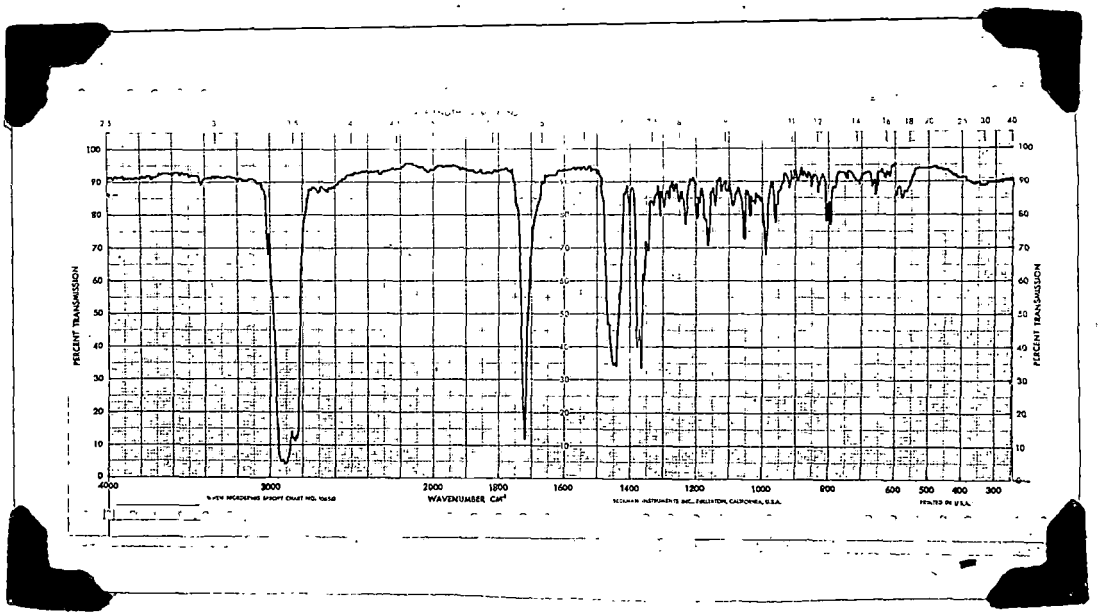

Fig. 9 : IR spectrum of the Ketone 23

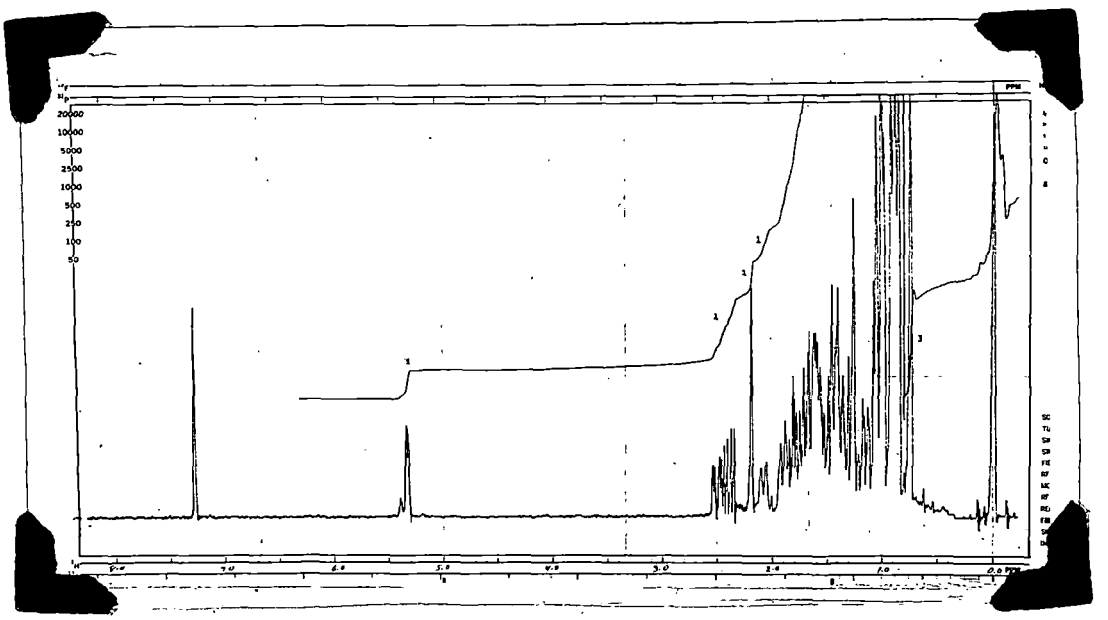

Fig. 10A : NMR spectrum of the Ketone 23 (300 MHz)

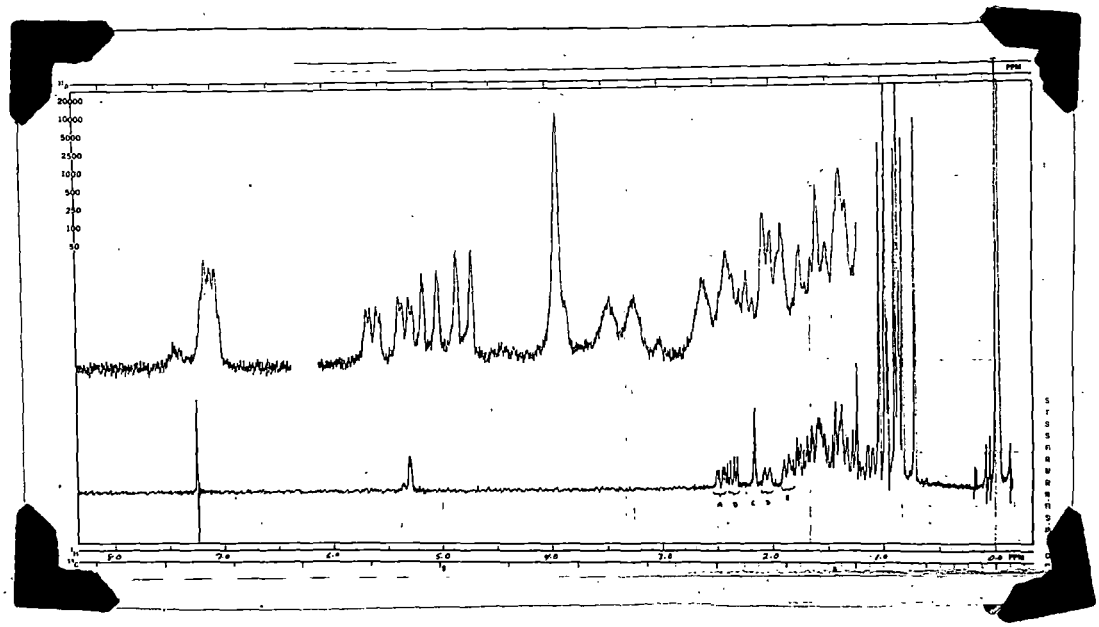

Fig. 10B : NMR spectrum of the Ketone 23 (300 MHz)

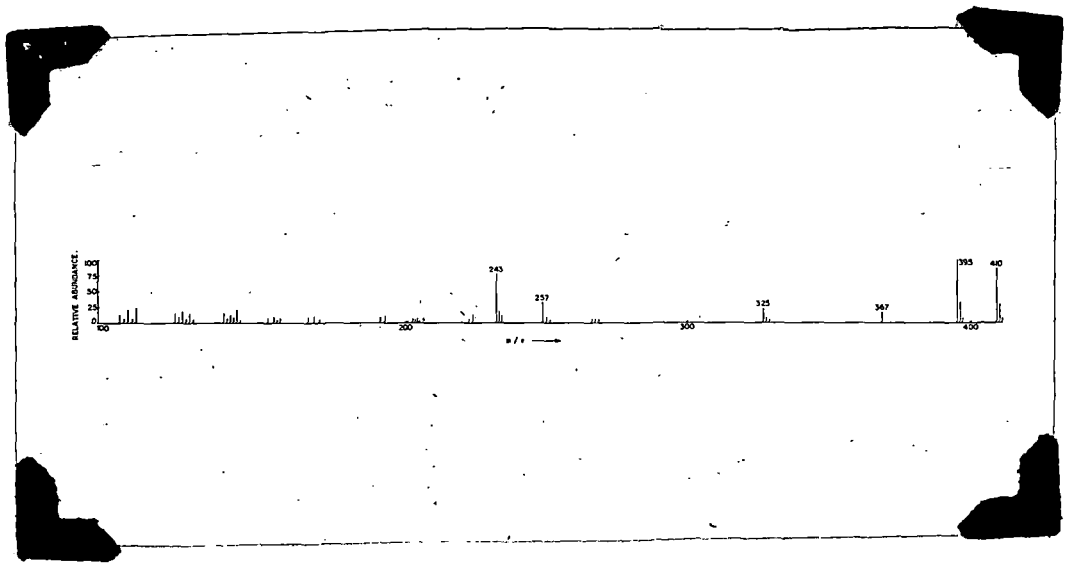

Fig. 11 : Mass spectrum of fern-9(11)-ene 24

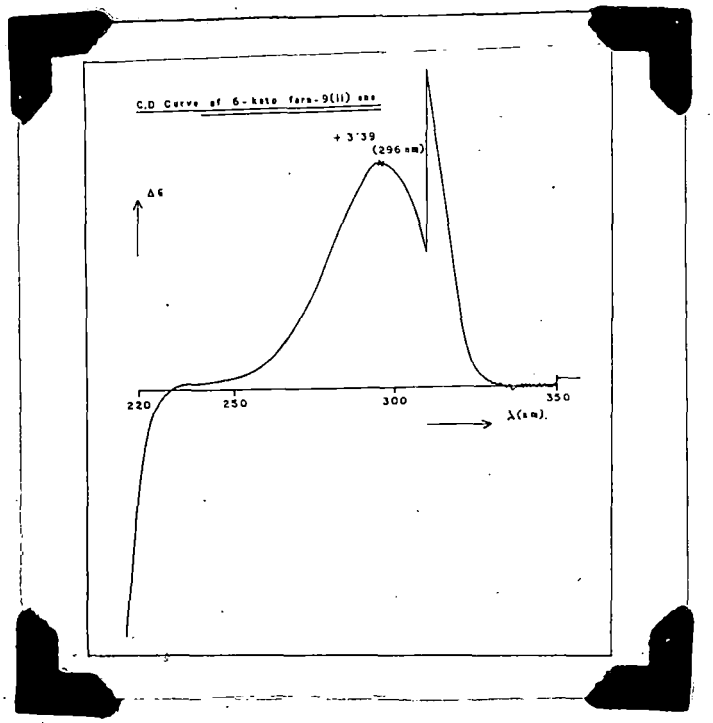

Fig. 12 : C. D. of the Ketone 23

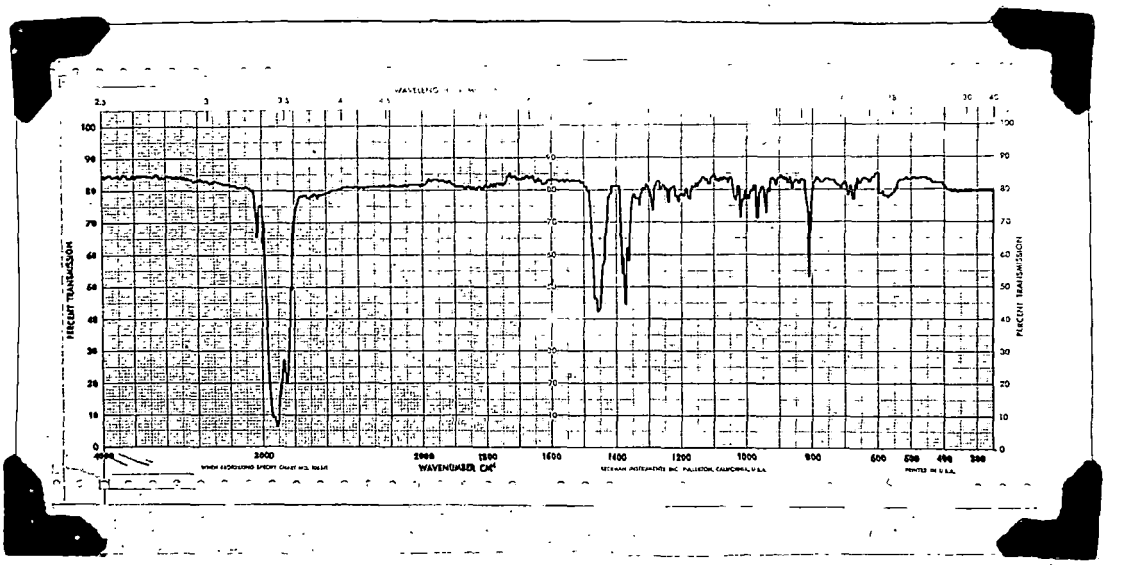

Fig. 13 : IR spectrum of the dehydrated product 25

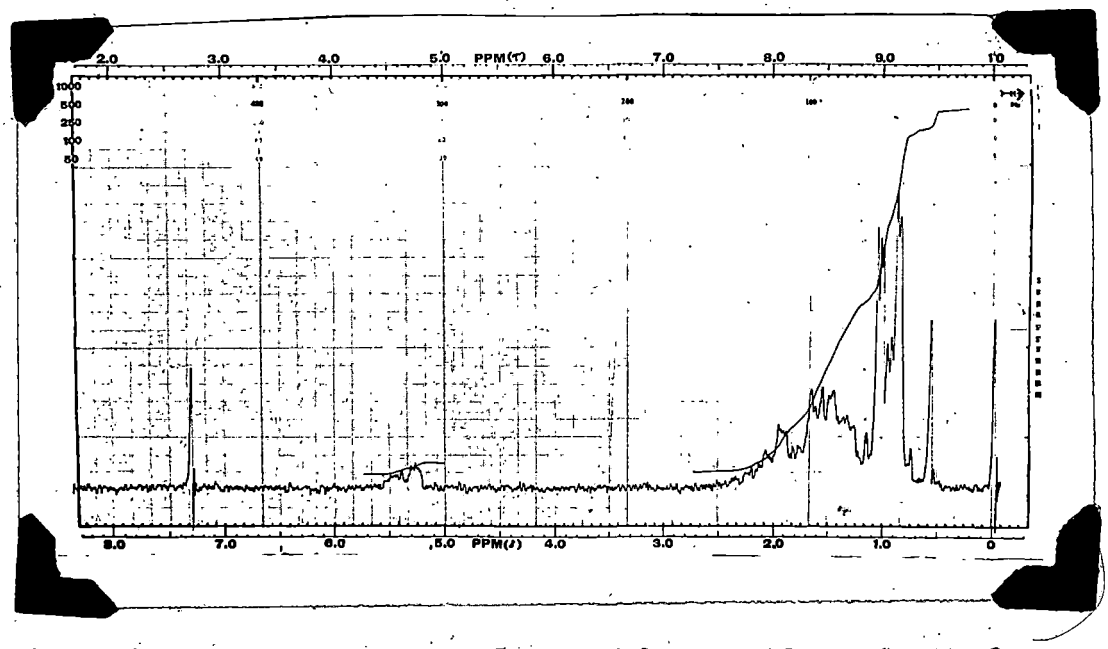

Fig. 14 : NMR spectrum of the dehydrated product 25

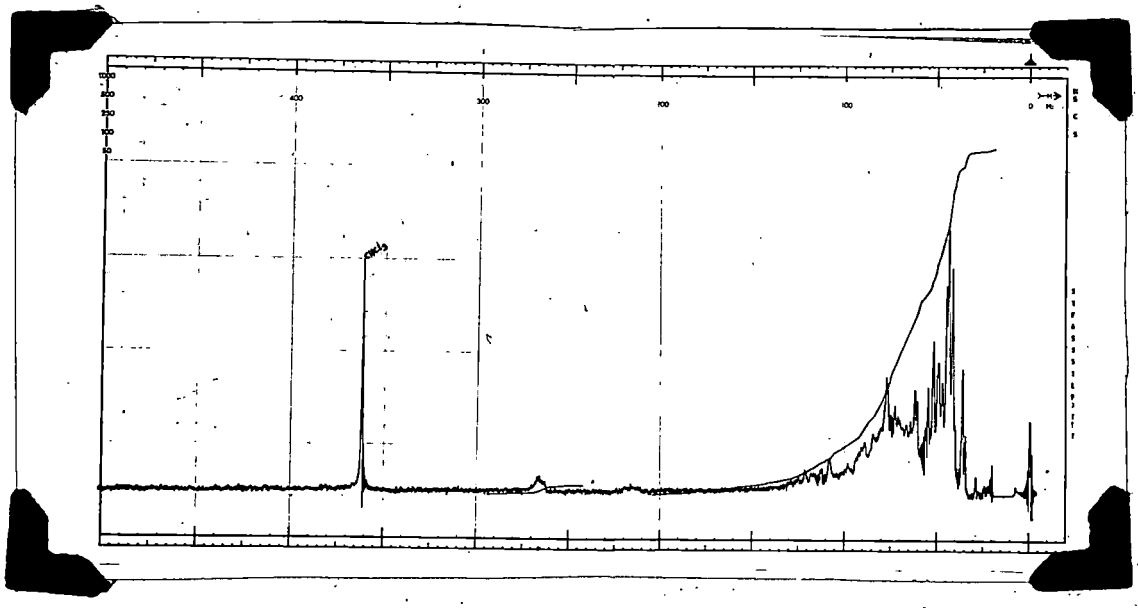

Fig. 15 : NMR spectrum of the epimeric alcohol 26

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 spectro-photometers. 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, 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 50 ml each	Residue on evaporation	Melting 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°, $(\alpha)_D$ - 89.13°

Found : C, 84.41; H, 11.85%

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

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

UV : no absorption in the region 200-300 mμ

Mass spectrum : M^+ 426

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

δ 5.40 (multiplet, IH, trisubstituted double bond)

δ 4.40 (multiplet, $-\overset{|}{C}H-C\underline{H}OH-CH_2-$)

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 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (170 mg) 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°, $(\alpha)_D$ - 83.72°.

Found : C, 81.93; H, 11.21%

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

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

Mass spectrum : M^+ 468 Fig - 5

NMR spectrum (60 Mc): δ 0.8-1.16 (8 methyl peaks)

δ 2.00 (3H, -O-CO-CH_3)

δ 5.40 (multiplet, I H, vinyl proton)

δ 5.20 (multiplet, IH, -$\overset{|}{C}$H-$\underline{C}$H(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 50 ml each	Residue on evaporation.
Petroleum	1 - 3	Solid (145 mg) m.p. 219-21°

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 of ketone 23, m.p. 221-23°, $(\alpha)_D$ 16.66°.

Found : C, 84.89; H, 11.35%

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

IR : 1720 cm^{-1} Fig - 9

Mass spectrum : M^+ 424 Fig - 8

NMR spectrum (300 MHz): δ 0.8-1.04 (8 methyl groups)

δ 5.40 (1H, vinyl proton)

Figs - 10A and 10B

Wolff-Kishner reduction[37] 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 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (150 mg) m.p. 165 - 68°

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

Fractions 1-3 were ~~crystallised~~ combined 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%

[redacted] :

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 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (130 mg) 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) : δ 5·28 (multiplet) δ 5·44 (multiplet) Fig - 14

Attempted acidisomerisation 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 50 ml each	Residue on 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 mμ.

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 50 ml each	Residue on evaporation
Petroleum	1 - 4	Solid (170 mg) 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 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (80 mg) 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 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (148 mg) 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_4 in presence of high pressure mercury lamp[33]:

Polypodinol A 21 (500 mg) in dry CCl_4 (100 ml) was stirred for 1 hour with finely divided HgO (900 mg) and iodine (1.07 mg) while being irradiated with a high pressure mercury lamp in the usual way[33b-33d]. 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 50 ml each	Residue on evaporation
Petroleum	1 - 5	Solid (425 mg) 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 neddles; 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 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 50 ml each	Residue on evaporation
Petroleum	1 - 3	Solid (160 mg) 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° which was found to be identical with the dehydration product 25 (m.m.p. and I.R).

Found : C, 88.31; H, 11.89%

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