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 Polypodinol B, $C_{30}H_{50}O$ along with fern-9(11)-ene, filicene, cyclolaudenol, nerifoliol and β- 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 ~~b~~ 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 rhizomes 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 hyd~~o~~roxide 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 of the residue
1.	Petroleum	Solid with oil	—
2.	Petroleum : benzene (4:1)	Solid (mixture)	—
3.	Petroleum : benzene (3:2)	Solid	240 - 43°
4.	Petroleum : 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 chomatography 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°, $(\alpha)_D$ 49.6° which was found to be identical with an authentic specimen of filicene[30] (m.m.p., co-TLC and IR). The mother-liquor from the crystallisation of filicene on concentration yielded another product, m.p. 169-71°, $(\alpha)_D$ - 16.3°. This compound was identified as fern-9(11)-ene[25] 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[31].

Fraction No. 2 : Isolation of two new triterpenes - Polypodinol A, $C_{30}H_{50}O$ and Polypodinol B, $C_{30}H_{50}O$ and detection of a new cyclolaudenol homologue, $C_{32}H_{54}O$ 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°, $(\alpha)_D$-89.13°. This compound has been characterized as a new triterpene alcohol, $C_{30}H_{50}O$, named as Polypodinol A. The chemistry and structure-elucidation of this compound has been described in chapter - IV (page-192).

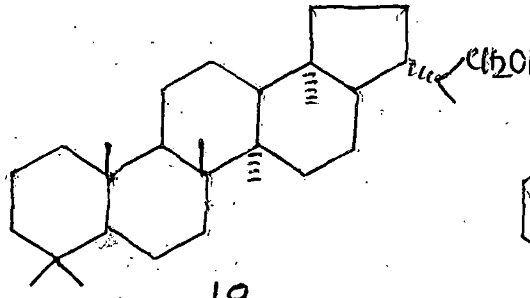
Elution with petroleum-benzene (3:2) gave a compound, m.p. 148-50°, $C_{30}H_{50}O$ in a very poor yield. Further work is in progress to isolate it in quantity to enable us to investigate its structure. Elution with the same solvent mixture gave another product, m.p. 162-64°. This compound on crystallisation from a mixture of chloroform and methanol afforded colourless needles, $C_{30}H_{50}O$, m.p. 165-66°, $(\alpha)_D$ 28.57°. 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° 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° 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°, which on crystallisation from methanol had m.p. 122-23°. 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).

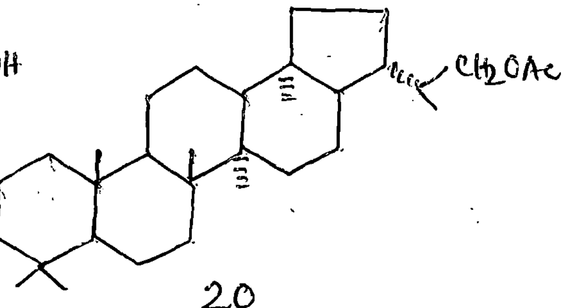
Fraction No. 3 : Isolation and identification of nerifoliol :

Rechromatography of the fraction no. 3 over a column of active alumina and elution with petroleum - benzene (2:3) gave an alchol, m.p. 244-46°, $(\alpha)_D$ 35.5°, (M^+ 428; Fig. 1A) ν_{max}^{nujol} 3340 cm^{-1} (OH; Fig-1B). NMR of the compound showed broad signal at τ 6.4 corresponding to two protons - CH_2OH and this signal was further resolved to AB quartets (J=11 cps) indicating that the carbon atom to which CH_2OH is bonded is asymmetric.

On acetylation, the alcohol furnished an acetate - 20. m.p. 195-96°, $(\alpha)_D$ 21.3°. (M^+ 470; Fig. 2A), ν_{max}^{nujol} 1725, 1230 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*[26] who isolated this compound from *Oleandra nerifolia* and established structure as shown in 19.



19



20

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

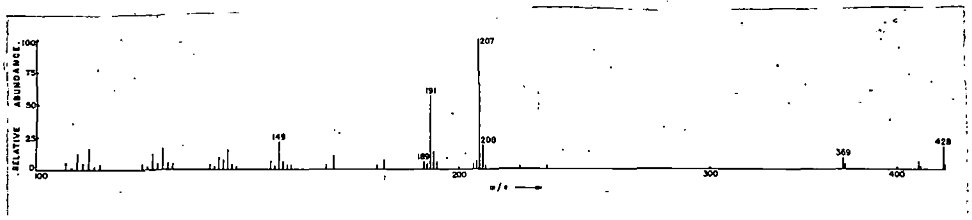


Fig. 1 A : Mass spectrum of nerifoliol **19**

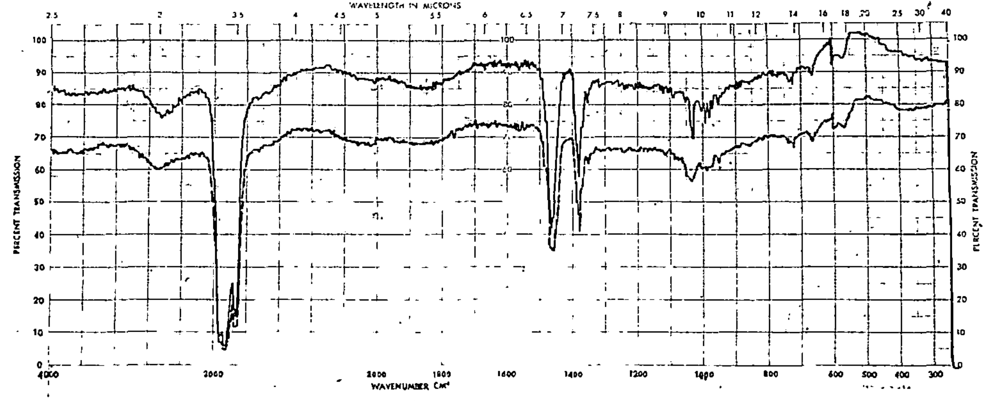


Fig. 1B : IR comparison of nerifoliol **19** (solid line) with an authentic sample (dotted line).

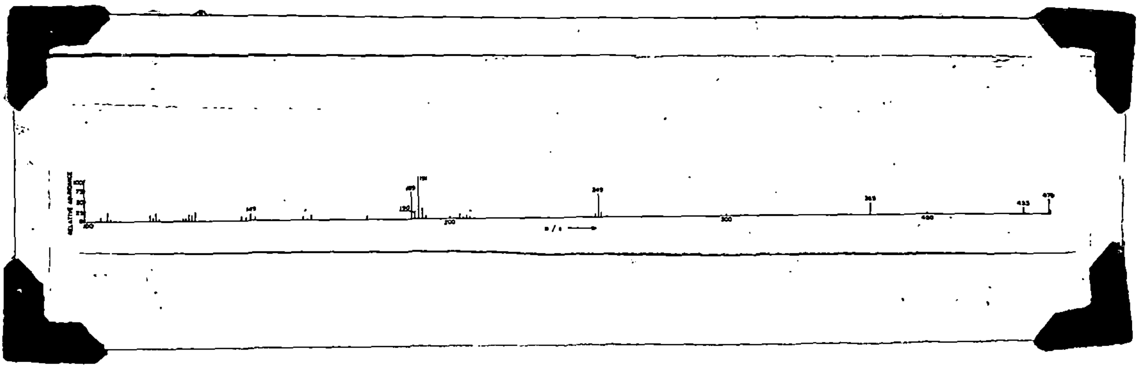


Fig. 2A : Mass spectrum of nerifoliol acetate 20

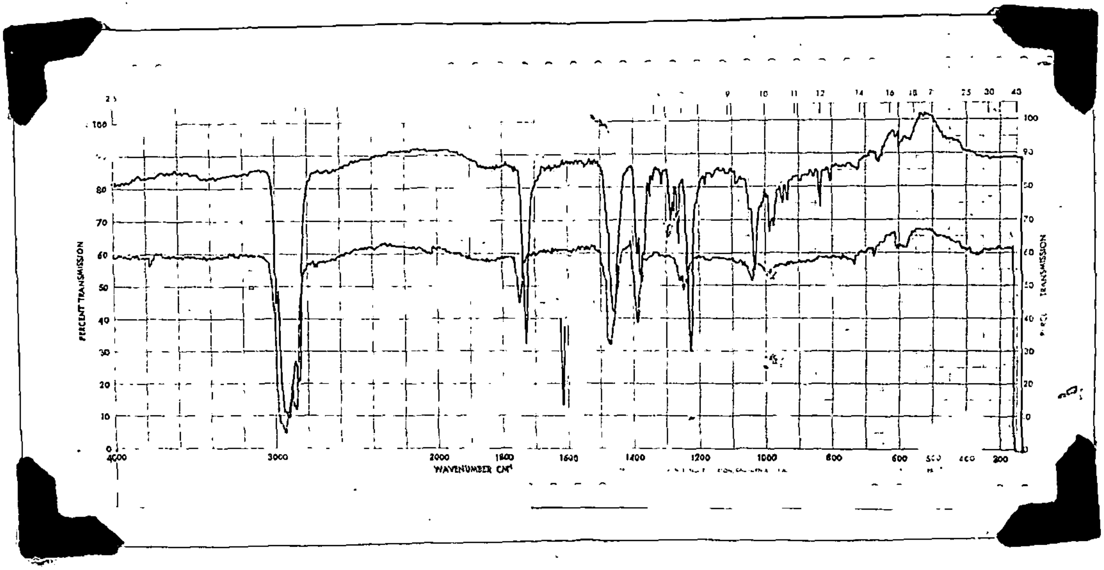


Fig. 2B : I R comparison of nerifoliol acetate 20 (solid line) with an authentic specimen (dotted line)

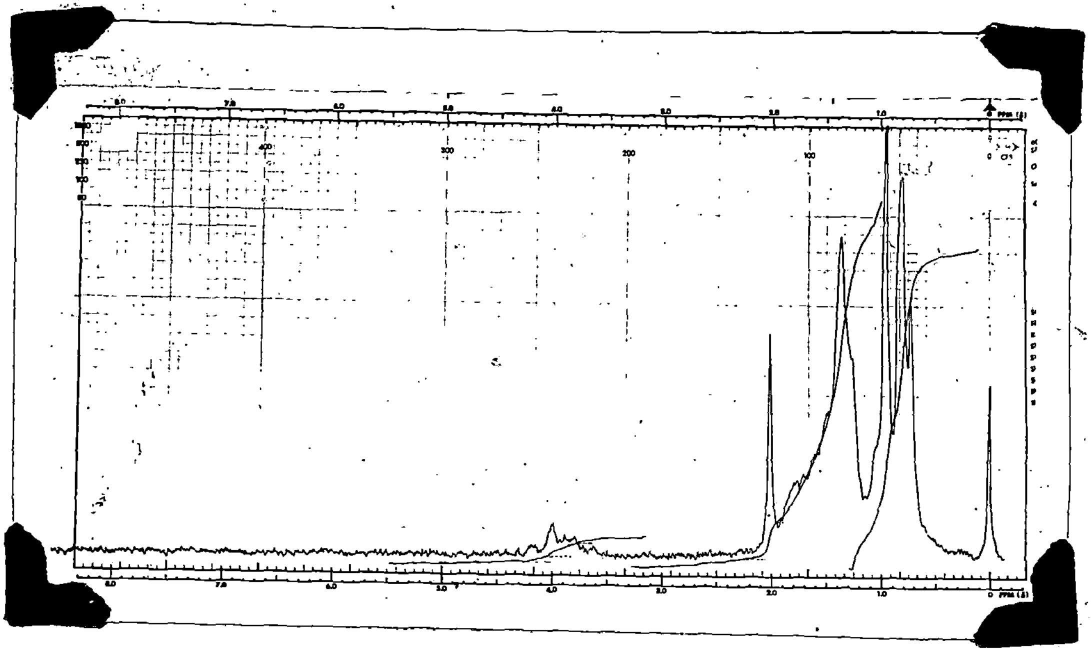


Fig.2C : NMR spectrum of nerifoliol acetate 20

Fraction No. 4 : Identification of β- 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 neede - shaped crystals, m.p. 136-37°, $(\alpha)_D$ - 32°. 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°, $(\alpha)_D$ - 40°. The alcohol and its acetate were identified as β-sitosterol and β- sitosterol acetate respectively by direct comparison with their respective authentic specimen.