CHAPTER-II

Section A: A Short Review on the Chemical Constituents of Ferns of Oleandraceae Family.

In recent years extensive works have been done in the field of Phytochemistry mainly due to two factors: (a) the development of modern techniques for structural investigations and (b) the potential usefulness of plants as a source of new therapeutic agents. But only a very limited studies have so far been done on the class of ferns. Of the fourteen families, with the exception of some genera of Polypodiaceae, very few individuals have been investigated by the Phytochemist. Some families, such as, Dipteridaceae and Salviniaceae are completely unexplored from the chemical point of view. However, recent discoveries of many interesting class of compounds such as new acylphlorglucinol derivatives and triterpenoids, indicate that the phytochemical investigation of ferns could be of great value both to the taxonomist, as an aid of classification, and to the natural product chemist in his search for new class of compounds with novel structures.

Only two species of the family Oleandraceae have so far been investigated. In this section is described the previous work done on the Oleandraceae family. It presents an up-to-date description of all organic compounds isolated from them . The work

on Oleandraceae family was mainly initiated by Pandey and Mitra.

Oleandra Wallichi (Hook) Pr.

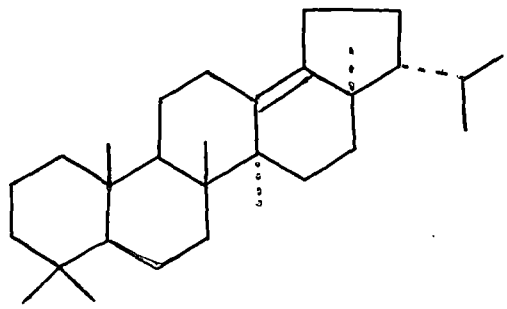
In 1967, Pandey and Mitra[2] reported the isolation of two triterpenic hydrocarbons from Oleandra Wallichi.

The residues obtained from the benzene extract of the rhizomes of O. Wallichi were partitioned between dilute alcohol (85%) and n-hexane. The n-hexane soluble fraction on chromatography gave two triterpene hydrocarbons, Wallichiene and Wallichienene.

The hydrocarbon Wallichiene, $C_{30}H_{50}$, m.p. 196-97°, $(\alpha)_D 0°$, gave positive Libermann-Burchard, Noller's and tetranitromethane tests. The IR spectra showed bands at 1381 and 1370 (gem-dimethyl group) 1210 and 1195 (quaternary gem-dimethyl group) and 1175 and 1149 cm^{-1} (isopropyl group). The NMR spectra showed signals for eight quaternary methyl groups at τ 9.2, 9.15, 9.11, 9.00 and 8.90 but no signal for olefinic protons. The IR spectra also indicated the absence of a trisubstituted double bond in Wallichiene. Wallichiene was subsequently shown to be identical with hopene-II, (1) prepared previously[3] from hydroxy hopane (2) by dehydration and isomerisation. This structure (1) of Wallichiene was confirmed by physical and chemical evidences.

The hydrocarbon Wallichienene, $C_{30}H_{48}$, m.p. 210-12°, $(\alpha)_D$ 42° gave positive Libermann-Burchard, Noller's and tetranitromethane tests. The IR spectra showed bands at 795 and 780 cm^{-1}

(di- or tri- substituted double bond). The characteristic UV absorption at 246 (log ε 4.37), 256 (log ε 4.43) and 265 nm (log ε 4.33) indicated the presence of a conjugated heteroannular diene system. Wallichienene, on hydrogenation, gave a dihydro compound $C_{30}H_{50}$, m.p. 196-97°, $(\alpha)_D$ 0° which was shown to be identical with Wallichiene or hopene-II, (1). Wallichienene was finally identified as neo-hopa-11,13(18)-diene[3,4], (3)



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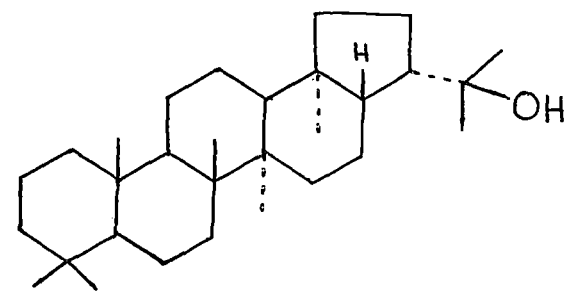

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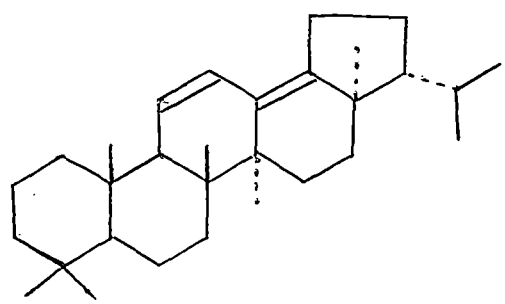


(3)

Though Wallichiene (hopene-II), (1), and Wallichienene [neo-hopa-11,13(18)-diene] (2), were prepared previously in the course of studies in the chemistry of related triterpenoids[3,4], Pandey and Mitra[2] reported their isolation for the first time from nature. The occurrence of pentacyclic triterpenoids with heteroannular conjugated diene system in nature is comparatively rare; however, a few such dienes have recently been reported[5]. Wallichienene was the first triterpene diene being isolated from a fern.

Oleandra nerifolia

Pandey and Mitra, in 1967, reported the isolation and structure elucidation of a new triterpene alcohol, nerifoliol, from the rhizomes of another fern oleandra nerifolia[6] [syn. oleandra pistillaris (Sw)C.Chr.]. Their works are described below.

The rhizomes of the fern O.nerifolia were extracted with benzene. The residue obtained from the neutral part was chromatographed over alumina. The n-hexane-benzene eluent yielded nerifoliol, $C_{30}H_{52}O$, m.p. 242-44°, $(\alpha)_D^{35°}$ (M^+428) which gave positive Libermann-Burchard test but negative test with tetranitromethane thus demonstrating that it might be a completely saturated triterpene. In addition, it was transparent in the

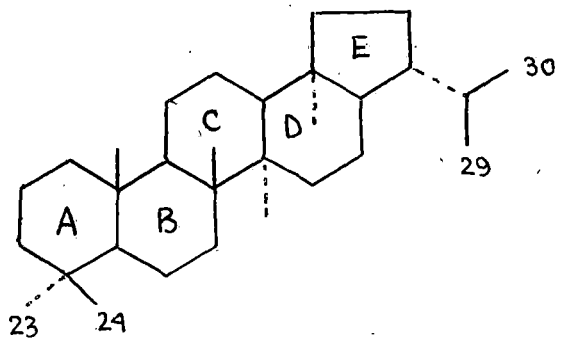
region 200 to 300 nm. The IR spectra showed bands at 3330 cm^{-1} with a supporting band at 1050 cm^{-1} indicating the presence of a primary hydroxyl function. The combination of peaks at 1390 and 1370 cm^{-1} indicated the presence of gem-dimethyl groups. The NMR spectra of the alcohol showed methyl signals at 44(3H), 49.50 (6H), 51.5(6H) and 58 (6H) cps, all corresponding to methyl groups.

On treatment with acetic anhydride and pyridine nerifoliol gave a monoacetate, $C_{32}H_{54}O_2$, m.p. 195-96°, $(\alpha)_D$ 20°, IR ν_{max}^{KBr} 1730, 1235 cm^{-1} (acetate). On oxidation with anhydrous chromium trioxide and pyridine nerifoliol furnished an aldehyde, nerifolial, $C_{30}H_{50}O$, m.p. 76°, IR ν_{max}^{KBr} 1730 cm^{-1}. Both nerifoliol and nerifolial, on oxidation with CrO_3 in acetic acid and benzene yielded nerifolic acid, $C_{30}H_{50}O_2$, m.p. 270-74°, $(\alpha)_D$ 16°, IR $_{max}^{KBr}$ 1730 cm^{-1} with an indefinite shoulder in the region 3571 to 3077 cm^{-1}. The corresponding methyl ester, methyl nerifoliate, $C_{31}H_{52}O_2$, m.p. 242-44°, $(\alpha)_D$ 8°, IR $_{max}^{KBr}$ 1730 cm^{-1} on reduction with lithium aluminium hydride in tetrahydrofuran gave back the original alcohol, nerifoliol.

From the above findings along with the biogenetic and chemotaxonomic considerations[7] Pandey and Mitra suggested[6] that nerifoliol was a pentacyclic triterpene alcohol having a hopane or modified hopane skeleton with a primary hydroxyl group. The presence of the primary hydroxyl group was also evidenced by the

NMR spectra of nerifoliol. The broad signal at τ 6.4 corresponding to two protons was attributed to the two protons of the $-CH_2OH$ group. Furthermore, this signal was resolved in two AB quartets (J = 11 cps) indicating that the carbon atom to which the group CH_2OH is bonded is asymmetric.

Nerifoliol when subjected to Huang-Minlon modification of W.K. reduction gave a hydrocarbon, $C_{30}H_{52}$, m.p. 190-92°, $(\alpha)_D$ 36°, which was shown[6] to be identical in all respects with hopane[3] (4). Therefore, Pandey and Mitra[6] concluded that the carbon skeleton of nerifoliol was the same as that of hopane. The nature of the hydroxyl group (primary) suggested that it was attached either to the gem-dimethyl or angular methyl groups or side chain.

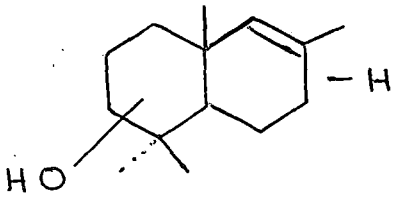


(4)

The relative ease with which methyl nerifoliate was hydrolysed (8% alcoholic Potassium hydroxide for 6 hours) excluded any of the angular positions as the possible site for the carboxyl group in nerifolic acid as there was no activating group present in the molecule. Evidently, Pandey and Mitra[6] suggested that C-23, 29 or 30 could be the possible positions for the carboxyl group as in case of medicagenic and desoxoglycyrrhetic acids[8,9]. The position C-24 (axial) was also excluded as the methyl ester would then be hydrolysed with great difficulty (cf. β-boswellic acid). They[6], therefore, concluded that the primary hydroxyl group in nerifoliol was attached to C-23, 29 or 30.

From a study of the mass fragmentation pattern of nerifoliol, Pandey and Mitra[6] concluded that the primary hydroxyl group was situated in the isopropyl side chain. The mass spectrum gave molecular ion peak, M^+ at m/e 428. The peaks at m/e 413 and 369 were due to the fragments formed from the molecular ion by the loss of 15 and 59 mass units. These were attributed to the ions formed by the loss of a methyl group (CH_3) and the isopropyl side chain carrying the hydroxyl function (C_3H_7O) respectively. Since there was no loss of 31 mass units (CH_2OH) to start with the mass spectra firmly excluded the possibility of the hydroxyl group being attached to C-23 or C-24 or any angular methyls and confirmed its presence at C-29 or C-30. The splitting of ring C gave rise to the fragments having m/e 207 and 191. The peak at

m/e 207 might belong to either to the left or right side of the molecule having oxygen in ring A (5) or ring E (6) or in the side



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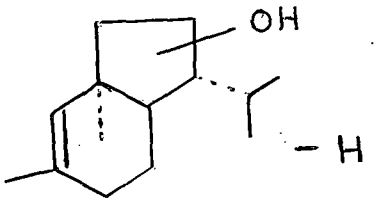

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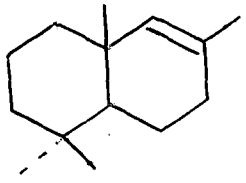

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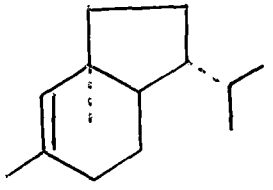
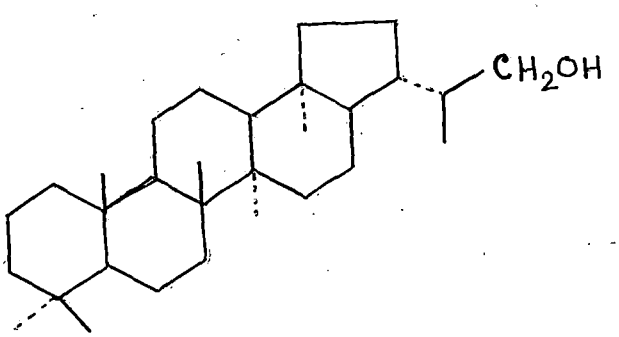


(8)

chain as shown. Similarly the peak at m/e 191 might belong to the left or right side of the molecule (7) or (8). That the succeeding lower mass peak at m/e 149 arose from the fragment

m/e 207 with the loss of 58 mass units was confirmed by the appearance of a metastable peak at m/e 107.2 (Calculated value 107.3) which was attributed to the loss of C_3H_6O unit, that is, the isopropyl side chain having the hydroxyl function, under transfer of one hydrogen. Thus the mass spectra of nerifoliol strongly favoured the position C-29 (or C-30) for the primary hydroxyl function in a hopane nucleus. From the above physical and chemical evidences Pandey and Mitra[6] assigned the structure (9) for nerifoliol.

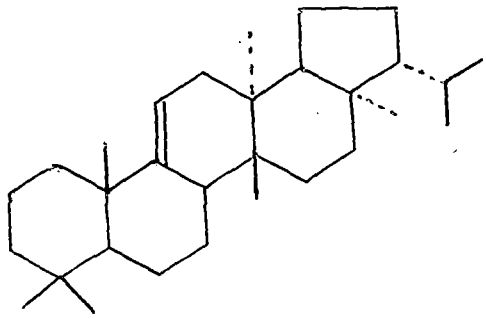


(9)

Incidentally, nerifoliol was the first triterpene primary alcohol isolated from a fern.

Section B: A Short Review on the Structure of Dryocrassol Isolated from the Aspidiaceous Fern.

In 1963, Ageta et al[10,11] reported the isolation of two triterpenoid hydrocarbons, fernene (10) and diploptene (hopene-b) (11) from the leaflets of the fern Dryopteris Crassirhizoma NAKAI (Aspidiaceae).



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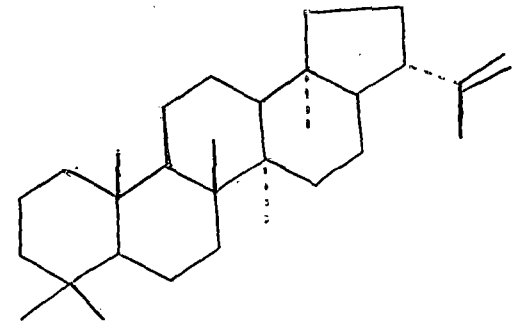
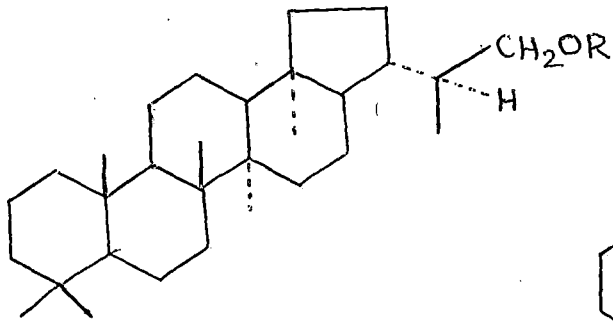


(11)

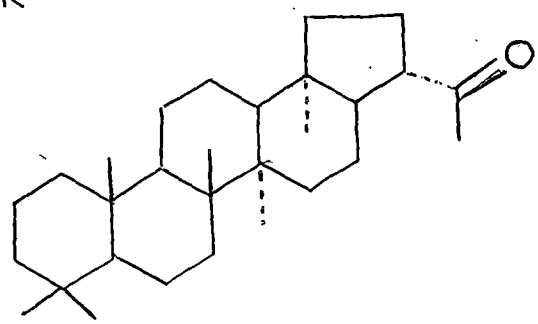
Further investigations on the triterpenoids from the same source were carried out by Ageta et al. They reported[12] the isolation of a new alcohol and its acetate, dryocrassol (12a)

and dryocrassol acetate (12b) along with 22-hydroxyhopane[13] (2), adiantone[14] (13), ferna-7, 9(11)-diene (14), fern-9(11)-ene-12 one[10,11] (15) and a sterol mixture. Dryocrassol acetate (12b) was also isolated from the leaves of Arachniodes standisii OHWI and Polystichum polyblepharum PR as the main triterpenoid constituent.



(12a) R = H

(12b) R = $COCH_3$



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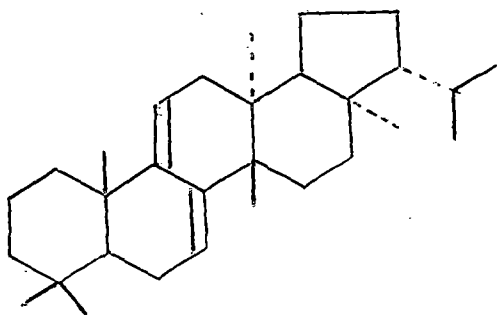

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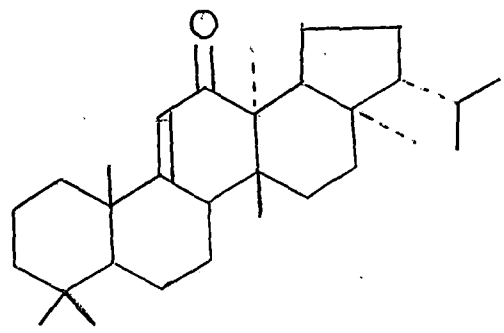
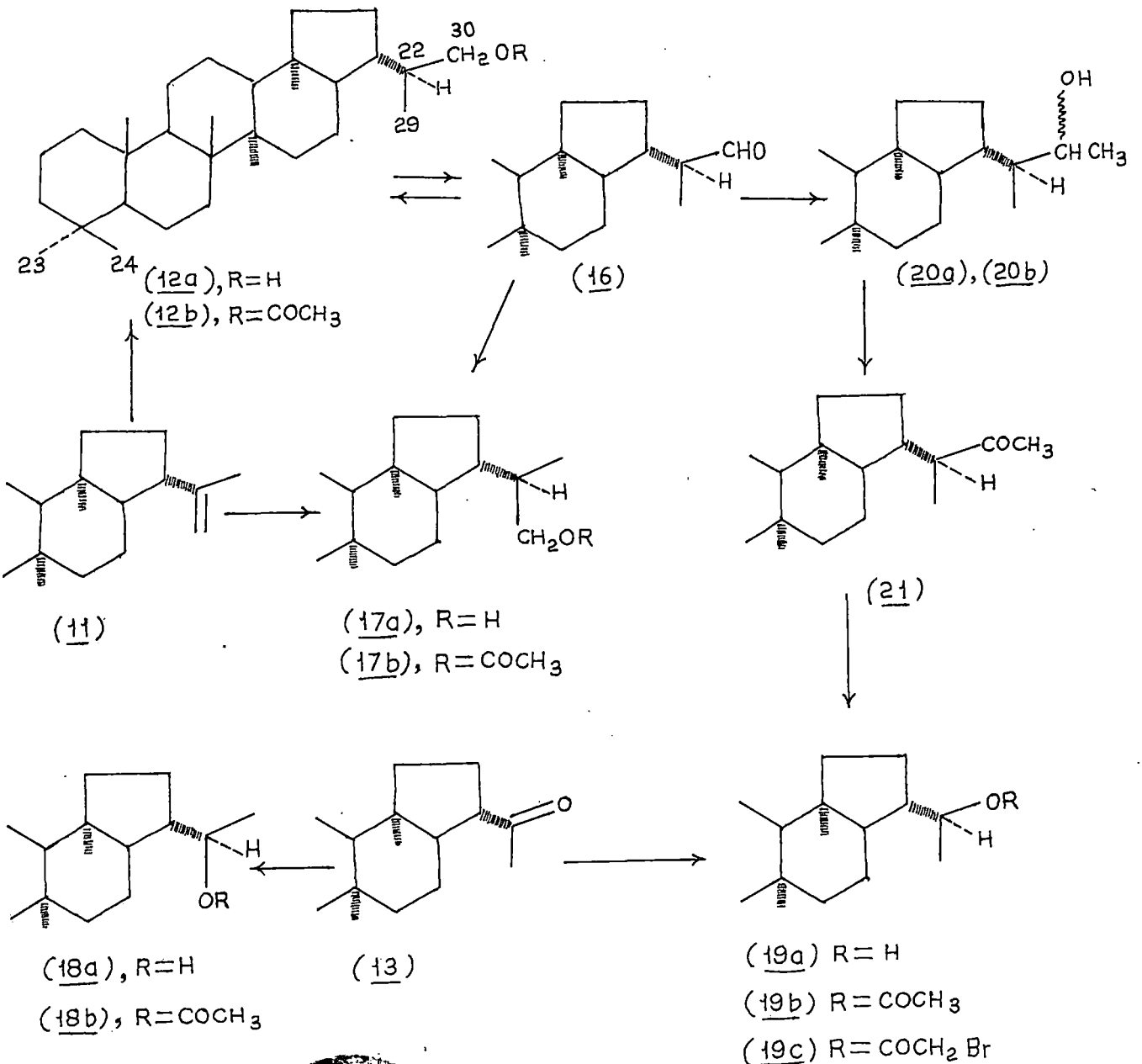


(15)

Dryocrassol (12a), $C_{30}H_{52}O$, m.p. 245-47°, $(\alpha)_D^{68°}$, IR ν_{max}^{KBr} 3330, 1026 cm^{-1} gave the acetate (12b), $C_{32}H_{54}O_2$, m.p. 196-98°, $(\alpha)_D^{58°}$, IR ν_{max}^{KBr} 1729, 1226 cm^{-1}. The NMR spectrum of dryocrassol showed signals each corresponding to three hydrogens at τ 9.15 (C-23), 9.21 (C-24), 9.19 (C-25), 9.04 (C-26), 9.04 (C-27), 9.27 (C-28), 8.97 (doublet, J = 6.5 Hz, C-29) and a multiplet corresponding to two hydrogens at τ 6.5. The mass spectrum of dryocrassol showed peaks at m/e 428 (M^+, 5%), 413 (2%), 369 (7%), 207 (100%) and 191 (64%). These NMR and mass spectra and also the same of dryocrassol acetate led Ageta et al[12] to suggest that dryocrassol was a triterpenoid of the hopane skeleton having a primary alcohol group in the side chain.

Chromic acid oxidation of dryocrassol in pyridine gave an aldehyde (16), m.p. 184-87°, $(\alpha)_D^{60°}$, IR ν_{max}^{KBr} 2700, 1725 cm^{-1} which was reduced into only dryocrassol (12a) with lithium aluminium hydride and into hopane[15] (4) by Wolff-Kishner method. Boiling of the aldehyde (16) with 5% methanolic potassium hydroxide afforded[12] unexpectedly a mixture of two alcohols, (12a) and (17a). The latter (17a), m.p. 242-44°, $(\alpha)_D^{35°}$ gave an acetate (17b), m.p. 214-16°. The alcohol (17a) was shown to be identical in all respects with nerifoliol[6]. Hydroboration of hop-22(29)-ene (11) gave also a 1:1 mixture of nerifoliol (17a) and dryocrassol (12a). Consequently, Ageta et al[12] concluded that either nerifoliol (17a) or dryocrassol (12a) should be hopane-29 (or 30)-ol having an epimeric centre at C-22.

Chart-1



Reduction of adiantone (13) with lithium aluminium hydride gave two isomeric alcohols adiantol A (less polar) (18a) m.p. 211-13°, $(\alpha)_D 40°$ [acetate (18b), m.p. 205-7°, $(\alpha)_D 35°$] and adiantol B (more polar) (19a), m.p. 252-56°, $(\alpha)_D 76°$ [acetate (19b), m.p. 222-24°, $(\alpha)_D 55°$]. The absolute configuration at C-22 of the latter alcohol (19a) was proved to be 22S by X-ray analysis of the corresponding bromoacetate[16] (19c). Grignard reaction of (16) with methyl magnesium iodide gave a mixture (1:1) of two alcohols (20a), m.p. 250-54°, IR ν_{max}^{KBr} 3430, 1127 cm^{-1} and (20b), m.p. 255-58°, IR ν_{max}^{KBr} 3500, 1090 cm^{-1} epimeric at C-30. Chromic acid oxidation of (20a) or (20b) in pyridine afforded the same methyl ketone (21), m.p. 239-42°, $(\alpha)_D 43°$, IR ν_{max}^{KBr} 1713 cm^{-1}. Baeyer-Villiger oxidation of the ketone (21) with perbenzoic acid yielded the acetate of an alcohol. This acetate was proved[12] to be identical with adiantol B acetate (19b). Ageta et al[12], therefore, concluded that the configuration at C-22 of (21), (20a), (20b), (16), (12a) and (12b) was 22S and that of (17a) and (17b) was 22R as shown in Chart-I. These workers also proposed the numbering of the side chain on the hopane skeleton. Thus nerifoliol (17a) was hopan-29-ol and dryocrassol (12a) was hopan-30-ol.