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NEW LIGNANS FROM PHYLLANTHUS NIRURI LINN.

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Phyllanthus niruri Linn. (Euphorbiaceae)[1,2] is a bitter plant often used locally in the treatment of jaundice, asthma and bronchial infections. Ottow[3] was the first to isolate a bitter principle which he named phyllanthin and assigned $C_{39}H_{37}O_8$ for its molecular formula. Later Pecklot[4] examined the plant but he could not isolate any crystalline material. Krishnamurty and Seshadri[5] made significant contribution in the study of this plant. They isolated the bitter component, phyllanthin, besides a non-bitter component, hypophyllanthin, for which $C_{21}H_{22}O_7$ and $C_{19}H_{22}O_6$ were the molecular formulas assigned. They were not able to relegate these compounds to any particular class of natural organic compounds.

In the present investigation, the isolation of phyllanthin and hypophyllanthin was very much improved. By mixing the leaf powder with slaked lime in the proportion of 10:3, the chlorophyll is effectively prevented from extraction by petroleum ether. The extract contained phyllanthin, hypophyllanthin, carotenoids and plant wax. To avoid waxes, methanol was added and phyllanthin and hypophyllanthin could thus be secured in a fairly pure condition. But their individual separation is better effected by fractional crystallisation from petroleum ether, hypophyllanthin separating out first. Fractionation on an alumina column using benzene as eluant was not very effective. However, several crystallisations from petroleum ether or methanol were found necessary to obtain pure samples of phyllanthin and hypophyllanthin.

Pure phyllanthin crystallises in the form of colourless short needles, m.p.96° (Found: C, 68.96*, 68.80; H, 8.30*, 8.47; O, 23.00*; C-Me, nil*; OMe, 41.32%; M.Wt. 387 (Rast's method)*; $C_{24}H_{34}O_6$ requires C, 68.90; H, 8.13; O, 22.97; 6-OMe, 44.49% and M.Wt. 418; U.V. λ max 230, 280 mµ, log ϵ 4.33 and 1.89; λ min 252 mµ, log ϵ 0.32). Hypophyllanthin similarly crystallises from petroleum ether as colourless long needles, m.p.128° (Found: C, 66.74*, 67.04; H, 6.97*, 7.08; O, 25.90*; C-Me, nil*; OMe, 34.5%; M.Wt. 395 (Rast's method)*; $C_{24}H_{30}O_7$ requires C, 66.97; H, 6.97; O, 26.04; 5-OMe, 36.04% and M.Wt.430; U.V. λ max 230.5, 280 mµ, log ϵ 4.56 and 2.23; λ min 255 mµ, log ϵ 0.97). A significant observation which was not formerly noted[5], is the optical activity of phyllanthin $(\alpha)_D^{30^\circ}$ +12.42°

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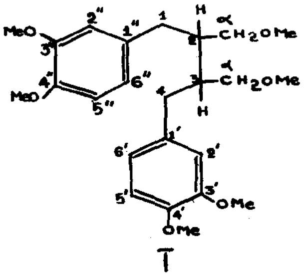
* Micro-analysis by Dr. W. Zimmerman, C.S.I.R.O., Melbourne.

(c, 1.45, $CHCl_3$) and hypophyllanthin $(\alpha)_D^{30^\circ}$ +3.9°. The new molecular formulas $C_{24}H_{34}O_6$ and $C_{24}H_{30}O_7$ for phyllanthin and hypophyllanthin are entirely supported by their chemical reactions, I.R., U.V., and N.M.R. data which reveal that these compounds belong to the class of lignans.

(i) Phyllanthin:

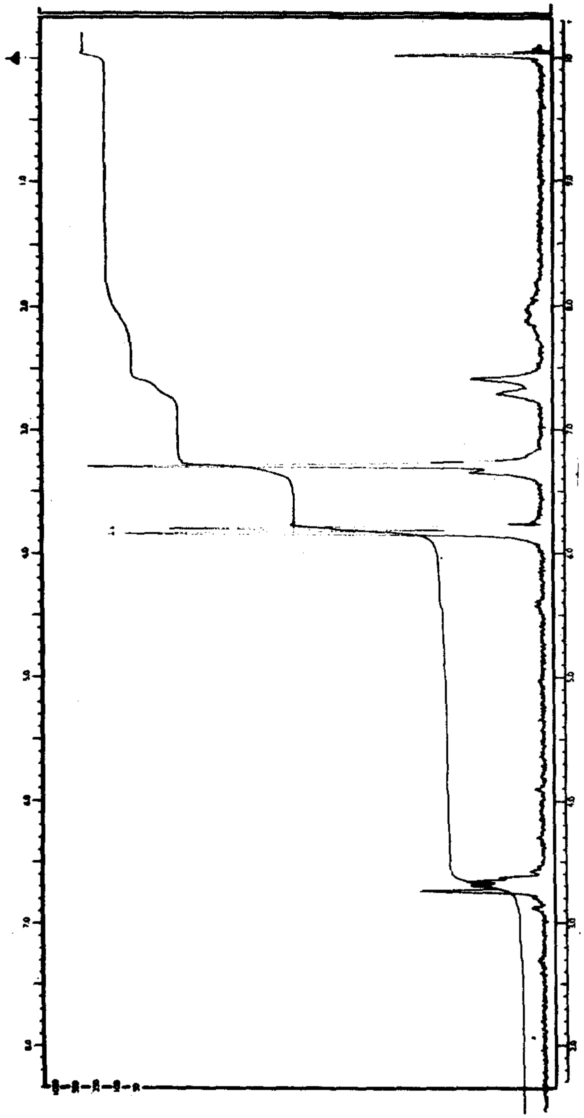
Phyllanthin and hypophyllanthin develop an emerald green colour with gallic acid and sulphuric acid suggesting the presence of a methylenedioxy group. But the N.M.R. spectrum of phyllanthin showed no proton absorption for methylenedioxy group at about τ 4.30 unlike hypophyllanthin (see below).[6,7]

A study of the N.M.R. data (Table I) and the proton assignments reveal that phyllanthin has the 2,3-diveratryl 1,4-butane structure (I).



I

(Lignan numbering is followed[8])



PHYLLANTHIN

Table I - Phyllanthin

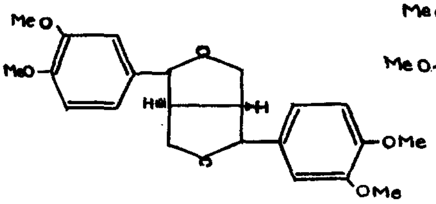
τ values	Multiplicity	Protons	Assignments
3.23-3.34	multiplets	6	2',5',6',2",5",6"-Ar.H.
6.13	singlet	6	4-Ar.OMe.
6.17	singlet	6	
6.66	doublet, J=4 c.p.s.	4	2α, 3α-2CH_2
6.68	singlet	6	2-Ali. OMe.
7.34	Doublet, J=7 c.p.s.	4	1,4-2CH_2
7.90	broad multiplet	2	3,2-2H

(N.M.R. spectra taken in $CDCl_3$ at 60 megacycles using tetramethyl silane as internal standard).

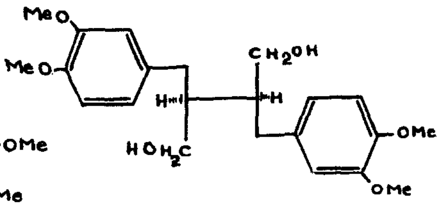
Phyllanthin is stable to neutral permanganate but yields veratric acid under mildly alkaline or neutral conditions whose isolation could be effected only with great difficulty (3.2 mg. from 500 mg. identified by I.R., m.p. and m.m.p., 178-80°). Oxidation with chromic acid-acetic acid or with alkaline hydrogen peroxide gave only intractable products. With bromine in chloroform, phyllanthin yields readily dibromo-phyllanthin m.p.136-7°, $(\alpha)_D^{30^\circ}$ +39.99° (c, 0.5, $CHCl_3$). (Found: C, 49.88; H, 5.32; OMe, 35.32; $C_{24}H_{32}O_6Br_2$ requires C, 50.00; H, 5.55; 6-OMe, 33.32%). It could be further nitrated in glacial acetic acid with a few drops of con. HNO_3 at 60° to give pale yellow needles of dinitrophyllanthin m.p.122°, $(\alpha)_D^{30^\circ}$+19.73° (c, 0.76, $CHCl_3$) (Found: C, 56.70; H, 6.21; OMe, 36.5; $C_{24}H_{32}O_6(NO_2)_2$ requires C, 56.69; H, 6.29; 6-OMe, 36.61%). The formation of dibromo - and dinitro - derivatives confirms our formulation of phyllanthin as a diphenyl butane (I) and in this respect it stimulates the behaviour of matairesinol[9] and hinokinin.[10]

The N.M.R. spectrum (Table I) supports the structure I for phyllanthin. The benzylic protons at 1 and 4 are indicated at τ 7.34 as a doublet with a splitting of J=7 c.p.s.[11,12] In addition to the four aromatic methoxyls (τ 6.13 and 6.17), two aliphatic methoxyls are indicated at τ 6.68. The doublet at τ 6.66 (J=4 c.p.s.) is correctly assigned to the two methylene groups at 2α and 3α positions each carrying an aliphatic methoxyl.[12]

The steric configuration at C-2 and C-3 as well as the structure I for phyllanthin are confirmed by an unambiguous synthesis from eudesmin (II) whose configuration was settled by Freudenberg and Sidhu.[13] Eudesmin was converted into tetrahydro eudesmin (III) by the action of Li+NH_3 in ethanol,[14] which was later methylated using methyl iodide and sodium hydride to give phyllanthin, absolutely identical (m.p., m.m.p.96° and I.R., $(\alpha)_D^{21^\circ}$ +13.1°. with natural sample, thus establishing the absolute configuration at C-2 and C-3 positions as shown in IV.



II



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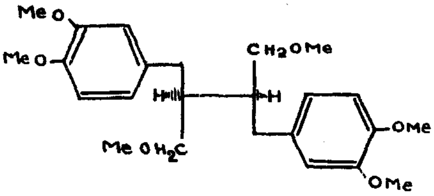
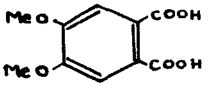


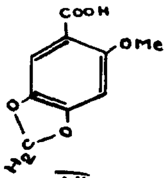
IV

(ii) Hypophyllanthin:

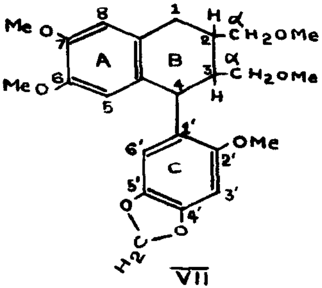
Hypophyllanthin, which is a minor component, could be oxidised by alkaline permanganate to m-hemipinic acid (V) (12.5 mg. from 350 mg. identified by m.p., m.m.p.174-5°, and I.R.) and 2-methoxy 4,5-methylenedioxy benzoic acid (VI)[15] (4.85 mg. from 350 mg., identified by m.p.150-1° and I.R.). Dehydrogenation of hypophyllanthin gave rise to a slightly impure product which has U.V.absorption at 225, 283, 298 and 305 mµ characteristic of 1-phenyl naphthalenes. These reactions suggest that hypophyllanthin is a 1-phenyl tetralin and may be represented by VII.



V

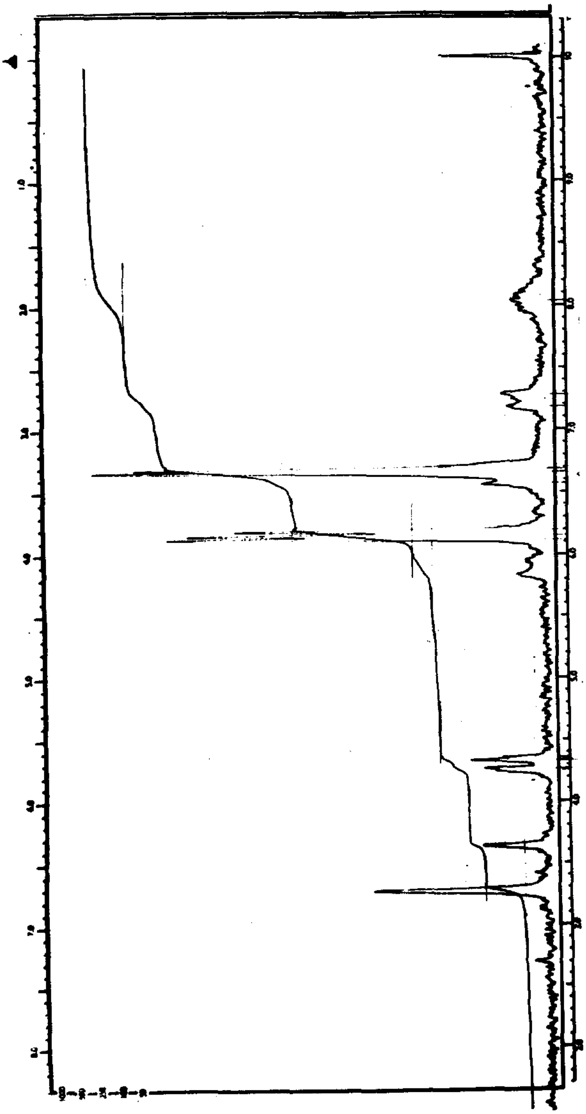


VI



VII

The N.M.R. spectrum (Table II) of hypophyllanthin which is closely similar to that of otobain[6], unequivocally suggests 1-phenyl tetralin structure (VII) with three aromatic methoxyls at τ 6.12, 6.15, 6.20 and two aliphatic methoxyls at τ 6.68 and 6.70. The doublet at τ 7.24 is indicative of the benzylic protons at C-1[12]. The I.R. spectrum of hypophyllanthin contains a strong band at 936 cm^{-1} characteristic of a methylenedioxy group along with other bands at 1356, 1258, 1124 and 1024 cm^{-1}. The presence of the above five methoxyls makes it difficult to confirm the presence of the methylenedioxy group.[16] The N.M.R. spectrum in this regard, showed a pair of doublets at τ 4.25 and 4.33 (J=1.20 c.p.s.) suggesting the presence of a methylenedioxy group. This doublet clearly shows the non-equivalence of the two protons in the methylenedioxy group as in dicentrin[17] and otobain[6] . Further, estimation of the methylenedioxy group follwing the method of Beroza[18] gave values of 1.13, 1.16 and 0.993 indicating one methylenedioxy group.



HYPOPHYLLANTHIN

Table II - Hypophyllanthin.

τ values	Multiplicity	Protons	Assignment
3.27	Singlet	3	8,5,6' - Ar. H
3.65	Singlet	1	3' - Ar. H
4.25,4.27 4.33,4.35	A pair of doublets (J=1.2 cps)	2	O-CH_2-O at 4',5'
5.82	Unresolved doublet	1	4 - H
6.12,6.15 6.20	Singlets	9	3 - Ar.OMe
6.58	Doublet (J=7 cps)	4	2α, 3α- 2-CH_2
6.68,6.70	Singlets	6	2 - Ali. OMe.
7.24	Doublet (J= 10 cps)	2	1 - CH_2
8.04	Broad multiplet	2	2,3 - 2H

(N.M.R.Spectra taken in $CDCl_3$ at 60 megacycles using tetramethyl silane as internal standard).

The unresolved doublet at τ 5.82 is certainly due to the doubly benzylic proton at C-4. This absorption is very similar to that of thymol or p-cymene[19] and closely simulates that of otobain[6] and sesangolin[15].

Further, the singlet at τ 3.65 may be assigned to a single aromatic proton at 3' in ring C and the absence of spin coupling indicates its para position to the only existing proton at 6'. These observations and the isolations of m-hemipinic acid (V) and 2-methoxy-4,5-methylenedioxy benzoic acid (VI) are taken to confirm the structure VII for hypophyllanthin. Further work is in progress.

Acknowledgment.

It is difficult to express our gratitude adequately to Prof. A.J. Birch F.R.S. for his kind encouragement during the course of this investigation. Our grateful thanks are also due to him for facilities to take N.M.R. and I.R. spectra and also for a generous supply of eudesmin.

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