

THE BITTER PRINCIPLE OF *PHYLLANTHUS NIRURI*

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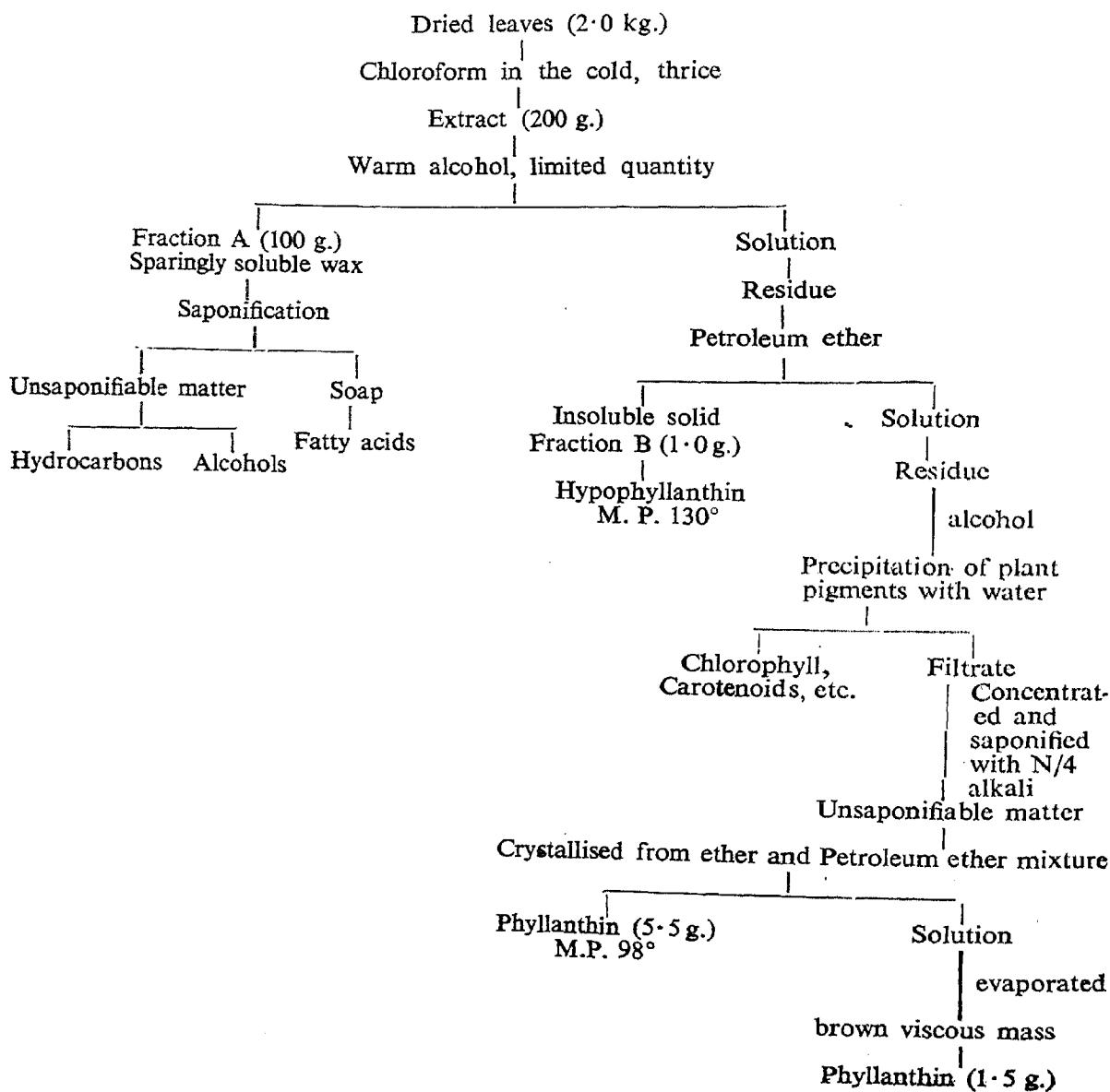
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DURING the war there was serious shortage of the cinchona alkaloids owing to the loss of Java and the ever-increasing demand of antimalarials for the fighting forces. Since synthetics were not available for civilians, attention was directed to the use of other plant drugs which had enjoyed reputation for the cure of intermittent fevers. *Phyllanthus niruri* is one such. It is also considered to have a wider therapeutic application,¹ for example a decoction of the leaves administered orally cures jaundice. The plant is an annual herb found as a weed all over India and all its parts and particularly the leaves are markedly bitter.

As early as 1891 it was reported that Ottow² isolated the toxic bitter principle of *Phyllanthus niruri* in a crystalline condition, and gave it the name 'phyllanthin' and the formula $C_{39}H_{37}O_8$; but no details were available. Later Peckolt³ recorded that he could obtain it only in an amorphous form and in a yield of 0.05%. From his description of its properties it is clear that it was impure. In the course of the work described in this paper successful attempts have been made to isolate phyllanthin in good yield and in a pure condition and study some of its important properties. Incidentally some other components of the drug have also been examined and their properties are described.

The dried leaves were employed for the extraction. Cold chloroform or hot ligroin could successfully extract the bitter principle. The first solvent was preferable. The concentrated extract contained also large amounts of wax and chlorophyll. The former was mostly left behind when the extract was taken up with a limited amount of alcohol. The alcoholic solution contained all the bitter component and a semi-solid product was obtained on removing the solvent. By treatment with petroleum-ether it could be separated into two fractions. (1) The sparingly soluble fraction was not bitter and it yielded after repeated crystallisation a colourless crystalline solid melting at 129-30°. Since it has considerable resemblance to phyllanthin in its chemical properties it is named hypophyllanthin. Hypophyllanthin and its bromoderivative are both stable to alkali, cold and hot. (2) The more soluble fraction contained the bitter principle along with

considerable amount of chlorophyll. Attempts to remove the colouring matter by chromatographic adsorption were not successful. Methods of fractional precipitation and crystallisation from solvents gave very poor yields and entailed considerable loss of phyllanthin. But separation could finally be achieved by treatment with alcoholic potash and this method is suited for the purification of larger quantities. Phyllanthin was thus obtained as a colourless crystalline substance melting at 97-98°. Its taste is extremely bitter and it is toxic to fish and frogs. It contains no nitrogen or halogen, has the approximate molecular formula $C_{21}H_{22}O_7$ and contains methoxyl and methylenedioxy groups. Five methoxy groups seem to be present in the molecule. It is stable to treatment with alkali and to oxidation with neutral permanganate. Demethylation with hydriodic acid was



unsuccessful leading to resinous products. By the action of concentrated hydrochloric acid at 125° a phenolic substance was formed. But it has not yet been obtained in a pure condition. Phyllanthin yields a monobromo derivative which is also stable to alkali. The properties of phyllanthin and its composition suggest that it should be aromatic. The above diagram explains the stages in the isolation of phyllanthin and hypo-phyllanthin.

EXPERIMENTAL

Fresh leaves were collected from the garden areas of Madras during the wet part of the year 1945, dried in air and powdered. 100.0 grams of the dried leaf powder were successively extracted in a Soxhlet with petroleum ether (80-100°), chloroform, acetone and alcohol using 6 hours of extraction for each solvent. Details of the various fractions are given below:

Solvent	Yield of extract	Nature of the product
Petroleum ether ..	7%	Dark green solid, waxy in consistency and bitter to taste. Mainly chlorophyll and wax along with the bitter principle.
Chloroform ..	3%	Green and waxy. Not bitter. Mostly chlorophyll and wax along with non-bitter materials.
Acetone ..	2%	Dark-brown and resinous ; contained mostly resin.
Alcohol ..	3%	Reddish-brown and astringent to taste. Mostly tannin with inorganic material.

Isolation of the various fractions.

The dry leaf powder (2.0 kg.) was extracted with chloroform thrice in the cold soaking each time for 24 hours. The major bulk of the solvent was recovered by ordinary distillation, the last portions being removed under reduced pressure. The residue (200.0 g.) was semi-solid in consistency and dark green in colour with a faint aromatic odour. To separate the wax, the residue was taken up with warm alcohol (500 c.c.) and allowed to stand in the cold for a day. It was then filtered and the solid waxy matter washed with a little alcohol. The filtrate was concentrated to about half the volume when some more of the wax separated. It was collected as before. The combined solid (100.0 g.) was crystallised from benzene-alcohol mixture twice when a colourless crystalline wax melting at 80° was obtained. This was marked (A); yield, 88.0 g.

The alcoholic solution was distilled to recover most of the solvent. The last traces were removed under reduced pressure and a dark green waxy solid was obtained. The material was then taken up with petroleum ether

(300 c.c.) and allowed to stand overnight in the refrigerator when a crystalline material was deposited. It was filtered and washed with a little petroleum ether when it became colourless. The petroleum ether solution was then concentrated to half its volume when a little more of the same crystalline solid separated out. It crystallised from alcohol as shining rectangular plates and prisms melting at 129-30°. This fraction (B) is named hypo-phyllanthin; yield, 1.0 g.

The petroleum ether solution was then distilled to recover the solvent. The dark green residue was taken up with alcohol and the colouring matter eliminated to a great extent by fractional precipitation with water. After filtration the aqueous alcoholic solution was distilled under reduced pressure and the solvent completely removed. The green residue was then dried over concentrated sulphuric acid *in vacuo*. It was taken up in ether-petroleum ether mixture (1:10) using the minimum volume and allowed to crystallise when a small quantity of a crystalline solid separated out. This was collected and washed with a little petroleum ether. When crystallised again from this solvent it came out as colourless narrow rectangular plates and prisms melting at 97-98°; yield, 0.05 g. It was bitter to taste. This substance (C) was given the original name, phyllanthin.

The mother-liquor was still intensely bitter and crystallisations using various solvents were unsuccessful as the bitter principle had presumably the same solubility as chlorophyll. Attempts at the elimination of chlorophyll were made using chromatographic adsorption on anhydrous alumina. Initially a petroleum ether solution was employed and the chromatogram developed with benzene-petrol mixture (1:8). Two coloured zones were formed, a lower orange-yellow layer, the colour being due to carotenoids and an upper greenish layer containing chlorophyll and other substances. The column was washed with the solvent mixture (benzene-petroleum ether, 1:6) and the washings collected in stages and examined. No substance was found in the colourless solution. Even when the orange-yellow zone was washed down there was no bitterness. But it was present in the chlorophyll layer and its extract contained all the bitter principle. Therefore the green extract was distilled and the solvent removed. The residue was dissolved in light petroleum and again subjected to chromatographic adsorption over a bigger column of anhydrous alumina, developed and eluted as before but a separation of chlorophyll and the bitter principle was not possible.

Recourse was then taken to eliminate chlorophyll by subjecting it to treatment with alkali. The solid residue containing the bitter principle and chlorophyll was dissolved in alcohol and after adding sufficient potash to make

it 1·4% (N/4), the alcoholic solution was refluxed for half an hour. The solvent was then removed and water added, whereby a pale yellow solid separated out. This was collected, washed with water and dried. The dried material was crystallised from ether-petroleum ether mixture (1:10) when a white crystalline bitter solid was obtained, identical with the above sample of phyllanthin; yield, 5·5 g. When the mother-liquor was evaporated, a brown viscous semi-solid appearing yellow in thin layers was left. It was equally bitter. On allowing to stand for several days, it underwent partial crystallisation. It was filtered after thinning with some ether. The process was repeated and about 1·5 g. more of phyllanthin obtained in a crystalline condition. The total yield of phyllanthin was 0·4% of the dry leaves.

Fraction A (Wax). It was a colourless amorphous solid melting at 80° and had acid No. 17 and saponification No. 92. It was saponified by boiling with 8% alcoholic potash and benzene. The unsaponifiable matter was separated into two fractions by means of cold petroleum ether. The less soluble portion after repeated crystallisation from ether-acetone mixture melted at 87°, and formed an acetate which crystallised from alcohol as colourless needles and melted at 72°. This fraction agreed with the description of myricyl alcohol which was shown by Chibnal *et al.*⁴ to be a mixture of the composition 40% C₃₀, 40% C₃₂ and 20% C₃₄ alcohols. The fraction more soluble in petroleum ether consisted of a small quantity of paraffin hydrocarbons which after purification by boiling with amyl alcoholic hydrochloric acid and by treatment with concentrated sulphuric acid at 130° melted at 59° (Found: C, 85·0; H, 14·9%). The melting point corresponded to an average chain length⁵ of C_{27·8} and hence this fraction should consist mainly of C₂₇ hydrocarbon and its near homologues. The fatty acid portion melted at 84° after crystallisation from alcohol. Its mean molecular weight as determined by titration with N/10 alkali was 408·1. This corresponded to an average chain-length of C₂₇ for the mixture and it should consist mainly of C₂₆ and C₂₈ acids and their near homologues. It could therefore be concluded that the wax consisted mostly of esters (about 85%) of the long-chain fatty acids and alcohols mentioned above, minor quantities of free fatty acids (about 10%) and still smaller quantities of hydrocarbons (about 5%).

Fraction B (Hypophyllanthin). It was a snow-white solid crystallising as rectangular plates and prisms and melting at 129-30°. It was tasteless and soluble in ether, benzene, chloroform and alcohol and insoluble in dilute acid or alkali. It gave no colouration with aqueous or alcoholic ferric

chloride. It did not respond to Molisch test for carbohydrates and Libermann-Burchard reaction for sterols. Nitrogen, halogens or sulphur were not present in it. No colour was produced when an alcoholic solution of hypophyllanthin was treated with magnesium and hydrochloric acid. It was recovered unchanged after boiling with 7% alcoholic sulphuric acid for 6 hours. It did not form any derivative with 2:4-dinitro-phenylhydrazine or semi-carbazide. With concentrated sulphuric acid and a little gallic acid it gave an emerald green colour on warming and thus indicated the presence of a methylene-dioxy group. [Found: C, 65.6; H, 6.4 and OCH_3 , 35.2%; $\text{C}_{19}\text{H}_{22}\text{O}_6$ requires C, 65.9; H, 6.4 and OCH_3 (4), 35.8%.]

The substance (0.5 g.) was boiled under reflux with 25 c.c. of absolute alcoholic potash (10%) for 7 hours. The solvent was later removed and water added when a solid was precipitated. It was filtered, washed free of alkali and crystallised from alcohol when it was found to be identical with hypophyllanthin.

Bromo-derivative.—When treated with bromine under conditions described for phyllanthin, hypophyllanthin yielded a bromo-derivative which crystallises from alcohol as narrow rectangular plates and prisms melting at 112–13°.

Substance C (Phyllanthin).—It was a colourless shining solid crystallising in narrow rectangular plates and prisms and melting at 97–98°. It had a marked bitter taste; it was soluble in ether, chloroform, benzene and alcohol, sparingly soluble in light petroleum and almost insoluble in dilute acid or alkali. No colour was produced on adding to its alcoholic solution ferric chloride or magnesium and hydrochloric acid. The substance dissolved in strong sulphuric acid producing an orange-red colour which changed to purple and was not thrown out on dilution. It did not contain nitrogen, sulphur or halogen. It did not give the test for sterols with the Libermann-Burchard reagent and for carbohydrates by the Molisch reaction. It was recovered unchanged after boiling with aqueous alcoholic sulphuric acid (7%) for 6 hours. No derivative was obtained with either phenylhydrazine or semi-carbazide. It was resistent to oxidation with neutral permanganate in acetone or with aqueous permanganate in alkaline medium. Like hypophyllanthin it gave an emerald green colour on warming with concentrated sulphuric acid and a little gallic acid and thus indicated the presence of a methylene-dioxy group. [Found: C, 66.0; H, 5.6; OCH_3 , 39.6% and molecular weight, 384 (Rast's micro-method) and 380 (depression of freezing point using benzene); $\text{C}_{21}\text{H}_{22}\text{O}_7$ requires C, 65.4; H, 5.7; OCH_3 (5), 40.2% and molecular weight 386.]

It was toxic to fish. In an aqueous solution containing 50 parts per million fresh water fish, *Haplochilus malarica* (about 1" in length and 1·0 g. in weight) showed symptoms of toxicity and lost equilibrium in 10 minutes. It was also toxic to frogs. Frogs weighing about 35·0 g. were employed for the purpose. By injecting into each about 0·1 c.c. of an aqueous alcoholic solution containing phyllanthin sufficient to produce a concentration of 100 parts per million body weight, they showed an apparent difficulty to preserve equilibrium in water and got overturned in 30 minutes. Further they turned pale and got depigmented in an hour and they regained the colour only after 20 hours.

Bromo-derivative.—0·5 g. of the substance was dissolved in chloroform (10 c.c.) and a solution of bromine in chloroform (4%) added little by little till the colour of bromine persisted even after five minutes. The reaction was attended with a copious evolution of hydrogen bromide gas. After half an hour the chloroform solution was allowed to evaporate in an open porcelain basin at the laboratory temperature. The residue was crystallised from alcohol when it came out as narrow rectangular plates and prisms melting at 139–40°. (Found: Br, 18·1%; $C_{21}H_{21}O_7Br$ requires Br, 17·2%).

0·5 g. of phyllanthin was boiled with acetic anhydride (5 c.c.) and hydriodic acid (5 c.c.) for 30 minutes. The mixture was cooled and aqueous sulphur dioxide added. A resinous material was precipitated and it could not be crystallised.

Phyllanthin was boiled with absolute alcoholic potash and also with 50% aqueous potash for 6 hours. It did not undergo fission and was recovered unchanged.

Demethylation of phyllanthin was attempted as follows: 0·5 g. of phyllanthin, 1·0 g. of resorcinol and 7 c.c. of concentrated hydrochloric acid were placed in a sealed tube and heated in a bomb furnace for an hour at 125°. The mixture containing some resin and a red coloured liquid was diluted with water and filtered. The insoluble substance was extracted with hot water several times. The combined filtrate was extracted with ether and the ether solution evaporated. The residue was taken up in water and treated with a solution of lead acetate and the lead salt filtered. It was decomposed with dilute hydrochloric acid and filtered. The filtrate was extracted with ether. When the solvent was removed a brown oily substance was obtained. It could not be crystallised. It gave a green colouration with a solution of ferric chloride.

SUMMARY

Phyllanthin, the bitter principle of the leaves of *Phyllanthus niruri* has been isolated in a crystalline condition and in good yield. It melts at 97-98°, has the molecular formula $C_{21}H_{22}O_7$ and contains methoxyl and methylene-dioxy groups. Its important properties are described. A related crystalline substance, hypophyllanthin, which is not bitter, has also been isolated in a small yield. The leaves contain about 5% of wax which consists mostly of esters.

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