CCLVII. THE ANTHELMINTHIC CONSTITUENT OF THE LEAVES OF *CALYCO- PTERIS FLORIBUNDA*.

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THE copper-coloured tender leaves of *Calycopteris floribunda* Lamk. are reputed to have laxative and anthelmintic properties [Nadkarni, 1927]; ground into paste and made into five-grain pills they are administered for the expulsion of round worms. According to Nadkarni (other authority not cited) an extract of the leaves exhibits the colour reactions of santonin.

Nadkarni states that *Colycopterus floribundo* (Malayalam *Chempullankhi*), belonging to the natural order Combretaceae, is a species found in Malabar and other parts of South India and is synonymous with *Combretum extensum* Roxb. *Colycopterus floribundo* is not listed in Hooker's *Flora of British India*, but *Calycopteris floribunda*, also belonging to the Combretaceae; no mention is made of its being identical with *Combretum extensum*; there are, indeed, no grounds for such a suggestion, and there is no possibility of confusion between the two plants.

The following description of *Calycopteris floribunda* Lamk. is given by Gamble (*Flora of the Presidency of Madras*, Pt III, p. 467). A straggling shrub, the wood of peculiar structure. Leaves opposite, elliptic or ovate. Flowers in dense axillary racemes, crowded in large panicles towards the ends of branches. Calyx-tube elongate, 5-striate, produced above the ovary; limb 5-lobed, the lobes persistent, recurved or spreading, accrescent. Petals 0. Stamens 10 in 2 series, short, 5 opposite to, 5 alternate with, the calyx lobes; filaments subulate; anthers didymous. Ovary inferior, 1-celled; ovules 3, pendulous; style subulate; stigma simple. Fruit narrow, ovoid, 5-ribbed, 1-seeded, surmounted by the enlarged calyx. Seed solitary; cotyledons convolute.

In a report on indigenous drugs made to the Government of Madras Rao Bahadur Dr M. C. Koman [1919] states that the plant will prove to be a good anthelmintic and a very efficient substitute for santonin.

The leaves were collected during the months of October and March in the reserve forests of the Kasargod and Kannothe Ranges of the Mangalore and Wynad Divisions respectively. We are greatly indebted to the District Forest Officer, Mangalore South, who arranged for the collection of the leaves and is responsible for the identification of the plant.

We have isolated from an acetone extract of the leaves a bright yellow crystalline substance to which, in accordance with usual practice, we propose to give the name *calycopterin*. 5.4 g. were obtainable from 4.5 kg. of the air-dried leaves, and preliminary examination readily revealed its nature as a colouring-matter of the flavone group. The substance was identified as the active constituent of the leaves and, so far as we are aware, this is the first example of a flavone with anthelmintic properties. Pharmacological tests by Dr K.

Venkatachalam, Pharmacology Research Officer, Medical College, Madras, who will publish them in detail elsewhere, have shown that calycopterin is toxic to round worms (*Ascaris lumbricoides*) suspended in a mixture of bile with aqueous sodium carbonate, approximating to the composition of the intestinal fluid in which the worms reside. It has no untoward effect on the circulatory or respiratory systems, and clinical trials conducted so far on patients in the General Hospital, Madras, have given encouraging results.

Calycopterin is a flavone of the molecular formula $C_{19}H_{18}O_8$, containing two hydroxyl and four methoxyl groups. Fusion with alkali gave *p*-hydroxybenzoic acid and a water-soluble phenol, whose constitution will be examined when more material is available. The stability of calycopterin in alkaline solution to aerial oxidation points to a methoxyl in position 3; three methoxyls and a hydroxyl are in the fused benzene ring, which is thus substituted in all the six positions (I). The improbability of a pentahydroxybenzene derivative occurring in nature is obvious, but the analytical evidence does not present an alternative.

Demethylation of calycopterin in the known manner with hydriodic acid in acetic anhydride solution gave a hexahydroxyflavone, different from the three already known, gossypetin, myricetin and quercetagenin, and to be designated as *calycopteretin*. Like myricetin, gossypetin and robinetin [Perkin, 1899; Baker, *et al.* 1929; Gulati and Venkataraman, 1933] calycopteretin undergoes a series of colour changes in weak alkaline solution. The substance crystallises in old gold-yellow needles, which slowly turn to dark green on exposure to air. Aeration of an alkaline solution effects decoloration and degradation.

EXPERIMENTAL.

Isolation of calycopterin.

Air-dried leaves (4.5 kg.) of *Calycopteris floribunda* were extracted with acetone in a Soxhlet apparatus, the extract filtered and the major part of the acetone removed *in vacuo*. The residue deposited yellow crystals, together with chlorophyll and resinous matter. The material was collected at the pump and washed successively with light petroleum and cold dilute alcohol. The undissolved substance was taken up in 5 % aqueous caustic soda, filtered, the filtrate acidified and extracted with ether. Two crystallisations of the residue after evaporation of the ether gave glistening golden yellow, prismatic needles, m.p. 225–226°. (Found: C, 60.8; H, 4.8; OCH_3 , 33.4, 33.6; OH, 9.2, 9.3 %; mol. wt. 365. $C_{19}H_{18}O_8$ requires: C, 60.9; H, 4.8; OCH_3 , 33.2; OH, 9.1 %; mol. wt. 374.) The molecular weight was determined by Rast's camphor method and is the average of several readings. The hydroxyl content was estimated by Verley and Bölsing's method [1901; see also Peterson and West, 1927; Marks and Morrell, 1931; Hopmann, 1931]; during many trials with known compounds it was found best to use 0.5 g. or more of the material and to heat at 110° for 3 hours.

Properties of calycopterin.

The substance is sparingly soluble in the common organic solvents in the cold, but dissolves readily in boiling alcohol, acetone, ethyl acetate and glacial acetic acid. It is insoluble in cold sodium carbonate solution, but dissolves completely on boiling; with caustic soda solution the crystals are coloured orange and dissolve to a yellow solution from which the substance is precipitated unchanged on acidification. It forms crystalline oxonium salts with the mineral acids; no compound is formed with semicarbazide, phenylhydrazine or 2:4-dinitrophenylhydrazine. The alcoholic solution gives a deep green coloration with ferric chloride, turning brown on the addition of ammonia; a bright orange, which changes rapidly to magenta, with magnesium and hydrochloric acid (in presence or absence of mercury); a pale green with sodium amalgam; a pale yellow turbidity with lead acetate solution; and with potassium acetate solution yellow needles are slowly deposited on standing. The yellow solution in glacial acetic acid turns dark red on the addition of a drop of concentrated sulphuric acid and an intense red, which becomes orange-brown on standing and deposits a yellow precipitate, on the addition of a drop of aqueous ferric chloride. Crystals of calycopterin are coloured crimson by concentrated nitric acid.

Derivatives of calycopterin.

Diacetylcalycopterin. The acetyl derivative, prepared by boiling the substance with 5 times its weight of acetic anhydride and a few drops of pyridine for 2 hours, crystallised from alcohol in colourless needles and thin elongated plates, m.p. 129°. (Found: C, 60.4; H, 5.0 %. $C_{23}H_{22}O_{10}$ requires: C, 60.3; H, 4.8 %.)

Dibenzoylcalycopterin. The benzoyl derivative (Schotten-Baumann method) crystallised from alcohol in thin, colourless, elongated, hexagonal plates, m.p. 165°. (Found: C, 67.6; H, 4.5 %. $C_{33}H_{26}O_{10}$ requires: C, 68.0; H, 4.5 %.)

Dimethylcalycopterin. To a mechanically stirred mixture of calycopterin (1 g.) and acetone (20 ml.) dimethyl sulphate (10 g.) and 30 % potassium hydroxide solution (30 g.) were added alternately from two dropping funnels, keeping the mixture always faintly alkaline. Finally more acetone (10 ml.) was added and the liquid heated on the water-bath for 30 minutes. On cooling and diluting with water, the precipitate was collected and twice crystallised from aqueous acetone. The shining pale yellow needles melted at 131° after sintering at 127°. (Found: C, 62.6; H, 5.6 %. $C_{21}H_{22}O_8$ requires: C, 62.7; H, 5.5 %.)

Calycopteretin. Calycopterin (1 g.) was suspended in acetic anhydride (15 ml.) and hydriodic acid (sp. gr. 1.7; 15 ml.) and heated (oil-bath) at 135–140° for 2 hours. After leaving overnight, the orange-red needles of calycopteretin hydriodide were collected on a sintered glass funnel and decomposed by boiling with glacial acetic acid (25 ml.) and water (25 ml.) for 30 minutes. After treatment with charcoal, it crystallised in long, glistening, old gold-yellow needles, which slowly changed to a dark green on exposure. (Found¹: C, 56.4; H, 3.4 %. $C_{15}H_{10}O_8$ requires: C, 56.6; H, 3.2 %.) The substance begins to sinter and darken at 294° and melts completely in the neighbourhood of 310°. Calycopteretin undergoes a series of colour changes in alkaline solution, the precise variations depending on the conditions of the reaction. Thus with dilute ammonia the colours are green → blue → green → yellow → orange → pale yellow; with dilute caustic soda solution greenish brown → deep green → brown → orange → pale magenta → yellow. When a few crystals are suspended in water, a drop of 10 %

¹ Microanalyses by Dr Friedrich Fischer, Chemisches Institut der Universität, Frankfurt-am-Main.

caustic soda solution added and the mixture shaken, a greenish yellow solution is formed, which rapidly changes to a dark malachite-green and then to a pale orange. The crystals are coloured bright orange by sulphuric acid and the yellow solution has a faint violet fluorescence. The alcoholic solution gives with ferric chloride an intense olive-green coloration; a magenta with magnesium and hydrochloric acid; and a dark green (and precipitation of a dark green product) with sodium amalgam.

Hexaacetylcalycopteretin. Colourless needles from alcohol, slowly changing to green on exposure; M.P. 212° after sintering at 207° . (Found¹: C, 57.0; H, 4.2 %. $C_{27}H_{22}O_{14}$ requires C, 56.8; H, 3.9 %.)

Degradation of calycopterin.

A mixture of calycopterin (1 g.) and caustic potash (5 g.) in water (10 ml.) was heated to gentle boiling for 2 hours in a current of hydrogen. On cooling, the mixture was acidified with sulphuric acid and extracted repeatedly with ether. The ether extract was extracted with saturated sodium bicarbonate solution. Removal of the ether, after drying over magnesium sulphate, gave an oily product from which a small amount of yellow needles, which proved to be unchanged calycopterin, separated after a time. The mixture was boiled with water and filtered to remove calycopterin; the residue obtained by slow evaporation of the water in a desiccator was crystallised from ethyl acetate-light petroleum and yielded cream-coloured needles, M.P. $132-133^{\circ}$ (sintering at 125°), the amount of which was insufficient for further examination. Acidification of the bicarbonate extract and ether extraction led to an acid, which crystallised from hot water in colourless needles (0.15 g.), M.P. $211-212^{\circ}$, undepressed by admixture with authentic *p*-hydroxybenzoic acid. (Found¹: C, 61.3; H, 4.5 %. $C_7H_6O_3$ requires: C, 60.9, H, 4.3 %.) The *acetyl* derivative crystallised from dilute alcohol in colourless needles, M.P. and mixed M.P. with *p*-acetoxybenzoic acid 185° .

SUMMARY.

1. The anthelmintic constituent, *calycopterin*, of the leaves of *Calycopteris floribunda* has been isolated.
2. Calycopterin is shown to be a dihydroxytetramethoxyflavone, which yields *p*-hydroxybenzoic acid on fusion with alkali.
3. Demethylation of calycopterin gives a new hexahydroxyflavone, *calycopteretin*.

We wish to thank Dr K. Venkatachalam for his interest in this investigation and for permission to publish the results.

¹ Microanalyses by Dr Friedrich Fischer, Chemisches Institut der Universität, Frankfurt-am-Main.

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