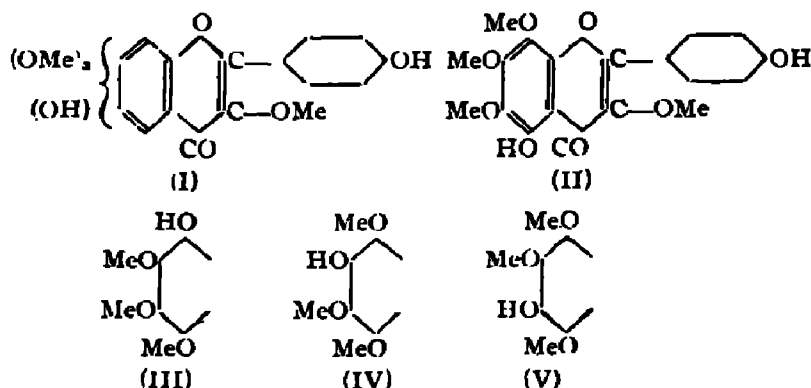


THE CONSTITUTION OF CALYCOPTERIN, THE YELLOW COLOURING MATTER OF THE LEAVES OF *CALYCOPTERIS FLORIBUNDA*

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The constitution of calycopterin, the yellow colouring matter of the leaves of *Calycopteris floribunda*, is shown to be 5:4'-dihydroxy-3:6:7:8-tetramethoxyflavone, since methylation with diazomethane under stated conditions gives a hydroxypentamethoxyflavone, one hydroxyl thus resisting methylation. Further, the dimethyl ether of calycopterin could be partially demethylated to a hydroxypentamethoxyflavone.

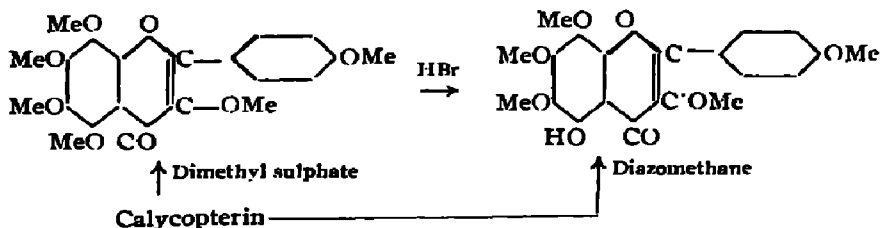
Having partially formulated calycopterin as (I) (Ratnagiriswāran, Sehra and Venkataraman, *Biochem. J.*, 1934, 28, 1964), four possibilities (II, III, IV, V) remained to be considered. The ease with which calycopterin gave a dimethyl ether, the latter being obtainable also by the action of methyl iodide and potassium hydroxide in methyl alcohol and the preparation



of a dibenzyl ether whose alcoholic solution did not exhibit a colouration with ferric chloride (*cf.* Gulati and Venkataraman, *J. Chem. Soc.*, 1936, 267) appeared to exclude formula (II). On methylation by diazomethane, however, only one hydroxyl group (4') was methylated, and the monomethylcalycopterin, characterised by the formation of an acetyl derivative, exhibited the characteristic properties of a 5-hydroxyflavone, such as intense colouration with alcoholic ferric chloride, insolubility in dilute alkali and the formation of sparingly soluble yellow potassium and sodium salts with alcoholic potassium and sodium hydroxides respectively. As the hydroxyl group is thus shown to be in the 5 position, the three methoxy groups must be in positions 6, 7, 8. Calycopterin was now, therefore, fully formulated

as 5:4'-dihydroxy-3:6:7:8-tetramethoxyflavone (II), the flavone thus falling into line with the suggestion of Bose and Nath (*J. Indian Chem. Soc.*, 1938, 15, 139) that in partially methylated polyhydroxyflavones occurring in nature, the hydroxyl in the 5-position is as a rule not to be found in the methylated condition.

Hydrogen Bromide as a reagent for Preferential Demethylation.—Dimethylcalycopterin was treated with hydrogen bromide in acetic acid at room temperature and the resulting compound was found to be identical with monomethylcalycopterin obtained by methylation of calycopterin with diazomethane, preferential demethylation of the 5-methoxyl group having taken place. This observation is of interest, as hydrogen bromide in acetic acid has not been previously employed for the preferential demethylation of the 5-methoxyl group in flavones, the reagent suggested



earlier being aluminium chloride (Gulati and Venkataraman, *Current Science*, 1933, 2, 50; Gulati and Venkataraman, *loc. cit.*; Mahal and Venkataraman, *ibid.*, 569). An interesting point with regard to this method is that it does not demethylate the 3-methoxyl group as aluminium chloride does (Mahal and Venkataraman, *Current Science*, 1935, 4, 311). In order to confirm that hydrogen bromide in the cold does not attack the methoxyl group in the 3-position, 3-methoxyflavone has been treated with it at room temperature when the ether remains unaffected, but when the reaction mixture is heated on the water-bath, 3-hydroxyflavone is formed. Hydrogen bromide is, therefore, a more facile reagent than aluminium chloride for preferential demethylation.

On the strength of a report from the Pharmacology Research Officer, Medical College, Madras, and the statement of Nadkarni (1927), anthelmintic properties were ascribed to calycopterin in a previous communication (Ratnagiriswaran *et al*, *loc. cit.*). Mahal (*Proc. Indian Acad. Sci.*, 1937, 3A, 186) has more recently studied the anthelmintic action of calycopterin, chrysin, genkwanin (Mahal and Venkataraman, *loc. cit.*), 7-hydroxyflavone and 6-hexyl-7-hydroxyflavone (Dhingra, Uppal and Venkataraman, *Proc. Ind. Acad. Sci.*, 1936, 3A, 206) *in vitro* by directly immersing round worms (*Ascaris lumbricoides*), tape worms (*Tenia serrata*) and leeches (*Hirudo*

medicinalis) in solutions of various concentrations. None of the flavones possesses any anthelmintic or germicidal properties. The Rideal-Walker coefficients are all less than unity.

EXPERIMENTAL.

Two slight modifications in the procedure for the isolation of calycopterin afforded an improved yield (0.23 g. from 100 g. of air-dried leaves). The leaves were not Soxhletted, but refluxed with two lots of acetone; the residue after removal of acetone was vigorously shaken with 150 c.c. of petroleum ether (b.p. 50°-60°) and filtered, the process being repeated thrice.

Calycopterin Dibenzyl Ether.—Benzylation of calycopterin (1 g.) with benzyl chloride (20 g.), potassium carbonate (16 g.) and acetone (150 c.c.) during 32 hours gave the dibenzyl ether, which crystallised from alcohol-acetic acid in pale yellow needles (1.25 g.), m.p. 185°. (Found: C, 71.0; H, 5.6. $C_{38}H_{30}O_8$ requires C, 71.4; H, 5.4 per cent).

Methylation of Calycopterin by Dimethyl Sulphate: Formation of Dimethylcalycopterin (3:4':5:6:7:8-Hexamethoxyflavone).—To a mixture of calycopterin (0.5 g.) and acetone (10 c.c.), dimethyl sulphate (5 g.) and potassium hydroxide solution (15 g. of 30%) were added alternately, keeping the mixture always alkaline. Finally more acetone (5 c.c.) was added and the mixture heated on the water-bath for about $\frac{1}{2}$ hour. The product, which separated on cooling and diluting the mixture with water, crystallised from aqueous acetone in pale yellow needles, m.p. 131°. Ratnagiriswaran and others (*loc. cit.*) recorded the melting point 131° after sintering at 127°.

Methylation of Calycopterin by means of Diazomethane: Formation of Monomethylcalycopterin (5-Hydroxy-3:6:7:8:4'-pentamethoxyflavone).—Calycopterin (0.3 g.) was dissolved in excess of methyl alcohol (30 c.c.); diazomethane in excess (3 mols) dissolved in dry ether was added and the reaction mixture kept at 0° overnight. The yellow solid obtained after allowing ether and alcohol to evaporate was treated with sodium hydroxide (2N). The solid was filtered off at the pump, washed with water and crystallised from absolute alcohol. The substance crystallised in brownish yellow needles, m.p. 124°. With alcoholic ferric chloride solution it gave a fairly deep green colouration. It was practically insoluble in dilute sodium hydroxide, but gave the sparingly soluble yellow potassium and sodium salts with alcoholic potassium and sodium hydroxides respectively. (Found: C, 62.2; H, 5.3. $C_{28}H_{20}O_8$ requires C, 61.9; H, 5.2 per cent).

Demethylation of Dimethylcalycopterin by Hydrogen Bromide in Acetic Acid.—A warm solution of dimethylcalycopterin (0.1 g.) in glacial acetic acid (1.5 c.c.) was treated with 50% hydrogen bromide in acetic acid (1 c.c.) and the reaction mixture was kept overnight. It was then diluted with water and extracted with ether. The ethereal extract was washed first with dilute sodium hydroxide solution and then with water. Ether was allowed to evaporate and the yellowish solid obtained was crystallised from alcohol. It crystallised in brownish yellow needles melting at 124°, not depressed by admixture with the compound obtained by methylation of calycopterin with diazomethane.

5-Acetoxy-3,6:7,8:4'-pentamethoxyflavone.—5-Hydroxy-3,6:7,8:4'-pentamethoxyflavone (0.2 g.) was heated under reflux on a sand-bath with acetic anhydride (1 c.c.) and a few drops of pyridine for 1½ hours. On cooling and diluting with water, the product crystallised from alcohol in colourless needles, m.p. 107°. (Found: C, 61.6; H, 5.0. $C_{22}H_{22}O_8$, requires C, 61.4; H, 5.1 per cent).

Demethylation of 3-Methoxyflavone.—(i) A mixture of 3-methoxyflavone (0.5 g.), glacial acetic acid (4 c.c.) and hydrogen bromide in acetic acid (5 c.c.) was kept for 48 hours at room temperature. It was then diluted with water and the separated product was crystallised from alcohol, m.p. 114°, not depressed by admixture with 3-methoxyflavone.

(ii) A mixture of 3-methoxyflavone (0.5 g.), glacial acetic acid (4 c.c.) and hydrogen bromide in acetic acid (5 c.c.) was heated on the water-bath for 1 hour. It was then diluted with water and the separated product was crystallised from alcohol, m.p. 168-69°; no depression was observed in the mixed melting point of this compound with 3-hydroxyflavone.

(iii) 3-Methoxyflavone (0.5 g.) was thoroughly mixed with excess of anhydrous aluminium chloride (1 g.), and the mixture was heated at 100° for 2 hours. The reaction mixture was then treated with dilute hydrochloric acid, and the dark coloured product was extracted with ether. The ethereal layer was shaken with dilute sodium hydroxide solution, and the alkaline layer acidified with dilute hydrochloric acid. The precipitate when crystallised from alcohol, had m.p. 169° and was identified as 3-hydroxyflavone.

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