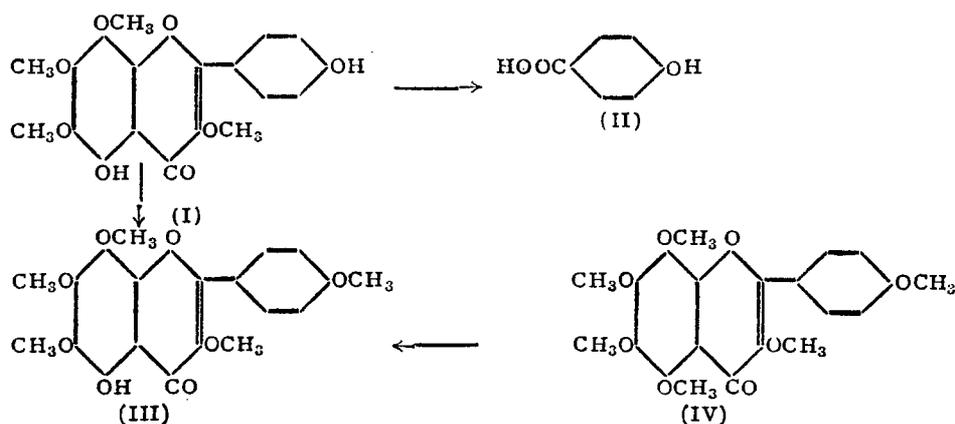


## CONSTITUTION OF CALYCOPTERIN

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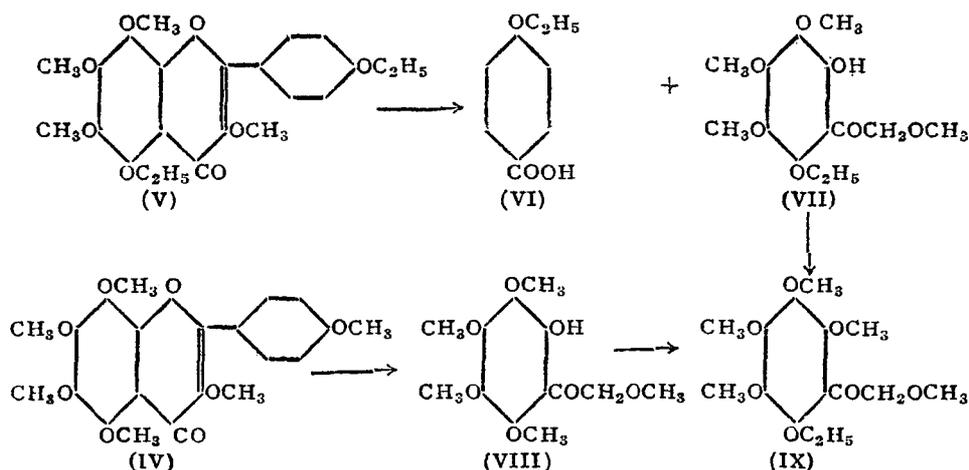
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CALYCOPTERIN is a tetramethyl ether of calycopteretin which is 3:5:6:7:8:4'-hexahydroxy-flavone. Its exact constitution was first investigated by Gulati and Venkataraman<sup>1</sup> and their conclusions were later modified by Shah, Venkataraman and Virkar.<sup>2</sup> Of the two free hydroxyl groups, one was easily located in the 4'-position by identifying *p*-hydroxybenzoic acid (II) as a product of alkaline fission. The position of the other hydroxyl has been finally fixed as 5, since this group is resistant to methylation and the monomethyl ether of calycopterin (III) gives reactions characteristic of a 5-hydroxyl. Further this monomethyl ether has been obtained by the partial demethylation of the dimethyl ether (IV) by means of hydrobromic acid. Consequently calycopterin is considered to be 5:4'-dihydroxy-3:6:7:8-tetramethoxy-flavone (I).



The above constitution has now been confirmed in a different way. Calycopterin is ethylated by means of ethyl iodide and potassium carbonate in acetone solution and the diethyl ether (V) obtained in good yield. When subjected to fission with alcoholic potash, it yields an acid product which is identified as *p*-ethoxybenzoic acid (VI) locating an ethoxyl in the 4'-position of the flavone. The other part is a ketone having the composition and the characteristic properties of 2-hydroxy-monoethoxy-tetramethoxy-acetophenone (VII). In order to determine its constitution it is further methylated by means of dimethyl sulphate and anhydrous potassium carbonate

in acetone solution. This product is found to be identical with the ethylation product (IX) of 2-hydroxy- $\omega$ :3:4:5:6-pentamethoxyacetophenone (VIII). The identity has been established not only by direct comparison but also by the preparation of derivatives. This is possible only if in calycopterin, a free hydroxyl exists in the 5-position which is substituted by an ethoxyl in the ethyl ether. The various transformations described above are diagrammatically represented below:



#### EXPERIMENTAL

##### *Ethylation of calycopterin:—Diethyl-calycopterin (V).—*

Calycopterin (1 g.) dissolved in acetone (20 c.c.) was boiled under reflux with excess of ethyl iodide (3.0 c.c.) and anhydrous potassium carbonate (10 g.) for a period of 15 hours. The solution which was originally orange red became practically colourless towards the end. The solvent was then removed by distillation, and the residue treated with ice-water. The ethyl ether which separated out as a liquid solidified during the course of half an hour. When it was crystallised from alcohol it came out as colourless rhombohedral prisms melting at 131–32° (Karrer<sup>3</sup>, mp. 130°). It was insoluble in dilute alkali and did not give any colour with alcoholic ferric chloride. The mixed melting point with dimethyl-calycopterin was depressed, 105–115°. (Found: C, 64.1; H, 6.1;  $C_{23}H_{26}O_8$  requires C, 64.0; H, 6.0%.) It was easily soluble in acetone, ethyl acetate and hot alcohol and very sparingly soluble in cold alcohol.

##### *Decomposition of diethyl calycopterin with alcoholic potash.—*

Diethyl-calycopterin (1.0 g.) was boiled under reflux with absolute alcoholic potash (30 c.c., 8%) for a period of six hours. At the end of the

operation, as much of the alcohol as possible was removed by distillation and the residue dissolved in water. The solution was then filtered from any suspended insoluble impurities and the clear filtrate was acidified with dilute sulphuric acid. It was then ether-extracted several times and the combined ether extract was shaken three times with 5% sodium bicarbonate solution to remove the acid part (A).

*The ketonic part.*—The ether solution was then washed with water and on distilling off ether it gave a pale yellow liquid which solidified during the course of a few hours. It was purified by crystallisation from light petroleum (B.P. 60°) when it came out in the form of pale yellow thin rectangular plates melting at 75–77° (Karrer<sup>3</sup> gives mp. 63–64°). It dissolved in alkali to give a yellow solution and gave an olive-green colour with alcoholic ferric chloride. It is highly soluble in water even in the cold. (Found: C, 55.9; H, 6.6; C<sub>14</sub>H<sub>20</sub>O<sub>7</sub> requires C, 56.0; H, 6.7%.)

*The acid part.*—The bicarbonate solution (A) on acidification with concentrated hydrochloric acid precipitated a crystalline material which was ether extracted. The residue obtained after removal of ether was purified by crystallisation from hot water or 10% alcohol when it came out in the form of colourless shining needles melting at 197–98°. The mixed melting point with a synthetic sample of *p*-ethoxy-benzoic acid (see below) was undepressed. (Found: C, 65.2; H, 6.2; OC<sub>2</sub>H<sub>5</sub>, 26.9; C<sub>9</sub>H<sub>10</sub>O<sub>3</sub> requires C, 65.1; H, 6.0; OC<sub>2</sub>H<sub>5</sub>, 27.1%.)

*p*-Hydroxy-benzoic acid (1.0 g.) was ethylated in acetone solution with ethyl iodide (3.0 c.c.) and anhydrous potassium carbonate by boiling for a period of 15 hours. The solvent was removed by distillation and the residue treated with water. The ether-ester that separated out as a liquid was twice extracted with ether. On removing the solvent it was again obtained as a liquid. It was then hydrolysed by boiling with 20% sodium hydroxide solution (20 c.c.) for half an hour and the acid obtained on acidification, was crystallised from 10% alcohol when it came out as colourless shining needles melting at 197–98°. Yield 0.9 g.

The aqueous potassium carbonate solution from which the ether had separated was acidified with dilute sulphuric acid when a crystalline precipitate separated out. It was crystallised from 10% alcohol and was found to be *p*-ethoxy-benzoic acid. Yield 0.1 g. Thus in the process of ethylation of *p*-hydroxy-benzoic acid some acid is formed along with the ester.

$\omega$ : 2: 3: 4: 5-pentamethoxy-6-ethoxy-acetophenone (IX).

The above ketone was methylated in acetone solution by boiling with dimethyl sulphate and anhydrous potassium carbonate for a period of ten

hours. The solvent was then removed by distillation, and the residue treated with water when an insoluble liquid separated out and it was extracted with ether. Removal of the solvent gave the methyl ether as a viscous liquid. It did not solidify on keeping in the ice-chest for a number of days and attempts at crystallisation were not successful. It was insoluble in aqueous alkali and did not give any colour with alcoholic ferric chloride. Hence it was considered to be the required ketone and was further identified as its 2:4-dinitro-phenyl-hydrazone. This was prepared by boiling the ketone with an alcoholic solution of dinitro-phenyl-hydrazine and was obtained as deep red rhombohedral prisms by crystallisation from alcohol or acetic acid melting at 198–99°. (Found: C, 57.7; H, 5.9;  $C_{21}H_{26}O_{10}N_4$  requires C, 57.5; H, 5.9%.) A mixed melting point with 2:4-dinitro-phenyl-hydrazine was depressed.

*Ethylation of 2-hydroxy- $\omega$ :3:4:5:6-pentamethoxy-acetophenone (VIII).—*

*$\omega$ :2:3:4:5-pentamethoxy-6-ethoxy-acetophenone (IX).—*

2-Hydroxy- $\omega$ :3:4:5:6-pentamethoxy-acetophenone<sup>4</sup> was ethylated in acetone solution by boiling with ethyl iodide and potassium carbonate. The product on working up came as a liquid which could not be solidified. It was insoluble in dilute aqueous alkali and did not give any colour with alcoholic ferric chloride. It was identified as its 2:4-dinitrophenylhydrazone. This was prepared as before and it crystallised from acetic acid as deep red rhombohedral prisms melting at 198–99°. A mixed melting point with the sample obtained previously was undepressed.

#### SUMMARY

The constitution of calycopterin as 5:4'-dihydroxy-3:6:7:8-tetramethoxy-flavone has been confirmed. Calycopterin diethyl ether has been shown to have ethoxyl groups in the 5 and 4'-positions, since on fission with alcoholic alkali, it yields *p*-ethoxy benzoic acid and 2-hydroxy- $\omega$ :3:4:5-tetramethoxy-6-ethoxy acetophenone. The constitution of the ketone has been established by methylation and comparison with a synthetic sample obtained by the ethylation of  $\omega$ :2:3:4:5-pentamethoxy-6-hydroxy-acetophenone.

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