

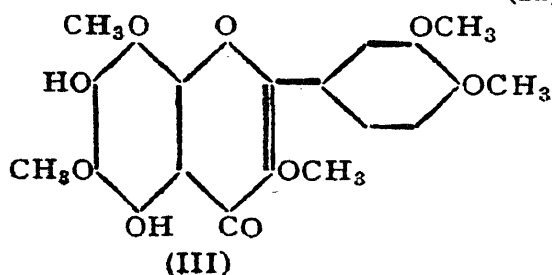
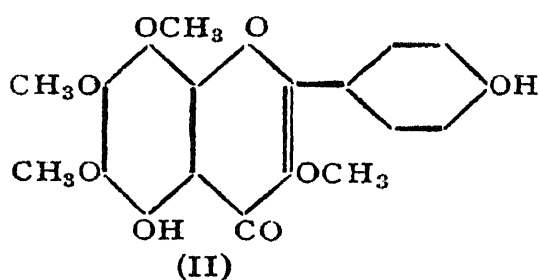
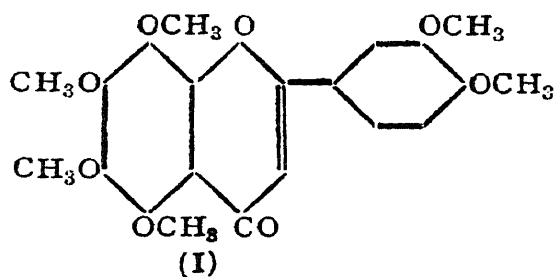
SYNTHESIS AND STUDY OF 5:6:7:8-HYDROXY-FLAVONOLS

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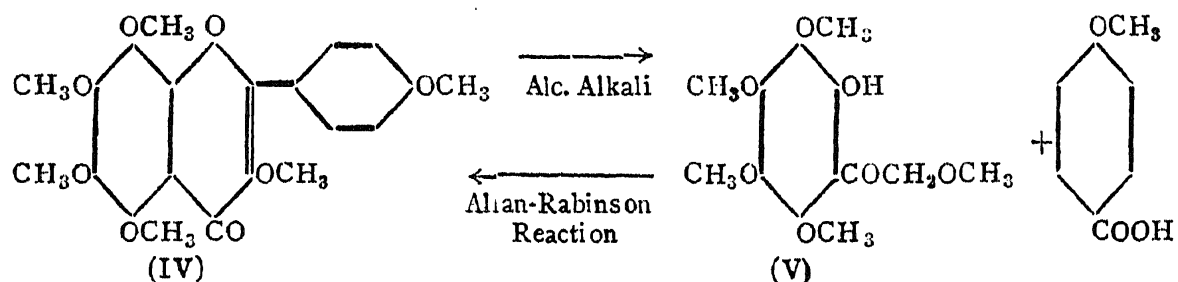
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FLAVONES and flavonols with hydroxyl groups in all the four positions, 5, 6, 7 and 8, seem to be more widely occurring in nature than originally expected. They are, however, recent discoveries and they do not occur free as such, but are found to be partially or completely methylated. Nobiletin (I) is a fully methylated flavone of this type and it was isolated by Tseng¹ from the peels of the Chinese Mandarin oranges (*Citrus nobilis*). Robinson and Tseng² established its constitution as 5:6:7:8:3':4'-hexamethoxy flavone. It has recently been synthesised by Horii³ by the condensation of 2-hydroxy-3:4:5:6-tetramethoxy-acetophenone with veratroyl chloride and the conversion of the product into the flavone according to the method of Baker and Venkataraman. Calycopterin (II) is a partially methylated flavonol, isolated earlier by Ratnagiriswaran, Sehra and Venkataraman⁴ from the coppered coloured leaves of *Calycopteris floribunda*. It was also found to be present in the leaves of *Digitatis thapsi* (Spanish digitalis) by Karrer⁵ and hence called also by the name thapsin. Its constitution has been recently given as 5:4'-dihydroxy-3:6:7:8-tetramethoxy-flavone (II)⁶. Erianthin (III) is also considered to be a flavonol of this series; it is partially methylated and has been recently obtained from the plant, *Blumea eriantha* D.C. by Bose and Dutt.⁷



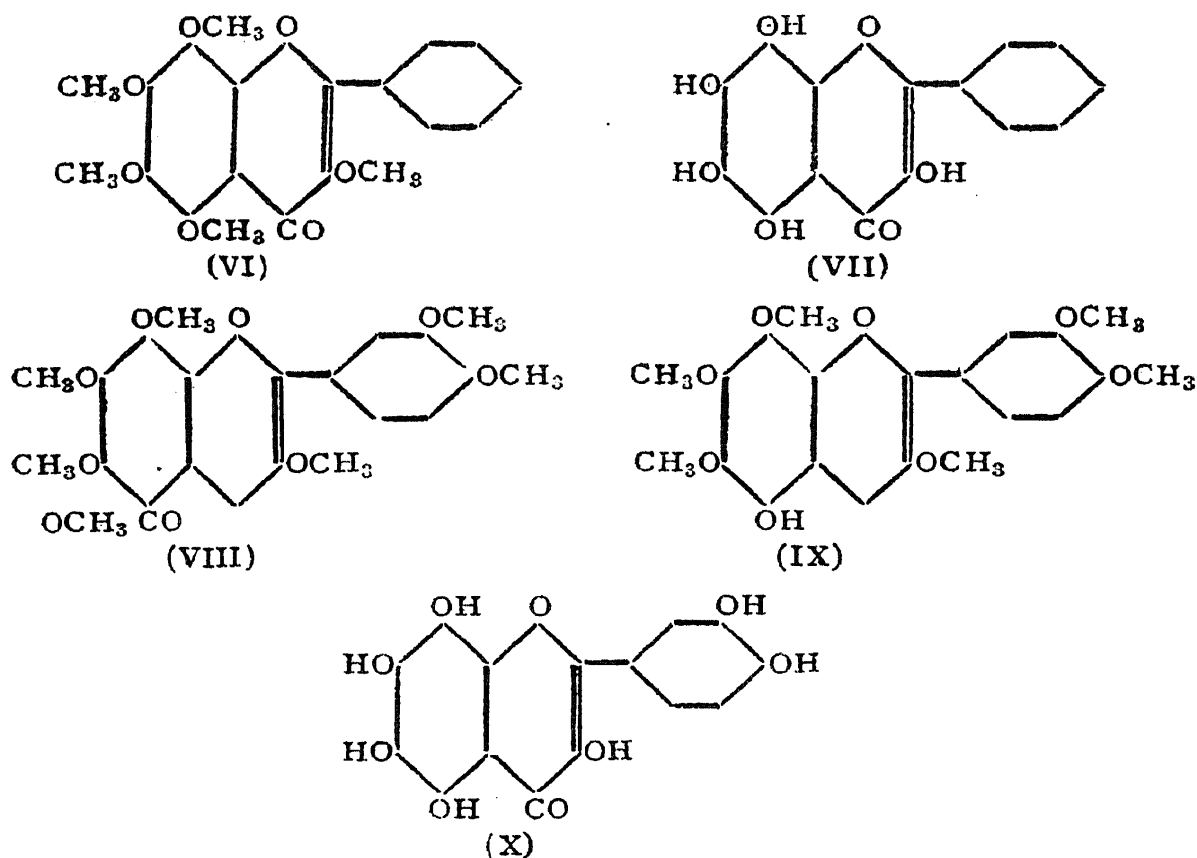
So far these are the only compounds that have been studied in detail. Some other substances isolated in this laboratory seem also to belong to this group. Full information is not available about these compounds enabling the identification of new members easy and this is particularly necessary since frequently these substances could be isolated only in very small quantities. A more detailed study of the hexahydroxy-flavone, calycopterin, has therefore been made now. Further, other members of this flavonol group have also been prepared and their reactions and derivatives studied in detail.

The dimethyl ether of calycopterin is more easily obtained pure by methylating calycopterin with dimethyl sulphate and potassium carbonate in anhydrous acetone solution. When subjected to hydrolytic fission with 8% absolute alcoholic potash, it yields, besides anisic acid, a ketonic product as a low melting solid which could also be characterised by the preparation of the 2:4-dinitrophenyl-hydrazone. Analytical data of these two substances and reactions show that the ketone is 2-hydroxy- ω : 3:4:5:6-pentamethoxy-aceto-phenone (V). Condensation of the ketone with anisic anhydride and sodium anisate yields dimethyl calycopterin. These experiments confirm the results of previous workers^{4,5,6} that calycopterin is a tetramethyl ether of 3:5:6:7:8:4'-hexahydroxyflavone.



Starting from the above ketone the other members of this flavonol group have been obtained. The following is a brief account of the results. The ketone condensed with benzoic anhydride and sodium benzoate smoothly yielding 3:5:6:7:8-pentamethoxyflavone (VI) as the sole product. On demethylation the corresponding penta-hydroxy compound, 6:8-dihydroxygalangin (VII) was obtained. By the condensation of the abovementioned ketone with the anhydride and sodium salt of veratric acid, two products were isolated, one of which separated out from alkaline solution and consisted essentially of the normal hepta-methoxy flavone (VIII) and the other separated from the solution after saturation with carbon-dioxide and consisted mainly of the partially demethylated product (IX) showing the properties of a free hydroxyl in the 5-position. Examination of these revealed that they were still mixtures with indefinite melting points and purification

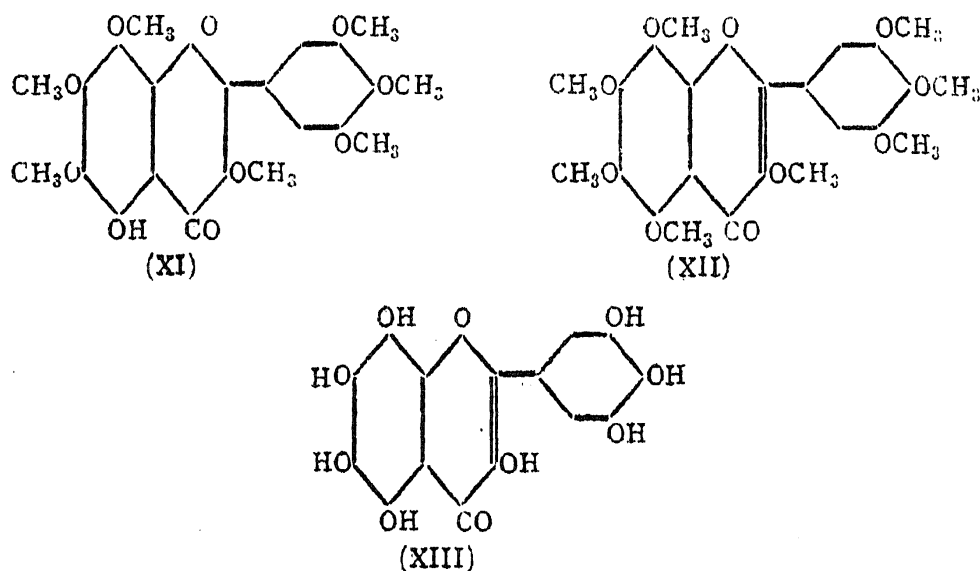
could not be achieved satisfactorily. However by methylating them a pure sample of the hepta-methoxy flavone (VIII) and by demethylating them the corresponding hepta-hydroxy flavone, 6 : 8-dihydroxyquercetin (X) were readily obtained. A pure sample of 5-hydroxy-3 : 6 : 7 : 8 : 3' : 4'-hexamethoxyflavone (IX) could, however, be obtained by ether-extracting the aqueous mother-liquor after the filtration of the impure sample of (IX) that first separated out. It gave a prominent ferric chloride colour and a sparingly soluble potassium salt when treated with alcoholic potash.



According to Bose and Dutt⁷ methyl erianthin should be identical with the abovementioned 5-hydroxy-hexamethoxy flavone (IX). But the recorded properties are not the same. Methyl erianthin is reported to crystallise in the form of needles and melt at 141°. The above hydroxy flavone (IX) crystallises in the form of plates and melts at 122°. The lower melting point of compound (IX) may be considered to be genuine from a comparison of analogous 5-hydroxy-compounds and the related fully methylated flavonols given in Table I. For example, monomethyl calycopterin melts at 124° whereas the dimethyl ether melts at 134°. The 5-hydroxy-compound (XI) described later on also melts 10° lower than the fully methylated ether.

When the anhydride and the sodium salt of trimethyl-gallic acid were used for the above Allan-Robinson condensation the product consisted entirely of the 5-hydroxy compound (XI). The octamethoxy flavone (XII)

was obtained from it by methylation and the octahydroxy compound (XIII), 6:8-dihydroxymyricetin by demethylation. This is the most highly hydroxylated compound of the flavone group known so far and is thus of special interest.



For the purpose of conveniently naming them, the new flavanols are considered to be derived from the series, galangin, k  mferol, quercetin and myricetin which have the 5:7-arrangement of hydroxyl groups and which seem to be more fundamental in evolution giving rise to the others by oxidation of the nuclear positions concerned.

The above hydroxyflavonols are in general yellow crystalline substances, the colour changing to green on storage. While 6:8-dihydroxygalangin is the most stable, retaining its yellow colour for several months, the others change from yellow to greenish yellow and green, the rapidity of the change reaching the maximum with the octa-hydroxy compound, 6:8-dihydroxymyricetin. With water, even in the cold, they give a green colour and on boiling, the colour intensifies to deeper green or blue. Even an alcoholic solution changes colour from yellow to green and blue on standing or heating. Crystallisation of the flavonols should not be done with solvents like alcohol and acetic acid and it is best effected by using pure dry ethyl acetate. The pure substances do not exhibit any fluorescence in alcoholic solution or even in concentrated sulphuric acid. Like most hydroxy-flavonols they all give bright red colour when treated with magnesium and hydrochloric acid in alcoholic solution.

The new series of flavonols can be viewed in two ways, (1) as 5:7:8-hydroxyflavonols with an additional hydroxyl group in the 6-position and (2) as 5:6:7-hydroxy flavonols with an additional hydroxyl group in the 8-position. In conformity with the first point of view, they exhibit colour

changes in alkaline solutions more remarkable than the members of the gossypetin series. These have been studied in detail using buffer solutions and the results may be useful for purposes of identifying samples. But they do not react with *p*-benzoquinone in the manner characteristic of gossypetin and its analogues. Bargellini's test yielding green flocks, which is characteristic of the quercetagenin series of flavonols is not given by the new flavonols. Thus the presence of one more hydroxyl group in them causes marked differences in properties.

The fully methylated ethers are colourless substances, insoluble in aqueous alkali and giving no colour with alcoholic ferric chloride. The ethers which have a free hydroxyl in the 5-position dissolve in aqueous alkali only with difficulty, form sparingly soluble potassium salts with alcoholic potash and give prominent ferric chloride colour.

The acetates are also colourless solids. Their crystallisation from boiling alcohol seems to bring about partial hydrolysis and the products assume colour on storage. It should therefore be carried out using anhydrous solvents or in the cold using acetone-alcohol mixture.

Table I gives some of the important data relating to this new series of flavonols and their derivatives.

TABLE I

Flavonols	6 : 8-Dihydroxy-galangin	Calycopteretin	6 : 8-Dihydroxy-quercetin	6 : 8-Dihydroxy-myricetin
1. Alc. Fe Cl ₃ ..	Olive green changing to brown	Bright green changing to brown	Deep green changing to brown	Deep green changing to brown
2. Mg + HCl ..	Orange red	Red	Deep red	Deep red
3. M.p. of the flavonols	257-58° (with decomp.)	318-20° (with decomp.)	Turns dark above 300; does not melt below 360°	Turns dark above 300°; does not melt below 360°
4. M.p. of the acetates	201-03°	218-20°	221° with sintering at 219°	225° with sintering 221°
5. M.p. of the fully methylated ethers	80-82°	133-34° 131° (Shah <i>et al</i> ⁶)	129-31°	122-23°
6. M.p. of partial methyl ethers with the 5-hydroxyl alone free	..	124° (Shah <i>et al</i> ⁶)	122-23°	113-15°

EXPERIMENTAL

Methylation of calycopterin : Dimethylcalycopterin (IV).—

Calycopterin (1.0 g.) was dissolved in dry acetone (25 c.c.), treated with dimethyl sulphate (1.0 c.c.) and anhydrous potassium carbonate (10.0 g.)

and boiled under reflux for a period of 20 hours. The solvent was then removed by distillation and the residue treated with water (150 c.c.). The methyl ether soon separated out. On crystallisation from alcohol, it came out in sheaves of colourless elongated needles melting at 133–34° (Cf. Shah *et al.*,⁶ pale yellow needles, m.p. 131°) and did not dissolve in aqueous alkali. (Found: C, 62.9; H, 5.7; OCH₃, 46.0; C₂₁H₂₂O₈ requires C, 62.7; H, 5.5; OCH₃, 46.2%.) Yield, 1.0 g.

Decomposition of the methyl ether with alcoholic potash : Isolation of anisic acid and ketone (V).—

Dimethyl calycopterin (1.0 g.) was boiled under reflux with 8% absolute alcoholic potash (30 c.c.) for a period of six hours. At the end of this period, as much of the alcohol as possible was removed by distillation and the residue dissolved in water. The solution was filtered from any suspended impurities, and then acidified with dilute sulphuric acid. The precipitated crystalline solid was filtered, washed with cold water and then crystallised from boiling water when it came out as colourless rectangular plates and prisms melting at 185°. It was found to be anisic acid. The mixed melting point with an authentic sample of anisic acid was undepressed. The filtrate was repeatedly ether-extracted and the combined ether solution washed with sodium bicarbonate solution (5%) to remove completely the acid part. The ether solution was then washed with water and the solvent evaporated. The residue was a pale yellow liquid which solidified on keeping in an ice-chest for a number of days. It crystallised from petroleum ether (b.p. about 60°) as pale yellow rectangular plates and prisms melting at 65–67° (Karrer⁵ gives mp. 66–67°). It gave a green colour with alcoholic ferric chloride. Yield, 0.38 g. (Found: C, 54.8; H, 6.1; OCH₃, 53.9; C₁₃H₁₈O₇ requires C, 54.6; H, 6.3; OCH₃, 54.2%.) The ketone was also characterised as its 2:4-dinitrophenylhydrazone in the following manner:

The ketone (0.2 g.) dissolved in alcohol (2 c.c.) was treated with 2:4-dinitrophenylhydrazine (0.2 g.) in alcohol (2.0 c.c.). The clear solution was then boiled under reflux on a water-bath for ½ hour. On cooling the alcoholic solution, the phenylhydrazone separated out. It was filtered and crystallised from dilute alcohol when it came out as short orange red rectangular prisms melting at 174–75°. (Found: C, 45.2; H, 5.0; OCH₃, 31.1; C₁₉H₂₂O₁₀N₄, 2H₂O requires C, 45.4; H, 5.2; OCH₃, 30.9%.) Loss on drying could not be estimated as the substance sublimed easily above 100° *in vacuo*.

Synthesis of dimethyl calycopterin (IV).—

An intimate mixture of sodium anisate (2 g.), anisic anhydride (4.0 g.) and the ketone (V) (0.75 g.) was heated in an oil-bath at 180° *in vacuo* for a

period of five hours. The hard mass was then broken up and dissolved in alcohol (50 c.c.). To the solution boiling under reflux was added aqueous potassium hydroxide solution (5 g. in 10 c.c. of water) during the course of half an hour and the boiling continued for another half-hour in order to decompose completely the unreacted anhydride. The alcohol was then removed by distillation under reduced pressure and the residue treated with excess of water. The methyl ether separated out as long needle-shaped colourless crystals. It was filtered and the product was crystallised from dilute alcohol when it came out as sheaves of colourless elongated needles melting at 133–34°. The mixed melting point with the sample obtained directly from calycopterin by methylation was undepressed. Yield, 0.5 g.

The clear alkaline solution from which the hexamethyl ether had separated out was then saturated with carbon dioxide. No product was obtained even by ether extraction.

Demethylation of Calycopterin: Calycopteretin (6:8-dihydroxy-kämpferol).

Calycopterin (0.5 g.) was dissolved in acetic anhydride (10.0 c.c.) and treated with hydriodic acid (10.0 c.c.; d. 1.7) with cooling. The clear solution was then boiled under reflux for half an hour, poured into ice-water and iodine present decomposed by passing sulphur-dioxide gas. The precipitated flavonol was twice recrystallised from ethyl acetate when it came out as bright yellow short rectangular plates, melting with decomposition at 318–20°. When crystallised from acetic acid it came in the form of golden yellow needles which soon turned green on exposure to air. (Found: C, 56.8; H, 3.4; $C_{15}H_{10}O_8$ requires C, 56.6; H, 3.2%.) It gave a bright green colour with alcoholic ferric chloride which rapidly changed to brown. The flavonol exhibited a series of colours in alkaline solution, the precise variations depending on the conditions of the reaction. Thus with 5% sodium bicarbonate it slowly dissolved to give a yellowish green and then green colour changing to greenish-blue and blue; it faded after 10 minutes to light green with a green precipitate. With 5% sodium carbonate the colours were yellow and deep green, quickly becoming blue on shaking; it then faded and became yellowish green within two minutes, brownish yellow and finally brown. With 5% caustic alkali the solution was reddish brown changing to red.

Calycopterin-hexaacetate.—

A small quantity of the flavonol was acetylated using acetic anhydride and a drop of pyridine and boiling for one hour. The product was then crystallised from cold acetone solution enough ethyl alcohol being added just to start the crystallisation. It came out as colourless needles melting at

218–20°. (Found: C, 56.9; H, 4.1; $C_{27}H_{22}O_{14}$ requires C, 56.8; H, 3.9%.)

Condensation of the ketone (V) with benzoic anhydride and sodium benzoate; preparation of pentamethoxy flavone (VI).—

An intimate mixture of benzoic anhydride (10 g.), sodium benzoate (2 g.) and the ketone (0.75 g.) was heated at 175–80° for a period of 5 hours. The product melted completely within the first one hour and later solidified. The reaction mixture was broken up in alcohol (50 c.c.) and while boiling under reflux, a solution of potassium hydroxide (15 g. in 25 c.c.) was added during the course of 20 minutes. The solution was boiled for a further 20 minutes to decompose the anhydride. The alcohol was then removed under reduced pressure and the residue treated with water. A viscous liquid separated out which did not solidify easily. Hence, the mixture was ether extracted, and the ether extract washed with water to remove alkali. After distilling off the solvent, the product was purified by dissolving in acetone and adding petroleum ether when coloured impurities separated out. The clear solution was then decanted; evaporation of the solvent left a viscous liquid which solidified during the course of 24 hours on keeping in an ice-chest. The product was further purified by crystallisation from dilute methyl alcohol when it came out as colourless rectangular prisms melting at 80–82°. It was insoluble in dilute alkali and did not give any colour with ferric chloride. (Found: C, 64.6; H, 5.3; $-OCH_3$, 41.6; $C_{20}H_{20}O_7$ requires C, 64.5; H, 5.4; $-OCH_3$, 41.7%.) Nothing was obtained on saturating the aqueous alkaline solution with carbon dioxide and extracting with ether.

6:8-Dihydroxy-galangin (VII).—

The methyl ether (0.5 g.) obtained above was demethylated according to the procedure described for calycopterin. The crude product came out as shining yellow needles. It was unaffected by water at ordinary temperature but on heating the solution turned green. Two crystallisations from ethyl acetate yielded yellow rectangular plates melting at 257–58°. It did not change colour on exposure to air. With alcoholic ferric chloride, it gave an olive green colour changing to brown. On treatment with concentrated sulphuric acid, the crystals turned orange red, later on dissolving to give a yellow solution. It did not exhibit any fluorescence in daylight. (Found: C, 59.3; H, 3.6; $C_{15}H_{10}O_7$ requires C, 59.6; H, 3.3%.) Yield 0.3 g. The flavanol exhibited the following colour changes in alkaline solution. With 5% sodium bicarbonate, the substance dissolved slowly to form a deep blue solution with a violet tinge which started fading after 12 minutes giving a green precipitate; with 5% sodium carbonate, it gave

immediately a blue colour and faded very fast to yellow and then to pale yellow. With caustic alkali, it immediately formed a reddish brown solution; on shaking with air it rapidly became yellow, yellowish green and finally yellow.

The flavonol (VII) was acetylated using acetic anhydride and one drop of pyridine. The acetate was purified by crystallisation from acetone-ethyl alcohol mixture as described previously when it came out in the form of colourless needles and rectangular plates melting at 201–03°. (Found: C, 58.7; H, 4.0; $C_{25}H_{20}O_{12}$ requires C, 58.6; H, 3.9%.)

Condensation of the ketone (V) with veratric anhydride and sodium vertrate.—

An intimate mixture of veratric anhydride (4 g.) and sodium vertrate (2 g.) and the ketone (0.75 g.) was heated *in vacuo* at 175–80° for a period of 5 hours. The product melted and formed a reddish-brown liquid which soon solidified. After the reaction was complete, it was broken up in alcohol (50 c.c.) and while boiling under reflux a solution of potassium hydroxide (5 g.) in 10 c.c. was added during the course of 20 minutes. The alcohol was then removed under reduced pressure and the residue dissolved in water. The product (A) that separated out was filtered and the clear filtrate was saturated with carbon dioxide when a yellow solid (B) separated out. (A) melted between 108 and 118° and (B) between 115 and 123°. Purification by fractional crystallisation from alcohol could not be effected successfully as the fractions continued to melt indefinitely and hence product (A) was directly used for complete methylation and product (B) for demethylation.

5-Hydroxy-hexamethoxyflavone (IX).—

The mother-liquor was extracted twice with ether and on evaporating the solvent, a viscous residue was obtained which solidified on cooling. It was crystallised from dilute alcohol when it appeared as yellow rectangular plates melting 122–23° (*cf.* methyl erianthin,⁷ yellow needles, m.p. 141°). It appeared to be exclusively the 5-hydroxy compound from the sharpness of its melting point. It gave a green colour with ferric chloride and formed a sparingly soluble potassium salt in alcoholic potash. Though it did not dissolve easily in cold aqueous potash, it did so on warming and formed a yellow solution. (Found: C, 60.5; H, 5.5; OCH_3 , 44.7; $C_{21}H_{22}O_9$ requires C, 60.3; H, 5.3; OCH_3 , 44.5%.) Yield, 90 mg.

3:5:6:7:8:3':4'-Heptamethoxy-flavone (VIII).—

Product (A) obtained from the above condensation was directly methylated using dimethyl sulphate and potassium carbonate in acetone solution. The heptamethoxy flavone was twice crystallised from alcohol when it came

out in the form of colourless rectangular plates, melting at 129–31°. (Found: C, 61.2; H, 5.9; OCH₃, 50.4; C₂₂H₂₄O₉ requires C, 61.1; H, 5.6; OCH₃, 50.2%.)

6:8-Dihydroxy-*quercetin* (X).—

Product (B) (0.5 g.) was demethylated using hydriodic acid (10 c.c.) in acetic anhydride solution by boiling for half an hour at 140–50°. The cooled solution was then poured into water, and iodine removed with sulphur dioxide. The flavonol was obtained as a yellow crystalline (rectangular plates) powder. It was filtered, washed with water and dried. The crystallisation was effected using a large excess of dry ethyl acetate when the compound came out as yellow rectangular plates and flat needles. On heating it darkened in colour above 340° and exhibited no other change till 360°. The crystals turned orange-red on treatment with concentrated sulphuric acid and then formed a yellow solution which did not exhibit any fluorescence in daylight. The alcoholic solution gave with ferric chloride an intense green colour which changed to brown. (Found: C, 53.7; H, 3.1; C₁₅H₁₀O₉ requires C, 53.9; H, 3.0%.) With 5% sodium bicarbonate it first gave a yellow solution which rapidly changed to greenish blue and deep blue within ½ minute and then faded to light green with a green precipitate; with 5% sodium carbonate the colours were yellow, emerald green, deep blue, fading and turning green, brownish yellow and finally brown; with 5% caustic alkali it immediately gave reddish brown changing to dark red.

The flavonol was acetylated by boiling with acetic anhydride and a drop of pyridine. The acetate was crystallised from acetone-ethyl alcohol mixture as previously described when it came out in the form of colourless rectangular plates and prisms melting at 221–22° with sintering at 219°. (Found: C, 55.6; H, 4.0; C₂₉H₂₄O₁₆ requires C, 55.4; H, 3.8%.)

5-Hydroxy-3:6:7:8:3':4':5'-heptamethoxy-flavone (XI).—

An intimate mixture of trimethyl gallic anhydride (5.0 g.), potassium trimethyl gallate (3.0 g.) and the ketone (V) (0.75 g.) was heated *in vacuo* at 175–80° for a period of 5 hours and the product worked up as before. There was no precipitate from the aqueous alkali solution that was first obtained, indicating absence of the fully methylated compound. However after saturation with carbon dioxide a yellow product separated out and it was extracted with ether. On removing the solvent, it came out as a viscous liquid which solidified on keeping in an ice-chest for 3 days. It crystallised from alcohol as bright yellow rectangular plates melting at 113–15°. It gave a brownish green colour with alcoholic ferric chloride and formed a

sparingly soluble potassium salt with alcoholic potash indicating the presence of a free hydroxyl in the 5-position. (Found: C, 59.2; H, 5.6; OCH₃, 48.6; C₂₂H₂₄O₁₀ requires C, 58.9; H, 5.4; OCH₃, 48.4%.)

3:5:6:7:8:3':4':5'-Octamethoxy-flavone (XII).—

The above methyl ether (XI) (0.2 g.) was further methylated in acetone solution using dimethyl sulphate and anhydrous potassium carbonate by boiling for 12 hours. The product was obtained as a liquid which solidified on keeping in the ice-chest for a number of days. It was crystallised from dilute alcohol when it came out as colourless rectangular plates melting at 122–23°. It did not give any colour with ferric chloride and did not dissolve in dilute alkali or impart any colour to the solution. (Found: C, 60.1; H, 5.7; OCH₃, 53.4; C₂₃H₂₆O₁₀ requires C, 60.0; H, 5.6; OCH₃, 53.7%.)

6:8-Dihydroxy-myricetin (XIII).—

The above 5-hydroxy-heptamethoxy-flavone (XI) (0.5 g.) was demethylated using hydriodic acid as already described. On pouring the cooled reaction mixture into ice-water and reducing iodine with sulphur dioxide, the flavonol separated out as a dark green solid. Direct crystallisation from dry ethyl acetate did not remove amorphous impurities completely. It was therefore dissolved in dry acetone and petroleum ether (b.p. 60°) added until some sticky impurity separated out and settled down; the clear solution was then decanted. On slow evaporation of the solvents, the flavonol separated out in a crystalline condition. Final purification was effected by crystallisation from dry ethyl acetate when it appeared as greenish yellow rectangular plates. The substance darkened in colour at 340° and did not melt below 360°. It gave a deep green colour changing to brown with alcoholic ferric chloride. It assumed a green colour rapidly on exposure to moisture. (Found: C, 51.2; H, 3.1; C₁₅H₁₀O₁₀ requires C, 51.4; H, 2.9%.) With 5% sodium bicarbonate, it gave immediately a blue solution turning deeper blue within 10 seconds; this rapidly faded to yellow with a green precipitate. With 5% sodium carbonate the colours were yellow, emerald green, blue, fading immediately to yellowish brown, red, yellowish brown and brown. With 5% caustic alkali it gave immediately a reddish brown solution changing to red.

The flavonol (XIII) (50 mg.) was acetylated using acetic anhydride and a drop of pyridine. The acetate was crystallised from acetone-ethyl alcohol mixture as already described when it came out as clusters of colourless rectangular plates melting at 225° with slight sintering at 221°. (Found: C, 54.2; H, 4.0; C₃₁H₂₆O₁₈ requires C, 54.2; H, 3.8%.)

Sodium amalgam in absolute alcohol.—

1. *6:8-Dihydroxy-galangin.*—The solution turns green and then the colour gradually fades to greenish yellow with no flocks.

2. *Calycopteretin.*—The solution becomes green and changes to greenish yellow and then yellowish brown with no flocks.

3. *6:8-Dihydroxy-quercetin.*—The solution turns green and immediately changes to brown and slowly precipitates brown flocks, while the solution becomes colourless.

4. *6:8-Dihydroxy-myricetin.*—Brown solution, rapidly precipitates brown-pinkflocks, the solution becoming colourless.

Gossypetone reaction.—

1. *6:8-Dihydroxy-galangin.*—The solution immediately becomes blue and rapidly changes to red; this fades on shaking to give a yellow solution changing finally to brown. After 24 hours it is coloured brown.

2. *Calycopteretin.*—The blue solution formed immediately changes to deep red and then to green, greenish yellow, brown and yellow. After 24 hours, it is yellow.

3. *6:8-Dihydroxy-quercetin.*—The blue solution immediately changes to brown red and fades to pale yellow. Pale yellow after 24 hours.

4. *6:8-Dihydroxy-myricetin.*—The alcoholic solution which was originally green changes to blue and turns into a stable intense red. It fades to brown during 24 hours.

Colour reactions with alkaline buffer solutions.—

1. *6:8-Dihydroxy-galangin.*—

pH 8.0 Substance sparingly soluble, pale yellow solution changing to dirty green, blue, and deep violet blue fading within three minutes and becoming pale blue within six minutes. The pale blue slowly changes and becomes pale yellow within 24 hours.

pH 8.6 Substance difficultly soluble, pale yellow solution changing to blue and then to violet blue and fading within two minutes. The pale blue slowly fades and finally after 24 hours it becomes pale yellow.

pH 9.2 Substance more easily soluble than before; yellow solution changes to blue and fades faster within the first minute and

becomes yellow by four minutes. After 24 hours it becomes pale yellow.

- pH 9·8 Substance dissolves quicker to a yellow solution and becomes blue, starts fading within 20 seconds and becomes pale blue in two minutes and yellow in four minutes. After 24 hours it becomes pale yellow.
- pH 10·4 Pale yellow solution, changes very fast to blue and becomes pale yellow within 15 seconds. After 24 hours there is no change.
- pH 11·0 Yellow solution quickly becoming yellowish green, pale blue and finally yellow. After 24 hours it is pale yellow.
- pH 11·6 Yellow solution changing fast to yellowish green and greenish blue, becomes yellow within 30 seconds and then golden yellow. Slowly it fades to pale yellow within 24 hours.
- pH 12·2 Same as above.
- pH 12·8 Same as above.
- pH 13·4 Same as above.

In general, the flavonol does not dissolve in the buffer solutions of lower pH readily. On boiling with distilled water, the substance forms a green solution which rapidly turns blue. With tap water the solution turns deeper blue than with distilled water and slowly fades and after 24 hours becomes pale yellow.

Calycopteretin.—

- pH 8·0 Yellow solution, quickly changes to dirty green and to deep blue within half minute, more intense blue within one minute and starts fading within 3 minutes to bluish green. This slowly fades within 10 minutes to yellowish green and becomes pale yellow within 18 minutes. After 24 hours the solution becomes pale yellow.
- pH 8·6 Yellow solution slowly changing to yellowish green, and blue in 20 seconds; starts fading after one minute and becomes greenish yellow within 8 minutes. After 24 hours it is pale yellow.
- pH 9·2 The initial yellow solution quickly changes to green, blue and intense blue within half minute and fades rapidly to yellow in two minutes. Within six minutes the yellow intensifies and becomes golden yellow. After 24 hours it is pale yellow.

- pH 9.8 Colour changes are very rapid; yellow solution turns quickly deep green and within half minute greenish yellow, becomes golden yellow within one minute and orange yellow within two minutes. The solution assumes a yellow colour after 24 hours.
- pH 10.4 Yellow solution rapidly changes to green, and emerald green and becomes yellowish green within half minute and orange yellow within 45 seconds. The colour fades and becomes golden yellow after 24 hours.
- pH 11.0 Same as above.
- pH 11.6 Yellow solution; colour changes same as above and faster.
- pH 12.2 Same as above. Changes faster than before giving orange yellow within 20 seconds and deeper yellow after 24 hours.
- pH 12.8 Same as above.
- pH 13.4 Yellow solution becomes greenish yellow and then fades to orange yellow. After 24 hours it becomes a deep yellow solution.

With distilled water, the substance becomes green changing to greenish blue. The solution is also of the same colour and changes to pure blue on boiling.

6:8-Dihydroxy-quercetin.—

- pH 8.0 Yellow solution becoming deep yellow; turns rapidly yellowish green, green, and emerald green and within 30 seconds turns blue and intensifies to deeper blue in 6 minutes. The blue fades slowly and turns to deep yellow in 24 hours.
- pH 8.6 Yellow solution going into deep green within 20 seconds; becomes blue within $\frac{1}{2}$ minute and intensifies to deep blue within one minute and fades after 3 minutes. Becomes pale yellow in 24 hours.
- pH 9.2 Yellow solution, quickly changes to green and blue within 20 seconds, starts fading within 2 minutes and becomes yellowish green after 6 minutes and yellow after 10 minutes, and pale orange after $\frac{1}{2}$ an hour. Pale yellow solution after 24 hours.
- pH 9.8 Yellow solution, changes very fast to greenish yellow and then blue, fades within 30 seconds and becomes greenish

yellow within one minute and yellow in 3 minutes. In 10 minutes becomes orange yellow which fades slowly and becomes yellow in 24 hours.

- pH 10·4 Very fast changes; yellow solution immediately turns green and emerald green and becomes greenish yellow and yellow within 20 seconds. It assumes a golden yellow colour within one minute and orange within 2 minutes; subsequently fades to paler orange within half an hour; yellow in 24 hours.
- pH 11·0 Same as above; within one minute deeper orange than above and fades within half an hour to pale orange; solution deep yellow after 24 hours.
- pH 11·6 Same as above; assumes orange with $\frac{1}{2}$ minute and orange red within one minute; fades after 30 minutes and becomes deep yellow in 24 hours.
- pH 12·2 Same as above; deep orange red within 45 seconds and starts fading to become deeper yellow in 24 hours.
- pH 12·8 Same as above; within 30 seconds deeper orange red; fades after one hour to give deep yellow in 24 hours.
- pH 13·4 Same as above. Solution deeper yellow after 24 hours.

Substance treated with distilled water gives a blue colour in the cold; the colour of the solution changes to green after boiling, fades after $\frac{1}{2}$ hour and becomes pale yellow after 24 hours.

6:8-Dihydroxy-myricetin.—

- pH 8·0 Yellow solution changes to yellowish green, green and emerald green and becomes deep blue within one minute; after 10 minutes becomes emerald green and fades after one hour. Deep yellowish brown after 24 hours.
- pH 8·6 Yellow solution changes to greenish yellow and then rapidly to green and blue within $\frac{1}{2}$ minute. Fades after 3 minutes to green and becomes pale yellowish green after 6 minutes; in one hour becomes pale yellow; yellow in 24 hours.
- pH 9·2 Yellow solution, changing fast to green and then blue within 20 seconds; starts fading after $1\frac{1}{2}$ minutes; yellowish green within 4 minutes and pale yellow in 24 hours.

pH 9.8 Yellow solution rapidly changing to green and emerald green; fades to green within 20 seconds and yellowish green in 2 minutes; brownish yellow in 24 hours.

pH 10.4 Yellow solution changing to green and emerald green and fading to yellowish brown within 40 seconds; colour turns brown within one minute, changes to brown red and reddish purple and fades to reddish brown after 2 minutes, becomes brown within 5 minutes and orange brown within 10 minutes. After 24 hours it is orange red.

pH 11.0 Yellow solution changing fast to yellowish green and to reddish purple within one minute and then brilliant purple; slowly fades to reddish brown in 12 minutes and orange red in 24 hours.

pH 11.6 Very rapid changes; initial yellow solution changes to yellowish green, yellowish brown and brown purple within 20 seconds and then to bluish purple; fades after one minute rapidly to reddish brown and becomes yellowish brown after 4 minutes; after 24 hours orange red.

pH 12.2 Faster changes, same as above.

pH 12.8 Yellow solution changing fast to green, emerald green and yellowish brown within 20 seconds, becomes brown and brown purple and bluish purple and fades to reddish brown in one minute and pale brown in 6 minutes; after 24 hours orange red.

pH 13.4 Same as above.

On treatment with distilled water the substance turns blue in the cold. The solution becomes deeper blue on warming and fades to pale green after 1½ hours. It is greenish yellow after 24 hours.

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SUMMARY

The constitution of calycopteretin has been confirmed by the decomposition of the fully methylated ether with alcoholic alkali and also by its synthesis from the decomposition products. Alkaline hydrolysis of the fully methylated ether yields anisic acid and 2-hydroxy- ω -3:4:5:6-pentamethoxyacetophenone. Condensation of this ketone with sodium anisate and anisic anhydride, regenerates calycopterin dimethyl ether. When the anhydride

and sodium salt of benzoic acid are used instead, 3:5:6:7:8-pentamethoxy flavone is readily formed. On demethylation, it yields 6:8-dihydroxy-galangin. When, however, the sodium salt and anhydride of veratric acid are used, partial demethylation takes place in the 5-position, giving rise to both hexamethyl and heptamethyl ethers. By complete demethylation, 6:8-dihydroxy-quercetin is obtained. Using gallic acid derivatives for the condensation, demethylation is found to be complete in the 5-position yielding 5-hydroxy-heptamethoxy-flavone. Methylation of this produces the octamethyl ether and demethylation the octahydroxy compound, 6:8-dihydroxy-myricetin. This is the most highly hydroxylated of flavones known so far.

A comparative study of the properties of the four flavonols of this group, their methyl ethers and acetates has been made. Colour reactions useful for rapid identification are also described. They are remarkable in several respects.

The properties of mono-methyl erianthin do not agree with those of 5-hydroxyhexamethoxy-flavone prepared in the course of this work.

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