

A METHOD OF DISTINGUISHING BETWEEN FLAVONES AND FLAVONOLS

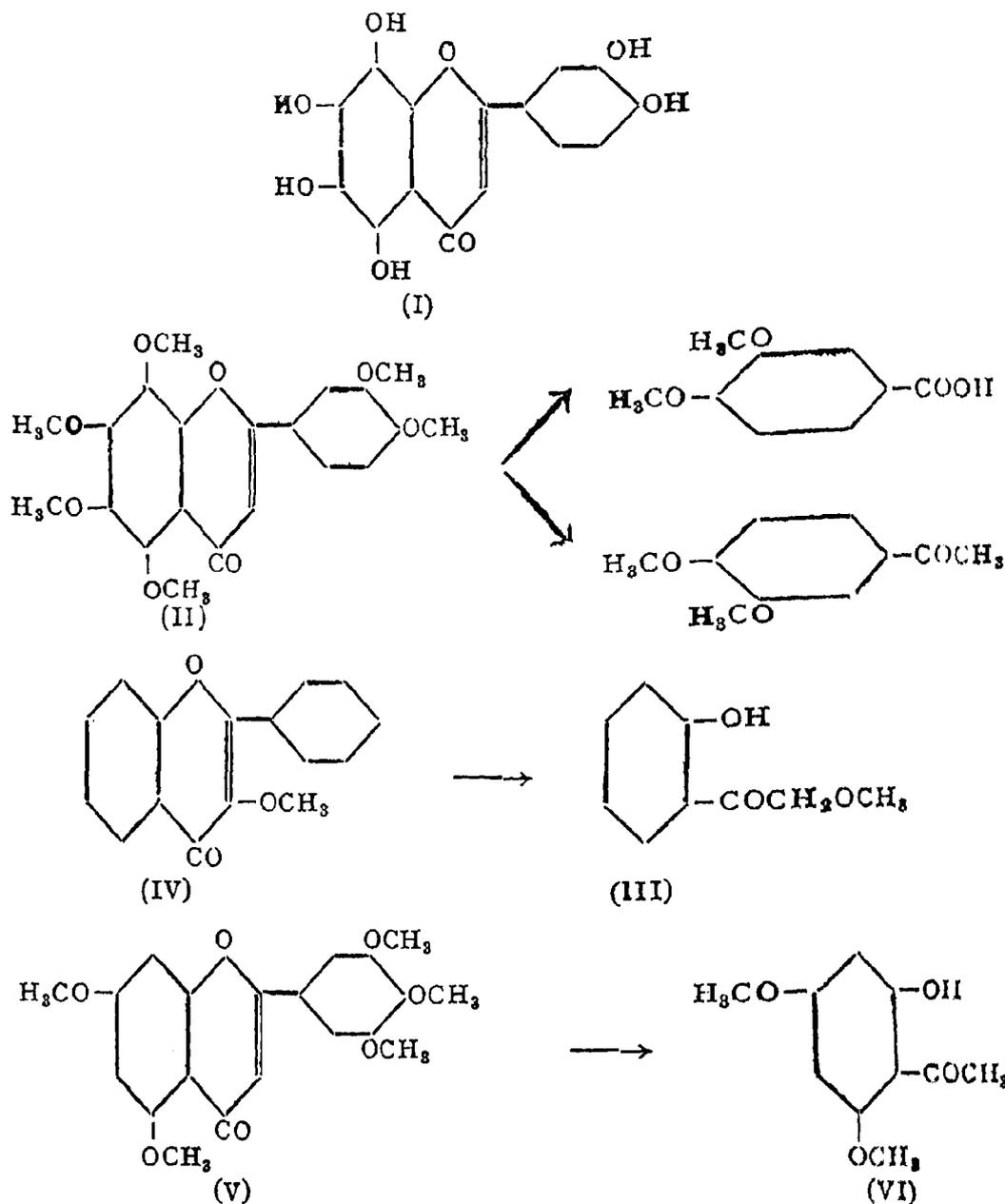
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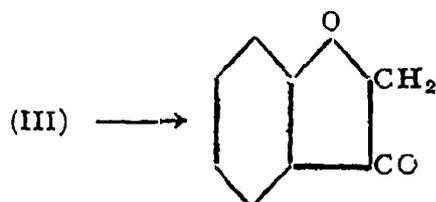
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IN the investigation of the naturally occurring anthoxanthins, a satisfactory method of distinguishing between flavones and flavonols was found necessary. Colour reactions, dyeing properties, absorption spectra and stability to aerial oxidation in alkaline solution have been used in the past fairly successfully, but their use becomes limited when the entire range of variation in types is taken into consideration. For example, stability to aerial oxidation could be mentioned. According to Perkin,¹ whereas as a rule hydroxy-flavones are not oxidised by air in alkaline solution and precipitated therefrom unchanged by acids, flavonols on the other hand are readily decomposed in this manner with the formation of water-soluble products. But when the molecule contains a large number of hydroxyl groups as in nor-nobiletin (flavone I), instability to this treatment arises even in flavones. A more satisfactory procedure is to effect complete methylation and subject the fully methylated ether to fission with aqueous or alcoholic alkali. The side-phenyl nucleus is obtained in both cases as a substituted benzoic acid satisfactorily. Under suitable conditions it has been possible to obtain this part of the molecule in the case of flavones as a substituted acetophenone also, in a small yield. In this manner Robinson and Tseng² could identify veratric acid and aceto-veratrone among the products of decomposition of nobiletin (II) with aqueous alcoholic potash and the formation of the ketone was considered to be a definite proof that nobiletin was a flavone. On the other hand, to obtain the condensed benzene ring as a substituted acetophenone (III) is more often a characteristic of flavonols (IV). Such a behaviour can however be found with flavones also. For example, tricinntrimethyl ether (V) yields phloracetophenone dimethyl ether (VI) by this method of hydrolysis.³

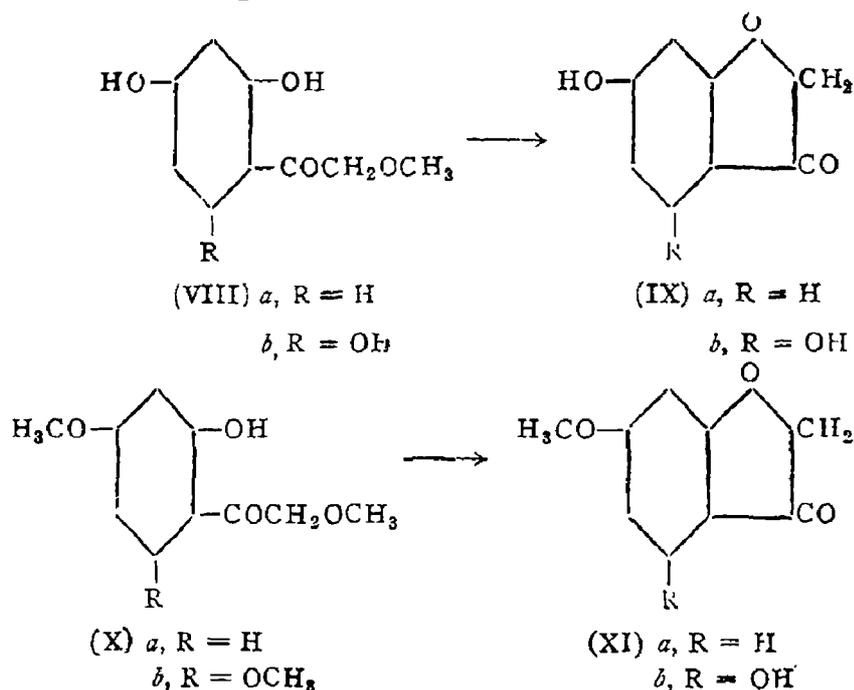
Though there is difficulty in making a distinction between flavones and flavonols simply from the manner of their fission with alkali it could be seen that the presence of an ω -methoxyl group in the ketonic fission product (III) representing the condensed benzene ring is definitely a characteristic of flavonols. The detection of this structural feature offers, therefore, an unailing method of distinction.



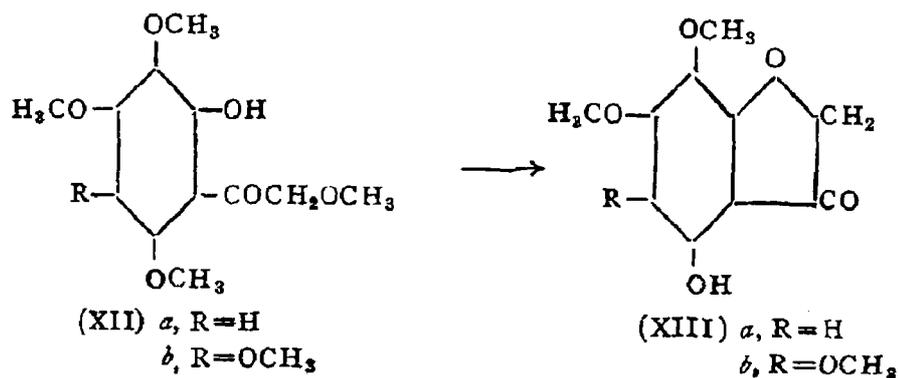
A satisfactory procedure has now been explored and shown to be suitable for this purpose. It consists in heating the ketonic fission product with aqueous hydrobromic acid (40%) on a water-bath at 100° for about 3 hours. ω -Methoxy-ketones form the corresponding coumaranones (VII) whereas the unsubstituted ones yield only resinous products. Stronger hydrobromic acid, hydriodic acid and aluminium chloride in various solvents are found to be unsuitable for this purpose since they cause considerable resin-formation.



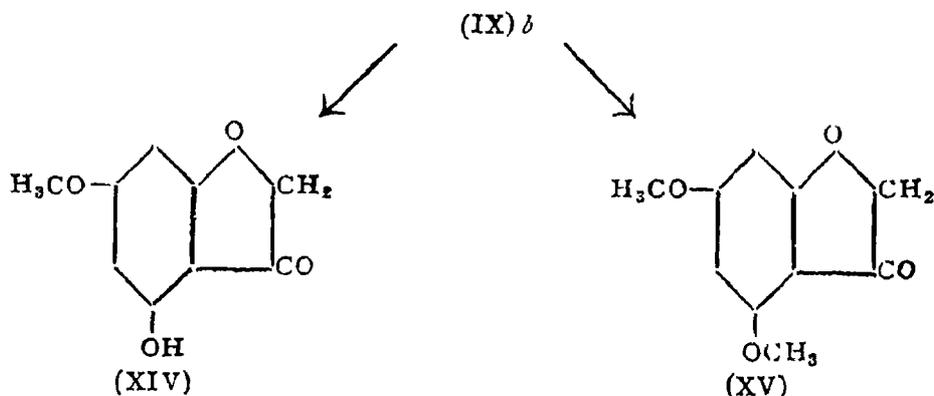
This reaction has been first examined using simpler cases like ω -methoxy-resacetophenone (VIII *a*) and ω -methoxy-phloracetophenone (VIII *b*) and also with the fission products of the fully methylated naturally occurring flavonols such as fisetol-dimethyl-ether (X *a*) and methoxy-fisetol-dimethyl-ether (X *b*). Whereas in the case of the first three, simple conversion into the coumaranones (IX *a*, IX *b* and XI *a*) takes place, in the case of the last, the product is found to be 4-hydroxy-6-methoxy-coumaranone (XI *b*) showing that there has been besides coumaranone formation partial demethylation in the susceptible 4-position.



A similar behaviour is noticed with gossypetol-tetramethyl-ether (XII *a*) which forms 4-hydroxy-6:7-dimethoxy-coumaranone (XIII *a*) and calycopteretol-pentamethyl-ether (XII *b*) which gives rise to 4-hydroxy 5:6:7-trimethoxy-coumaranone (XIII *b*). The 4-position in these coumaranones corresponds to the 5-position in flavones and flavonols and due to the influence of the neighbouring carbonyl group it suffers easy demethylation. The hydroxyl in this position is again resistant to methylation owing to the existence of chelation.

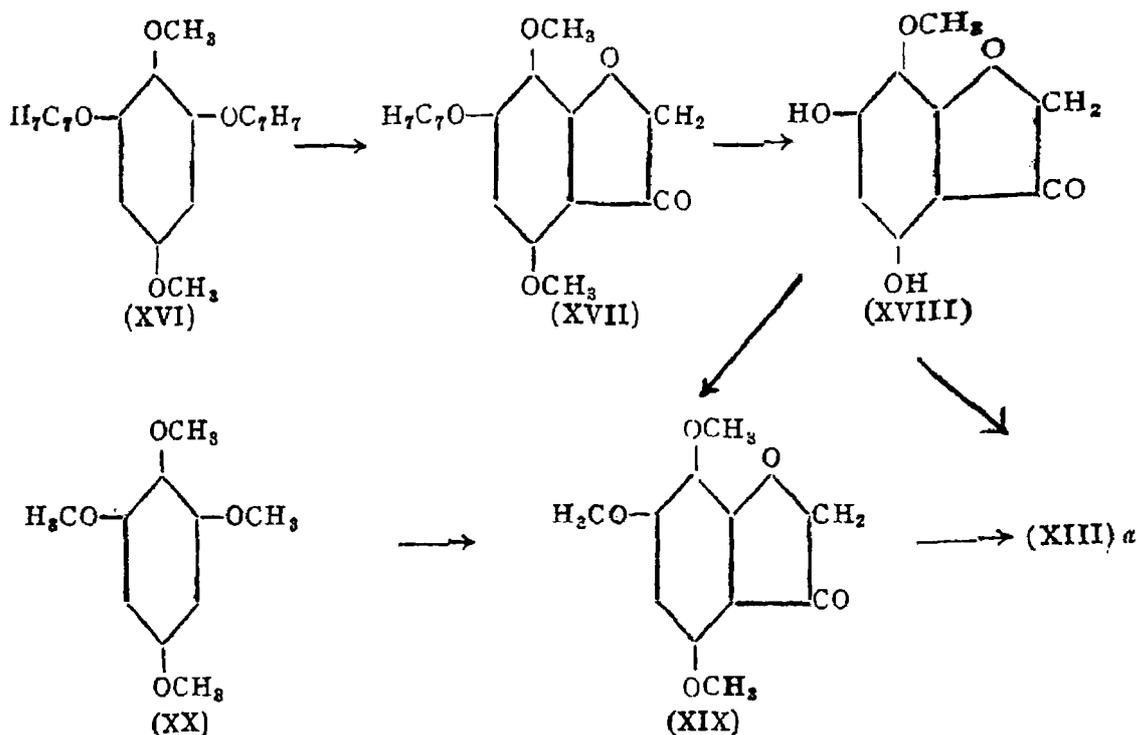


That these products are coumaranones can be established by their condensation with benzaldehyde forming well-defined benzylidene derivatives. In most of the cases obtained in the present study comparison has been effected with compounds obtained synthetically by well-established methods of coumaranone formation. For example, 4:6-dihydroxy coumaranone (IX *b*) was prepared by the condensation of phloroglucinol with chloracetyl chloride and subsequent ring closure with sodium acetate. Earlier workers reported difficulty in methylating hydroxy-coumaranones. The action of dimethyl sulphate or methyl iodide in aqueous alkali generally produced resinous products. But by means of dimethyl sulphate and potassium carbonate in anhydrous acetone medium the reaction could be carried out in stages yielding either partially methylated (XIV) or fully methylated (XV) products.

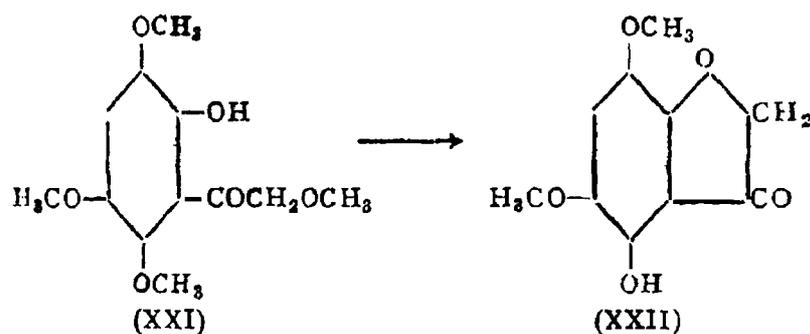


For the synthesis of coumaranone (XIII *a*) obtained from gossypetol-tetramethyl-ether (XII *a*) the starting material was 1:4-dimethoxy-2:6-dibenzoyloxy-benzene (XVI). This condenses with chloracetyl chloride with partial debenzoylation and the product (ortho-hydroxy-chloro-ketone) subsequently reacts with sodium acetate and yields 4:7-dimethoxy-6-benzyloxy-coumaranone (XVII). Treatment with hydrobromic acid in glacial acetic acid solution removes not only the benzyl group but also the methyl group in the 4-position yielding (XVIII). But this could be remethylated partially to form 4-hydroxy-6:7-dimethoxy coumaranone (XIII *a*) and completely to produce 4:6:7-trimethoxy-coumaranone (XIX). In this connection it may be stated that 1:2:3:5-tetramethoxy-benzene (XX) undergoes this condensation with chloracetyl chloride in the cold rather poorly and only low yields of 4:6:7-trimethoxy coumaranone (XIX) could be obtained by further treatment with sodium acetate. This when demethylated with hydrobromic acid formed the dimethyl ether (XIII *a*) with the 4-hydroxyl group free.

An important case where a definite proof that it was a flavonol was needed at an earlier date was gardenin. Its constitution has recently been



conclusively established as 5-hydroxy-3:6:8:3':4':5'-hexamethoxy-flavone by synthetic methods.⁴ The ketonic fission product (XXI) of methyl gardenin is now shown to undergo coumaranone conversion with hydrobromic acid. Here again the coumaranone has a methoxyl group less due to partial demethylation affecting position 4 and it is therefore considered to be 4-hydroxy-5:7-dimethoxy-coumaran-3-one (XXII). In conformity with this constitution it gives a marked colour with ferric chloride.



EXPERIMENTAL

6-Hydroxy-coumaran-3-one (IX a)

ω -Methoxy-resacetophenone (1.0 g.) was treated with an aqueous solution of hydrobromic acid (20 c.c.; 40%). The mixture was heated at 100° over a boiling water-bath for 3 hours. It was filtered through cotton-wool to remove uncrystallisable resinous matter that separated out during the reaction. The clear aqueous solution was left in the refrigerator for 48 hours. Since nothing separated out, it was extracted with ether and the ether solution concentrated when a brown solid was obtained. It crystallised

from aqueous alcohol as pale yellow rectangular plates melting at 239–40°. Yield, 0.1 g. (Found: C, 63.7; H, 4.3; C₈H₆O₃ requires C, 64.0; H, 4.0%.)

By heating it at 60° with concentrated sulphuric acid and benzaldehyde it gave the benzal derivative which crystallised from alcohol as pale yellow prisms melting at 261–62°. This gave a blood-red colour with concentrated sulphuric acid.

For purposes of comparison 6-hydroxy-coumaranone was prepared according to the procedure of Arima and Okamoto⁵ with modifications. To an ice-cooled solution of resorcinol (11.0 g., 1 mol.) in dry ether (50 c.c.) anhydrous aluminium chloride (40 g., 3 mols.) was added in small lots followed by chloracetyl chloride (8 c.c., 1 mol.) in drops and the mixture was allowed to stand overnight. Water and hydrochloric acid were added carefully and the ether was driven off over a hot water-bath. The heating was continued for a further 15 minutes and the mixture cooled. The solid that separated out was filtered and boiled with a 10% aqueous solution of sodium acetate. On cooling the resulting solution, a yellow solid was obtained. It crystallised from alcohol as yellow rectangular plates melting at 239–40° and gave a benzylidene derivative melting at 261–62°.

There was complete identity between the two sets of samples and the mixed melting points were undepressed.

6-Methoxy-coumaran-3-one (XI a)

(a) ω : 4-Dimethoxy-2-hydroxy-acetophenone (1.0 g) was treated with hydrobromic acid (20 c.c.; 40%). The reaction was carried out and the product worked up as in a previous experiment. It crystallised from alcohol as straw-coloured needles melting at 119–20°. Yield, 0.1 g. (Found: C, 66.2; H, 5.0; C₉H₈O₃ requires C, 65.9; H, 4.9%.) It did not give any colour with alcoholic ferric chloride and was insoluble in aqueous alkali.

(b) 6-Hydroxy-coumaranone was methylated using excess of dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium. The product was found to be identical with the above sample.

(c) 6-Methoxy-coumaranone was also prepared by modification of the method of Blom and Tambor⁶ using monomethyl resorcinol and chloracetyl chloride and dry ether. The earlier authors used bromacetyl bromide and carbon disulphide. The product agreed with the sample described above and with the description of Blom and Tambor.

4:6-Dihydroxy-coumaran-3-one (IX b)

(a) ω -Methoxy-phloracetophenone (1.0 g.) was heated with hydrobromic acid (20 c.c.; 40%) for 3 hours at 100°. After filtering the reaction mixture through cotton, the clear filtrate was left in the refrigerator for 48 hours. The yellow solid that separated out crystallised from hot water as pale yellow rectangular plates melting at 248–49°. Yield, 0.1 g. (Found: C, 57.6; H, 3.9; $C_8H_6O_4$ requires C, 57.8; H, 3.6%.)

With alcoholic ferric chloride it gave a purple colour and was soluble in aqueous alkali. When heated with benzaldehyde and concentrated sulphuric acid it gave the benzylidene derivative which melted at 297–98°.

(b) The dihydroxy-coumaranone was also prepared from phloroglucinol by condensation with chloracetyl chloride and dry ether and subsequent treatment of the ω -chloroketone with sodium acetate. It melted at 248–49° and was identical with the sample described under (a). The mixed melting point was also undepressed. This substance was prepared earlier by Slater and Stephen⁷ by the condensation of phloroglucinol with hydroxy-acetonitrile. The older and the present descriptions of the compound are in agreement.

4-Hydroxy-6-methoxy-coumaran-3-one (XI b)

(a) ω :4:6-Trimethoxy-2-hydroxy-acetophenone (1.0 g.) was heated with hydrobromic acid (20 c.c.; 40%). The product crystallised from alcohol as colourless needles melting at 147–48°. Yield, 0.1 g. (Found: C, 60.4; H, 4.6; OCH_3 , 17.6; $C_9H_8O_4$ requires C, 60.0; H, 4.4; OCH_3 , 17.2%.)

With alcoholic ferric chloride it gave a brown colour and was sparingly soluble in aqueous alkali though the solution was coloured yellow.

(b) The above coumaranone was also obtained by the partial methylation of 4:6-dihydroxy-coumaranone (IX b) using the requisite quantity of dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium and boiling for 6 hours only.

4:6-Dimethoxy-coumaranone (XV)

4:6-Dihydroxy-coumaranone (IX b) (1.7 g.) was boiled with dimethyl sulphate (4.0 c.c.) and anhydrous potassium carbonate (10 g.) in dry acetone medium (50 c.c.) for 30 hours. The dimethyl ether crystallised from alcohol as rectangular plates and prisms melting at 101–02°. (Found: C, 62.2; H, 5.3; $C_{10}H_{10}O_4$ requires C, 61.9; H, 5.2%.) With alcoholic ferric chloride it did not give any colour and was insoluble in aqueous alkali.

4-Hydroxy-6:7-dimethoxy-coumaranone (XIII a)

First method.—Gossypetol-tetramethyl ether⁸ (1.0 g.) obtained by the fission of hexamethyl-gossypetin was heated at 100° for 3 hours with hydrobromic acid (20 c.c.; 40%) and the product worked up as in similar cases. It crystallised from aqueous alcohol as colourless rectangular plates melting at 138–39°. Yield, 0.1 g. (Found: C, 57.5; H, 5.1; OCH₃, 29.9; C₁₀H₁₀O₅ requires C, 57.2; H, 4.9; OCH₃, 29.5%.) With alcoholic ferric chloride it gave a brown colour and was sparingly soluble in aqueous alkali.

Second method.—

4:7-Dimethoxy-6-benzyloxy-coumaranone (XVII)

To an ice-cold dry ether solution of 2:6-dibenzyloxy-1:4-dimethoxy benzene⁹ (7.0 g.) anhydrous aluminium chloride (7.5 g.) was added in small lots and chloracetyl chloride (1.5 c.c.) drop by drop. After 2 hours, water (100 c.c.) and concentrated hydrochloric acid (50 c.c.) were added carefully. Ether was distilled off and the mixture was heated at 100° over a boiling water-bath for 15 minutes. On cooling the ω -chloro-ketone separated out as a dark coloured semi-solid. It was treated with an aqueous solution (10%, 100 c.c.) of sodium acetate and the mixture was heated on a water bath for one hour. The product crystallised from alcohol as grey prisms melting at 153–54°. (Found: C, 68.0; H, 5.9; C₁₇H₁₆O₅ requires C, 68.0; H, 5.5%.) With alcoholic ferric chloride it did not give any colour and was insoluble in aqueous alkali.

4:6-Dihydroxy-7-methoxy-coumaranone (XVIII)

The above dimethoxy-benzyloxy-coumaranone (0.8 g.) was treated with a mixture of glacial acetic acid (5.0 c.c.) and a solution of hydrobromic acid in glacial acetic acid (5.0 c.c., 60%). The mixture was heated at 100° over a water-bath for one hour. On cooling and diluting the solution with water a dark coloured solid separated out. It crystallised from alcohol as pale brown prisms melting at 251–52°. (Found: C, 55.5; H, 3.5; C₉H₈O₅ requires C, 55.1; H, 3.3%.) With alcoholic ferric chloride it gave a brown colour and was easily soluble in aqueous alkali.

4-Hydroxy-6:7-dimethoxy-coumaranone (XIII a)

The above dihydroxy compound (0.2 g.) was methylated using dimethyl sulphate (0.1 c.c.) and anhydrous potassium carbonate (5.0 g.) in dry acetone medium (20 c.c.) and boiling for 6 hours. The partial methyl ether crystallised from alcohol as colourless rectangular plates melting at 138–39°. Mixed

melting point with the compound obtained by the first method was undepressed.

Third method.—1:2:3:5-Tetramethoxy-benzene and chloracetyl chloride were condensed together in dry ether medium in the presence of aluminium chloride and the product worked up and treated with sodium acetate as in a similar reaction described above. 4:6:7-Trimethoxy coumaranone (XIX) was first obtained as a viscous liquid; this liquid was also obtained by the complete methylation of (XVIII). It was subjected to partial demethylation using hydrobromic acid in glacial acetic acid medium according to the procedure described previously. The demethylated compound, 4-hydroxy-6:7-dimethoxy-coumaranone melted at 138–39° and the mixed melting point with the compound obtained by the first method was undepressed.

4-Hydroxy-5:6:7-trimethoxy-coumaranone (XIII b)

Calycopteretol-pentamethyl ether¹⁰ (XII b) (1.0 g.) obtained from hexamethyl-calycopterin was heated for 3 hours at 100° with hydrobromic acid (20 c.c.; 40%). The coumaranone crystallised from alcohol as colourless rectangular prisms melting at 126–27°. Yield, 0.1 g. (Found: C, 55.2; H, 4.8; OCH₃, 38.9; C₁₁H₁₂O₆ requires C, 55.0; H, 5.0; OCH₃, 38.8%.) With alcoholic ferric chloride it gave a brown colour and was sparingly soluble in aqueous alkali.

4-Hydroxy-5:7-dimethoxy-coumaranone (XXII)

2-Hydroxy- ω :3:5:6-tetramethoxy-acetophenone⁴ (XXI) was prepared by the alkali fission of methyl gardenin. The ketone (1.5 g.) was heated with an aqueous solution of hydrobromic acid (50 c.c.; 40%). After cooling, the mixture was extracted with ether and the ether solution dried over sodium sulphate. When the solvent was removed by evaporation, a semi-solid was left behind. It crystallised from petroleum ether as very pale grey flakes melting at 51–52°. Yield, 0.2 g. (Found: OCH₃, 30.0; C₁₀H₁₀O₅ requires OCH₃, 29.5%.) With alcoholic ferric chloride it gave a green colour and it was sparingly soluble in aqueous alkali. Its benzylidene derivative crystallised from alcohol as yellowish brown tiny prisms and melted at 183–4°.

SUMMARY

A method of distinguishing between a flavone and a flavonol and characterising the latter is described. It consists in subjecting the ketonic fission product of the fully methylated anthoxanthin, representing the condensed benzene nucleus to the action of 40% aqueous hydrobromic acid,

ω -Methoxy-ketones derived from flavonols yield coumaranones which could be converted into benzylidene derivatives. Simpler examples and more difficult cases such as gossypetol-tetramethyl-ether and calycopteretol pentamethyl-ether have been used for the investigation. The nature of the products has been proved by independent synthesis. The ketone obtained by the fission of methyl gardenin undergoes this reaction and hence supports the constitution of gardenin as a flavonol. Ortho-hydroxy-ketones which are unsubstituted in the ω -position do not yield any crystallisable product in this reaction.

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