NUCLEAR OXIDATION IN THE FLAVONE SERIES

Part I. A New Synthesis of Gossypetin and 8-Hydroxy-Galangin

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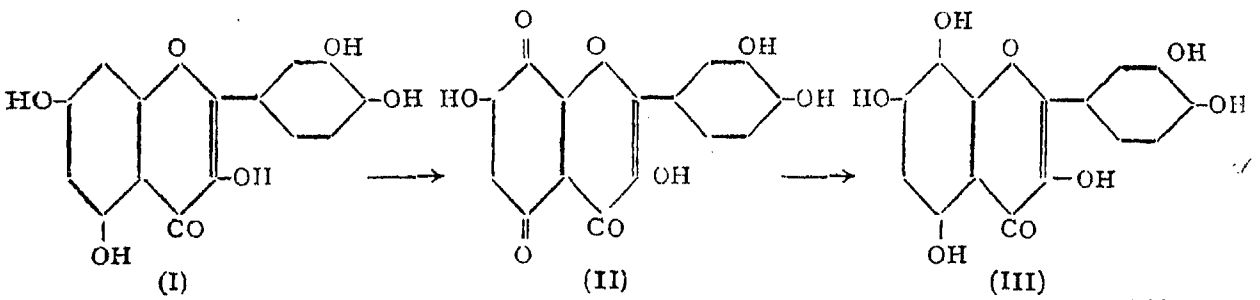
Robinson's theory of the Biogenesis of Anthocyanins and Anthoxanthins and its special application to the naturally occurring group of flavones and flavonols was discussed in an earlier publication.[1] In that connection it was necessary to provide experimental support regarding the possibility of nuclear oxidation taking place in these compounds. Attempts made by others in the past in this direction appear to have been unsuccessful.

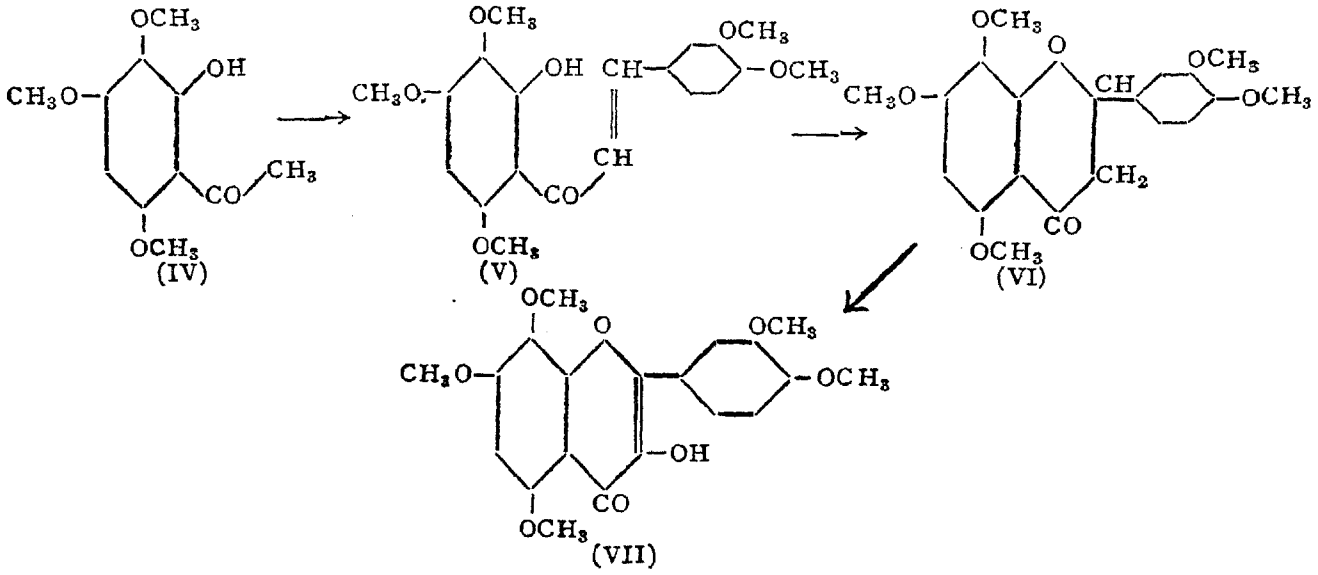
As early as 1911 when anthocyanins were still considered to be oxidation products of hydroxy-flavones, Nierenstein and Wheldale[2] subjected quercetin (I) to oxidation with chromic acid in acetic acid solution in the cold in order to obtain an anthocyanin-like substance. The deep red product was called quercetone and given the acceptable 5 : 8-quinone structure (II). By subjecting it to reductive acetylation and subsequently hydrolysing the intermediate acetate they obtained what was considered to be 8-hydroxy-quercetin (III) and degradation experiments showed that the side phenyl nucleus was unaffected. But the properties of the compound were different from those of gossypetin and quercetagetin. It melted at 352–53° and was insoluble in chloroform, acetone and pyridine and gave with cold alkali only a yellow colour. Its penta-methyl ether melted at 147–49° and the hexamethyl ether at 142–43°. This work threw into some confusion the constitution of gossypetin and quercetagetin till the classical paper of Baker, Nodzu and Robinson[3] settled the question finally.

Nierenstein[4] brought forward synthetic support for his oxidation experiments. Starting from what was considered to be 2-hydroxy-3 : 4 : 6-trimethoxy



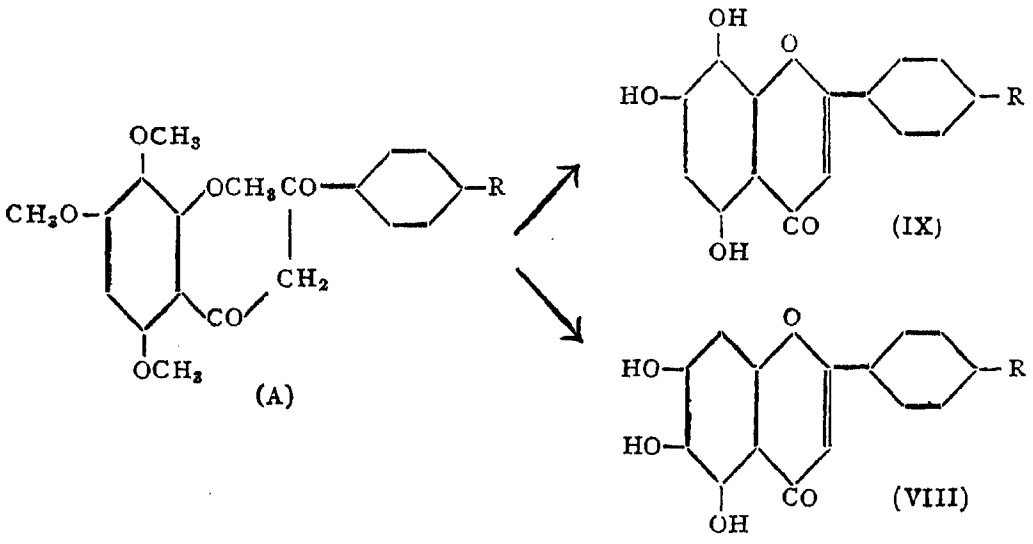
acetophenone and adopting Kostanecki's method of synthesis he prepared 8-hydroxy-quercetin which was found to be identical with his oxidation product. However in view of the later work of Baker *et al.*[3] establishing the constitution of gossypetin and quercetagetin by synthesis, it was definite that Nierenstein's oxidation had gone wrong. Even the straight-forward method of synthesis failed in his hands for the reason given below. It appears to be a further error that the final products from the two methods were considered to be identical. For the failure of the synthesis the starting ketone (IV) for which he gave a melting point of 125–26° and which was prepared by a method not free from ambiguity seems to have been primarily responsible. Recently we had occasion to carry out this synthesis since the pentamethyl gossypetin (VII) with the 3-hydroxyl free was required in connection with the constitution of gossypin. The required ketone was obtained by an unambiguous method (Sastri and Seshadri)[5] and it melted at 113–14°. The synthesis went smoothly yielding a gossypetin derivative. The following table gives a comparison of the various compounds obtained by Nierenstein and by us:

Name of the compound	Nierenstein's data	Ours
1 2-hydroxy-3:4:6-trimethoxy-acetophenone (IV)	125–26°	113–14°
2 Chalkone (V) ..	143°	143–45°
3 Acetate of chalkone ..	168°	135–36°
4 Flavanone (VI) ..	186–87°	175–76°
5 Flavonol (VII) ..	147–49°	228–30°
6 Fully methylated ether of (VII) ..	142–43° (different from hexa-methyl-gossypetin)	170–72° (identical with hexa-methyl gossypetin)



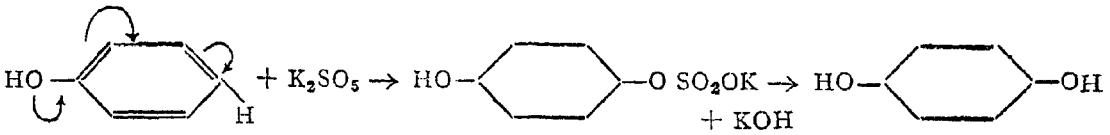
Another error which seems to have passed unnoticed, was present in the above work of Nierenstein. He reported that the pentamethoxy-flavonol (VII) was found to be identical with the pentamethyl-ether obtained by the partial methylation of his hydroxy-quercetin using dimethyl sulphate and alkali. This involved the idea that the particular hydroxyl in flavonols which was resistant to methylation was the one located in the 3-position. It was obviously wrong since even in flavones having no hydroxyl in the 3-position this characteristic had been observed. It is now well known that the 3-hydroxyl is in fact readily methylated and the resistant hydroxyl is present in the 5-position.

Nierenstein's[6] oxidation of chrysin to chrysone and its subsequent conversion to hydroxy chrysin seems to have met with no better success. The hydroxy-chrysin melted at 304–05° and its properties showed no agreement with those of norwogonin.[5,7] However, earlier this experiment was used for arriving at the correct constitution of scutellarein. Bargellini[8] synthesised scutellarein by heating with hydroiodic acid 2:3:4:6:4′-pentamethoxy-dibenzoylmethane (A, R = OCH_3). Of the two possible constitutions for the product he preferred the 5:6:7-arrangement (VIII, R= OH) based on certain analogies. In support of this he repeated the reaction using the simpler compound 2:3:4:6-tetramethoxy-dibenzoyl-methane (A, R = H) and for this product he definitely ruled out the 5:7:8-structure (IX, R = H) since it did not agree with the hydroxy-chrysin of Nierenstein in its properties. In order to provide further evidence for the constitution of scutellarein he[9] oxidised apigenin with chromic acid and reported the formation of a red substance probably analogous to chrysone. But the promised report of further work has not appeared so far.

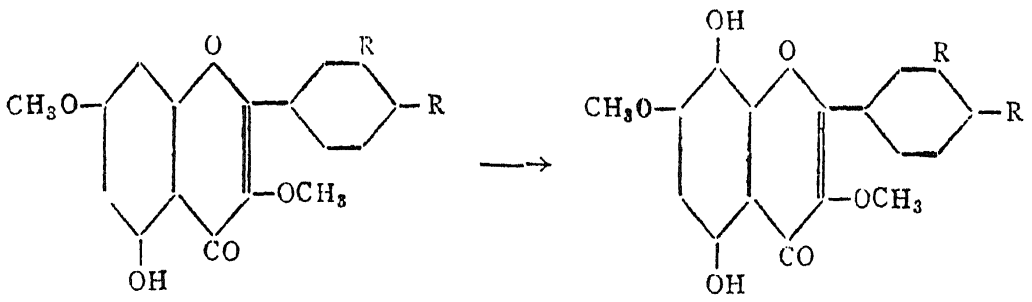


More recently Venkataraman[10] reported that attempts made in his laboratory to oxidise chrysin and 5-hydroxy-6-benzyl-7-benzyloxy-flavone using chromic anhydride, nitric acid, potassium persulphate and selenium dioxide were unsuccessful; they did not lead to any homogeneous material other than the starting substances.

In spite of these failures the theory of biogenesis was so well supported from other directions that a reinvestigation of nuclear oxidation seemed to be justified. Potassium persulphate was chosen as the most convenient and direct reagent whose action is most closely approximating to biological nuclear oxidation. It is well established that this oxidising agent readily attacks the nuclear para position to an existing hydroxyl and introduces a fresh hydroxyl. As an intermediate stage a sulphate seems to be formed. Since this is soluble in water it is readily separated from impurities, particularly the unchanged material, and the oxidation product is then liberated by acid hydrolysis. It is clear that the oxidation involves anionoid (nucleophilic) activity of the concerned para position and the stages could be represented as given below taking the simplest phenol as example.

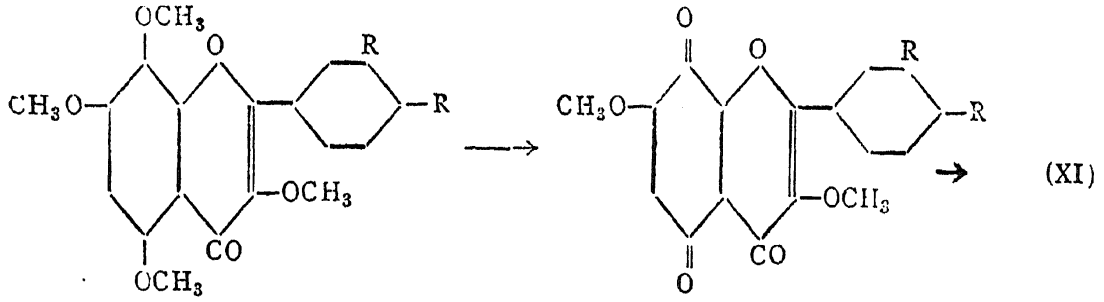


In the experiments described later in this paper the conversion of galangin and quercetin into 8-hydroxy-galangin and gossypetin has been achieved. Prior to oxidation, the 5:7-hydroxy-flavonols are converted into their partial methyl ethers leaving the hydroxyl in the 5-position alone free (X). This is intended to give protection to the molecule against general oxidation under the alkaline conditions employed. 3:7-O-dimethyl-galangin (X, R = H) and 3:7:3′:4′-O-tetramethyl-quercetin (X, R = OCH_3) are then oxidised with potassium persulphate. A difficulty arises here from the sparing solubility of these partial methyl ethers in aqueous alkali, but it has been got over by the judicious addition of pyridine. Satisfactory yields of the 5:8-dihydroxy compounds (XI) are obtained. These compounds give the characteristic reactions of quinols and readily yield the corresponding quinones by treatment with *p*-benzo quinone. Methylation with dimethyl sulphate in anhydrous acetone medium converts them into the tetramethyl ether of 8-hydroxy-galangin[11] (XII, R = H) and gossypetin hexamethyl ether[12] (XII, R = OCH_3) respectively and demethylation yields the free hydroxy flavonols.



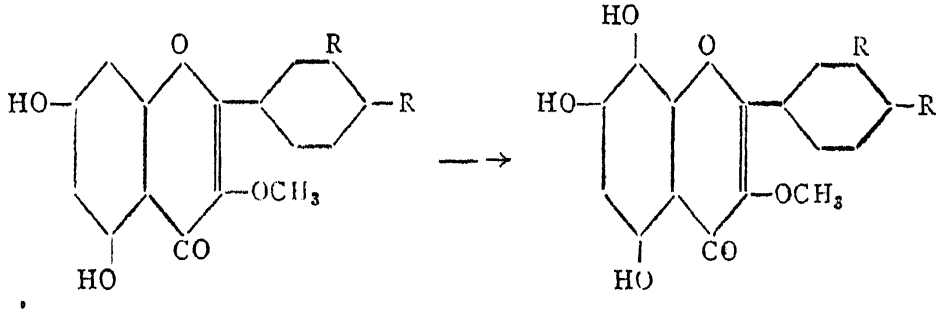
(X) R = H or OCH_3 (XI)

For purposes of comparison the 5: 8-quinones (XIII) and quinols (XI), have been prepared from authentic samples of the fully methylated ethers of 8-hydroxy-galangin and gossypetin (XII) by oxidation with nitric acid and subsequent reduction with sodium sulphite or sulphur dioxide.[13]



(XII) R = H or OCH_3 (XIII)

From the point of view of the easy synthetic preparation of these 5: 7: 8-hydroxy-flavonols, the oxidation of the dihydroxy compounds (XIV), galangin-3-methyl ether (R = H) and quercetin-3: 3′: 4′-trimethyl ether (R = OCH_3) becomes important, because these dihydroxy compounds form the earliest products of Allan-Robinson synthesis. Their oxidation has been found to proceed smoothly, their ready solubility in aqueous alkali being an added convenience. The products are trihydroxy compounds (XV) and are again obtained in good yields. This constitutes a remarkably easy method of synthesis of these 5: 7: 8-hydroxy-flavonols and makes them readily available for experimental purposes. Further it marks a stage in the attempt to minimise the protection of the hydroxyl groups before effecting oxidation and to approximate more closely to biogenetic conditions.



(XIV) R = H or OCH_3 (XV)

Experimental

5 : 8-Dihydroxy-3 : 7-dimethoxy-flavone

To a stirred solution of 5-hydroxy-3 : 7-dimethoxy-flavone (1 g.) in a mixture of pyridine (20 c.c.) and aqueous potassium hydroxide (1 g. in 25 c.c.) were added simultaneously aqueous potassium persulphate solution (2 g. in 100 c.c.) and aqueous potash (1 g. in 25 c.c.) dropwise during the course of two hours. After keeping the deep olive brown reaction mixture overnight it was neutralised to congo red and extracted twice with ether. The clear brown aqueous solution was then treated with sodium sulphite (2 g.) and concentrated hydrochloric acid (20 c.c.) and kept in a boiling water-bath for 30 minutes. The glistening brownish yellow crystalline solid that separated out was filtered off and washed well with water; some more of it was obtained by ether extracting the mother liquor; yield, 0.55 g. After recrystallisation from ethyl acetate it came out as bright yellow long and thin rectangular plates melting at 221–23° (Found: C, 65.3; H, 4.8; $C_{17}H_{14}O_6$ requires C, 65.0; H, 4.5%).

It was sparingly soluble in alcohol and ethyl acetate and moderately in glacial acetic acid. It was not easily soluble in sodium carbonate solution. In 5% aqueous sodium hydroxide it readily dissolved to give a deep red solution which changed to bluish-violet in a few minutes. With ferric chloride in alcoholic solution it gave a pale green colour which quickly changed to brown and with *p*-benzoquinone a red solution was obtained which slowly deposited an orange red crystalline solid.

The dihydroxy compound (0.2 g.) was acetylated with acetic anhydride (3 c.c.) and a few drops of pyridine. The acetate crystallised from alcohol in the form of colourless matted soft needles melting at 191–93°. It was sparingly soluble in alcohol but readily in chloroform.

A solution of the dihydroxy compound (0.2 g.) in dry acetone (20 c.c.) was treated with dimethyl sulphate (0.5 c.c.) and anhydrous potassium carbonate (5 g.). After refluxing for 6 hours the solvent was distilled off and the residue treated with water. The pale brown solid that separated out was filtered, washed and crystallised from a mixture of benzene and ligroin from which it separated out as colourless rectangular prisms melting at 162–64° identical with 3 : 5 : 7 : 8-tetramethoxyflavone[11]; the mixed melting point was undepressed. The dihydroxy compound was demethylated by means of boiling hydriodic acid. The product crystallised from ethyl acetate in the form of fibrous needles melting at 231–33°. It gave all the reactions of 8-hydroxy galangin[11] and the mixed melting point was undepressed. The acetate was also prepared. It crystallised from dilute

alcohol as colourless narrow rectangular prisms and melted at 173–74° alone or mixed with an authentic sample of the acetate of 8-hydroxy-galangin.

3 : 7-*Dimethoxy-flavoquinone*

3 : 5 : 7 : 8-Tetramethoxy-flavone (0.2 g.) was treated with nitric acid (d. 1.25; 5 c.c.) with vigorous stirring. On keeping for 15 minutes at 15–20° the solid dissolved to a yellow solution which changed to orange and finally red and a deep red product separated out which soon solidified. It was filtered, washed with nitric acid (d. 1.25) followed by water thoroughly and dried. Crystallised twice from alcohol it separated out as shining orange-red rectangular plates and prisms melting at 220-21° (Found: C, 65.4; H, 4.0; $C_{17}H_{12}O_6$ requires C, 65.4; H, 3.8%). It was sparingly soluble in alcohol. In aqueous sodium hydroxide it readily dissolved to a bluish-violet solution.

A solution of the above quinone (0.1 g.) in glacial acetic acid (0.5 c.c.) was treated with sodium sulphite (0.5 g.). The deep red solution immediately changed to bright yellow. On dilution with water a yellow solid separated out which was filtered, washed and crystallised from alcohol from which it came out in the form of golden yellow shining rectangular plates melting at 221–23° alone or in admixture with the quinol prepared by the oxidation of 5-hydroxy-3 : 7-dimethoxy-flavone.

5 : 7 : 8-*Trihydroxy*-3-*methoxy-flavone*

A stirred solution of 5 : 7-dihydroxy-3-methoxy-flavone (1 g.) in aqueous potassium hydroxide (0.75 g. in 25 c.c.) was slowly treated with a solution of potassium persulphate (2 g. in 100 c.c. of water) and aqueous potash (0.75 g. in 25 c.c.) simultaneously during the course of 2 hours while keeping the reaction mixture at a temperature of 15–20°. After allowing the deep brown solution to stand for 24 hours at room temperature, it was neutralised (Congo Red) and the brown solid that separated out was removed by extraction with ether twice. The clear brown aqueous layer was treated with sodium sulphite (2 g.) and concentrated hydrochloric acid (20 c.c.) and kept in a boiling water-bath for 30 minutes. A yellow solid began to separate during the course of the heating and on cooling more of it was obtained. It was filtered and washed free from acid; ether extraction of the mother liquor yielded some more of the substance; yield 0.5 g. It crystallised from a mixture of absolute alcohol and benzene in the form of tiny yellow prisms melting at 245–47° (Found: C, 64.1; H, 4.3; $C_{16}H_{12}O_6$ requires C, 64.0; H, 4.0%).

It was readily soluble in alcohol, acetone or ethyl acetate and sparingly in ether. In 5% aqueous sodium hydroxide it dissolved to a deep brown-red solution which changed rapidly to pale blue on shaking with air. With 5% sodium carbonate the solution was yellowish-brown changing to pale yellow. In alcoholic solution it gave a deep reddish-brown colour with ferric chloride, a yellow precipitate with lead acetate and a red colour with *p*-benzoquinone with a gradual separation of a reddish-brown solid.

The substance (0.2 g.) on acetylation with acetic anhydride (3 c.c.) and a few drops of pyridine gave rise to the acetyl derivative which crystallised from a mixture of benzene and ligroin in the form of colourless thin plates melting at 195–97°.

On methylation with dimethyl sulphate (0.5 c.c.) and anhydrous potassium carbonate (5 g.) in anhydrous acetone medium the trihydroxy compound yielded the methyl ether which crystallised from benzene petroleum ether mixture in the form of colourless thick rectangular prisms melting at 162–64°. Mixed melting point with an authentic sample of 3 : 5 : 7 : 8-tetramethoxy-flavone was not depressed.

5 : 8-*Dihydroxy*-3 : 7 : 3′ : 4′-*tetramethoxy-flavone*

A stirred solution of 5-hydroxy-3 : 7 : 3′ : 4′-tetramethoxy-flavone (1 g.) in a mixture of pyridine (20 c.c.) and aqueous potash (0.5 g. in 25 c.c.) was treated with aqueous potassium persulphate (1.5 g. in 50 c.c. of water), and potassium hydroxide solution (0.5 g. in 25 c.c.) during the course of two hours. After leaving the deep olive green solution for 24 hours it was neutralised with concentrated hydrochloric acid and the solid which separated out was extracted with ether. The clear brown aqueous layer was heated on a boiling water-bath for 30 minutes after the addition of sodium sulphite (2 g.) and concentrated hydrochloric acid (20 c.c.). The yellow crystalline solid that was deposited was filtered off and washed; some more of it was obtained on extracting the mother liquor with ether; yield, 0.45 g. When crystallised twice from ethyl acetate it separated in the form of lustrous golden yellow thick rectangular prisms melting at 250–52° (Found: C, 60.6; H, 4.6; $C_{19}H_{18}O_8$ requires C, 61.0; H, 4.8%).

It was sparingly soluble in alcohol and ethyl acetate and moderately soluble in glacial acetic acid. It was not easily soluble in sodium carbonate. In 5% aqueous sodium hydroxide it readily dissolved to a deep red solution which changed to bluish-violet in a few minutes. In alcoholic solution it gave with ferric chloride a transient green colour which quickly changed to brown; with *p*-benzoquinone a red colour was produced and a deep red solid slowly separated out.

The dihydroxy compound (0.1 g.) was methylated using dimethyl sulphate (0.5 c.c.) and anhydrous potassium carbonate (5 g.) in dry acetone medium. The methyl ether crystallised from alcohol in the form of colourless needles metling at 170–72° alone or in admixture with the hexamethyl ether of gossypetin.

A solution of the dihydroxy compound (0.2 g.) in dry acetone (25 c.c.) was treated with ethyl iodide (0.5 c.c.) and anhydrous potassium carbonate (5 g.). After refluxing for 12 hours the solvent was completely removed, the residue treated with water and the white solid which separated out was filtered off. On crystallisation from alcohol the diethyl ether separated out in the form of colourless narrow rectangular plates melting at 143–45° (Found: C, 61.3; H, 6.3; $C_{23}H_{26}O_8$, H_2O requires C, 61.6; H, 6.3%). It was readily soluble in alcohol, benzene or acetone, and insoluble in sodium hydroxide.

5 : 7 : 8-*Trihydroxy*-3 : 3′ : 4′-*trimethoxy-flavone*

To a stirred solution of 5 : 7-dihydroxy-3 : 3′ : 4′-trimethoxy-flavone (1 g.) in aqueous potassium hydroxide (0.5 g. in 25 c.c.) were added dropwise potassium persulphate solution (1.5 g. in 50 c.c.) and aqueous potash (0.5 g. in 25 c.c.) during the course of 2 hours. After keeping the deep brown solution for 24 hours it was neutralised with hydrochloric acid and extracted twice with ether. The clear brown aqueous layer was treated with sodium sulphite (2 g.) and concentrated hydrochloric acid (20 c.c.). On heating the solution at 100° for 30 minutes a yellow solid separated out which was filtered and washed; some more of it was obtained by extracting the mother liquor with ether; yield, 0.45 g. On crystallisation from alcohol it separated in the form of yellow microscopic prisms melting at 244–46°. (Found: C, 57.2; H, 4.9; $C_{18}H_{16}O_8$, H_2O requires C, 57.2; H, 4.8%). It was easily soluble in alcohol and the solution gave a deep greenish brown colour with ferric chloride, a yellow precipitate with lead acetate and a red colour with *p*-benzoquinone. In 5% aqueous sodium hydroxide it readily dissolved to a deep brown red solution; the colour faded rapidly to a pale blue which was fairly stable. With aqueous sodium carbonate the initial brown-red faded to a pale yellowish-green.

The trihydroxy compound (0.1 g.) was methylated in anhydrous acetone medium with dimethyl sulphate (0.5 c.c.) and anhydrous potassium carbonate (5 g.). The methyl ether crystallised from alcohol in the form of colourless needles melting at 170–72°. The mixed melting point with an authentic sample of hexamethyl gossypetin was not depressed.

Summary

Nuclear oxidation leading to the preparation of 8-hydroxy-galangin and gossypetin has been carried out. 3 : 7-dimethyl-ether of galangin and 3 : 7 : 3′ : 4′-tetramethyl-ether of quercetin have been oxidised by means of potassium persulphate to the corresponding 5 : 8-dihydroxy-compounds (quinols). Even the 5 : 7-dihydroxy-compounds, 3-O-methyl-galangin and 3 : 3′ : 4′-O-trimethyl-quercetin could be oxidised to the corresponding 5 : 7 : 8-trihydroxy-derivatives in good yields. Subsequent methylation yields the fully methylated ethers of 8-hydroxy-galangin and gossypetin and demethylation the free hydroxy-flavonols. These experiments not only illustrate facile nuclear oxidation in the flavone series in support of the theory of biogenesis, but also constitute simple and elegant methods for the synthesis of 5 : 7 : 8-hydroxy-flavonols.

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