THE FACTORS THAT AFFECT ALKALI COLOUR REACTIONS OF FLAVONOLS: A STUDY OF FLAVONOLS OF UNCOMMON TYPES

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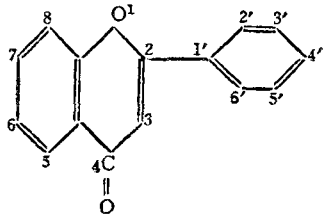
IN a number of earlier papers it has been pointed out that colour changes in alkaline buffer solutions can serve as useful indications of the constitution of flavonoids. Flavonols of the gossypetin series having the 5: 7: 8-arrangement of hydroxyl groups in the condensed benzene ring exhibit prominent colours and also a display of colours.[1] This is more marked in the case of flavonols of the calycopterin series having the 5: 6: 7: 8-tetra-hydroxy benzene ring.[2] Both these groups of compounds also undergo ready conversion into quinones when treated with *p*-benzoquinone. Somewhat similar properties though less marked are possessed by myricetin[3] and robinetin,[4] 6-hydroxy myricetin[5] and 6-hydroxy robinetin[6] in which the side phenyl nucleus carries three hydroxyls in the 3′, 4′ and 5′ positions. Since the corresponding flavones do not show such prominent colours, the hydroxyl in the 3-position, characteristic of flavonols, seems to be an essential requisite.



The quercetagetin series of flavonols in which the 5: 6: 7-arrangement of contiguous hydroxyl groups is present do not give prominent alkali colour reactions except the highest member having the 3′: 4′: 5′-hydroxy groups.[5] In the case of nor-gardenin[7] (3: 5: 6: 8: 3′: 4′: 5′- heptahydroxy flavone) the colour reaction is again not prominent and there is no display of colours though in the condensed benzene nucleus of this flavonol there is a hydroxy quinol system involving the 5: 6: 8-positions and the side phenyl nucleus has three hydroxyl groups as in robinetin. It appeared therefore that more factors are involved than known before; probably it was essential to have

a hydroxyl free in the 7-position and the exact distribution of the hydroxyl groups was an important consideration. In order to throw more light on these questions, a number of new flavonols have been prepared and studied during the past several years and the results are collected in this paper.

A few flavonols without a 7-hydroxyl group but otherwise having favourable structures have now been examined. They were prepared from some of the intermediates in the synthesis of gardenin[10] and they are 3: 5: 3′: 4′: 5′-pentahydroxy flavone and 3: 5: 8: 3′: 4′: 5′-hexahydroxy flavone. Though they do not give as deeply coloured solutions as the corresponding flavonols having the 7-hydroxyl group, they are somewhat better than nor-gardenin. Hence it would appear that the 5: 6: 8-arrangement of hydroxyl groups is a disadvantage.

As already mentioned prominent colours are exhibited by myricetin and robinetin which have the three hydroxyl groups in the side phenyl nucleus in the 3′: 4′: 5′-positions. In an earlier paper[8] flavonols having a quinol-system in the side phenyl nucleus, *i.e.*, 2′: 5′-dihydroxy compounds were reported and were shown to exhibit very feeble alkali colour changes as compared with the 5: 8-dihydroxy compounds, *e.g.*, 3-hydroxy primetin,[9] though both were quinols. To study further the part played by the side phenyl nucleus in alkali colour reactions, 3: 5: 7: 2′: 3′: 4′-hexahydroxy and 3: 7: 2′: 3′: 4′-pentahydroxy flavones have now been synthesised. As compared with myricetin and robinetin, they give feeble alkali colour reactions and lack colour display. The isomers of quercetin and fisetin having 2′: 3′-dihydroxy groups have also been prepared and compared with quercetin and fisetin. The 2′: 3′-combination reduces appreciably the intensity of colour reactions.

Fluorescence in alcohol and particularly in sulphuric acid solutions appears to be of good diagnostic value. A characteristic feature of fisetin, quercetin, robinetin and myricetin is their marked capacity to exhibit fluorescence in concentrated sulphuric acid solutions while their methyl ethers show only diminished fluorescence. This is markedly absent in the isomeric compounds and their methyl ethers reported in this paper.

Experimental

(All compounds were dried in vacuo at 110° C. over phosphorus pentoxide before analysis.)

3: 5: 3′: 4′: 5′-*Pentahydroxy flavone.*—5-Hydroxy-3: 3′: 4′: 5′-tetramethoxy flavone[10] (0·5 g.) was demethylated by refluxing with a mixture of acetic anhydride (15 c.c.) and hydriodic acid (10 c.c.) for 1·5 hrs. The

product (0·2 g.), yellow prisms from a mixture of ethyl acetate and petroleum ether, melted at 278–80° with earlier darkening at 272° (Found: C, 59·7; H, 3·9; $C_{15}H_{10}O_7$ requires C, 59·6; H, 3·3%). It gave a green colour with alcoholic ferric chloride and dissolved very slowly in aqueous sodium bicarbonate (5%) giving an orange yellow solution. Its pale green solution in aqueous sodium carbonate (5%) quickly changed to purple red and then slowly to brown. In aqueous sodium hydroxide (5%) it showed a transient green colour changing to purple which on shaking rapidly became brown.

The penta acetate, colourless rectangular plates from ethanol, melted at 207–8°.

3:5:3′:4′:5′-*Pentamethoxy flavone.*—Methylation of the above 5-hydroxy flavone (0·5 g.) with dimethyl sulphate (1 c.c., excess) and potassium carbonate (2 g.) in acetone for 30 hrs. yielded the pentamethyl ether (0·4 g.). It came out of ethyl acetate as thin rectangular plates, m.p. 152–53° (Found: C, 64·8; H, 5·2; $C_{20}H_{19}O_7$ requires C, 64·7; H, 5·1%).

3:5:8:3′:4′:5′-*Hexahydroxy flavone.*—Obtained by the demethylation of 5:8-dihydroxy-3:3′:4′:5′-tetramethoxy flavone[10] (0·5 g.) with a mixture of hydriodic acid (10 c.c.) and acetic anhydride (15 c.c.), the hexahydroxy flavone crystallised from a mixture of ethyl acetate and petroleum ether as thick yellow prisms, m.p. 297–300° (Found: C, 56·0; H, 3·5; $C_{15}H_{10}O_8$ requires C, 56·6; H, 3·2%). It gave a green colour with alcoholic ferric chloride deepening with excess of the reagent. With aqueous sodium bicarbonate (5%) it gave a yellow colour. Its yellow solution in aqueous sodium carbonate (5%) changed to brown and finally to deep red. Its deep red solution in aqueous sodium hydroxide (5%) changed to dark brown.

The hexa acetoxy flavone crystallised from ethyl acetate as colourless, small rectangular plates melting at 236–37°.

3:5:8:3′:4′:5′-*Hexamethoxy flavone.*—5:8-Dihydroxy-3:3′:4′:5′-tetramethoxy flavone[10] (0·3 g.) was completely methylated by dimethyl sulphate (1 c.c.) and potassium carbonate (3 g.) in acetone when refluxed for 30 hrs. It crystallised from a mixture of ethyl acetate and petroleum ether as colourless, thin plates melting at 170–71° (Found: C, 62·7; H, 5·5; $C_{21}H_{22}O_8$ requires C, 62·7; H, 5·5%).

o-Veratric anhydride.—A mixture of *o*-veratric acid[11] (20 g.), anhydrous ether (200 c.c.) and dry pyridine (20 c.c.) was well cooled in ice and salt mixture and treated with thionyl chloride (5 c.c.) in small quantities with good mixing. After keeping overnight in the refrigerator the anhydride was isolated by

the usual method. Colourless needles (16 g.) from petroleum ether, m.p. 93°. Heilbron[12] gives the same melting point.

7-Hydroxy-3 : 2′ : 3′-trimethoxy flavone.—An intimate mixture of ω-methoxy resacetophenone (3 g.), *o*-veratric anhydride (16 g.) and sodium *o*-veratrate (6 g.) was heated at 180-90° under reduced pressure for 6 hrs. and the product was worked up by the general procedure of Allan-Robinson condensations. Pale yellow, thick prisms from methanol (1·2 g.), m.p. 210–11° (Found: C, 66·2; H, 4·9; $C_{18}H_{16}O_6$ requires C, 66·7; H, 4·9%). It gave no colour with alcohoic ferric chloride. Its solution in concentrated sulphuric acid was yellow with no fluorescence. The acetatee (acetic anhydride-sulphuric acid method) crystallised as colourless, small prisms from ethyl acetate-petroleum ether, m.p. 125–26°.

Methylation with dimethyl sulphate and potassium carbonate in dry acetone medium gave 3 : 7 : 2′ : 3′-tetramethoxy flavone, colourless prisms from benzene-petroleum ether, m.p. 82–83° (Found: C, 66·4; H, 4·9; $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3%). Its solution in strong sulphuric acid was yellow and showed no fluorescence. Fisetin tetramethyl ether gave a yellow solution in concentrated sulphuric acid showing feeble green fluorescence.

3 : 7 : 2′ : 3′-Tetrahydroxy flavone.—Demethylation of the 7-hydroxy-3 : 2′ : 3′-trimethoxy flavone (0·3 g.) by refluxing for 2 hrs. in acetic anhydride (5 c.c.) with hydriodic acid (5 c.c.; d., 1·7) gave the tetrahydroxy flavone (0·15 g.), yellow prisms from ethyl acetate-benzene, m.p. 290–2° (decomp.) (Found: C, 62·8; H, 3·7; $C_{15}H_{10}O_6$ requires C, 62·9; H, 3·5%).

It gives a reddish-brown ferric reaction turning brownish-green with more reagent. In dilute sodium carbonate it dissolves to a bright yellow solution unchanged on keeping; fisetin gives a deep yellow solution turning brown gradually. With strong sulphuric acid it gives a pale yellow solution without fluorescence whereas fisetin yields a yellow solution showing bluish-green fluorescence.

The tetra-acetate was prepared with acetic anhydride and two drops of concentrated sulphuric acid; colourless long, thin plates from methyl alcohol, m.p. 204–5°.

5 : 7-Dihydroxy-3 : 2′ : 3′-Trimethoxy flavone.—A mixture of ω-methoxy phloracetophenone (4 g.), *o*-veratric anhydride (28 g.) and sodium *o*-veratrate (10 g.) was heated under reduced pressure at 170–80° for 7 hrs. The product (1·3 g.) crystallised from methanol as clusters of long, thin needles and tapering plates, m.p. 196–97° (Found: C, 62·3; H, 5·2; $C_{18}H_{16}O_7$ requires

C, 62·8; H, 4·7%). The substance gave a greenish-brown colour with alcoholic ferric chloride; its solution in concentrated sulphuric acid was yellow without any fluorescence. The diacetate crystallised from ethyl acetate and petroleum ether as colourless prisms, m.p. 118–19°.

Methylation by the dimethyl sulphate-potassium carbonate method gave 3:5:7:2′:3′-pentamethoxy flavone, colourless, thick rhombs (ethyl acetate-petroleum ether), m.p. 170–1°, giving a yellow solution in strong sulphuric acid without fluorescence (Found: C, 64·5; H, 5·7; $C_{20}H_{20}O_7$ requires C, 64·5; H, 5·4%). Quercetin pentamethyl ether dissolved in concentrated sulphuric acid to a yellow solution having feeble green fluorescence.

3:5:7:2′:3′-*Pentahydroxy flavone.*—The above dihydroxy flavone was demethylated by refluxing with acetic anhydride and hydriodic acid for 2 hrs. The product crystallised from alcohol as yellow, small prisms, m.p. 300° (decomp.) (Found: C, 59·1; H, 3·7; $C_{15}H_{10}O_7$ requires C, 59·6; H, 3·3%). It gives a greenish-brown ferric chloride colour. In concentrated sulphuric acid it forms a yellow solution without fluorescence while quercetin gives a yellow solution showing bluish-green fluorescence.

2:3:4-*Trimethoxy benzoic acid.*—No convenient preparative method for this compound seems to have been described before.

Method I.—Dry pyrogallol carboxylic acid[13] (50 g.) was dissolved in dry acetone (500 c.c.) and the solution was refluxed with dimethyl sulphate (140 c.c.) and potassium carbonate (200 g.) for 40 hrs. The product isolated as usual, was taken up in ether and washed with aqueous alkali to remove any phenolic material; removal of the solvent gave the methyl 2:3:4-trimethoxy benzoate as an oil. This was refluxed with aqueous sodium hydroxide (10%; 200 c.c.), the alkaline solution extracted with ether and acidified yielding the 2:3:4-trimethoxy benzoic acid (30 g.), colourless needles (from hot water), m.p. 99°.

Method II.—Gallacetophenone[14] (50 g.) was completely methylated by refluxing with dimethyl sulphate (100 c.c.) and potassium carbonate (200 g.) for 40 hrs. The product was dissolved in ether and the ethereal solution freed from phenolic impurities by washing with dilute alkali. Evaporation of the solvent gave the trimethoxy acetophenone (62 g.) as a reddish oil. It was directly employed for the next stage.

To gallacetophenone trimethyl ether (30 g.) was added alkaline hypochlorite solution (250 c.c.; prepared by passing chlorine from 25 g. of potassium permanganate and 40 c.c. of concentrated hypochloric acid into

10% sodium hydroxide solution) in small quantities with vigorous shaking; the mixture was maintained at 60–75°, during this addition and for a further period of 3 hrs. It was cooled and the unoxidised ketone removed by extraction with ether. Acidification of the alkaline solution gave the 2: 3 : 4-trimethoxy benzoic acid as a colourless solid. The recovered ketone was again oxidised; total yield, quantitative. This sample was purer than that prepared by the first method.

2: 3: 4-*Trimethoxy benzoic anhydride.*—A mixture of 2: 3: 4-trimethoxy benzoic acid (30 g.), dry ether (200 c.c.) and dry pyridine (30 c.c.) was cooled in freezing mixture and treated with thionyl chloride (8 c.c.) in small lots with good mixing. After keeping overnight the anhydride was worked up. Crystallisation from benzene-petroleum ether gave colourless, thick prisms (26 g.), m.p. 86° (Found: C, 59·3; H, 5·6; $C_{20}H_{22}O_9$ requires C, 59·1; H, 5·4%).

7-*Hydroxy*-3: 2′: 3′: 4′-*Tetramethoxy flavone.*—A mixture of ω-methoxy resacetophenone (3 g.), 2: 3: 4-trimethoxy benzoic anhydride (26 g.) and the sodium salt of 2: 3: 4-trimethoxy benzoic acid (10 g.) was fused under reduced pressure at 170–80° for 6 hrs. and the product worked up as in similar cases. The flavone (0·8 g.) formed almost colourless prisms from methanol, m.p. 226–27° (Found: C, 64·1; H, 5·0; $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0%). It did not give any colour with alcoholic ferric chloride; its solution in concentrated sulphuric acid was yellow without any fluorescence. The acetate crystallised as long, thin needles and plates from ethyl acetate-petroleum ether, m.p. 120–21°.

3: 7: 2′: 3′: 4′-*Pentamethoxy flavone* was prepared by methylation of the 7-hydroxy compound with methyl sulphate and potassium carbonate in acetone solution; colourless, thick prisms from methanol, m.p. 145–46° (Found: C, 64·2; H, 5·3; $C_{20}H_{20}O_7$ requires C, 64·6; H, 5·4%). Its yellow solution in concentrated sulphuric acid did not have any fluorescence. Robinetin pentamethyl ether gave a yellow solution with a green fluorescence.

3: 7: 2′: 3′: 4′-*Pentahydroxy flavone.*—The above 7-hydroxy compound was demethylated with acetic anhydride-hydriodic acid and the product crystallised from ethyl acetate. Yellow, small prisms, decomposing at 314–15° (Found: C, 59·5; H, 3·6; $C_{15}H_{10}O_7$ requires C, 59·6; H, 3·3%). The following are the more important colour reactions shown by this compound:

Aqueous sodium carbonate (5%).—Deep yellow solution. On shaking with air turns orange red and then brown which fades off. Under these

conditions robinetin forms a blue solution changing to green, purple, brown which fades gradually.

Aqueous sodium hydroxide (2%).—Brown-red solution turning orange and finally pale brown. Robinetin gives a blue solution becoming green which changes to brown.

Alcoholic ferric chloride.—Greenish-brown, becomes bluish-green with more of the reagent and changes to violet on standing. With robinetin a greenish-brown colour develops turning brown with more of the reagent.

Concentrated sulphuric acid.—Yellow solution without fluorescence while robinetin dissolves to a yellow solution with green fluorescence.

The penta-acetate crystallised as colourless plates from ethyl acetate-petroleum ether, m.p. 185–86°.

5:7-*Dihydroxy*-3:2′:3′:4′-*Tetramethoxy flavone.*—A mixture of ω-methoxy phloracetophenone (3 g.), 2:3:4-trimethoxy benzoic anhydride (28 g.) and sodium salt of 2:3:4-trimethoxy benzoic acid (10 g.) was heated at 180–90° for 6 hrs. under reduced pressure and the product worked up as in the previous cases; the crude flavone was purified by chromatography over alumina in benzene solution and elution with benzene-acetone (1:1) mixture. Pale yellow, thick prisms from alcohol (1·1 g.), m.p. 174–75° (Found: C, 61·3; H, 5·4; $C_{19}H_{18}O_8$ requires C, 61·0; H, 4·8%). It gave an olive-brown ferric reaction and a yellow solution in strong sulphuric acid without fluorescence. The diacetate (acetic anhydride and a drop of sulphuric acid) formed colourless prisms from ethyl acetate-petroleum ether, m.p. 146–47°.

The dihydroxy flavone was completely methylated by the methyl sulphate-potassium carbonate method giving 3:5:7:2′:3′:4′-hexamethoxy flavone as colourless, small prisms from alcohol, m.p. 153–54° (Found: C, 62·4; H, 5·6; $C_{21}H_{22}O_8$ requires C, 62·7; H, 5·5%). It dissolved to a yellow solution in strong sulphuric acid without any fluorescence. Myricetin hexamethyl ether gave a yellow solution with green fluorescence.

3:5:7:2′:3′:4′-*Hexahydroxy flavone.*—Demethylation of the above dihydroxy compound by the acetic anhydride-hydriodic acid method gave the hexahydroxy flavone as brownish-yellow prisms from methanol, decomposing at 308–10° (Found: C, 56·8; H, 3·4; $C_{15}H_{10}O_8$ requires C, 56·6; H, 3·2%). It showed the following colour reactions:

Aqueous sodium carbonate (5%).—Yellow solution becoming brown. Myricetin gives a deep green solution turning violet-brown, purple, red, orange and brown.

Aqueous sodium hydroxide (2%).—Yellow solution turning orange red. Myricetin dissolves to a green solution becoming blue and then brown.

Alcoholic ferric chloride.—Olive-green solution which turns brown on keeping. With myricetin an olive-green colour is obtained which becomes brown with more of the reagent. On standing the solution turns blue and a blue-black precipitate settles down.

Concentrated sulphuric acid.—Yellow solution without fluorescence while myricetin gives a yellow solution with bluish-green fluorescence.

SUMMARY

In order to get fuller information on the influence of hydroxyl groups on alkali colour reactions a number of flavonols of types uncommon in Nature have been prepared and examined. Though the correlation between structure and alkali colour reactions is complex, when applied with care the reactions can be used for diagnostic purposes. The alkali colour reactions of the flavonols with 2′ : 3′ and 2′ : 3′ : 4′ disposition of hydroxyl groups are not prominent and their fluorescence properties are also very feeble.

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