## 905. *Chelate Systems. Part I.*

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The application of paper partition chromatography to the study of chelate systems is discussed. It has been shown that the pyrone carbonyl group of flavones forms stronger hydrogen bonds with the 5- than with the 3-hydroxyl group. The ability of the carbonyl group to form bonds with both hydroxyl groups simultaneously has been observed and electronic mechanisms are suggested. Some previously unreported flavones have been synthesised and alternative methods of preparation of some known compounds described.

Consden, Gordon, and Martin (*Biochem. J.*, 1944, **38**, 224) have shown that if adsorption and diffusion effects are neglected a partition chromatogram may be considered as a liquid–liquid extraction system and that in consequence the term $1/R_F - 1$ is directly related to the partition coefficient, $\alpha$. Further, Martin (*Symposia Biochem. Soc.*, 1949, **3**, 4) has considered that the addition of a given group to a solute molecule should change $\ln \alpha$ and hence $\ln (1/R_F - 1)$, in a manner determined by the natures of the added group and the two solvent phases but independent of the nature of the rest of the molecule. Bate-Smith and Westall (*Biochem. Biophys. Acta*, 1950, **4**, 427) have proposed the symbol $R_M$ for the term $\log (1/R_F - 1)$ and have shown that analysis of the $R_M$ values of a number of naturally occurring phenols supports Martin's conclusions.

The aim of the present investigation was to study the usefulness of partition chromatography as a simple and direct method of detecting chelation and estimating the resulting suppression of hydrophilic behaviour. This is of some importance since the accepted methods, *e.g.*, the analysis of infra-red absorption data, the depression of melting point on admixture with water, and the consideration of solubilities in polar and non-polar solvents, are tedious, demand rigid purification of material, and, for the last two, are only qualitative.

A group of 30 flavones, selected to provide most of the possible combinations of hydroxyl and methoxyl substitution in the 3-, 5-, 7-, and 4′-positions, and a few simple phenols have been run on paper partition chromatograms. $R_M$ values have been determined, with three solvents, under substantially the same conditions as those recommended by Bate-Smith and Westall (*loc. cit.*). The solvents were chosen to give the maximum useful spread of $R_M$ values and comprised the water-poor components of the mixtures indicated at the foot of Table 1. $R_M$ values numerically greater than $+1{\cdot}19$ and $-1{\cdot}09$ have been ignored since beyond those limits, small errors of $R_F$ measurement involve disproportionate errors in $R_M$.

From Table 1 it has been possible to calculate $\Delta R_M$ values relating to changes in molecular structure, *e.g.*, the difference between the $R_M$ values of 7 : 4′-dihydroxyflavone (no. 2) and 7-hydroxyflavone (no. 1) gives a value for $\Delta R_M$ corresponding to the addition of a hydroxyl group to the 4′-position of the nucleus. $\Delta R_M$ values corresponding to changes in the degree of hydroxylation are given in Table 2.

Comparison of $\Delta R_M$ values for the addition of hydroxyl groups to the 7- and the 4′-position of the flavone nucleus with the corresponding values for the simple phenols shows that hydroxyl groups in these positions make a normal phenolic contribution to chromatographic behaviour. The substantial agreement between these figures provides additional experimental evidence supporting the theoretical conclusions of Martin and of Consden, Gordon, and Martin (*loc. cit.*). In the case of hydroxyl groups in the 3- or the 5-position, however, not only is there a suppression of the normal positive $R_M$ contribution of the hydroxyl group, but a negative $R_M$ change is observed. This may be explained by the well-known chelation of the 5-hydroxyl group and the not so widely recognised chelation of the 3-hydroxyl group with the carbonyl group in position 4. The ring structures thus produced are relatively hydrophobic and favour a transfer of solute from the aqueous to the organic phase.

It is unlikely that the van der Waals forces between the organic solvent and the 6- and the 5-membered ring are appreciably different in magnitude or that these chelate structures

TABLE 1. $R_M$ *values of hydroxyflavones and simple phenols.*

| No. | Compound | $\Delta R_M$ in solvent: A | B | C |
|---|---|---|---|---|
| | (a) *Flavones.* | | | |
| 1 | 7-Hydroxy- | −0·71 | −0·53 | −0·18 |
| 2 | 7 : 4′-Dihydroxy | +0·49 | >+1·19 | >+1·19 |
| 3 | 4′-Hydroxy-7-methoxy- | −0·70 | −0·43 | +0·11 |
| 4 | 7-Hydroxy-4′-methoxy- | −0·65 | −0·30 | +0·12 |
| 5 | 5 : 7-Dihydroxy- | −1·0 | −0·93 | −0·75 |
| 6 | 5-Hydroxy-7-methoxy | >−1·09 | >−1·09 | >−1·09 |
| 7 | 7-Hydroxy-5-methoxy- | −0·33 | −0·03 | +0·70 |
| 8 | 5 : 7 : 4′-Trihydroxy- | +0·05 | +0·67 | +0·80 |
| 9 | 5 : 4′-Dihydroxy-7-methoxy- | −0·87 | −0·82 | −0·52 |
| 10 | 5 : 7-Dihydroxy-4′-methoxy- | −0·90 | −0·82 | −0·46 |
| 11 | 4′-Hydroxy-5 : 7-dimethoxy- | −0·29 | +0·17 | +1·11 |
| 12 | 3 : 7-Dihydroxy- | −0·82 | −0·51 | −0·21 |
| 13 | 3-Hydroxy-7-methoxy- | >−1·09 | >−1·09 | >−1·09 |
| 14 | 7-Hydroxy-3-methoxy- | −1·0 | −0·82 | −0·49 |
| 15 | 3 : 7 : 4′-Trihydroxy- | +0·30 | +1·02 | +1·17 |
| 16 | 3 : 4′-Dihydroxy-7-methoxy- | −0·92 | −0·49 | −0·03 |
| 17 | 3 : 7-Dihydroxy-4′-methoxy- | −0·81 | −0·43 | −0·07 |
| 18 | 3 : 5 : 7-Trihydroxy- | −0·92 | −0·76 | −0·69 |
| 19 | 5 : 7-Dihydroxy-3-methoxy- | >−1·09 | −1·06 | −0·93 |
| 20 | 3 : 5 : 7 : 4′-Tetrahydroxy- | +0·15 | +0·78 | +0·82 |
| 21 | 5 : 7-Dihydroxy-3 : 4′-dimethoxy- | >−1·09 | −1·04 | −0·78 |
| 22 | 3 : 5 : 7-Trihydroxy-4′-methoxy- | −0·86 | −0·65 | −0·47 |
| 23 | 4′-Hydroxy- | −0·70 | −0·49 | −0·10 |
| 24 | 3 : 5-Dihydroxy-7-methoxy- | >−1·09 | >−1·09 | >−1·09 |
| 25 | 7-Hydroxy-3 : 4′-dimethoxy- | −1·0 | −0·82 | −0·30 |
| 26 | 7-Hydroxy-3 : 5-dimethoxy- | −0·77 | −0·48 | +0·31 |
| 27 | 5 : 4′-Dihydroxy- | −0·92 | −0·87 | −0·62 |
| 28 | 5-Hydroxy-4′-methoxy- | >−1·09 | >−1·09 | >−1·09 |
| 29 | 4′-Hydroxy-5-methoxy- | −0·27 | +0·10 | +0·84 |
| | (b) *Simple phenols.* | | | |
| 30 | Resorcinol | −0·06 | +0·52 | +0·43 |
| 31 | 4-Methylresorcinol | −0·30 | +0·13 | +0·03 |
| 32 | 2 : 4-Dimethylresorcinol | −0·65 | −0·43 | −0·43 |
| 33 | Phloroglucinol | +1·13 | >+1·19 | >+1·19 |
| 34 | *C*-Methylphloroglucinol | +0·82 | >+1·19 | >+1·19 |
| 35 | *C*-Dimethylphloroglucinol | +0·43 | +1·09 | +0·96 |

Solvent A: Nitromethane, 100 vols.; water, 100 vols. Solvent B: Benzene, 60 vols.; nitromethane, 40 vols.; water, 100 vols. Solvent C: Benzene, 100 vols.; pyridine, 1 vol.; water, 100 vols.

TABLE 2. *Values of* $R_M$ *changes resulting from addition of hydroxyl groups.*

| Hydroxyl group added to: | $R_M$ derived from $R_M$'s of compounds nos. | $\Delta R_M$ A | Mean | B | Mean | C | Mean |
|---|---|---|---|---|---|---|---|
| *Flavone.* | | | | | | | |
| 7-Position | 2—23 | +1·19<br>+0·97 | +1·08 | — <br> +1·54 | +1·54 | — <br> +1·42 | +1·42 |
| 4′-Position | 2—1 | +1·20 | | — | | — | |
| | 8—5 | +1·05 | Mean, | +1·60 | Mean, | +1·55 | Mean, |
| | 15—12 | +1·12 | +1·11 | +1·53 | 1·55 | +1·38 | +1·48 |
| | 20—18 | +1·07 | | +1·54 | | +1·51 | |
| 5-Position | 5—1 | −0·29 | | −0·40 | | −0·57 | |
| | 19—14 | — | | −0·24 | | −0·44 | |
| | 8—2 | −0·44 | | — | | — | |
| | 9—3 | −0·17 | Mean, | −0·39 | Mean, | −0·63 | Mean, |
| | 10—4 | −0·25 | −0·27 | −0·52 | −0·36 | −0·58 | −0·53 |
| | 21—25 | — | | −0·22 | | −0·48 | |
| | 27—23 | −0·22 | | −0·38 | | −0·52 | |
| 3-Position | 12—1 | −0·11 | | +0·02 | | −0·03 | |
| | 17—4 | −0·16 | Mean, | −0·13 | Mean, | −0·19 | Mean, |
| | 16—3 | −0·22 | −0·17 | −0·06 | −0·05 | −0·14 | −0·12 |
| | 15—2 | −0·19 | | — | | — | |
| *Simple phenol nucleus.* | 33—30 | +1·19 | | — | | — | |
| | 34—31 | +1·12 | Mean, | — | Mean, | — | Mean, |
| | 35—32 | +1·08 | +1·13 | +1·52 | +1·52 | +1·39 | +1·39 |

interfere with the normal intermolecular bonding of water molecules to any significantly different extent. The fact that the $R_M$ changes caused by the addition of hydroxyl groups to position 5 are appreciably higher than to position 3 suggests therefore that the hydrogen bonding in the six- is stronger than in the five-membered chelate ring. This is in agreement with indirect chemical evidence, *e.g.*, the well-known, easier acetylation and methylation of the 3-hydroxyl group, and the greater resistance of the 3-methoxyl group to demethylation.

Table 3 gives the values of $R_M$ changes caused by methylation of flavone hydroxyl groups. Here again, the difference between the various hydroxyl groups is apparent,

TABLE 3. *Values of $R_M$ changes resulting from the methylation of flavone hydroxyl groups.*

| Position of OH | $\Delta R_M$ derived from $R_M$'s of compounds nos. | $\Delta R_M$ A | | $\Delta R_M$ B | | $\Delta R_M$ C | |
|---|---|---|---|---|---|---|---|
| 7- | 3—2 | −1·19 | Mean, | — | Mean, | — | Mean, |
| | 16—15 | −1·22 | −1·11 | −1·51 | −1·50 | −1·20 | −1·26 |
| | 9—8 | −0·92 | | −1·49 | | −1·32 | |
| 4′- | 4—2 | −1·14 | | — | | — | |
| | 10—8 | −0·95 | Mean, | −1·49 | Mean, | −1·26 | Mean, |
| | 17—15 | −1·11 | −1·05 | −1·45 | −1·46 | −1·24 | −1·26 |
| | 22—20 | −1·01 | | −1·43 | | −1·29 | |
| 5- | 7—5 | +0·67 | | +0·90 | | +1·45 | |
| | 11—9 | +0·58 | Mean, | +0·99 | Mean, | +1·63 | Mean, |
| | 26—19 | — | +0·63 | +0·58 | +0·86 | +1·24 | +1·44 |
| | 29—27 | +0·65 | | +0·97 | | +1·46 | |
| 3- | 14—12 | −0·18 | | −0·31 | | −0·28 | |
| | 19—18 | — | Mean, | −0·30 | Mean, | −0·24 | Mean, |
| | 21—22 | — | −0·19 | −0·39 | −0·35 | −0·31 | −0·26 |
| | 25—27 | −0·19 | | −0·39 | | −0·23 | |

methylation of the 5-hydroxyl being responsible for a positive $\Delta R_M$ and that of the 3-hydroxyl a much smaller negative $\Delta R_M$ than that caused by methylation of hydroxyl groups in the 7- or 4′-position. This increase in hydrophilic character may be ascribed to the opening of the chelate rings with consequent loss of their characteristic negative $\Delta R_M$ contributions. Comparison of the $\Delta R_M$ values for methylation of the 3-hydroxyl with those for the 5-hydroxyl group provides further evidence for the suggestion that the carbonyl–5-hydroxyl bond is considerably stronger than the carbonyl–3-hydroxyl linkage. In part, however, the differences in $\Delta R_M$ values resulting from the various methylations may be ascribed to the dissimilar $R_M$ contributions of the resulting methoxyl group. This will be discussed in a later communication.

TABLE 4. *$R_M$ changes caused by addition of a hydroxyl group to the 5-position if the 3-position is already hydroxylated.*

| $\Delta R_M$ derived from $R_M$'s of compounds nos. | $\Delta R_M$ A | | $\Delta R_M$ B | | $\Delta R_M$ C | |
|---|---|---|---|---|---|---|
| 20—15 | −0·15 | Mean, | −0·24 | Mean, | −0·35 | Mean, |
| 22—17 | −0·05 | −0·10 | −0·22 | −0·24 | −0·40 | −0·41 |
| 18—12 | −0·10 | | −0·25 | | −0·48 | |

*$R_M$ changes caused by addition of a hydroxyl group to the 3-position of the 5-position is hydroxylated.*

| | | | | | | |
|---|---|---|---|---|---|---|
| 20—8 | +0·10 | Mean, | +0·11 | Mean, | +0·02 | Mean, |
| 22—10 | +0·04 | +0·07 | +0·17 | +0·15 | −0·01 | +0·02 |
| 18—5 | +0·08 | | +0·17 | | +0·06 | |

Comparison of the figures in Table 4 with the $\Delta R_M$ values given in Table 2 clearly shows that the pyrone carbonyl group is able to form bonds with both 3- and 5-hydroxyl groups at the same time. This recalls the analogous double chelation reported by Flett (*J.*, 1948, 1441) for 1 : 8-dihydroxyanthraquinone. In this respect hydroxy-flavones and -anthraquinones differ from the simple phenolic ketones, phloracetophenone and 2 : 6-dihydroxyacetophenone, which are able to form bonds with only one hydroxyl group at a time. This difference may be ascribed to the fact that in the first pair of compounds, but not in the

second pair, the carbonyl oxygen atom is held centrally in the plane of the ring and may therefore be approached simultaneously by both hydroxyl groups.

The fact that the addition of a hydroxyl group to position 5 causes a decrease, and to position 3 an increase, in hydrophilic behaviour provides further evidence supporting the suggestion that hydrogen bonding is stronger in the former than in the latter system.

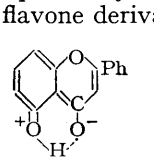
TABLE 5. *Values of* $R_M$ *changes caused by addition of hydroxyl groups to the* 3- *and the* 5-*position of the flavone nucleus.*

| Parent structure | | A | B | C |
|---|---|---|---|---|
| 7-Hydroxyflavone ..... | $\Delta R_M$ on addition of OH to 5-position | −0·29 | −0·40 | −0·57 |
| | ,, ,, 3- ,, | −0·11 | +0·02 | −0·03 |
| ∴ Expected | ,, ,, ,, | −0·40 | −0·38 | −0·60 |
| Observed (from $R_M$ values of compounds 18 and 1) | | −0·21 | −0·23 | −0·51 |
| 7-Hydroxy-4′-methoxyflavone... | $\Delta R_M$ on addition of OH to 5-position | −0·25 | −0·52 | −0·58 |
| | ,, ,, 3- ,, | −0·16 | −0·13 | −0·19 |
| ∴ Expected | ,, ,, ,, | −0·41 | −0·65 | −0·77 |
| Observed (from $R_M$ values of compounds 22 and 4) | | −0·21 | −0·35 | −0·59 |
| 7 : 4′-Dihydroxyflavone ..... | $\Delta R_M$ on addition of OH to 5-position | −0·44 | | |
| | ,, ,, 3- ,, | −0·19 | | |
| ∴ Expected | ,, ,, ,, | −0·63 | | |
| Observed (from $R_M$ values of compounds 20 and 2) | | −0·34 | | |

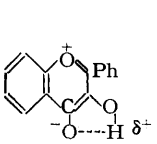
Table 5 gives a comparison of the $\Delta R_M$'s actually resulting from the simultaneous addition of hydroxyl groups to positions 3 and 5 of the flavone nucleus with the calculated values, on the assumption that no interaction of the chelate systems occurs. This clearly shows that the addition of a second chelating hydroxyl group to an existing chelate system is accompanied by an overall loss of hydrogen bonding.

It is now possible to consider the electronic mechanisms responsible for the formation of these chelate structures. The bonding of the 5-hydroxyl group is clearly related to the well-known hydrogen bonding of *o*-hydroxy-carbonyl compounds and may be considered as arising from the contribution of structure (I) to the resonance state of the molecule. The system may, in addition, show some measure of degeneracy in the position of the hydrogen atom, but it is unlikely that the resulting energy makes any large contribution to the stability of the linkage (cf. Ketelaar, *Rec. Trav. chim.*, 1941, **60**, 523).

The hydrogen bonding between the carbonyl and the 3-hydroxyl group differs from the chelation of the 5-hydroxyl group in that there is in the former structure no mechanism for the transfer of electrons from the hydroxyl to the carbonyl group. It seems likely that in this case the electrostatic attraction constituting the hydrogen bond is established between the hydrogen atom carrying a fractional positive charge arising from the $-I$ effect of the hydroxyl-oxygen atom and the carbonyl-oxygen atom carrying a negative charge derived from structure (II) and similar quinonoid contributions from hydroxyl groups in the 7-, 2′-, and 4′-positions. This formulation is similar to that proposed by Briggs and Locker (*J.*, 1951, 3136) to explain why the acidity of 7- or 4′-hydroxyflavonols is higher than that of the corresponding flavone derivatives.




(I)




(II)

This formulation explains the observation that the carbonyl group chelates more strongly with the 5- than with the 3-hydroxyl group; of the structures leading to hydrogen bonding, (I) must be expected to make the greatest contribution to the state of the molecule since it involves the smallest separation of electrostatic charges. The deduction that the simultaneous chelation of 3- and 5-hydroxyl groups is weaker than expected is accounted for by the fact that structures (I) and (II) are cross-conjugated, *i.e.*, that the two chelate systems are formed by mutually opposing mechanisms. This may not, however, be the the only explanation, since it is also likely that the formation of the first hydrogen bond involves a deformation of the electron cloud of the carbonyl-oxygen atom, and renders its electrons less available for bonding with the second hydroxyl group.

The compounds required in this investigation were synthesised by the well-known oxidative cyclisation of chalkones and Allan–Robinson methods. Towards the end of this work the use of the latter procedure was abandoned, since it was found that complete hydrolysis of the contaminant 3-acyl derivatives involved serious losses of the required flavones.

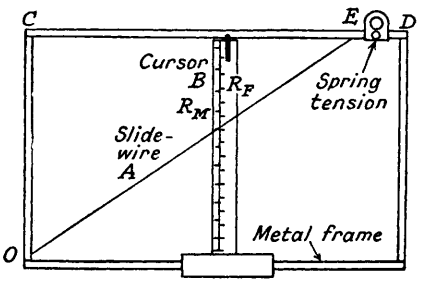
## Experimental

(a) *Partition Chromatography.*—These experiments were carried out according to the general procedure of Bate-Smith and Westall (*loc. cit.*). In order to achieve thorough equilibration of the filter-paper strips, the following precautions were observed. The tanks carried dummy filter papers which were irrigated by the aqueous phase during a 24-hour equilibration period, and the tank walls were swathed with filter-paper saturated with organic phase. The lids of the chromatography tanks were fitted into liquid seals to prevent the escape of solvent vapours.

The filter paper used was Whatman's No. 1, and the sheets were large enough to accommodate all the compounds at the same time, thus avoiding the difficulties of relating $R_M$ values measured on different samples of paper. All experiments were carried out at 20° ± 0·5°

After irrigation of the chromatograms the compounds were located by viewing the papers in ultra-violet light and by spraying them with bisdiazotised benzidine solution.

The flavones studied showed serious " tailing " on the chromatograms. These slender tails were characterised by the sharp leading and trailing edges, and appeared to be due to the low




solubility of the compounds investigated in one or both of the liquid phases. $R_M$ values were therefore measured at the leading edges of the spots.

The time-consuming calculation of $R_F$ and $R_M$ values was avoided by the use of a chromatogram-analysing device based on simple geometrical principles. This is illustrated in the Figure and consists of a rectangular metal frame fitted with a swinging arm, *A*, pivoted at one corner, *O*, and a cursor, *B*, with a hair-line parallel to the two short sides. The swinging arm is a fine steel wire tensioned by a spring-loaded drum *E* carried on the opposite long side and the transparent cursor is engraved to read $R_F$ and $R_M$.

The instrument is used by setting the side *OC* to the line of origin of the chromatogram and adjusting the movable drum so that the wire, *A*, passes through the point of intersection of the side *CD* and the solvent front. The cursor is moved so that the hair line cuts the spot under examination, and $R_F$ and $R_M$ values are read directly off the scales at the point of intersection of the wire and hair-line.

In order to avoid resetting the point *O* to the origin of each succeeding spot in cases where the solvent front had not remained parallel with the base line, the cursor was used to help in setting the wire correctly.

(b) *Preparation of Compounds for Chromatography.*—Unless otherwise indicated, the flavones required for this investigation were prepared by methods described in the literature.

M. p.s were determined with a Kofler block. Analyses were by Drs. Weiler and Strauss.

*4'-Hydroxy-7-methoxyflavone.* To a solution of 2-hydroxy-4-methoxyacetophenone (5 g.) and *p*-benzyloxybenzaldehyde (9 g.) in alcohol (100 ml.) aqueous sodium hydroxide (10 g. in water, 20 ml.) was added. Next day, the sodium salts were precipitated by the addition of an excess of ether, collected, and decomposed with aqueous acetic acid. Recrystallised from alcohol, *4-benzyloxystyryl 2-hydroxy-4-methoxyphenyl ketone* formed stout yellow needles (7 g.), m. p. 126—128°, giving a dark red colour with ferric chloride and alcohol (Found : C, 76·4; H, 5·6. $C_{23}H_{20}O_4$ requires C, 76·7; H, 5·6%). This compound (3·5 g.) was dehydrogenated by selenium dioxide (re-sublimed, 3·5 g.) in boiling amyl alcohol (50 ml.) during 12 hours. The

resulting mixture was filtered and the residue washed with hot amyl alcohol. The combined filtrate and washings were steam-distilled to remove the solvent, and the resulting residue recrystallised from alcohol. *4'-Benzyloxy-7-methoxyflavone* formed lustrous yellow plates (3 g.), m. p. 194° (Found: C, 77·1; H, 5·4. $C_{23}H_{18}O_4$ requires C, 77·1; H, 5·1%). Debenzylation of this material (3 g.) was effected by acetic acid (20 ml.) and concentrated hydrochloric acid (15 ml.) on the water-bath for 1 hour. After steam-distillation of the mixture and recrystallisation of the resulting residue from alcohol, *4'-hydroxy-7-methoxyflavone* was obtained in slender, pale yellow prisms (1·8 g.), m. p. 267°, showing a faint blue fluorescence in sulphuric acid (Found: C, 71·2; H, 4·7; OMe, 11·6. $C_{15}H_9O_3 \cdot OMe$ requires C, 71·6; H, 4·5; OMe, 11·6%). The *acetate*, prepared in pyridine, separated from aqueous alcohol in colourless needles, m. p. 153—155° (Found: C, 69·5; H, 4·6. $C_{18}H_{14}O_5$ requires C, 69·7; H, 4·5%).

*4'-Hydroxy-5-methoxyflavone.* Condensation of 2-hydroxy-6-methoxyacetophenone (7 g.) with *p*-benzyloxybenzaldehyde (10 g.) and aqueous sodium hydroxide (10 g. in water, 20 ml.) in alcohol (75 ml.) during 24 hours yielded *4-benzyloxystyryl 2-hydroxy-6-methoxyphenyl ketone* (8 g.). Recrystallised from alcohol, this formed yellow plates, m. p. 127—128°, giving a red-brown colour with ferric chloride and alcohol (Found: C, 76·4; H, 5·5. $C_{23}H_{20}O_4$ requires C, 76·7; H, 5·6%). Dehydrogenation of this compound (6 g.) was accomplished by heating it under reflux for 18 hours with selenium dioxide (6 g.) and amyl alcohol (60 ml.). The resulting mixture was worked up in the usual way and yielded *4'-benzyloxy-5-methoxyflavone* in pale yellow needles (4·5 g.), m. p. 182° (from alcohol) (Found: C, 77·1; H, 5·0. $C_{23}H_{18}O_4$ requires C, 77·1; H, 5·1%). This material (3 g.) was debenzylated by acetic acid (26 ml.) and concentrated hydrochloric acid (20 ml.) on the water-bath for 1 hour. The resulting mixture was steam-distilled to remove benzyl chloride and excess of acetic acid, and the residue recrystallised from alcohol. *4'-Hydroxy-5-methoxyflavone* formed pale yellow needles (1·5 g.), m. p. 270—271° (Found: C, 71·6; H, 4·5; OMe, 11·4. $C_{15}H_9O_3 \cdot OMe$ requires C, 71·6; H, 4·5; OMe, 11·6%). The *acetate* separated from alcohol in colourless rectangular plates, m. p. 184—186° (Found: C, 69·4; H, 4·4. $C_{18}H_{14}O_5$ requires C, 69·7; H, 4·5%). Demethylation of the above-named ether (1·1 g.) by a boiling mixture of acetic acid (5 ml.) and hydriodic acid (*d* 1·7) (16 ml.) yielded 5 : 4'-dihydroxyflavone (0·7 g.) (Syed and Wheeler, *J.*, 1936, 1714), in pale yellow needles, m. p. 240°. Methylation of the compound (0·5 g.) with methyl sulphate (0·25 g.) and potassium carbonate in boiling acetone (50 ml.) during 3 hours then yielded 5-hydroxy-4'-methoxyflavone in pale yellow needles, m. p. 157—158° (from alcohol).

*7-Hydroxy-3 : 5-dimethoxyflavone.* Interaction of 5 : 7-dihydroxy-3-methoxyflavone (3·4 g.) (Kalff and Robinson, *J.*, 1925, 181), benzyl bromide (2·25 g.), and excess of anhydrous potassium carbonate in boiling acetone (50 ml.) during 8 hours resulted in a product which was worked up in the usual way and yielded *7-benzyloxy-5-hydroxy-3-methoxyflavone.* Recrystallised from alcohol, this formed slender, pale yellow needles (3·9 g.), m. p. 125—127°, and gave a violet colour with ferric chloride and alcohol (Found: C, 73·9; H, 4·8. $C_{23}H_{18}O_5$ requires C, 73·8; H, 4·9%). The *acetate* was prepared in pyridine and separated from alcohol in rosettes of colourless needles, m. p. 133—134° (Found: C, 72·4; H, 5·2. $C_{25}H_{20}O_6$ requires C, 72·10; H, 4·8%). Methylation of the benzyl ether (2·1 g.) with an excess of methyl sulphate and anhydrous potassium carbonate in boiling acetone was complete in 24 hours and yielded *7-benzyloxy-3 : 5-dimethoxyflavone.* Recrystallised from aqueous alcohol and finally from benzene–light petroleum, it formed slender, cream-coloured needles (2 g.), m. p. 123—124·5°, giving no colour with ferric chloride and alcohol (Found: C, 74·5; H, 5·4. $C_{24}H_{20}O_5$ requires C, 74·2; H, 5·2%). Debenzylation of this compound (1 g.) by acetic acid (10 ml.) and concentrated hydrochloric acid (11 ml.) for 1 hour resulted in a product which on recrystallisation from acetic acid yielded *7-hydroxy-3 : 5-dimethoxyflavone* in rectangular, cream-coloured plates (0·5 g.), m. p. 287—290° [Found: C, 68·8; H, 4·8; OMe, 20·3. $C_{15}H_8O_3(OMe)_2$ requires C, 68·5; H, 4·7; OMe, 20·8%]. The *acetate*, purified by recrystallisation from aqueous alcohol and finally from benzene–light petroleum (b. p. 80—100°), formed colourless, felted needles, m. p. 122—122·5° (Found: C, 66·7; H, 5·0. $C_{19}H_{16}O_6$ requires C, 67·0; H, 4·8%).

5 : 7-*Dihydroxy-4'-methoxyflavone* (*acacetin*). The brown product resulting from the fusion of phloracetophenone (5 g.), anisic anhydride (50 g.), and sodium anisate (6 g.) and subsequent hydrolysis according to the directions of Robinson and Venkataraman (*J.*, 1925, 2344) was more easily purified by the following modified procedure. The mixture was repeatedly extracted with boiling ether, giving a solution which on cooling deposited 3-p-*methoxyphenoxy*-5 : 7-*dihydroxy-4'-methoxyflavone* in yellow needles (6 g.). Recrystallised from aqueous alcohol, this formed pale yellow prisms, m. p. 235°, and gave an intense purple colour with ferric chloride and

alcohol (Found : C, 69·1; H, 4·3. $C_{24}H_{18}O_7$ requires C, 68·9; H, 4·3%). Its *diacetate*, prepared in pyridine, separated from benzene–light petroleum (b. p. 60—80°) in colourless prisms, m. p. 205—206° (Found : C, 66·7; H, 4·5. $C_{28}H_{22}O_9$ requires C, 66·9; H, 4·4%). The 3-*p*-methoxybenzoylflavone was hydrolysed by boiling 5% aqueous sodium carbonate for 3 hours. On being saturated with carbon dioxide, the resulting pale brown solution deposited a cream-coloured solid which on recrystallisation from aqueous alcohol yielded acacetin in pale yellow, lustrous needles, m. p. 203°.

3 : 4′-*Dihydroxy*-7-*methoxyflavone* (cf. Anand, Iyer, and Venkataraman, *Proc. Indian Acad. Sci.*, 1949, **29**, 203). To a well stirred solution of 4-benzyloxy-2-hydroxy-4-methoxyphenyl styryl ketone (1 g.) in alcohol (50 ml.) and aqueous sodium hydroxide (5 g. in 15 ml. water), hydrogen peroxide was added until the violent reaction ceased and a clear, pale yellow solution solution was obtained. The solid precipitated on acidification was recrystallised from acetic acid. 4′-*Benzyloxy*-3-*hydroxy*-7-*methoxyflavone* formed pale yellow, irregular prisms (0·8 g.), m. p. 175—176°, giving an apple-green fluorescence with sulphuric acid and a dark violet colour with ferric chloride and alcohol (Found : C, 73·5; H, 4·9. $C_{23}H_{18}O_5$ requires C, 73·8; H, 4·9%). Its *acetate* separated from alcohol in colourless, lustrous plates, m. p. 162° (Found : C, 71·9; H, 4·9. $C_{25}H_{20}O_6$ requires C, 72·1; H, 4·8%). This compound (1·1 g.) was debenzylated by acetic acid (20 ml.) and concentrated hydrochloric acid (15 ml.) on the steam-bath for 1 hour. 3 : 4′-Dihydroxy-7-methoxyflavone separated from alcohol in yellow needles (0·65 g.), m. p. 270° (decomp.).

3 : 7-*Dihydroxy*-4′-*methoxyflavone* (cf. Nadkarni and Wheeler, *J.*, 1938, 1320). 4-Benzyloxy-2-hydroxyphenyl 4-methoxystyryl ketone (Mahal, Rai, and Venkataraman, *J.*, 1935, 866) (1 g.) was oxidised as in the previous experiment. 7-*Benzyloxy*-3-*hydroxy*-4′-*methoxyflavone* crystallised from alcohol in slender, pale yellow needles (0·8 g.), m. p. 195°, giving a green-brown colour with alcohol and ferric chloride and a green fluorescence with concentrated sulphuric acid (Found : C, 73·4; H, 5·0. $C_{23}H_{18}O_5$ requires C, 73·8; H, 4·9%). The *acetate* crystallised from alcohol in colourless, rectangular plates, m. p. 171—173° (Found : C, 71·8; H, 4·8. $C_{25}H_{20}O_6$ requires C, 72·1; H, 4·8%). On debenzylation with acetic acid (20 ml.) and concentrated hydrochloric acid (15 ml.) on the water-bath for 1 hour, this compound (3 g.) yielded 3 : 7-dihydroxy-4′-methoxyflavone in cream-coloured needles (1·9 g.), m. p. 286—290°, from methyl alcohol.

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