Rearrangement in the Demethylation of 2'-Methoxyflavones. Part II. Further Experiments and the Determination of the Composition of Lotoflavin.*

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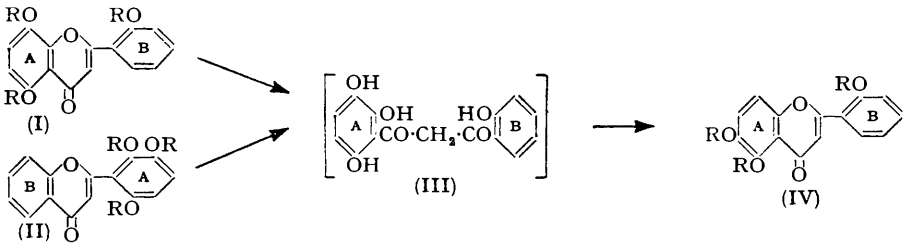
Both 5 : 8 : 2'-trimethoxyflavone (I; R = Me) and the 2' : 3' : 6'-isomer (II; R = Me) yield 5 : 6 : 2'-trihydroxyflavone (IV; R = H) on demethylation by hydriodic acid. This result confirms previous views (see Gallagher *et al.*, *J.*, 1953, 3770) that the diketone produced by decyclisation, *e.g.*, (III), is intermediate in the rearrangement of both 5 : 8-dihydroxy- and 2'-hydroxyflavones. The presence of hydrogen ion is necessary for the 5 : 8 ⟶ 5 : 6 rearrangement of flavones.

Experiments related to the structure of lotoflavin, which Dunstan and Henry (*Phil. Trans.*, 1901, **194**, *B*, 515) thought to be 5 : 7 : 2' : 4'-tetrahydroxyflavone (V; R = H) have shown that 7 : 2' : 4' : 6'-tetramethoxyflavone (VI; R = Me) rearranges to form (V; R = H) on treatment with hydriodic acid. Lotoflavin as represented by the samples available is now found to be quercetin containing some kæmpferol.

Gallagher, Hughes, O'Donnell, Philbin, and Wheeler (Part I *) found that certain 2'-methoxyflavones are rearranged during demethylation by hydriodic acid under sufficiently drastic conditions to give the related 2'-hydroxyflavones in which the 2-phenyl group and the fused aromatic ring of the original flavone are interchanged (compare I and IV with II). Their suggestion that an intermediate disalicyloylmethane of the type (III) occurs in the rearrangement has been confirmed; both 5 : 8 : 2'-trimethoxyflavone (I; R = Me) and the 2' : 3' : 6'-isomer (II; R = Me) give 5 : 6 : 2'-trihydroxyflavone (IV; R = H) on treatment with hydriodic acid. Cyclisation of the diketone (III) can occur in three ways to form

* Part I, *J.*, 1953, 3770.

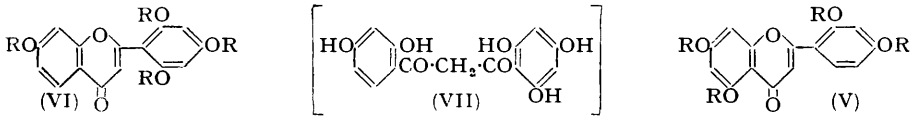
(I; R = H), (II; R = H) or (IV; R = H). As shown by Gallagher *et al.* (*loc. cit.*) the more stable of two related 2'-hydroxyflavones, *e.g.*, (II and IV; R = H), is that in which the 2-phenyl side chain contains the smaller number of hydroxyl groups. The greater stability of a 5 : 6-dihydroxyflavone, *e.g.*, (IV; R = H), than of a 5 : 8-dihydroxyflavone, *e.g.*, (I; R = H), is well known.



Demethylation Experiments.—Gallagher *et al.* (*loc. cit.*) also suggested that the 5 : 8 ⟶ 5 : 6 (I ⟶ IV) and the 2'-rearrangement (II ⟶ IV) involve the formation of a hydrogen bond between the hydroxyl-oxygen atom at positions 8 or 2', and the oxide-oxygen atom at position 1. Support is afforded to this view as regards the (I ⟶ IV) change by the results of some experiments (Table 1) on the demethylation of 5 : 8-dimethoxyflavone by various reagents. The presence of hydrogen ion is found to be necessary to produce rearrangement of 5 : 8- to 5 : 6-dihydroxyflavone. Experiments on the yields obtained in the demethylation of 7-methoxyflavone by various reagents are also included.

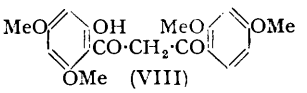
Composition of Lotoflavin

5 : 7 : 2' : 4'- *and* 7 : 2' : 4' : 6'-*Tetrahydroxyflavone.*—Dunstan and Henry (*Phil. Trans.*, 1901, **194**, *B*, 515; Henry, *J. Soc. Chem. Ind.*, 1938, **57**, 248) claimed that a flavone which they had isolated from *Lotus arabicus*, and which they called " Lotoflavin," was 5 : 7 : 2' : 4'-tetrahydroxyflavone (V; R = H). Synthesis of this tetrahydroxyflavone by Cullinane, Algar, and Ryan (*Sci. Proc. Roy. Dublin Soc.*, 1928, **19**, 77; cf. Gupta and Seshadri, *Proc. Indian Acad. Sci.*, 1953, **37**, *A*, 611) and by Robinson and Venkataraman (*J.*, 1929, 61) showed that it has not the properties of lotoflavin. Since both methods of synthesis involve demethylation by hydriodic acid in the final stage, it seemed possible that the product (V; R = H) might have undergone the 2'-type rearrangement to the related 7 : 2' : 4' : 6'-tetrahydroxyflavone (VI; R = H) with intermediate formation of the diketone (VII). However, this view proved untenable for, in line with the results on stability given in Part I (*e.g.*, system IV more stable than II), it has now been confirmed that it is (VI; R = H) which rearranges to form (V; R = H) (see also Gupta and Seshadri, *loc. cit.*). Further, the pentamethyl ether (IX) cyclises with hydriodic acid to (V; R = H), and does not give (VI; R = H) as Cullinane *et al.* (*loc. cit.*) supposed.

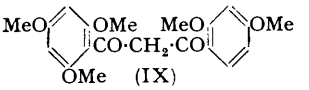


The synthesis of the ether (V; R = Me) required the cyclisation of 2 : 4-dimethoxybenzoyl-(2-hydroxy-4 : 6-dimethoxybenzoyl)methane (VIII). This diketone was obtained with m. p. 120° both by the Claisen ester-ketone condensation, and by an Allan–Robinson fusion of 2-hydroxy-4 : 6-dimethoxyacetophenone and 2 : 4-dimethoxybenzoic anhydride in presence of triethylamine (cf. Kuhn and Löw, *Ber.*, 1944, **77**, 197) followed by alkaline hydrolysis of the product. Previous workers in attempting the synthesis of the diketone (VIII) by the Claisen method obtained in fact the pentamethyl ether (IX), m. p. 151°, owing to the presence of methyl sulphate in the methyl 2 : 4-dimethoxybenzoate used in the synthesis.

The exclusion of the possibility that 5 : 7 : 2' : 4'-tetrahydroxyflavone had rearranged to 7 : 2' : 4' : 6'-tetrahydroxyflavone on treatment with hydriodic acid led to a re-examination of the lotoflavin problem. The structure which Dunstan and Henry (*loc. cit.*) assigned to lotoflavin depended mainly on the result of an alkaline fusion which, these workers



(VIII)



(IX)

thought, gave β-resorcylic acid and phloroglucinol. The evidence for the occurrence of these products was weak. The identification of the acid rested chiefly on the observed m. p. (207°). The m. p. of some of the dihydroxybenzoic acids is indefinite; Beilstein records 194—217° for β-resorcylic acid and 194—205° for protocatechuic acid. Even if Dunstan and Henry's identification of the degradation products were correct, the fact remains that 5 : 7 : 2' : 4'- and 7 : 2' : 4' : 6'-tetrahydroxyflavone would give the same products on alkaline fusion. However, the properties of 7 : 2' : 4' : 6'-tetrahydroxyflavone and of its tetra-acetate (see Experimental section) differ from those described for lotoflavin.

Scrutiny of the literature did not reveal a tetrahydroxyflavone with properties like those recorded for lotoflavin. Derivatives of 3 : 5 : 7 : 3'-tetrahydroxyflavone (Shaw and Simpson, *J.*, 1952, 5031) which gave a tetra-acetate with m. p. near that recorded for lotoflavin tetra-acetate, and of the 5 : 7 : 3' : 5'-isomer, which is new, were synthesised, with similar negative results.

Examination of Lotoflavin Specimens.—Dr. Henry (personal communication) stated that no specimen of lotoflavin remains with him. However, a sample (35 mg.), labelled " lotoflavin," was kindly made available by Professor J. Algar. Dr. N. M. Cullinane (personal communication) states that it was supplied by Dr. Harold Brown of the Imperial Institute, where Dunstan and Henry worked. It was found to be identical with a second authentic specimen (*ca.* 5 mg.) which was obtained later through the courtesy of Sir Robert Robinson. Comparative chromatographic runs were made on paper between a number of flavones including 5 : 7 : 2' : 4'-tetrahydroxyflavone, 5 : 7 : 4'-trihydroxyflavonol (kæmpferol), 5 : 7 : 2' : 4'-tetrahydroxyflavonol (morin), 5 : 7 : 3' : 4'-tetrahydroxyflavonol (quercetin), and the lotoflavin specimen.

The specimen gave two spots in all solvent systems tried, the major one of which had R_F values and colour reactions identical with those of quercetin; the second component, present only in trace amounts, had R_F values and colour reactions indistinguishable from those of kæmpferol (Table 2). The major component was strongly retarded on borate-buffered filter paper in butan-1-ol–water, showing the presence of an *o*-dihydroxy-grouping (Wachtmeister, *Acta Chem. Scand.*, 1951, **5**, 976; Swain, *Biochem. J.*, 1952, **53**, 200). The presence of this grouping was confirmed on identification by paper chromatography of protocatechuic acid together with phloroglucinol as the products of alkaline degradation of lotoflavin on a microscale (cf. Bate-Smith and Swain, *J.*, 1953, 2187). The same degradation products were given by quercetin. The ultraviolet spectra of lotoflavin in ethanol, 0·002M-sodium ethoxide (Mansfield, Swain, and Nordström, *Nature*, 1953, **172**, 23), and 0·1% ethanolic aluminium chloride (Swain, *Chem. and Ind.*, 1954, 1480) were identical with those of quercetin (Table 3), and the infrared spectra of the two compounds in the double bond (5·5—7 μ) and " finger print " regions (8—12 μ) were also indistinguishable. Further, the m. p. of acetylated lotoflavin was not depressed by the addition of quercetin penta-acetate and the ultraviolet spectra of these two derivatives in ethanol and 0·1% ethanolic aluminium chloride had identical absorption maxima. Robinson and Venkataraman (*loc. cit.*) commented on the similarity in dyeing properties of lotoflavin and quercetin.

It is clear, therefore, that lotoflavin, as represented by the two samples available, is quercetin with, probably, 1—2% (see the Experimental section for estimation of percentage) of kæmpferol. It is interesting that these two flavonols occur together in tea leaves (Oshima and Goma, *J. Agric. Chem. Soc. Japan*, 1933, **9**, 948; Oshima and Ka, *ibid.*, 1936, **12**, 975), and in strawberries (*Fragaria chiloensis*) (Williams and Wender, *J. Amer. Chem. Soc.*, 1952, **74**, 5919).

Experimental

Ethanol was employed for crystallisation if no solvent is mentioned.

5 : 8 : 2′-, 2′ : 3′ : 6′-, and 5 : 6 : 2′-Trimethoxyflavone : Preparation and Demethylation by Aluminium Chloride.—(1) *5 : 8 : 2′-Trihydroxyflavone* (I; R = H). 2-o-*Anisoyloxy*-3 : 6-*dimethoxyacetophenone*, which was prepared from 2-hydroxy-3 : 6-dimethoxyacetophenone (Baker, Brown, and Scott, *J.*, 1939, 1924) by the pyridine–acid chloride method (see Nowlan, Slavin, and Wheeler, *J.*, 1950, 342), crystallised in plates, m. p. 90° (Found : C, 65·2; H, 5·5; OMe, 28·0. $C_{18}H_{18}O_6$ requires C, 65·5; H, 5·5; 3OMe, 28·2%). This ester was shaken in pyridine with powdered potassium hydroxide (1·5 mols.) for 3 hr. and the mixture was poured on ice and hydrochloric acid. The crude product, which separated as an oil, crystallised from methanol and gave o-*anisoyl*-3 : 6-*dimethoxysalicyloylmethane* in yellow plates (70% yield), m. p. 84—86° (Found : C, 65·5; H, 5·5; OMe, 27·9%. Required : as for the ester), and gave a greenish-brown colour with ethanolic ferric chloride. The hydroxy-diketone was boiled for 3 min. with glacial acetic acid containing a few drops of hydrochloric acid. Controlled addition of water precipitated *5 : 8 : 2′-trimethoxyflavone* (I; R = Me) which crystallised in plates, m. p. 200—201° (Found : C, 68·7; H, 4·9; OMe, 30·8. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1; 3OMe, 29·8%). This flavone was refluxed ($CaCl_2$ guard) in benzene for 1 hr. with anhydrous aluminium chloride (5 pts.), and the insoluble residue was treated with ice and 10% hydrochloric acid. The mixture was heated on a steam-bath for 15 min. and cooled. *5 : 8 : 2′-Trihydroxyflavone* crystallised in pale yellow needles, m. p. *ca.* 310° (Found : C, 66·3; H, 3·9. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%), and gave an olive-green colour with ethanolic ferric chloride [*triacetate* (acetic anhydride–perchloric acid method), needles, m. p. 189—190° (Found : C, 63·9; H, 4·2. $C_{21}H_{16}O_8$ requires C, 63·6; H, 4·0%)]. To confirm the structure of the trihydroxyflavone it was refluxed in anhydrous acetone for 6 hr. with methyl sulphate (10% excess) and potassium carbonate. The mixture was filtered. The product which separated on evaporation of the acetone did not after crystallisation depress the m. p. of an authentic sample of 5 : 8 : 2′-trimethoxyflavone.

Some hydroxyflavones give on methylation in this way coloured sulphates which, however, are readily decomposed by treatment with boiling water, or by crystallisation from aqueous ethanol.

(2) *2′ : 3′ : 6′-Trihydroxyflavone* (II; R = H). A mixture of methyl *O*-benzylsalicylate (3·5 g.) and 2 : 3 : 6-trimethoxyacetophenone (2 g.) was refluxed with sodium powder (0·4 g.) in xylene (20 ml.) for 45 min. The product when cold was treated with damp ether and with 2% aqueous sodium hydroxide. O-*Benzylsalicyloyl*-2 : 3 : 6-*trimethoxybenzoylmethane*, which separated from the alkaline solution on acidification, gave a red ethanolic ferric colour and crystallised from methanol in prisms (1·5 g.), m. p. 100—101° (Found : C, 71·1; H, 6·0. $C_{25}H_{24}O_6$ requires C, 71·4; H, 5·7%). The diketone (1 g.) was heated for 1 hr. at 100° with a mixture of hydrochloric acid (5 ml.) and acetic acid (10 ml.). *2′ : 3′ : 6′-Trimethoxyflavone* (II; R = Me) which separated when the product was poured into water crystallised from methanol in prisms (0·25 g.), m. p. 158—159° (Found : C, 69·2; H, 5·3; OMe, 29·9. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1; 3OMe, 29·8%). Demethylation with aluminium chloride in benzene [see (1)] yielded 2′ : 3′ : 6′-trihydroxyflavone, which formed yellow needles, m. p. >340°, and gave a green ethanolic ferric colour (Found : C, 67·1; H, 3·8. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%). Remethylation of the trihydroxyflavone re-formed the trimethoxyflavone, m. p. and mixed m. p. 158°.

(3) *5 : 6 : 2′-Trihydroxyflavone* (IV; R = H). 2-o-*Anisoyloxy*-5 : 6-*dimethoxyacetophenone* was prepared from 2-hydroxy-5 : 6-dimethoxyacetophenone (Baker, *J.*, 1939, 960) and was purified by extraction of its solution in benzene with 1% aqueous sodium hydroxide. It crystallised from ligroin in plates, m. p. 76° (Found : C, 65·2; H, 5·5; OMe, 27·5. $C_{18}H_{18}O_6$ requires C, 65 5; H, 5·5; 3OMe, 28·2%). The corresponding diketone was obtained as described under (1), but the mixture of ester, pyridine, and potassium hydroxide was heated for 15 min. on a steam-bath, and mechanically shaken for 24 hr. The product obtained on acidification was extracted from ether by 2% aqueous sodium hydroxide and precipitated by carbon dioxide. o-*Anisoyl*-5 : 6-*dimethoxysalicyloylmethane* separated from methanol in yellow needles, m. p. 59—61° (Found : C, 65·7; H, 5·6; OMe, 28·0. Required : as for the ester). For cyclisation this diketone (1·5 g.) was heated at 100° with sodium acetate (2 g.) and acetic acid (20 ml.) for 4 hr. The solid which was precipitated on dilution with water was washed with 3% aqueous sodium hydroxide and with water, and was crystallised from methanol (charcoal). *5 : 6 : 2′-Trimethoxyflavone* (IV; R = Me) separated in plates (1·1 g.), m. p. 124—125° (Found : C, 69·3; H, 5·2; OMe, 29·5. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1; 3OMe, 29·8%), and on demethylation by aluminium chloride yielded *5 : 6 : 2′-trihydroxyflavone* which crystallised in pale yellow needles,

m. p. 274—278° (decomp.) (Found: C, 66·6; H, 4·0. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%), and gave a green colour with ethanolic ferric chloride. The structure was confirmed by remethylation [*triacetate*, needles, m. p. 144—146° (Found: C, 63·8; H, 4·0. $C_{21}H_{16}O_8$ requires C, 63·6; H, 4·0%)].

Demethylation of Trimethoxyflavones by Hydriodic Acid.—Formation of 5 : 6 : 2'-trihydroxyflavone from 5 : 8 : 2'-trimethoxyflavone. 5 : 8 : 2'-Trimethoxyflavone (0·5 g.) was heated for 2 hr. with hydriodic acid (*d* 1·7; 12 ml.) and phenol (6 ml.) in a sealed tube at 160—170° and the product was poured into saturated aqueous sodium hydrogen sulphite. The precipitate on crystallisation gave 5 : 6 : 2'-trihydroxyflavone in pale yellow needles, m. p. and mixed m. p. *ca.* 279° (decomp.). This result was confirmed by acetylation and remethylation to yield 5 : 6 : 2'-triacetoxy- and 5 : 6 : 2'-trimethoxy-flavone respectively (mixed m. p. confirmation).

Formation of 5 : 6 : 2'-trihydroxyflavone from 2' : 3' : 6'-trimethoxyflavone. 2' : 3' : 6'-Trimethoxyflavone was refluxed for 1½ hr. with a mixture of hydriodic acid and acetic anhydride. The product when purified in the usual way did not depress the m. p. of authentic 5 : 6 : 2'-trihydroxyflavone [see (3)]; its identity was confirmed by acetylation and remethylation. 5 : 6 : 2'-Trimethoxyflavone was demethylated without rearrangement when treated with hydriodic acid and phenol in a sealed tube at 160—170° for 1½ hr. The product was identified as 5 : 6 : 2'-trihydroxyflavone by acetylation and remethylation.

Demethylation of 7-methoxy- and 5 : 8-dimethoxy-flavone. Table 1 summarises results of experiments on the demethylation of these flavones. Standard methods of purifying the products were employed.

TABLE 1.

Demethylating agent	Time of reaction	Temp. of reaction	Yield of (1), (2), or (3) in g. from 1 g. [(1) 7-Hydroxy-, (2) 5 : 8-dihydroxy-, (3) 5 : 6-dihydroxy-flavone]
HI (*d* 1·7) in Ac_2O	1 hr.	Reflux	0·7 (1)
70% H_2SO_4	1½ hr.	,,	0·7 (1); 0·5 (3)
C_5H_5N,HCl [a]	6 hr.	200°	0·6 (1); 0·6 (2)
C_5H_5N,H_2SO_4	6 hr.	200°	0·5 (1)
$AlCl_3$ in C_6H_6	¾ hr.	Reflux	0·8 (1); 0·7 (2)
$AlBr_3$ in C_6H_6	¾ hr.	,,	0·7 (1); 0·5 (2)
$AlCl_3$–NaCl [b]	2 min.	180°	0·8 (1); 0·8 (2)

[a] No reaction at 150°. In one instance when damp pyridine hydrochloride was employed, rearrangement of 5 : 8-dimethoxyflavone to give 5 : 6-dihydroxyflavone occurred. [b] See Bruce, Sorrie, and Thomson, *J.*, 1953, 2403.

Composition of lotoflavin

(4) 5 : 7 : 2' : 4'-*Tetrahydroxyflavone* (V; R = H).—2 : 4-*Dimethoxybenzoyl*-(2-*hydroxy*-4 : 6-*dimethoxybenzoyl*)*methane* which was required for the preparation of this flavone was prepared in three ways:

(*a*) 2-Hydroxy-4 : 6-dimethoxyacetophenone was condensed by the Claisen method with methyl 2 : 4-dimethoxybenzoate as described at (2) above. The alkaline extract of the diketone was saturated with carbon dioxide. The yellow solid thus obtained formed, after several recrystallisations, yellow needles, m. p. 120—121°, not altered by purification through the cuproderivative (Found: C, 63·1; H, 5·6; OMe, 34·8. $C_{19}H_{20}O_7$ requires C, 63·3; H, 5·6; 4OMe, 34·4%). The ethanolic ferric reaction was olive-green.

(*b*) 2-Hydroxy-4 : 6-dimethoxyacetophenone was heated at 150° with methyl 2 : 4-dimethoxybenzoate in presence of sodium sand as described by Cullinane, Algar, and Ryan (*loc. cit.*; cf. Gupta and Seshadri, *loc. cit.*). The identity of the product (VIII), m. p. 120°, was confirmed by a mixed m. p. determination with the compound obtained as in (*a*) above.

(*c*) A mixture of 2-hydroxy-4 : 6-dimethoxyacetophenone (3 g.), 2 : 4-dimethoxybenzoic anhydride (30 g.), and freshly distilled triethylamine (12 ml.) was heated in nitrogen for 4 hr. at 170°. The product was refluxed with aqueous potassium hydroxide (23 g. in 35 ml. of H_2O) until a clear solution was produced (7 min.). The yellow solid obtained on treatment of the solution with carbon dioxide gave on repeated crystallisation the required diketone in yellow needles (1·0 g.), m. p. 120—121°, not depressed by addition of the diketone prepared as in (*a*) above.

The effect of addition of methyl sulphate in the Claisen condensation [see (*c*) above] was tested as follows: 2-Hydroxy-4 : 6-dimethoxyacetophenone (1·7 g.), methyl 2 : 4-dimethoxybenzoate (2·5 g.), sodium sand (0·3 g.), and methyl sulphate (0·3 ml.) were heated at 150° for

2 hr. The product was treated with damp ether and with water. 2 : 4 : 6-Trimethoxyacetophenone (0·5 g.) was obtained from the ethereal layer. The aqueous portion yielded 2 : 4-dimethoxybenzoyl-(2 : 4 : 6-trimethoxybenzoyl)methane (0·15 g.), m. p. 153° (Found : C, 64·1; H, 5·9; OMe, 41·0. Calc. for $C_{20}H_{22}O_7$: C, 64·2; H, 5·9; 5OMe, 41·4%). This was the compound (m. p. 151°) (IX) obtained by Cullinane *et al.* and by Gupta and Seshadri (*locc. cit.*) who considered it to be (VIII) which, however, has m. p. 120°. We are indebted to Professor Seshadri for a sample of his material (IX), m. p. 151°, which enabled us to confirm its constitution.

5 : 7 : 2′ : 4′-Tetramethoxyflavone (V; R = Me) was obtained by cyclisation of 2 : 4-dimethoxybenzoyl-(2-hydroxy-4 : 6-dimethoxybenzoyl)methane as described at (1). It formed needles, m. p. 179—180° (Found : C, 66·6; H, 5·4; OMe, 36·3. Calc. for $C_{19}H_{18}O_6$: C, 66·7; H, 5·3; 4OMe, 36·2%). Cullinane *et al.* (*loc. cit.*) give m. p. 186° for tetramethoxyflavone. The ethanolic ferric reaction was negative. Demethylation of the tetramethoxyflavone by aluminium chloride in benzene yielded 5 : 7 : 2′ : 4′-tetrahydroxyflavone which after purification through the tetra-acetate had m. p. 334—336° (Found : C, 62·9; H, 3·8. Calc. for $C_{15}H_{10}O_6$: C, 62·9; H, 3·5%). The ethanolic ferric reaction was reddish-brown [tetra-acetate, m. p. 159° (Found : C, 60·5; H, 4·1. Calc. for $C_{23}H_{18}O_{10}$: C, 60·8 H, 4·0%)]. Robinson and Venkataraman (*J.*, 1929, 67) give m. p. 332—335° for the flavone and m. p. 159° for the tetra-acetate.

(5) 7 : 2′ : 4′ : 6′-*Tetrahydroxyflavone* (VI; R = H).—*Methyl 2-benzyloxy-4-methoxybenzoate*, which was prepared from the corresponding 2-hydroxy-compound by using potassium carbonate, acetone, and benzyl chloride, separated from methanol in needles, m. p. 64° (Found : C, 71·1; H, 6·1. $C_{16}H_{16}O_4$ requires C, 70·6; H, 5·9%), and gave a purple ethanolic ferric colour. A mixture of the above ester (3 g.) and 2 : 4 : 6-trimethoxyacetophenone (2 g.) in dry ether was added to sodium sand (0·6 g.). The solvent was evaporated and the residue heated for 2 hr. on a steam-bath ($CaCl_2$ guard). The product was treated with damp ether and purified as described at (2). *O-Benzyl-4-methoxysalicyloyl-(2 : 4 : 6-trimethoxybenzoyl)methane* separated from methanol in needles, m. p. 141° (Found : C, 69·2; H, 6·1. $C_{26}H_{26}O_7$ requires C, 69·3; 5·8%). The ethanolic ferric colour was red. On debenzylation and cyclisation [see (2)], the diketone yielded 7 : 2′ : 4′ : 6′-*tetramethoxyflavone* (VI; R = Me) which crystallised in plates, m. p. 197° (Found : C, 66·5; H, 5·4; OMe, 36·0. $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3; 4OMe, 36·2%). This flavone (0·2 g.) was heated at 210—220° for 5 min. with aluminium chloride (3 g.) and sodium chloride (1·5 g.) (Bruce, Sorrie, and Thomson, *J.*, 1953, 2403). The product, when worked up as in normal demethylation by aluminium chloride [see (1) above], gave 7 : 2′ : 4′ : 6′-*tetrahydroxyflavone*, which separated from ethyl acetate or aqueous acetic acid in pale yellow, hygroscopic, aggregates (0·1 g.), m. p. 303—304° (decomp.). The flavone exhibited a dark blue fluorescence in sulphuric acid (Found : C, 62·3; H, 3·8. $C_{15}H_{10}O_6$ requires C, 62·9; H, 3·5%) [*tetra-acetate*, m. p. 136—137° (from ligroin) (Found : C, 60·6; H, 4·0. $C_{23}H_{18}O_{10}$ requires C, 60·8; H, 4·0%)]. Remethylation (potassium carbonate–methyl sulphate–acetone) gave the tetramethoxyflavone (mixed m. p. confirmation).

Formation of 5 : 7 : 2′ : 4′-*Tetrahydroxyflavone from* 7 : 2′ : 4′ : 6′-*Tetramethoxyflavone.*—7 : 2′ : 4′ : 6′-Tetramethoxyflavone was refluxed for 2 hr. at 160° with hydriodic acid and phenol in an atmosphere of carbon dioxide. The product was purified in the usual way and acetylated. The tetra-acetate thus obtained had m. p. 159°. This m. p. was not depressed by the addition of an authentic sample of 5 : 7 : 2′ : 4′-tetra-acetoxyflavone.

Formation of 5 : 7 : 2′ : 4′-*Tetrahydroxyflavone* (V; R = H) *from* 2 : 4-*Dimethoxybenzoyl-(2 : 4 : 6-trimethoxybenzoyl)methane* (IX).—2 : 4-Dimethoxybenzoyl-(2 : 4 : 6-trimethoxybenzoyl)-methane (Cullinane *et al.*, *loc. cit.*) was refluxed with hydriodic acid (*d* 1·94) for 4 hr. The crude flavone which separated when the mixture was poured into aqueous sodium hydrogen sulphite was crystallised and acetylated (acetic anhydride–perchloric acid). The product when crystallised from ethanol and then from ethyl acetate had m. p. 159°, not depressed by addition of authentic 5 : 7 : 2′ : 4′-tetra-acetoxyflavone. Cullinane *et al.* (*loc. cit.*) considered that the product (m. p. 171°) which they obtained by the mild action of hydriodic acid on (IX) was 7 : 2′ : 4′ : 6′-tetramethoxyflavone (m. p. 197°; see above). As it gave a green ethanolic ferric colour it was most probably 5 : 7 : 2′ : 4′-tetramethoxyflavone (m. p. 180°) with the m. p. depressed by some demethylation in the 5-position. 5 : 7 : 2′ : 4′-Tetramethoxyflavone was demethylated without rearrangement when treated for 2 hr. with hydriodic and phenol at 170° in a sealed tube. This result was confirmed by acetylation and remethylation (cf. Gupta and Seshadri, *loc. cit.*).

(6) 5-*Hydroxy*-3 : 7 : 3′-*Trimethoxyflavone.*—5 : 7-Dihydroxy-3 : 3′-dimethoxyflavone, m. p. 240°, was prepared by the Allan–Robinson fusion method as described by Shaw and Simpson (*J.*, 1952, 5031) (Found : C, 65·0; H, 4·6; OMe, 19·0. Calc. for $C_{17}H_{14}O_6$: C, 65·0; H, 4·5; 2OMe, 19·0%) [diacetate, m. p. 143°]. Shaw and Simpson (*loc. cit.*) give m. p. 245—246° for the flavone

and m. p. 143° for the diacetate. It was found advantageous to use triethylamine (Kuhn and Löw, *Ber.*, 1944, **77**, 196) in place of sodium *m*-methoxybenzoate in the fusion. The *m*-methoxybenzoic anhydride required was prepared (60% yield) by the process described by Robinson and Venkataraman (*J.*, 1929, 63) for the preparation of 2 : 4-dimethoxybenzoic anhydride. This flavone on partial methylation by methyl sulphate–potassium carbonate–acetone (Sastri and Seshadri, *Proc. Indian Acad. Sci.*, 1946, **23**, *A*, 262) yielded *5-hydroxy-3 : 7 : 3'-trimethoxyflavone* in yellow needles, m. p. 99—101° (Found : C, 65·9; H, 4·9; OMe, 28·0. $C_{18}H_{16}O_6$ requires C, 65·9; H, 4·9; 3OMe, 28·4%). The ethanolic ferric reaction was green. *5-Acetoxy-3 : 7 : 3'-trimethoxyflavone* had m. p. 164—165° (Found : C, 64·7; H, 4·8. $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9%). Dunstan and Henry (*Phil. Trans.*, 1901, **194**, *B*, 515) give m. p. 125° and 175° for the dimorphic trimethyl ether of lotoflavin and m. p. 147° for the monoacetyl derivative of the ether. 3 : 5 : 7 : 3'-Tetra-acetoxyflavone had m. p. 180—182°. Shaw and Simpson (*loc. cit.*) give m. p. 177—180° for this compound and Dunstan and Henry (*loc. cit.*) give m. p. 176—178° for lotoflavin tetra-acetate.

(7) *5 : 7 : 3' : 5'-Tetrahydroxyflavone.* — *2-(3 : 5-Dimethoxybenzoyloxy)-4 : 6-dimethoxyacetophenone* (prep. by the pyridine–acid chloride method) separated in plates, m. p. 136—137° (Found : C, 63·4; H, 5·6; OMe, 34·3. $C_{19}H_{20}O_7$ requires C, 63·3; H, 5·6; 4OMe, 34·4%). This ester was transformed by treatment with potassium hydroxide in pyridine at 60° for 4 hr. The resulting diketone, *3 : 5-dimethoxybenzoyl-(2-hydroxy-4 : 6-dimethoxybenzoyl)methane*, formed yellow needles, m. p. 139—140° (Found : C, 62·9; H, 5·5; OMe, 34·8. Required : as for the ester). The ethanolic ferric reaction was green. *5 : 7 : 3' : 5'-Tetramethoxyflavone* crystallised in needles, m. p. 201—202° (Found : C, 66·3; H, 5·4; OMe, 36·6. $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3; 4OMe, 36·2%). *5 : 7 : 3' : 5'-Tetrahydroxyflavone* was obtained by demethylation of the tetramethoxyflavone for 2 hr. at 170° by hydriodic acid and phenol. This tetrahydroxyflavone separated from aqueous acetic acid in pale yellow needles, darkening *ca.* 280°, m. p. above 330° (Found : C, 55·9, 56·1; H, 4·4, 4·4. $C_{15}H_{10}O_6,2H_2O$ requires C, 55·9; H, 4·3%). Attempts to dry this flavone at temperatures between 100° and 160° resulted in a product with indefinite amounts of water. *5 : 7 : 3' : 5'-Tetra-acetoxyflavone*, prepared by the sodium acetate–acetic anhydride method, crystallised in plates, m. p. 198—200° (Found : C, 60·5; H, 4·0. $C_{23}H_{18}O_{10}$ requires C, 60·8; H, 4·0%).

Chromatographic and spectrographic comparison of lotoflavin and other flavones. Samples of the specimen of lotoflavin made available by Professor Algar were run on paper (cf. Nordström and Swain, *J.*, 1953, 2764) against 5 : 7 : 4'-trihydroxyflavone (apigenin), 5 : 7 : 2' : 4'-tetrahydroxyflavone, 5 : 7 : 3' : 4'-tetrahydroxyflavone (luteolin), 5 : 7 : 4'-trihydroxyflavonol (kæmpferol), 5 : 7 : 2' : 4'-tetrahydroxyflavonol (morin), 5 : 7 : 3' : 4'-tetrahydroxyflavonol (quercetin), 5 : 3' : 4'-trihydroxy-7-methoxyflavonol (rhamnetin), 5 : 7 : 4'-trihydroxy-3'-methoxyflavonol (*iso*rhamnetin), and 5 : 7 : 3' : 4' : 5'-tetrahydroxyflavonol (myricetin). The solvent systems were : (1) acetic acid–water–concentrated hydrochloric acid (30 : 10 : 3) (Bate-Smith, *Biochem. J.*, 1954, **58**, 122); (2) butanol–acetic acid–water (6 : 1 : 2) (Nordström and Swain, *loc. cit.*); (3) butanol–water.

Rhamnetin and *iso*rhamnetin were not run with solvent system (2). The chromatograms were inspected in ultraviolet light before and after treatment with ammonia vapour. Lotoflavin gave two spots; the major component ran with quercetin and the minor with kæmpferol.

Lotoflavin, kæmpferol, quercetin, rhamnetin, and *iso*rhamnetin were also run, in solvent (3), on plain and borate-buffered papers. Quercetin and the main constituent from lotoflavin did not travel. The minor component ran normally with kæmpferol; rhamnetin moved slightly, and *iso*rhamnetin normally.

The ultraviolet absorption maxima of lotoflavin and quercetin are recorded in Table 2.

TABLE 2. $\lambda_{max.}$ *of lotoflavin and quercetin and their acetates in* EtOH, 0·002M-NaOEt, *and ethanolic* 0·1% $AlCl_3$.

	In EtOH		In 0·002M-NaOEt	In 0·1% $AlCl_3$	
Lotoflavin	255	370	335	270	430
,, acetate	252	298	—	252	298
Quercetin	255	370	335	270	430
,, acetate	252	298	—	252	298

The infrared spectra of lotoflavin and quercetin as paraffin oil mulls were determined with a Grubb-Parsons S3A single-beam spectrometer, corresponding peaks being obtained as follows : 6·05, 6·24, 6·43, 6·59, 8·24, 8·36, 8·57, 8·88, 9·19, 9·88, 10·22, 10·70, 11·57, 11·86, 11·94 μ.

Microdegradation of lotoflavin. Lotoflavin (*ca.* 0·1 mg.) was fused with potassium hydroxide (100 mg.) for 1 min., and the product was rapidly cooled and dissolved in water. The solution was acidified to approx. pH 3 by dilute hydrochloric acid and extracted with ether. The ethereal solution was washed with water (0·5 ml.), and the solvent removed. The residue was dissolved in ethanol and run on paper [solvent system: butan-1-ol–acetic acid–water (6 : 1 : 2)] against phloroglucinol, phloracetophenone, protocatechuic acid, and β-resorcylic acid. The spots from the degradation product and from quercetin, on similar treatment, ran with phloroglucinol and protocatechuic acid. For inspection the papers were sprayed with diazotised *p*-nitroaniline and over-sprayed with 0·1N-sodium hydroxide (Bate-Smith and Swain, *J.*, 1953, 2187; Swain, *Biochem. J.*, 1952, **53**, 200).

Acetylation of lotoflavin. Lotoflavin (19m g.) was acetylated by acetic anhydride–perchloric acid. The product separated in needles, m. p. 190° [Found: C, 59·1, 58·7; H, 4·0, 3·9. Calc. for $C_{15}H_5O_2(OAc)_5$: C, 58·6; H, 3·9. Calc. for $C_{15}H_6O_2(OAc)_4$: C, 60·8; H, 4·0%]. This acetate did not depress the m. p. of authentic quercetin penta-acetate. The ultraviolet spectra of both acetates were determined in ethanol and ethanolic 0·1% aluminium chloride solution. The results are given in Table 2.

Approximate percentage of kæmpferol in lotoflavin. Ethanolic solutions of mixtures (0·5 mg.) of kæmpferol (1, 5, 10%) and quercetin were run on No. 3 Whatman paper (3·5 cm. strip) in solvent system (1). Each kæmpferol strip was eluted with spectroscopic ethanol, and the elution volume was made up to 25 ml. The absorption (at 368 mμ) of the solution was determined and compared with a curve showing absorption (at 368 mμ) against concentration for ethanolic solutions of kæmpferol. A relation between kæmpferol taken and kæmpferol found by chromatography was thus obtained. Lotoflavin when chromatographed under the same conditions as the synthetic mixtures of kæmpferol and quercetin showed 1—2% of kæmpferol.

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