258. *Derivatives of* 5 : 6 : 4′- *and* 5 : 8 : 4′-*Trihydroxyflavones, and a Note on the Structure of Ginkgetin.*

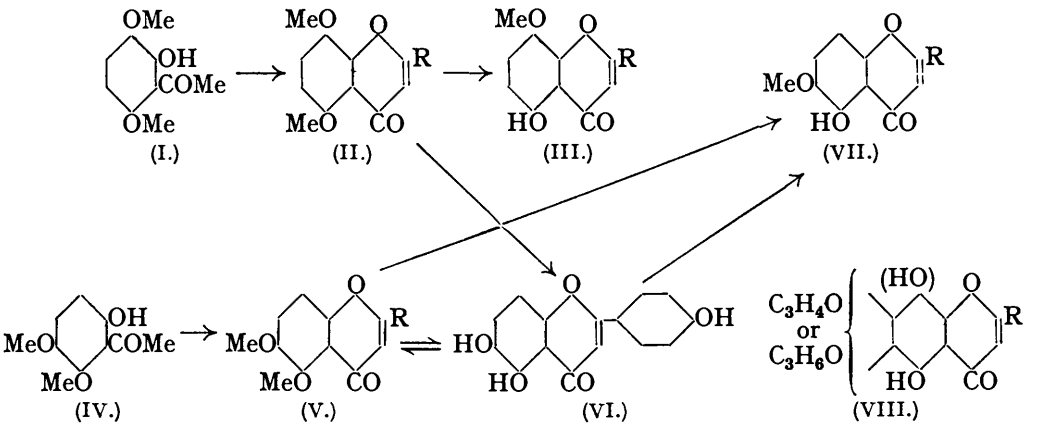
By Wilson Baker and W. H. C. Simmonds.

Ginkgetin, isolated from the leaves of the Maidenhair tree, has previously been regarded as 5 : 8-dihydroxy-4′-methoxyflavone. The dimethyl ether (II) of this substance and the isomeric 5 : 6 : 4′-trimethoxyflavone (V), as well as a number of derivatives of both compounds, have been synthesised by established methods, and their properties prove that ginkgetin can be neither 5 : 8- nor 5 : 6-dihydroxy-4′-

methoxyflavone. A review of the available evidence indicates that ginkgetin is not a simple flavone and is probably best represented in the present state of our knowledge by the expression (VIII).

THE suggestion was made by Furukawa that the yellow, phenolic compound isolated from the autumnal leaves of the Maidenhair tree (*Ginkgo biloba* L.) was probably 5 : 8-dihydroxy-4′-methoxyflavone (*Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1932, **19**, 27; 1933, **21**, 278). This substance, which may be termed *ginkgetin*, showed many of the properties of a flavone, and the formula proposed became more probable when it was shown that primetin, isolated from the leaves of *Primula modesta*, was 5 : 8-dihydroxyflavone (Baker, *Nature*, 1939, **143**, 900; J., 1939, 956; Baker, Brown, and Scott, J., 1939, 1922; Nakazawa, *J. Pharm. Soc. Japan*, 1939, **59**, 524; Horii, *ibid.*, p. 552).

We have accordingly synthesised 5 : 8 : 4′-*trimethoxyflavone* (II), but the compound differs widely from ginkgetin dimethyl ether, and a further comparison of certain derivatives of (II) with the corresponding ginkgetin derivatives proves conclusively that ginkgetin is not 5 : 8-dihydroxy-4′-methoxyflavone (see Table). As little evidence was adduced by Furukawa that ginkgetin was 5 : 8- rather than 5 : 6-dihydroxy-4′-methoxyflavone, both of which are derivatives of hydroxyquinol (cf. previous confusion over the structure of primetin, originally thought to be 5 : 6-dihydroxyflavone), 5 : 6 : 4′-*trimethoxyflavone* (V) also has been synthesised, but the properties of this compound and derivatives (see Table) prove that ginkgetin cannot be 5 : 6-dihydroxy-4′-methoxyflavone.



R = ·C_6H_4·OMe (*p*).

The synthesis of 5 : 8 : 4′-trimethoxyflavone (II) was carried out by a series of reactions parallel to those described by Baker, Brown, and Scott (*loc. cit.*). 2-Hydroxy-3 : 6-dimethoxyacetophenone (I) was converted into its O-*anisoyl* derivative, and this compound when treated with sodamide in toluene underwent molecular rearrangement to 2-*hydroxy*-3 : 6 : 4′-*trimethoxydibenzoylmethane*; the final ring closure to 5 : 8 : 4′-*trimethoxyflavone* (II) was effected by heating with sodium acetate in acetic acid. Partial demethylation of (II) with aluminium chloride in ether gave 5-*hydroxy*-8 : 4′-*dimethoxyflavone* (III), convertible into an *acetyl* derivative, but complete demethylation of (II) with hydrobromic acid in acetic acid gave 5 : 6 : 4′-*trihydroxyflavone* (VI), reorientation of the hydroxyl groups having occurred through opening and subsequent closing of the flavone ring in the alternative direction. Methylation of the 5 : 6 : 4′-trihydroxyflavone (VI) so formed gave 5 : 6 : 4′-trimethoxyflavone (V) and not 5 : 8 : 4′-trimethoxyflavone (II). A similar change of orientation occurs in the formation of 5 : 6-dihydroxyflavone by demethylation of 5 : 8-dimethoxyflavone with hydrobromic acid in acetic acid (Baker, Brown, and Scott, *loc. cit.*). Attempts to demethylate 5 : 8 : 4′-trimethoxyflavone to 5 : 8 : 4′-trihydroxyflavone proved unsuccessful (cf. similar attempts to demethylate primetin dimethyl ether without conversion into 5 : 6-dihydroxyflavone; Baker, J., 1939, 956), and this reorientation even occurred when hydriodic acid (*d* 1·7) was used at 130°, conditions which Hattori found

(*Ber.*, 1939, **72**, 1914) were successful in converting 5 : 7-dihydroxy-8-methoxyflavone into 5 : 7 : 8-trihydroxyflavone without rearrangement.

5 : 6 : 4′-*Trimethoxyflavone* (V) was synthesised independently from 2-hydroxy-5 : 6-dimethoxyacetophenone (IV), *via* the stages 2-*anisoyloxy*-5 : 6-*dimethoxyacetophenone* and 2-*hydroxy*-5 : 6 : 4′-*trimethoxydibenzoylmethane*, as in the synthesis of (II) (cf. Baker, J., 1939, 956). Demethylation of (V) with aluminium chloride in ether gave 5-*hydroxy*-6 : 4′-*dimethoxyflavone* (VII), which yielded a *monoacetyl* derivative, whereas demethylation with hydrobromic acid in acetic acid gave 5 : 6 : 4′-trihydroxyflavone (VI), best characterised as its *triacetate*.

The following Table gives the melting points of the derivatives of 5 : 6 : 4′- and 5 : 8 : 4′-trihydroxyflavones, and of the corresponding ginkgetin compounds.

	Trihydroxy.	Triacetate.	Trimethyl ether.	Dimethyl ether.	Dimethyl ether acetate.
5 : 6 : 4′-	298°	209°	164°	173°	182·5°
5 : 8 : 4′-	—	—	161	146	200
Ginkgetin derivative ...	330	237—238	225—227	277—278	257—259

It is now possible to draw certain conclusions as to the probable structure of ginkgetin, a problem of considerable interest in view of the fact that the Maidenhair tree is possibly the oldest living plant, having flourished unchanged since the Cretaceous or even Jurassic period (see Seward, *Science Progress*, 1937, **32**, 420). Furukawa showed that it contains one methoxyl and two phenolic hydroxyl groups, one of which is difficult to methylate but gives a reddish ferric chloride reaction and an acetyl derivative. Ginkgetin gives a crystalline oxonium salt with hydrochloric acid in acetic acid, and on alkaline hydrolysis yields anisic acid, *p*-hydroxybenzoic acid, and *p*-hydroxyacetophenone. This establishes the partial structure 5-hydroxy-4′-methoxyflavone (see formula VIII), and because ginkgetin gives a green ferric chloride reaction and its absorption spectrum indicates the absence of a hydroxyl group in position 6 the second phenolic group is most likely to occupy position 8. We have now shown that ginkgetin can be neither 5 : 6- nor 5 : 8-dihydroxy-4′-methoxyflavone, nor can it be the known 5 : 7-dihydroxy-4′-methoxyflavone (acacetin), and are thus forced to the conclusion either that it is not an individual substance, a conclusion which does not seem justified by the known facts, although this possibility is mentioned by Furukawa (probably because in one alkaline fusion he isolated what appeared to be phloroglucinol), or that it possesses a higher molecular weight than previously supposed. The latter conclusion is strongly supported by the high melting points of ginkgetin derivatives (see Table), and it is significant that the only molecular-weight determination carried out was on the diacetyl derivative, which gave a value of 384, whilst Furukawa's formula, $C_{16}H_{10}O_3(OAc)_2$, requires a molecular weight of 368. Careful examination of the recorded analytical data for ginkgetin and its six derivatives shows that the formulæ $C_{19}H_{14}O_6$ and $C_{19}H_{16}O_6$ for the parent substance agree very satisfactorily with the analyses, giving as a possible structure for ginkgetin the partial formula (VIII). The C_3H_4O, or C_3H_6O, group must possess an ether oxygen atom and is most likely to be a methylfurano- or chromeno-group, or one of the corresponding saturated groupings. In support of this structure it may be mentioned that karangin has been shown to be a simple furanoflavonol derivative (Manjunath, Seetharamiah, and Siddappa, *Ber.*, 1939, **72**, 93), and kellin is 5 : 8-dimethoxy-2-methyl-6 : 7-2′ : 3′-furanochromone (Fontl and Salem, *Biochem. Z.*, 1930, **226**, 166; Späth and Gruber, *Ber.*, 1938, **71**, 106), whilst rotenone is a more complex derivative of dihydrofurano*iso*flavanone. Osajin and pomiferin appear to be complex derivatives of 5 : 4′-dihydroxy- and 5 : 3′ : 4′-trihydroxy-flavone respectively (Wolfram, Morgan, and Benton, *J. Amer. Chem. Soc.*, 1940, **62**, 1484).

Experimental.

2-*Anisoyloxy*-3 : 6-*dimethoxyacetophenone*.—2-Hydroxy-3 : 6-dimethoxyacetophenone (I) (Baker, Brown, and Scott, *loc. cit.*) (3·5 g.), pyridine (15 c.c.), and anisoyl chloride (3·3 g.) were heated on the water-bath for 20 minutes, dilute hydrochloric acid added, and the solid (5·4 g.) collected, washed, and dried. The *anisoyl* derivative separated from alcohol in colourless needles, m. p. 131° (Found: C, 65·3; H, 5·5. $C_{18}H_{18}O_6$ requires C, 65·4; H, 5·4%).

2-*Hydroxy*-3 : 6 : 4′-*trimethoxydibenzoylmethane*.—The preceding compound (3·5 g.) was added to sodamide (7 g.) finely powdered under toluene (35 c.c.), and the mixture heated on the water-bath for 4 hours. The solids were collected, washed with hot benzene, dried, and cautiously added to ice-water, and the solution acidified with dilute acetic acid. The solid *product* (1·3 g.) separated from benzene in orange prisms, m. p. 138—139° (Found : C, 65·8; H, 5·6. $C_{18}H_{18}O_6$ requires C, 65·4; H, 5·4%). Its solution in dilute sodium hydroxide was pale yellow, and it gave a greenish-yellow colour with alcoholic ferric chloride.

5 : 8 : 4′-*Trimethoxyflavone* (II).—2-Hydroxy-3 : 6 : 4′-trimethoxydibenzoylmethane (1 g.) was heated on the water-bath for 3 hours with glacial acetic acid (10 c.c.) and anhydrous sodium acetate (1 g.), water added, and the solid collected, washed, and dried (0·6 g.). 5 : 8 : 4′-*Trimethoxyflavone* separated from dilute alcohol in pale yellow, microcrystalline prisms, m. p. 161° (Found in material dried at 120° under diminished pressure : C, 69·1; H, 5·3. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1%). In concentrated sulphuric acid it gave a yellow solution without fluorescence.

5-*Hydroxy*-8 : 4′-*dimethoxyflavone* (III).—To a solution of aluminium chloride (2 g.) in anhydrous ether (12 c.c.) was added 5 : 8 : 4′-trimethoxyflavone (0·2 g.), and the mixture refluxed for 18 hours. Water was now added, and the solid orange aluminium complex collected and decomposed by boiling for a few minutes with acetic acid (5 c.c.) and concentrated hydrochloric acid (2 c.c.). The yellow precipitate obtained on dilution separated from methyl alcohol in pale yellow needles, m. p. 146° (Found : C, 68·6; H, 5·0. $C_{17}H_{14}O_5$ requires C, 68·4; H, 4·7%). It was insoluble in cold dilute sodium hydroxide solution, but gave an intense apple-green colour with alcoholic ferric chloride. The *acetyl* derivative, prepared by boiling with acetic anhydride for 2 hours and adding water, crystallised from methyl alcohol in colourless needles, m. p. 200° (Found : C, 67·4; H, 4·5. $C_{19}H_{16}O_6$ requires C, 67·0; H, 4·7%).

6-*Benzyloxy*-2-*methoxyacetophenone and* 2-*Hydroxy*-6-*methoxyacetophenone*.—2-Hydroxy-6-benzyloxyacetophenone (28 g.; Baker, Brown, and Scott, *loc. cit.*) was dissolved in a solution of potassium hydroxide (30 g.) in water (120 c.c.) containing acetone (20 c.c.) and stirred at room temperature while methyl sulphate (30 c.c.) was added during 1 hour. Further similar quantities of alkali and methyl sulphate were then added, and after 12 hours the solution was diluted and extracted with ether. The extract yielded the crude product (25·7 g.), which separated from light petroleum (b. p. 60—80°) in needles, m. p. 74° (Found : C, 74·8; H, 6·3. $C_{16}H_{16}O_3$ requires C, 75·0; H, 6·3%). Hydrolysis of the 6-*benzyloxy*-2-*methoxyacetophenone* (23·4 g.) in glacial acetic acid (80 c.c.) and concentrated hydrochloric acid (40 c.c.) at 60° for 1 hour gave, after dilution, extraction with ether, and isolation of the phenolic material, 2-hydroxy-6-methoxyacetophenone (11 g.), the overall yield from 2 : 6-dihydroxyacetophenone by this method being 40%. Direct methylation of 2 : 6-dihydroxyacetophenone gives 2-hydroxy-6-methoxyacetophenone in 70% yield (Baker, J., 1939, 959).

2-*Anisoyloxy*-5 : 6-*dimethoxyacetophenone*.—Molecular quantities of 2-hydroxy-5 : 6-dimethoxyacetophenone (Baker, *loc. cit.*) and anisoyl chloride were heated in pyridine, and the product isolated as in the case of the isomeric 3 : 6-dimethoxy-compound. The *anisoyl* derivative separated from alcohol in needles, m. p. 99° (Found : C, 65·6; H, 5·3. $C_{18}H_{18}O_6$ requires C, 65·5; H, 5·4%).

2-*Hydroxy*-5 : 6 : 4′-*trimethoxydibenzoylmethane*.—The molecular rearrangement of the preceding compound (0·75 g.) was carried out as described in the case of the isomeride, twice the volume of toluene being employed. The crude *product* (0·54 g.), also isolated as described, separated after several crystallisations from light petroleum (b. p. 40—60°) in orange-red, nodular, crystalline masses, m. p. 69° (Found : C, 66·7; H, 5·3. $C_{18}H_{18}O_6$ requires C, 65·5; H, 5·4%). Its solution in aqueous sodium hydroxide was yellow, and in alcoholic ferric chloride it gave a reddish-brown colour.

5 : 6 : 4′-*Trimethoxyflavone* (V).—2-Hydroxy-5 : 6 : 4′-trimethoxydibenzoylmethane (0·26 g.), acetic acid (4 c.c.), and anhydrous sodium acetate (0·4 g.) were heated on the water-bath for 4 hours, water added, and the solid collected, washed, and dried (0·21 g.). 5 : 6 : 4′-*Trimethoxyflavone* separated from alcohol in colourless needles, m. p. 164° (Found in material dried at 110° in a vacuum : C, 69·6; H, 5·4. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1%). The mixed m. p. with 5 : 8 : 4′-trimethoxyflavone (m. p. 161°) was *ca.* 136°. Its yellow solution in concentrated sulphuric acid was without fluorescence.

5-*Hydroxy*-6 : 4′-*dimethoxyflavone* (VII).—(a) *By demethylation of* 5 : 6 : 4′-*trimethoxyflavone*. 5 : 6 : 4′-Trimethoxyflavone (0·15 g.) was refluxed for 18 hours in a solution of aluminium chloride (1·5 g.) in absolute ether (6 c.c.), and the product isolated as in the case of the isomeride (III) (yield 0·07 g.). 5-*Hydroxy*-6 : 4′-*dimethoxyflavone* separated from methyl alcohol in yellow

needles, m. p. 173° (Found: C, 68·4; H, 5·0. $C_{17}H_{14}O_5$ requires C, 68·4; H, 4·7%). It was insoluble in dilute sodium hydroxide solution, and gave an apple-green coloration with alcoholic ferric chloride. The *acetyl* derivative formed colourless needles from alcohol, m. p. 182·5° (Found: C, 66·8; H, 4·8. $C_{19}H_{16}O_6$ requires C, 67·0; H, 4·7%).

(b) *By methylation of* 5 : 6 : 4′-*trihydroxyflavone.* 5 : 6 : 4′-Trihydroxyflavone (below) (0·05 g.) in 50% methyl alcohol (2 c.c.) was shaken at 20° in coal gas with the alternate addition of methyl sulphate and dilute sodium hydroxide solution. After 12 hours dilution with water precipitated a greenish solid, which separated from alcohol in yellow needles, m. p. and mixed m. p. 173°.

5 : 6 : 4′-*Trihydroxyflavone* (VI).—(a) *From* 5 : 6 : 4′-*trimethoxyflavone* (V). 5 : 6 : 4′-Trimethoxyflavone (0·04 g.) was heated for 8 hours (oil-bath at 145—150°) with acetic acid (2 c.c.) and hydrobromic acid (2 c.c.; *d* 1·5), water added, and the yellow solid collected and crystallised from dilute acetic acid. It separated in spherulitic growths of yellow needles, m. p. 298°, which, with the small amount of material available, could not be obtained quite pure (Found: C, 65·7; H, 3·4. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%). 5 : 6 : 4′-*Trihydroxyflavone* gave an orange-yellow solution in aqueous sodium hydroxide, a non-fluorescent yellow solution in concentrated sulphuric acid, and an apple-green solution in alcoholic ferric chloride. The *triacetyl* derivative, prepared by boiling with acetic anhydride for 2 hours and adding water, separated from alcohol in colourless needles, m. p. 209° (Found: C, 63·3; H, 4·1. $C_{21}H_{16}O_8$ requires C, 63·6; H, 4·0%).

(b) *From* 5 : 8 : 4′-*trimethoxyflavone* (II). Demethylation (of 0·2 g.) was effected as under (a), and the resulting crude product (0·15 g.) was characterised as 5 : 6 : 4′-trihydroxyflavone by conversion into the triacetyl derivative (m. p. and mixed m. p. 209°), and by methylation at the b. p. with a large excess of methyl sulphate and 15% aqueous potassium hydroxide in presence of acetone, which yielded 5 : 6 : 4′-trimethoxyflavone (V), m. p. and mixed m. p. 164°.

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, August 15th,* 1940.]