280. *The Structures of the Naturally Occurring Biflavonyls.*

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The determination of the structures of the biflavonyls bilobetin, ginkgetin, isoginkgetin, sciadopitysin, and kayaflavone (XVIc, d, e, f, and g) is described. Complete methylation of these compounds gave in each case ginkgetin tetramethyl ether, whose structure (VII), first proposed on spectroscopic and biosynthetic grounds, was established by degradation to the acids (III, IV, and V). The positions of the *O*-methyl groups in the structures (XVIc—g) were determined by (*a*) the study of the effect of base upon their ultraviolet spectra, (*b*) their oxidative degradation with alkaline hydrogen peroxide, and (*c*) their alkaline hydrolysis to " ketoflavones " (XIV).

The biflavonyls form a new group of natural products, representatives of which are widely distributed among the Gymnosperms; their biosynthesis undoubtedly involves oxidative coupling.

THE chemistry and structures of members of the recently recognised class of the biflavonyl pigments [1] has been reviewed by Baker and Ollis.[2] That article showed how our work, at that time reported only in preliminary form, led, partly in conjunction with the complementary work of Nakazawa, of Kariyone, and of Kawano in Japan, to the establishment of the structures of ginkgetin, isoginkgetin, sciadopitysin, sotetsuflavone, and kayaflavone. The present paper gives details of our work on ginkgetin, isoginkgetin, and other biflavonyls including bilobetin which is a new member of the class. Thus, including amentoflavone, the number of known biflavonyls having a 3′,8″-carbon–carbon linkage is seven. The investigations now described provide an essentially independent derivation of the structures of the biflavonyls. As the work in other laboratories has been reviewed elsewhere,[2] reference is made to these other studies only when they are immediately relevant to our structural arguments.

[1] For preliminary reports see *Proc. Chem. Soc.*, 1959, **91**, 269.
[2] Baker and Ollis, " Recent Developments in the Chemistry of Natural Phenolic Compounds," ed. W. D. Ollis, Pergamon Press, London, 1961, p. 152.

The early work on impure ginkgetin by Furukawa,[3] and by Baker *et al.*,[4,5] including syntheses of compounds whose structures had been proposed for the pigment,[4,6] showed only that it had a somewhat complex flavonoid structure.[2] However, the studies by Nakazawa in 1941,[7] which for nine years were generally unknown owing to war-time conditions, led to the isolation of pure ginkgetin, $C_{30}H_{12}O_4(OH)_4(OMe)_2$, through its sparingly soluble potassium salt, and it was suggested that ginkgetin was possibly derived from two molecules of the apigenin (5,7,4′-trihydroxyflavone) type.

Our initial studies on ginkgetin, begun in 1946,[5] had also led to the isolation of non-homogeneous material, but eventually a crystalline acetate was obtained having a molecular weight [8] approximately twice that required for the formula $C_{16}H_{10}O_3(OAc)_2$. Separation of individual pigments was finally achieved by a lengthy countercurrent distribution between ethyl methyl ketone and a borate buffer, the location of the biflavonyls being indicated by the yellow colour of their alkaline solutions. The progress of the fractionation was found, however, to be best followed by isolation of the contents of individual tubes and examination of their ultraviolet spectra in ethanol and in N/500- and N/50-ethanolic sodium hydroxide. Three main fractions which possessed significantly different spectral characteristics were obtained. The spectra and their change on addition of alkali were later recognised (see Table) as being characteristic of ginkgetin, isoginkgetin, and bilobetin, and the appropriate fractions subsequently yielded these biflavonyls. The ginkgetin, obtained in 0·03% yield from the dried leaves, was shown to be identical with a sample of ginkgetin generously supplied by Professor Nakazawa; the two new biflavonyls isoginkgetin and bilobetin were obtained in 0·04% and 0·016% yield from the leaves. It was later found that isoginkgetin could be directly isolated from a crude leaf extract (material A; see Experimental) by crystallisation from acetone, and that the more soluble ginkgetin which was retained in the acetone mother-liquors was precipitated as its very sparingly soluble potassium salt on addition of aqueous potassium carbonate; recrystallisation of this salt from aqueous potassium carbonate and subsequent acidification gave ginkgetin.

Analyses * confirmed Nakazawa's partial formula for ginkgetin, $C_{30}H_{12}O_4(OH)_4(OMe)_2$, and showed that isoginkgetin could also be represented by this formula. The two compounds gave identical tetramethyl ethers and on demethylation the same hexahydroxy-compound, which was characterised as its hexa-acetyl derivative. Complete methylation of the hexahydroxy-compound regenerated ginkgetin tetramethyl ether, showing that no rearrangement had occurred during demethylation. Ginkgetin and isoginkgetin, however, gave different tetra-acetyl derivatives and different tetraethyl ethers. These facts proved that the two compounds possessed the same skeletal structure and oxygenation pattern and differed only in the positions of one or both of the *O*-methyl groups.

Comparison of the ultraviolet spectra (see Table) of ginkgetin, isoginkgetin, and their tetra-acetates and tetramethyl ether with those of apigenin (Ia), acacetin (Ib), and genkwanin (Ic) and their derivatives suggested very strongly that ginkgetin and isoginkgetin were derivatives of 5,7,4′-trihydroxyflavone (Ia). Although the positions of maximal absorption were very similar, the molecular extinction coefficients of ginkgetin and isoginkgetin and their derivatiyes were approximately double those of the corresponding

* Difficulty was experienced in obtaining entirely satisfactory analytical figures for some of the biflavonyls and their derivatives. This appears to be caused by tenacious retention of water or solvent of crystallisation.

[3] Furukawa, Abs. from *Bull. Inst. Phys. Chem. Res., Tokyo*, 1929, **2**, 5; *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, 1932, **19**, 27; 1933, **21**, 278.

[4] (*a*) Baker and Simmonds, *J.*, 1940, 1370; (*b*) Baker and Flemons, *J.*, 1948, 2138; Baker, Flemons, and Winter, *J.*, 1949, 1560.

[5] Butt, Dissertation, Bristol, 1948.

[6] Horii, *J. Pharm. Soc. Japan*, 1940, **60**, 222; Ikawa, *J. Chem. Soc. Japan*, 1941, **62**, 1052; Kogure, *ibid.*, 1952, **73**, 271, 308; Kogure and Kubota, *J. Inst. Polytechnics, Osaka City Univ.*, 1952, **2**, 70, 76.

[7] Nakazawa, *J. Pharm. Soc. Japan*, 1941, **61**, 90, 174, 228 (*Chem. Abs.*, 1950, **44**, 9441).

[8] Baker, Ollis, and Zealley, *J.*, 1951, 208.

Ultraviolet spectra of flavones and biflavonyls. ($\lambda_{max.}$ in mμ; figures in parentheses are ε.)

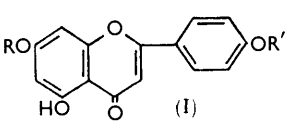
No.		Ethanol			
			Band I		Band II
1	Ginkgetin (Xc or Xd) = (XVId)	212 (76,000)	271·5 (42,200)		335 (40,000)
2	Isoginkgetin (Xb) = (XVIe)	213 (90,000)	271·5 (42,000)		330 (36,500)
3	Apigenin (Ia)		269 (18,800)	300 * (13,500)	340 (20,900)
4	Acacetin (Ib)	~210	269 (20,300)	298 * (16,400)	330 (20,800)
5	Genkwanin (Ic)		269 (17,000)	300 * (12,000)	337 (19,600)
6	Ginkgetin tetra-acetate	211 (63,500)	248—258 * (34,500)		317 (47,000)
7	Isoginkgetin tetra-acetate	220 (74,000)	250 * (47,000)		324 (50,500)
8	Acacetin diacetate	222 * (21,200)	258 (13,300)		325 (26,300)
9	Ginkgetin tetramethyl ether	212 (63,000)	267 (48,000)		328 (45,500)
10	Apigenin trimethyl ether	212 (37,000)	265 (21,200)		325 (24,000)
11	Sciadopitysin (Xa) = (XVIf)		271·5 (37,600)		330 (35,000)
12	Kayaflavone (XVIg)		271·5 (44,100)		329 (41,000)
13	Bilobetin (XVIc)		272 (44,500)		337 (35,200)

	N/50-Ethanolic NaOEt			N/500-Ethanolic NaOEt			N/5000-Ethanolic NaOEt		
No.	Band I		Band II	Band I		Band II	Band I		Band II
1	284 (46,500)		397 (30,800)	271 (38,500)		340 (30,000)	270 (38,800)		343 (32,000)
2	280 (53,500)		376·5 (24,300)	279·5 (54,500)	295 * (42,500)	378 (25,700)	274 (45,000)		345 (29,000)
3				277 (21,900)	330 (13,000)	400 (31,700)			
4	279 (31,300)		371 (13,300)	278 (32,600)	295 * (21,000)	376 (14,200)	278 (26,600)		347 (14,500)
5				269 (13,600)	292 * (10,500)	397 (23,800)			
11	287 (50,800)		378 (16,000)	273 * (34,000)		348 (26,200)	272 (35,000)		317 (30,000)
12	281 (58,000)		378 (20,800)	276 (51,600)		353 (24,200)	274 (48,000)		308 (32,000)
13	278·5 (49,400)	295 * (34,400)	395 (36,200)	278·5 (50,400)	295 * (34,400)	395 (36,200)	279 (51,500)	295 (36,600)	395 (36,600)

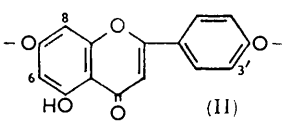
* Point of inflexion.

simple flavones. It was therefore considered probable that ginkgetin and isoginkgetin contained two 5,7,4′-trioxygenated flavone units joined in such a way that interaction between the chromophores was very slight.

	R	R′
(a)	H	H
(b)	H	Me
(c)	Me	H
(d)	Me	Me



(I)



(II)

The infrared spectra of ginkgetin and isoginkgetin showed strong sharp bands at 1660 cm.$^{-1}$, as did those of apigenin (Ia), acacetin (Ib), and apigenin 7,4′-dimethyl ether (Id). This band is characteristic of 5-hydroxyflavones, and although this hydroxyl group is internally hydrogen-bonded the effect of 5-*O*-alkylation and 5-*O*-acylation is opposite to

that shown in the case of simple *o*-hydroxy-ketones. Because of internal hydrogen bonding in *o*-hydroxy-ketones, the carbonyl bands of these compounds show a shift to higher frequencies on either *O*-alkylation or *O*-acylation. However, a similar comparison of the infrared spectra of 5-hydroxyflavones and other 5-hydroxychromones with the spectra of their 5-*O*-alkyl and 5-*O*-acyl derivatives shows a shift in the opposite direction, that is, to lower frequencies. The reason for this anomaly has been considered elsewhere.[9] In practice this effect is very useful in diagnosing the presence of a 5-hydroxyflavone structure (see II). The infrared spectra of derivatives of ginkgetin, isoginkgetin, and appropriate simple flavones [ginkgetin tetramethyl ether (1644 $cm.^{-1}$), ginkgetin tetraethyl ether (1643 $cm.^{-1}$), isoginkgetin tetraethyl ether (1640 $cm.^{-1}$), apigenin trimethyl ether (1645 $cm.^{-1}$), acacetin 5,4′-diethyl ether (1645 $cm.^{-1}$), 5-*O*-ethyl-7,4′-di-*O*-methylapigenin (1641 $cm.^{-1}$), tetra-*O*-acetylisoginkgetin (1650 $cm.^{-1}$), tri-*O*-acetylapigenin (1650 $cm.^{-1}$), and di-*O*-acetylacacetin (1645 $cm.^{-1}$)] all showed that they had absorption bands at significantly lower wavelengths than those of ginkgetin and isoginkgetin which absorbed at 1660 $cm.^{-1}$. Thus the ultraviolet and infrared evidence pointed towards the presence in ginkgetin and isoginkgetin of *two* 5-hydroxyflavonoid units (II).

Considerations based upon current views[10] on the biogenetic importance of the oxidative coupling of phenols in the *ortho*- and *para*-positions suggested that the biflavonyls are formed from precursors of apigenin type (II). This restricts the interflavonyl linkage to positions 3′, 6, and 8, giving six possible biflavonyl structures having 6-6, 6-8, 8-8, 3′-6, 3′-8, or 3′-3′ carbon–carbon linkages. Carbon–oxygen coupling might occur to give biflavonyl ethers, and such structures were also considered.

Proof that a 3′-8 linkage is present was obtained by degradation of ginkgetin tetramethyl ether with alkaline hydrogen peroxide. A model experiment with apigenin trimethyl ether (VI) yielded the expected[11] 2-hydroxy-4,6-dimethoxybenzoic acid (III) and *p*-anisic acid (IV), and under similar conditions ginkgetin tetramethyl ether gave the same two acids and a dicarboxylic acid, $C_{12}H_5O(OMe)_3(CO_2H)_2$ (pK'_a 5·3 and 6·8 in 50% aqueous ethanol), giving a strong ferric chloride reaction. The infrared spectrum of this new acid showed two bands ($\nu_{max.}$ 1685 and 1640 $cm.^{-1}$) in the carbonyl region attributable to isolated aromatic carboxyl and *o*-hydroxy-carboxyl groups, respectively. This acid could therefore be formulated as a derivative of biphenyl, $C_{12}H_4(OH)(OMe)_3(CO_2H)_2$. Its ultraviolet spectrum [$\lambda_{max.}$ (ε) 222 mμ (26,700), 247 mμ (27,100), 256 mμ (26,800), 299 mμ (3900)] was in satisfactory agreement with the composite curve [$\lambda_{max.}$ ($\varepsilon_{max.}$) 218 mμ (35,000), 257 mμ (27,100), 264 mμ (22,400), 294 mμ (4300)] obtained by summation of the spectra of 2-hydroxy-4,6-dimethoxybenzoic acid (III) and *p*-anisic acid (IV), thus confirming its identification as a biphenyl. It followed that a biphenyl residue exists in ginkgetin and, it being borne in mind that the acid possesses three methoxyl groups rather than four or two, it followed that the flavone units must be directly linked between carbon 3′ of one flavonoid unit and carbon 6 or 8 of the other. This led to two possible structures for ginkgetin tetramethyl ether, of which that (VII) with a 3′-8 interflavonyl link was almost certainly correct because the other having a 3′-6 link would involve in ginkgetin a severely sterically hindered 5″-hydroxyl group whose methylation and acetylation would, contrary to what is observed, require abnormally vigorous conditions. Hence formula (VII) was proposed for ginkgetin tetramethyl ether and in consequence structure (V) for the derived biphenyldicarboxylic acid. Partial stuctures of the type (VIII) follow for ginkgetin and isoginkgetin. These structures (VII), (V), and (VIII) were not compatible with the structure for ginkgetin previously proposed by Nakazawa.[7]

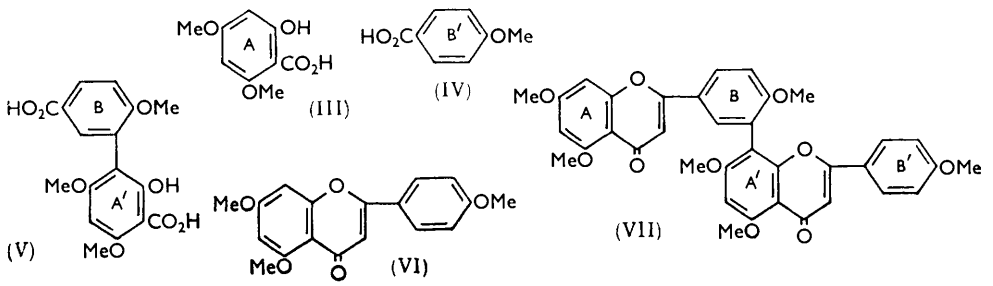
At this stage in our studies the extensive investigations by Kariyone and Kawano on

[9] Looker and Hanneman, *J. Org. Chem.*, 1962, **27**, 381; Briggs and Colebrook, *Spectrochim. Acta*, 1962, **18**, 939; Briggs and Ollis, forthcoming publication.

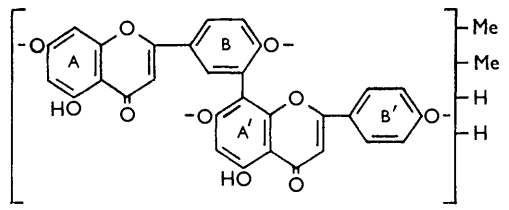
[10] Barton and Cohen, "Festschrift Arthur Stoll," Birkhäuser, Basel, 1957, p. 117; Erdtman and Wachtmeister, *op. cit.*, p. 144; Hassall and Scott, "Recent Developments in the Chemistry of Natural Phenolic Compounds," ed. W. D. Ollis, Pergamon Press, London, 1961, pp. 119.

[11] Molho, *Bull. Soc. chim France*, 1956, **23**, 39.

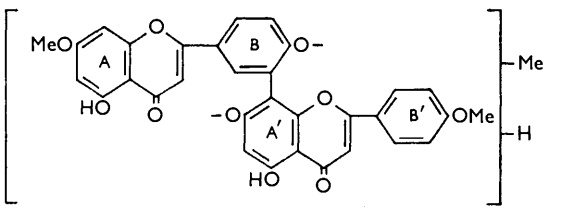
sciadopitysin were reported.[12] These authors showed that sciadopitysin was a biflavonyl closely related to ginkgetin, in that sciadopitysin trimethyl ether was identical with ginkgetin tetramethyl ether, but the structure proposed by Kariyone and Kawano [12] for sciadopitysin was not compatible with structure (VII) for its trimethyl ether. It



(III) (IV) (V) (VI) (VII)



(VIII)



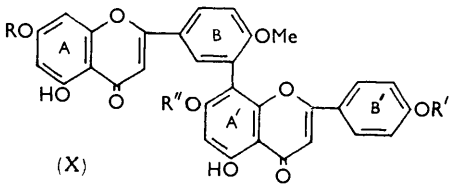
(IX)

was, however, possible to provide an alternative interpretation [1,2] of all their degradative results in terms of formula (VII) for sciadopitysin trimethyl ether and a corresponding formula for sciadopitysin. Kariyone and Kawano [12] showed that vigorous alkaline hydrolysis of sciadopitysin yielded amongst other products *p*-anisic acid and 4-methoxy- and 2,6-dihydroxy-4-methoxy-acetophenone. This established the position of the two methoxyl groups in the partial structure (IX) for sciadopitysin, an observation in harmony with the partial structure (VIII) already deduced for ginkgetin and isoginkgetin. As sciadopitysin is much more accessible than either ginkgetin or isoginkgetin, being obtained in 0·5% yield from the dried leaves of the umbrella pine, *Sciadopitys verticillata* Sieb. and Zucc., by simple extraction with trichloroethylene, much of our subsequent work was carried out with this substance. The necessary plant material was kindly collected by Mr. C. Puddle, of Bodnant Gardens, Denbighshire.

Final acceptance of the partial structure (VIII) for ginkgetin, isoginkgetin, and sciadopitysin required the location of the *O*-methyl groups and a rigid proof of the positions of the oxygen atoms on rings B and A′ so far provisionally allocated on biosynthetic and spectroscopic evidence. A study of the oxidation of these three biflavonyls with alkaline hydrogen peroxide provided further evidence. All three biflavonyls yielded 4-methoxyisophthalic acid, thus proving the presence of a methoxyl group in position 4′ of ring B. The other acidic products were *p*-anisic acid from isoginkgetin and sciadopitysin, and *p*-hydroxybenzoic acid from ginkgetin; these acids clearly arise from ring B′. It followed that sciadopitysin must have structure (Xa), isoginkgetin (Xb), and ginkgetin (Xc) or (Xd).

[12] Kariyone and Kawano, *J. Pharm. Soc. Japan*, 1956, **76**, 448, 451, 453; Kawano, *ibid.*, p. 457.

The same structure (Xa) for sciadopitysin was later proposed on largely different evidence by Kawano,[13] who made the important observation that sciadopitysin trimethyl ether could be degraded by a series of steps to 2,4,6,2′-tetramethoxybiphenyl, thus rigorously establishing the orientation of the oxygen atoms attached to rings A′ and B.



(X)

	R	R′	R″
(a)	Me	Me	H
(b)	H	Me	H
(c)	Me	H	H
(d)	H	H	Me

A study of the effect of base upon the ultraviolet spectra of the biflavonyls (see Table) allowed a decision to be made between the two structures (Xc) and (Xd) for ginkgetin. This effect of alkali upon the spectra of simple hydroxyflavones was already known,[14] and in the Table are given the results with the biflavonyls and some model hydroxyflavones. These show certain common features which were of value in the determination of the structure of the biflavonyls.

The spectra in neutral ethanol showed two maxima, band I (~270 mμ) and band II (~330—340 mμ). The effect of base upon the position and intensities of these two bands is as follows.

(*a*) When a 7-hydroxyl group is present (see XI):
band I, shift (~270 ⟶ 280 mμ) with greatly increased intensity;
band II, shift (~330 ⟶ 370 mμ) with reduced intensity.
(*b*) When a 4′-hydroxyl group is present (see XII):
band I, shift (~270 ⟶ 280 mμ) with reduced intensity;
band II, large shift (~330 ⟶ 400 mμ) with increased intensity.

The effect of alkali upon 7- or 4′-hydroxyflavones is to generate the mesomeric anions (XI) and (XII). When a flavone contains both 7- and 4′-hydroxyl groups the effect of weak base (case *a*) is predominantly to remove a proton from the more acidic 7-hydroxyl group. Nevertheless, in a 7,4′-dihydroxyflavone even with weak alkali some effect (case *b*) due to ionisation of the 4′-hydroxyl group should also be apparent because an equilibrium will be established involving both types (XI and XII) of anion. In a 5-hydroxyflavone the effects associated with ionisation of this group will be small[15] because it is internally hydrogen-bonded, and hence its ionisation is suppressed.

The effect of base upon the ultraviolet spectra of three of the biflavonyls recorded in the Table may be summarised as follows.

Isoginkgetin (Xb):
band I, shift (271·5 ⟶ 280 mμ) with marked increase in intensity;
band II, shift (330 ⟶ 376·5 mμ) with considerable decrease in intensity.

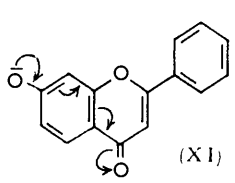
Sciadopitysin (Xa):
band I, shift (271·5 ⟶ 287 mμ) with marked increase in intensity.
band II, shift (330 ⟶ 378 mμ) with very considerable decrease in intensity.

Ginkgetin (Xc or Xd):
band I, shift (271·5 ⟶ 284 mμ) with moderate increase in intensity.
band II, shift (335 ⟶ 397 mμ) with a decrease in intensity appreciably less than for isoginkgetin and sciadopitysin.

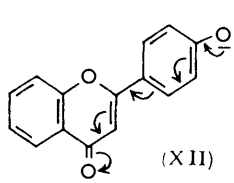
[13] Kawano, *Chem. and Ind.*, 1959, 368, 852; *Chem. and Pharm. Bull. Japan*, 1959, **7**, 698, 821.
[14] Mansfield, Swain, and Nordström, *Nature*, 1953, **172**, 23; Nordström and Swain, *J.*, 1953, 2764.
[15] Briggs and Locker, *J.*, 1951, 3136.

The interpretation of these results is unequivocal. In the case of isoginkgetin the effect is clearly of type (*a*), and because band II does not shift beyond 376·5 mμ even with N/50-base it follows that hydroxyl groups cannot be located at positions 4′ and 4‴. The hydroxyl groups must, therefore, be located at positions 7 and 7″, confirming the structure (Xb) proposed on other grounds.

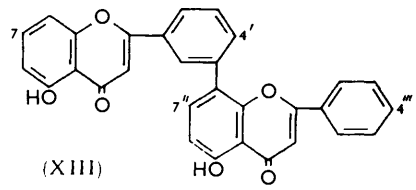
The spectral changes shown by sciadopitysin are also of type (*a*), except that the spectra in N/5000- and N/500-base still exhibit features characteristic of un-ionised sciadopitysin. These do not disappear until N/50-base is used. This shows that a 7-hydroxyflavone group is present and in order to account for its reduced acidity it must occupy a special position. Of the positions 7 and 7″ [see (XIII)], position 7″ is special in that it is sterically hindered, whereas 7 is not. This leads to the structure (Xa) for sciadopitysin. A similar steric inhibition of the development of the normal spectral characteristics of phenoxide anions has been noticed for other *ortho*-substituted phenols in alkaline solution;[16] the effect is probably due to the difficulty of solvating the anion rather than an interference with removal of a proton.



(XI)



(XII)



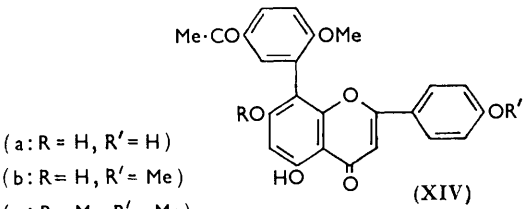
(XIII)

It was now possible to decide between structures (Xc) and (Xd) for ginkgetin. The spectral shifts on addition of alkali showed some of the characteristics of a (*b*)-type change, but although band II showed a shift to 397 mμ it was associated with a decrease in intensity. This was clearly due to the one hydroxyl group located in the 4‴-position, but in order to account for the intensity decrease the other hydroxyl group must be in either the 7- or the 7″-position. As with sciadopitysin, the spectral characteristics of the undissociated ginkgetin molecule are still apparent in N/5000- and N/500-base and disappear only in N/50-base. There must therefore be a sterically protected hydroxyl group of the 7-hydroxyflavone type present, thus giving the structure (Xc) for ginkgetin.

Supplementary and confirmatory evidence for these structures for sciadopitysin (Xa), isoginkgetin (Xb), and ginkgetin (Xc) was acquired by an examination of the degradation products which have been referred to as " ketoflavones." These and other products are obtained by hydrolysis of biflavonyls with 30% aqueous potassium hydroxide. Nakazawa[7] had obtained a ketoflavone, $C_{23}H_{15}O_6$·OMe, from ginkgetin by alkaline hydrolysis and a similar product, $C_{23}H_{14}O_5(OMe)_2$, had been obtained by Kariyone and Kawano[12] from sciadopitysin. It was established that the second compound was a monomethyl ether of the first, but the structures first proposed for these ketoflavones were based upon incorrect formulæ for ginkgetin and sciadopitysin. A re-investigation of the ketoflavone from ginkgetin (Xc) confirmed the formula $C_{23}H_{15}O_6$·OMe, and its infrared spectrum showed two carbonyl bands ($\nu_{max.}$ 1658 and 1645 cm.$^{-1}$) and hydroxyl absorption (3260 cm.$^{-1}$). On empirical grounds this ketoflavone must have the structure (XIVa). As well as this ketoflavone, 2,6-dihydroxy-4-methoxyacetophenone and 4-hydroxyacetophenone were detected chromatographically, and the latter ketone was also characterised as its 2,4-dinitrophenylhydrazone. These hydrolysis products fully supported the structure (Xc) for ginkgetin. Alkaline hydrolysis of sciadopitysin (Xa) yielded the corresponding ketoflavone (XIVb) and this proved to be identical with the product similarly obtained from isoginkgetin (Xb), thus confirming the structural relation between sciadopitysin (Xa) and isoginkgetin (Xb).

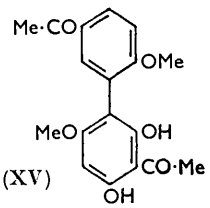
[16] Coggeshall and Glessner, *J. Amer. Chem. Soc.*, 1949, **71**, 3150.

Kayaflavone $C_{30}H_{12}O_4(OH)_3(OMe)_3$ had been isolated from the leaves of the gymnosperm, *Torreya nucifera* Sieb. and Zucc., by Kariyone and Sawada [17] who demonstrated that it was a biflavonyl since trimethylation gave sciadopitysin trimethyl ether (VII).



(a: R = H, R′ = H)
(b: R = H, R′ = Me)
(c: R = Me, R′ = Me)

(XIV)



(XV)

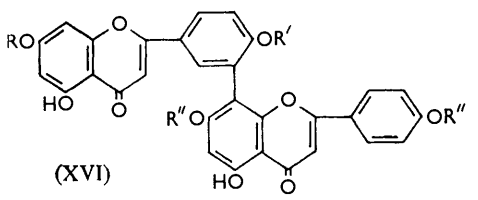
However, the structure they then proposed [18] for kayaflavone was based upon their incorrect structure for sciadopitysin. We therefore re-examined kayaflavone. It was isolated from the leaves of *Torreya nucifera* obtained from the Westonbirt arboretum through the kindness of Mr. G. Leyshon and Mr. R. F. Wood of the Forestry Commission. The identity of kayaflavone trimethyl ether with sciadopitysin trimethyl ether (VII) was confirmed, and oxidation of kayaflavone with alkaline hydrogen peroxide gave 4-methoxyisophthalic acid and *p*-anisic acid. Since kayaflavone is an isomer of sciadopitysin (Xa) it follows that it must have the structure (XVIg). This was supported by its infrared spectrum which showed one carbonyl band ($\nu_{max.}$ 1662 cm.$^{-1}$) and by the effect of base on its ultraviolet spectrum (see Table). The latter is of type (*a*), showing the presence of a 7-hydroxy-group which, in contrast with that in sciadopitysin, is relatively easily ionised; this behaviour is expected with the structure (XVIg) for kayaflavone. Kariyone and Sawada [17] obtained a ketoflavone, $C_{23}H_{13}O_4(OMe)_3$, and a diketone, $C_{18}H_{18}O_6$, by alkaline hydrolysis of kayaflavone and these compounds must be formulated as (XIVc) and (XV), respectively. This structure (XVIg) for kayaflavone is confirmed by Kawano's subsequent study [19] of the hydrolysis of its triethyl ether with aqueous barium hydroxide.

The biflavonyl, sotetsuflavone, isolated from the leaves of the cycad, *Cycas revoluta* Thunb. and from other Gymnosperms, was shown by Kariyone and Sawada [20] to have the molecular formula, $C_{30}H_{12}O_4(OH)_5{\cdot}OMe$, and pentamethylation gave ginkgetin tetramethyl ether (VII). It had already been stated [20] that the ketoflavone, $C_{23}H_{15}O_6{\cdot}OMe$, formed from sotetsuflavone was identical with the ketoflavone from ginkgetin and, having determined the structure of ginkgetin, we then proposed a structure for sotetsuflavone.[1] Later work showed that the claim [20] on which this structural proposal was based was not correct and the structure (XVIb) has now been firmly established for sotetsuflavone.[21]

We have now shown that bilobetin which accompanies ginkgetin and isoginkgetin is an isomer of sotetsuflavone. Bilobetin has the molecular formula $C_{30}H_{12}O_4(OH)_5{\cdot}OMe$ and we are grateful to Dr. R. I. Reed of Glasgow University for a mass-spectrometric determination of its molecular weight which is compatible with this formula. Bilobetin gave a penta-acetyl derivative, and a pentamethyl ether identical with ginkgetin tetramethyl ether (VII). Degradation of bilobetin with alkaline hydrogen peroxide yielded 4-methoxyisophthalic acid and *p*-hydroxybenzoic acid, thus leading directly to the structure (XVIc). A similar oxidation of sotetsuflavone (XVIb) kindly supplied by Dr. N. Kawano yielded 4-hydroxyisophthalic acid and *p*-hydroxybenzoic acid. It may be noted that the ultraviolet spectrum of bilobetin is in accord with this structure (XVIc), but it was not possible to interpret the effect of base in detail because of the many phenolic hydroxyl groups present.

[17] Kariyone and Sawada, *J. Pharm. Soc. Japan*, 1958, **78**, 1010, 1016.
[18] Kariyone, Proc. Phytochemical Symposium, Kuala Lumpur, 1957, p. 160.
[19] Kawano, *Chem. and Pharm. Bull. Japan*, 1961, **9**, 358.
[20] Kariyone and Sawada, *J. Pharm. Soc. Japan*, 1958, **78**, 1013, 1016.
[21] Kawano and Yamada, *J. Amer. Chem. Soc.*, 1960, **82**, 1505; *J. Pharm. Soc. Japan*, 1960, **80**, 1577.

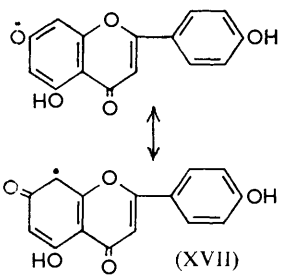
The seven known biflavonyls, which all have the same carbon–oxygen skeleton, are shown at (XVI); the list includes amentoflavone studied by Sawada.[22] The carbon–oxygen skeleton is finally supported by Nakazawa's synthesis of ginkgetin tetramethyl ether by an Ullmann reaction between 3'- and 8-iodoapigenin trimethyl ether.[23]



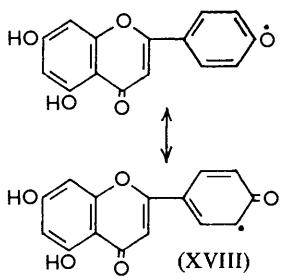
(XVI)

	R	R'	R''	R'''
(a) Amentoflavone	H	H	H	H
(b) Sotetsuflavone	H	H	Me	H
(c) Bilobetin	H	Me	H	H
(d) Ginkgetin	Me	Me	H	H
(e) Isoginkgetin	H	Me	H	Me
(f) Sciadopitysin	Me	Me	H	Me
(g) Kayaflavone	H	Me	Me	Me

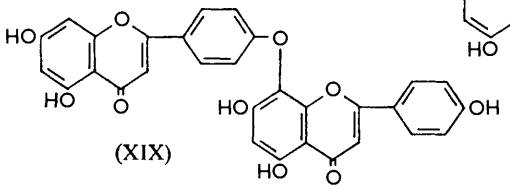
The structures (XVI) of the biflavonyls strongly support the suggestion that their biosynthesis involves the oxidative coupling of free radicals (*e.g.*, XVII and XVIII) derived either from apigenin or a closely related compound, in association with *O*-methylation. Their unsymmetrical structure does, however, suggest another possibility that they could arise as the result of an electrophilic attack of the radical (XVIII) upon apigenin or a derivative thereof at position 8. In this connection it may be noted that the radical (XVIII) might be expected to be relatively more stable than (XVII), and position 8 in apigenin is normally associated with electrophilic substitution. The positions of the *O*-methyl groups would be expected, as with the simpler flavanoids, to show some phytochemical regularity, and indeed, although the biflavonyls are widely distributed among the Gymnosperms, there is a recognisable relationship between the taxonomic classification of plants which contain biflavonyls and the types of biflavonyl present.[24] Hinokiflavone, which often occurs with biflavonyls, has been shown to be a biflavonyl ether (XIX),[25] and its formation probably also involves a related oxidative coupling leading to a biphenyl ether.



(XVII)



(XVIII)



(XIX)

The isolation of ginkgetin, isoginkgetin, and bilobetin from the leaves of *Ginkgo biloba* L. is noteworthy in view of the considerable botanical and taxonomic interest of this tree.[26,27]

[22] Quoted by Kawano, *Chem. and Ind.*, 1959, 368, and by Chang, Chen, Ueng, Choong, and Chen, *J. Formosan Sci.*, 1960, **14**, 1.
[23] Nakazawa, *Chem. and Pharm. Bull. Japan*, 1959, **7**, 748.
[24] Sawada, *J. Pharm. Soc. Japan*, 1958, **78**, 1023; Kawano, *ibid.*, 1960, **80**, 1647.
[25] Kariyone and Sawada, *J. Pharm. Soc. Japan*, 1958, **78**, 1020; Fukui and Kawano, *J. Amer. Chem. Soc.*, 1959, **81**, 6331; Kariyone and Fukui, *J. Pharm. Soc. Japan*, 1960, **80**, 746; Kawano and Fukui, *ibid.*, p. 749; Fukui, *ibid.*, pp. 752, 756.
[26] Seward, *Nature*, 1937, **139**, 741.
[27] Seward, *Sci. Progr.*, 1937, **32**, 420.

Experimental

Unless otherwise stated, infrared spectra were determined for Nujol mulls because of the low solubility of biflavonyls and their derivatives in the usual solvents.

Paper-chromatographic Methods, Solvent Mixtures, and Sprays.—Whatman No. 1 paper was used for normal chromatograms and Whatman No. 3 (chromatography grade) for thick-paper separations. The following solvent mixtures and sprays were used. BAAC solvent (top layer of 1 : 1 : 1 v/v butan-1-ol–1·5N-aqueous ammonia–5N-aqueous ammonium carbonate); EA solvent (90 : 5 : 5 v/v ethanol–15N-aqueous ammonia–water); Methyl Red spray phosphate-buffered at pH 7 (mixture, 1 : 2 v/v, of 0·1% ethanolic Methyl Red and phosphate buffer).

Preliminary Extraction of Ginkgo biloba *Leaves.*—The fallen, air-dried, yellow, autumnal leaves (1 kg.) were extracted four times at daily intervals with ether (2 l. each) at room temperature with occasional stirring. The combined extracts were filtered through cotton wool, concentrated (to 2 l.), then filtered through Celite and shaken with saturated aqueous potassium hydrogen carbonate until the washings were colourless. The ethereal solution was then extracted with aqueous 2N-potassium carbonate (5 × 200 ml.), and the yellow crystals of the potassium salt of ginkgetin which separated at the interface were added to the carbonate washings. The combined alkaline extracts were first shaken with ether and then acidified to pH 4 with 2N-hydrochloric acid at 60°. The precipitate (2·3 g.) was collected by centrifugation, washed, and dried. This material (2·0 g.) was extracted with warm ethyl acetate (2 × 75 ml.), and the extracts were shaken with aqueous potassium hydrogen carbonate and with water. Evaporation of the ethyl acetate gave a buff-coloured solid (1·5 g.) which was chromatographed on a silica-gel column (28 × 4 cm.). Elution with ethyl acetate gave a main fraction (972 mg.) which crystallised from pyridine (15 ml.) and water (10 ml.), giving a mixture *A* (543 mg.) which sintered about 195° and had m. p. about 220°.

Isolation of Ginkgetin and Isoginkgetin.—(*a*) Material *A* (1 g.) was boiled with acetone (2 l.); the mixture was filtered and concentrated to 140 ml.; after storage, crystals (324 mg.) were collected, and the mother-liquors yielded a second fraction (198 mg.). The combined materials were recrystallised from acetone, yielding yellow *isoginkgetin* (358 mg.) which after being dried at 170° melted from 210°, effervesced at 226°, resolidified at 245—257°, and had a final m. p. 353° (decomp.) [Found: C, 65·6; H, 4·35; OMe, 9·6. $C_{30}H_{16}O_8(OMe)_2,H_2O$ requires C, 65·75; H, 4·1; OMe, 10·6%].

The above acetone mother-liquors yielded a residue which was dissolved in hot aqueous 10% potassium carbonate, decanted from a little dark oil, and on storage deposited bright yellow crystals (377 mg.). These recrystallised from 10% potassium carbonate solution (16 ml.), giving the potassium salt of ginkgetin (281 mg.) as yellow octahedra. This was dissolved in warm potassium carbonate solution and poured into 2N-sulphuric acid. The precipitate was collected by centrifugation, washed, and crystallised from acetone, yielding ginkgetin as yellow platelets, m. p. 350° (decomp.). [Found: C, 67·3; H, 4·0; OMe, 10·6. Calc. for $C_{30}H_{16}O_8(OMe)_2$: C, 67·8; H, 3·9; OMe, 10·95%]. Comparison by mixed m. p. and ultraviolet and infrared spectra showed that this substance was identical with a specimen of ginkgetin isolated by Nakazawa.[7]

(*b*) Material *A* (150 mg.) was dissolved in hot 10% aqueous potassium carbonate, the solution decanted from a little oil, and the potassium salt (63 mg.) of ginkgetin which separated on storage was recrystallised as described, and finally yielded ginkgetin (33 mg.). Addition of potassium chloride to the original mother-liquors gave a precipitate. This was collected and dissolved in the minimum quantity of 10% aqueous potassium carbonate and, after removal of a precipitate (10 mg.), acidification and crystallisation from acetone gave isoginkgetin (46 mg.).

Countercurrent Examination of Material A. *Isolation of Bilobetin, Ginkgetin, and Isoginkgetin.*—A series of experiments showed that the best results were obtained by using ethyl methyl ketone and borate buffers.[28] The contents of the tubes were examined spectroscopically; fractions containing biflavonyls showed three types of ultraviolet spectra which, with the shifts produced in N/500- and N/50-ethanolic sodium hydroxide, were characteristic of ginkgetin, isoginkgetin, and bilobetin (see Table).

Material *A* (1·4 g.) in ethyl methyl ketone (50 ml.; equilibrated) was subjected to countercurrent distribution between the ketone and a borate buffer (pH 9·8); after 133 transfers the

[28] " Hydrogen Ions," by Britton, Vol. 1, p. 310 (Chapman and Hall, London, 1942).

following fractions were collected, the solids being obtained by acidification, extraction with the help of ethyl acetate, and evaporation: (1) tubes 99—95 and material discharged from last tube (504 mg.) (no biflavonyls present); (2) tubes 94—75 (191 mg.; $K = 1{\cdot}46$; ginkgetin-type spectrum); (3) tubes 74—58 (175 mg.; no peak; ginkgetin-type spectrum); (4) tubes 57—41 (255 mg.; $K = 0{\cdot}52$; isoginkgetin-type spectrum); (5) tubes 40—0 (580 mg.; no peak; bilobetin-type spectrum). Fractions (2) and (3) yielded ginkgetin (308 mg.). Fraction (5) (464 mg.) was again subjected to similar countercurrent distribution with a borate buffer (pH 9·1), yielding, after 243 transfers, fractions (6) tubes 99—74 (80 mg.; $K = 0{\cdot}47$; isoginkgetin-type spectrum); (7) tubes 73—54 (130 mg.; $K = 0{\cdot}35$; bilobetin-type spectrum); (8) tubes 53—42 (74 mg.; $K = 0{\cdot}25$; bilobetin-type spectrum); (9) tubes 41—24 (113 mg.; no peak; bilobetin-type spectrum); (10) tubes 23—0 (109 mg.), not further examined. Fraction (6) gave isoginkgetin (79 mg.). Fraction (7), whose distribution showed excellent agreement with the calculated curve, yielded a solid which was crystallised from ethanol (15 ml.) (charcoal); the first material was discarded and concentration then yielded *bilobetin* (51 mg.), yellow needles, which softened at 245—253°, resolidified about 278°, and finally melted at 320° (decomp.). After being heated at 100°/0·5 mm. for 2 hr., this compound had m. p. (rapid heating) 345—347° (Found: C, 62·3; H, 4·5; OMe, 5·7. $C_{31}H_{17}O_9{\cdot}OMe,2{\cdot}5H_2O$ requires C, 62·3; H, 4·2; OMe, 5·2%).

Several similar countercurrent distributions of material *A* (1·4 g.) were carried out; average yields were ginkgetin 310 mg., isoginkgetin 420 mg., and bilobetin 130 mg.

Isolation of Sciadopitysin and Kayaflavone.—These compounds were isolated as previously described [12,17] from the leaves of *Sciadopitys verticillata* and *Torreya nucifera*. The former gave sciadopitysin as pale yellow rods, m. p. 295—297° (decomp.), from acetone [Found: C, 68·2; H, 3·9; OMe, 14·8. Calc. for $C_{30}H_{15}O_7(OMe)_3$: C, 68·3; H, 4·2; OMe, 16·0%].

Kayaflavone was obtained as pale yellow needles, m. p. 335° (decomp.), from acetone [Found: C, 68·45; H, 4·5; OMe, 14·2. Calc. for $C_{30}H_{15}O_7(OMe)_3$: C, 68·3; H, 4·2; OMe, 16·0%].

Acetyl Derivatives of Ginkgetin, Isoginkgetin, Bilobetin, and Sciadopitysin.—Ginkgetin (25 mg.), anhydrous sodium acetate (25 mg.), and acetic anhydride (4 ml.) were heated at 100—110° for 4 hr., cooled, and poured into water. The solid was washed, dried, and recrystallised from ethyl acetate, giving the tetra-acetyl derivative of ginkgetin (28 mg.) as pale yellow, thick, rhombic plates, m. p. 265·5—266·5°, $\lambda_{max.}$ (in EtOH) 211 (ε 63,500), 248—258 infl. (ε 34,500), 317 mμ (47,000) [Found: C, 65·4; H, 4·9; OMe, 8·7; *C*-Me, 8·1; Ac, 24·4%; *M*, ebullioscopic in chloroform, Menzies–Wright,[8] 757. Calc. for $C_{30}H_{12}O_4(OMe)_2(OAc)_4$: C, 64·9; H, 4·7; OMe, 8·45; *C*-Me, 8·2; Ac, 23·5%; *M*, 735].

Isoginkgetin similarly gave its *tetra-acetyl derivative* as colourless prisms, m. p. 251—252° (from ethyl acetate), $\lambda_{max.}$ (in EtOH) 220 (ε 74,000), 250 (infl. ε 47,000), 324 mμ (ε 50,000) [Found: C, 65·2; H, 4·3; OMe, 9·0. $C_{30}H_{12}O_4(OMe)_2(OAc)_4$ requires C, 65·4; H, 4·1; OMe, 8·45%].

Bilobetin gave the *penta-acetyl derivative* as colourless prisms, m. p. 183—184° (from ethyl acetate), $\lambda_{max.}$ (in EtOH) 252 (ε 32,000), 314 mμ (ε 37,500) [Found: C, 60·2; H, 4·6; OMe, 3·6. $C_{30}H_{12}O_4(OMe)(OAc)_5,3H_2O$ requires C, 60·3; H, 4·5; OMe, 3·8%].

Sciadopitysin triacetate formed colourless prisms, m. p. 271—273°, from ethanol [Found: C, 65·7; H, 4·2; OMe, 13·05. Calc. for $C_{30}H_{12}O_4(OMe)_3(OAc)_3$: C, 66·3; H, 4·3; OMe, 13·2%].

Hydrolysis of the tetra-acetyl derivatives of ginkgetin and isoginkgetin with 0·5N-aqueous sodium hydroxide at room temperature for 24 hr. regenerated the phenolic biflavonyls.

Methylation of Ginkgetin, Isoginkgetin, Bilobetin, Sciadopitysin, and Kayaflavone.—Ginkgetin (240 mg.), anhydrous potassium carbonate (4 g.), methyl iodide (1 ml.), and dry acetone (25 ml.) were boiled for 10 hr., with further addition of methyl iodide (0·5 ml.) and potassium carbonate (2 g.) after 4 hr. and again after 8 hr. The filtered solution yielded a product (63 mg.) which was added to that (150 mg.) obtained from the solids by dissolution in water, acidification, and extraction with chloroform. Crystallisation from ethanol gave a *hydrate* of ginkgetin tetramethyl ether (191 mg.) as pale yellow crystals, m. p. 227—228° [Found: C, 66·4; H, 5·3; OMe, 24·9. $C_{30}H_{12}O_4(OMe)_6,1\frac{1}{2}H_2O$ requires C, 66·6; H, 5·1; OMe, 28·6%]. Recrystallisation from benzene gave a *benzene solvate*, m. p. 209° (with effervescence) [Found: C, 70·9; H, 5·2; OMe, 23·9. $C_{30}H_{12}O_4(OMe)_6,\frac{1}{2}C_6H_6$ requires C, 70·8; H, 5·0; OMe, 28·1%]. The hydrate, m. p. 227—228°, when heated at 190°/10^{-5} mm. for 1 day gave ginkgetin tetramethyl ether, final

m. p. 246°, $\lambda_{max.}$ (in EtOH) 212 (ε 63,000), 267 (ε 48,000), 328 mμ (ε 45,500) [Found: C, 69·3; H, 4·8; OMe, 30·0. Calc. for $C_{30}H_{12}O_4(OMe)_6$: C, 69·45; H, 4·9; OMe, 29·8%].

Similar methylation of isoginkgetin, bilobetin, sciadopitysin, and kayaflavone gave ginkgetin tetramethyl ether. Identity of the five specimens was established by m. p., mixed m. p., and ultraviolet and infrared spectra, and in the case of isoginkgetin its tetramethyl ether gave the same *X*-ray powder photograph (for which we thank Dr. T. H. Bevan) as ginkgetin tetramethyl ether.

Ginkgetin and Isoginkgetin Tetraethyl Ethers.—Ginkgetin (250 mg.), anhydrous potassium carbonate (4 g.), ethyl iodide (1 ml.), and acetone (50 ml.) were boiled for 40 hr., with additions of ethyl iodide (1 ml.) and potassium carbonate (1 g.) after 6, 24, and 30 hr. After evaporation, water (200 ml.) was added and extraction with chloroform yielded a solution which was filtered through alumina and then chromatographed on silica gel. The major chloroform eluate yielded ginkgetin tetraethyl ether as a *monohydrate* (322 mg.) as pale yellow needles, m. p. 237—239°, from ethanol (lit.,[7] m. p. 175°) (Found: C, 68·4; H, 5·8. $C_{40}H_{38}O_{10},H_2O$ requires C, 68·9; H, 5·8%). Dehydration was effected at 195°/10^{-5} mm. for 200 hr. (Found: C, 71·0; H, 5·7. Calc. for $C_{40}H_{38}O_{10}$: C, 70·8; H, 5·6%)

Similar ethylation of isoginkgetin gave *isoginkgetin tetraethyl ether monohydrate* as pale yellow needles (from ethanol), m. p. 242—244° (Found, in material heated at 195°/10^{-5} mm. for 200 hr.: C, 68·6; H, 5·8. $C_{40}H_{38}O_{10},H_2O$ requires C, 68·9; H, 5·8%).

5-Ethoxy-7,4′-dimethoxyflavone and Acacetin Diethyl Ether.—Ethylation of 5-hydroxy-7,4′-dimethoxyflavone [29] (300 mg.) as in the preceding experiment gave *5-ethoxy-7,4′-dimethoxyflavone* (240 mg.) as colourless needles, m. p. 162° (from ethanol) (Found: C, 69·5; H, 5·6. $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·6%). Acacetin diethyl ether, colourless needles m. p. 196° (from ethanol), was prepared similarly (Found: C, 70·7; H, 6·2. Calc. for $C_{20}H_{20}O_5$: C, 70·6; H, 5·9%) (lit.,[30] m. p. 194°).

Demethylation of Ginkgetin and Isoginkgetin.—Isoginkgetin (60 mg.), glacial acetic acid (6 ml.), and 48% hydrobromic acid (6 ml.) were boiled for 12 hr. and then poured into water. The precipitate was collected, dissolved in ethyl acetate, shaken with water, and recovered, giving a product which was then heated with acetic anhydride (8 ml.) and anhydrous sodium acetate (0·5 g.) at 105—110° for 4 hr. Crystallisation from ethyl acetate gave the hexa-acetate (27 mg.) as colourless needles, m. p. 242·5—244·5°, $\lambda_{max.}$ (in EtOH) 256 (ε 37,400) and 303 mμ (ε 42,500) (lit.,[7] m. p. for the hexa-acetyl derivative of demethylated ginkgetin, 239—240°).

Similar demethylation of ginkgetin and acetylation gave the same hexa-acetate, identified by m. p., mixed m. p., and infrared spectrum. Methylation as described above of demethylginkgetin gave ginkgetin tetramethyl ether.

Oxidation of Ginkgetin Tetramethyl Ether with Alkaline Hydrogen Peroxide.—40% Aqueous potassium hydroxide (40 ml.), 30% hydrogen peroxide (9 ml.), and a solution of ginkgetin tetramethyl ether (420 mg.) in ethanol (60 ml.) were stirred at room tmeperature for 50 hr., more hydrogen peroxide (9 ml.) being added after 10 hr. and again after 20 hr. The excess of peroxide was then removed by addition of a little manganese dioxide and, after acidification with dilute sulphuric acid, the solution was extracted continuously with ether. The extract yielded a solid (380 mg.) giving neutral (93 mg.) and acidic (260 mg.) fractions on extraction of its solution in chloroform with 10% aqueous potassium hydrogen carbonate, acidification, and again continuous extraction with ether. The acidic product was chromatographed on Whatman No. 3 paper buffered with 0·1M-sodium borate [31] and eluted with butan-1-ol saturated with water. The main bands (R_F 0·31, 0·15, and ~0·0) were located by spraying strips of the dried paper with (*a*) diazotised *p*-nitroaniline followed by 10% aqueous sodium carbonate,[31] (*b*) 2% aqueous ferric chloride, (*c*) phosphate-buffered (pH 7) Methyl Red spray, and the paper associated with each band was cut out. The material of R_F 0·31 was extracted with hot ethanol, yielding a solid which was chromatographed on silica gel and eluted with benzene–chloroform (3 : 2 v/v). This gave *p*-anisic acid (82 mg.), m. p. and mixed m. p. 180—182° (from water); it was identified by its infrared spectrum.

The material of R_F 0·15 was similarly extracted and chromatographed on silica gel. Elution with benzene and crystallisation of the main fraction from ethanol yielded 2-hydroxy-4,6-dimethoxybenzoic acid (37 mg.). This was identified by m. p. and mixed m. p. 160—162°,

[29] Czajkowski, Kostanecki, and Tambor, *Ber.*, 1900, **33**, 1994.
[30] Nakazawa, *J. Pharm. Soc. Japan*, 1941, **61**, 182.
[31] Wachtmeister, *Acta Chem. Scand.*, 1951, **5**, 976; Swain, *Biochem. J.*, 1953, **53**, 200.

and by comparison of its infrared spectrum and chromatographic behaviour with an authentic specimen obtained by oxidation of apigenin trimethyl ether (see below).

The paper bearing the material having R_F ~0 was treated with dilute sulphuric acid and extracted with hot ethyl acetate, and the product (84 mg.) recrystallised from aqueous ethanol, yielding *2-hydroxy-4,6,6'-trimethoxybiphenyl-3,3'-dicarboxylic acid* as prisms, m. p. 240—241·5°, $\lambda_{max.}$ (in EtOH) 222, 247, 253—256 (infl.) and 299 mμ (ε 26,700, 27,100, 26,800, and 3900, respectively), $\lambda_{max.}$ (N/500-NaOH–EtOH) 222, 239, and 297 mμ (ε 31,400, 27,800, and 3400, respectively), $\nu_{max.}$ 1680 (aromatic CO_2H) and 1640 cm.$^{-1}$ (*o*-hydroxy-aromatic acid CO_2H) [Found: C, 58·6; H, 4·75; OMe, 25·9. $C_{14}H_7O_5(OMe)_3$ requires C, 58·6; H, 4·6; OMe, 26·7%]. Potentiometric titration with N/20-sodium hydroxide in 50% aqueous ethanol gave apparent pK_a values of 5·3 and 6·8 [Found: equiv., 171·5. $C_{15}H_{14}O_4(CO_2H)_2$ requires equiv., 174].

Oxidation of Apigenin Trimethyl Ether with Alkaline Hydrogen Peroxide.—Apigenin trimethyl ether (100 mg.) was oxidised and the acidic products were isolated and separated chromatographically as described in the case of ginkgetin tetramethyl ether. There were obtained *p*-anisic acid (12 mg.), m. p. 180—182°, and 2-hydroxy-4,6-dimethoxybenzoic acid (14 mg.), m. p. 160—162°.

4-Hydroxyisophthalic Acid.—4-Methoxyisophthalic acid (230 mg.) and freshly distilled pyridine hydrochloride (600 mg.) were heated at 200° for 5 min., then cooled, water (5 ml.) was added, and the mixture acidified by 12N-sulphuric acid. After being kept overnight at 0°, the precipitate was collected and crystallised from water (charcoal), giving 4-hydroxyisophthalic acid (136 mg.) as needles, m. p. 311° (decomp.) (lit.,[32] m. p. 310°). It gave a strong purple colour with ethanolic ferric chloride.

Oxidation of Ginkgetin, Isoginkgetin, Bilobetin, Sotetsuflavone, Sciadopitysin, and Kayaflavone with Alkaline Hydrogen Peroxide.—30% Hydrogen peroxide (10 ml.) was added to a stirred solution of ginkgetin (100 mg.) in 30% aqueous potassium hydroxide (10 ml.) at room temperature. Further quantities of hydrogen peroxide (5 × 10 ml.) were added after 1, 3, 5, 20, and 22 hr. After a total period of 24 hr., the acidic material (48 mg.) was isolated and separated by paper chromatography as described for the degradative oxidation of ginkgetin tetramethyl ether, except that EA solvent was used and the acids were then located by ferric chloride and Methyl Red sprays and were extracted with ethanol. The acid of R_F 0·41 was sublimed (170°/0·1 mm.) and crystallised from water, giving *p*-hydroxybenzoic acid (16 mg.), m. p. and mixed m. p. 214—215°. The other acid, having R_F 0·14, was crystallised from water, giving 4-methoxyisophthalic acid (15 mg.), m. p. and mixed m. p. 278—279°. The acids were also shown to be identical with authentic specimens by their chromatographic behaviour and infrared spectra.

Similar oxidation of isoginkgetin, sciadopitysin, and kayaflavone yielded *p*-anisic acid, m. p. 183—184°, and 4-methoxyisophthalic acid, m. p. 278—279°, identified with authentic specimens by mixed m. p., chromatographic behaviour (EA solvent, *p*-anisic acid, R_F 0·54, and 4-methoxyisophthalic acid, R_F 0·15) and infrared spectra. Oxidation of bilobetin gave 4-methoxyisophthalic acid and *p*-hydroxybenzoic acid, whereas sotetsuflavone gave 4-hydroxyisophthalic acid (R_F 0·24; purple colour with ferric chloride) and *p*-hydroxybenzoic acid. Chromatographic comparisons were also made with the BAAC mixture, giving the R_F values: *p*-hydroxybenzoic acid, 0·19; *p*-methoxybenzoic acid, 0·41; 4-hydroxyisophthalic acid, 0·04; and 4-methoxyisophthalic acid, 0·01.

Alkaline Hydrolysis of Ginkgetin.—Ginkgetin (200 mg.) was boiled with 30% aqueous potassium hydroxide (4 ml.) for 1 hr. in a nitrogen atmosphere, diluted, and acidified with 10% sulphuric acid. The precipitate (120 mg.) was collected and the aqueous filtrate extracted continuously with ether. The precipitate was crystallised from ethanol, giving the ketoflavone (XIVa) (39 mg.), m. p. 287·5—288·5° after drying at 100°/10^{-5} mm. for 9 hr. and at 185°/10^{-5} mm. for 36 hr., $\nu_{max.}$ 3260 (OH), 1658 (conjugated CO), and 1645 cm.$^{-1}$ (chelated conjugated CO) (Found: C, 68·6; H, 4·6; OMe, 7·8. Calc. for $C_{23}H_{15}O_6$·OMe: C, 68·9; H, 4·3; OMe, 7·4%). The ether extract yielded an oil (72 mg.), and paper chromatography with BAAC solvent showed a component (R_F 0·78) having a ferric chloride reaction identical with that of 2,6-dihydroxy-4-methoxyacetophenone (R_F 0·78). Neither distillation nor chromatography yielded a crystalline product, but with Brady's reagent the oil gave 4-hydroxyacetophenone

[32] Fosdick and Fancher, *J. Amer. Chem. Soc.*, 1941, **63**, 1277; Hunt, Jones, and Lindsey, *J.*, 1956, 3099.

2,4-dinitrophenylhydrazone, identified by m. p. and mixed m. p. 264—265°, and infrared spectra. 2,6-Dihydroxy-4-methoxyacetophenone (R_F 0·78) and 4-hydroxyacetophenone (R_F 0·77) are not separated by using the BAAC solvent.

Alkaline Hydrolysis of Sciadopitysin and Isoginkgetin.—A similar hydrolysis of sciadopitysin (200 mg.) gave a precipitate (122 mg.) after acidification, which was dissolved in chloroform and shaken with aqueous potassium hydrogen carbonate. Chromatography of the chloroform-soluble material on silica gel gave a main fraction (94 mg.) on elution with chloroform. This was fractionally crystallised from acetone, giving first sciadopitysin (8 mg.), m. p. 287—293°, and concentration and addition of ether gave the ketoflavone (XIVb) (44 mg.), pale yellow rhombs (from acetone), m. p. 267—268°, $\nu_{max.}$ ~3300 (no distinctive absorption), 1675 (conjugated CO), 1650 cm.$^{-1}$ (chelated conjugated CO) [Found: C, 68·9; H, 4·6; OMe, 13·95. Calc. for $C_{23}H_{14}O_5(OMe)_2$: C, 69·4; H, 4·7; OMe, 14·35%].

A similar alkaline hydrolysis of isoginkgetin (200 mg.) gave the same ketoflavone (XIVb) (23 mg.), identified by m. p., mixed m. p., and infrared spectra.

Paper-chromatographic Behaviour of Some Hydroxyacetophenones.—The following ketones may be separated on Whatman No. 1 paper; they give the indicated R_F values in BAAC and EA solvent mixtures, respectively: 4-hydroxy-, 0·77, ~1; 4-methoxy-, 0·84, ~1; 2,4,6-trihydroxy-, 0·63, 0·40; 2,6-dihydroxy-4-methoxy-, 0·78, 0·66; and 2-hydroxy-4,6-dimethoxyacetophenone, 0·86, 0·80. The ketones all appear as dark spots in ultraviolet light, and the *o*-hydroxy-ketones give dark spots with 2% ethanolic ferric chloride, and red spots with phosphate-buffered (pH 7) Methyl Red. With Brady's reagent as spray, the simpler ketones rapidly give orange-red spots, whereas the *o*-hydroxy-ketones give orange spots relatively slowly.

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