# [2',2']-(+)-Catechin-(+)-taxifolin from Commercial Willow Bark: Structure, Bonding Positions and Oxidative Cleavage

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[2',2']-(+)-Catechin-(+)-taxifolin, a novel biflavonoid from commercial willow bark (*Salix* spp.), is characterized by spectrometric methods. The bonding positions are established by nuclear Overhauser effect difference spectroscopy of the methyl ether acetates of the bi-(+)-taxifolin analogues, which represent products of oxidation of the methylene function of the (+)-catechin moiety and which undergo methylene insertion reactions with diazomethane. Photoinduced oxidative cleavage of the biphenyl-type interflavonoid linkage involving singlet oxygen, accompanied by intermolecular transfer of an acetyl group of the peracetate, yielding a range of flavan-3-ols and dihydroflavonols, provides the first example of this type of biflavonoid photofragmentation.

Apart from biflavanoids of black [1] and green [2] tea possessing an interflavanoid linkage between the two B-rings of flavan units this type of natural C–C bonding is atypical, the biphenyl moiety being commonly found only in biflavonoids [3] and hydrolysable tannins.[4] The range of natural biflavonoids is now extended by the isolation of novel B-ring, B-ring coupled analogues from commercial willow bark (*Salix* spp.). Reinvestigation of the ethyl acetate-soluble portion of the bark extract, comprising a mixture of flavonoids, condensed tannins, and phenolic glycosides,[5–9] revealed a novel biflavonoid as second representative [10] of a flavano-flavonol biflavonoid, but the first in which the monomeric units are linked through a carbon-carbon bond at the B-rings.

Evidence for the first natural biflavonoid (**1**) with different monomeric constituent units (flavan and dihydroflavonol moieties, respectively) coupled *via* B-rings, reported in a preliminary communication,[11] was obtained by acetylation of the appropriate fraction from chromatography on Sephadex LH-20. The free phenol was accompanied by several flavonoids and (+)-catechin (**5**), the latter serving as precursor of associated condensed tannins. Isolation of the acetate (**2**) led to its characterization as a derivative of a [2',2']-(+)-catechin-(+)-taxifolin biflavonoid.

Analysis of the 300 MHz $^1$H n.m.r. spectrum of the acetate derivative (**2**) revealed *meta*-coupled doublets (A-ring) at δ 6.66 and 6.60 (*J* 2.2 Hz) in addition to the characteristic ABXY system of the catechin moiety [3-H(C), m, δ 5.20; 2-H(C), d, δ5.11, *J* 6.5 Hz; 4-$H_2$(C), dd, δ 2.66 and 2.91, *J* 6.5 and 5.0 Hz; *cf.* Table 1]. The presence of a second aromatic spin-system was evident from resonances at δ 6.61 (d, *J* 2.0 Hz) and 6.81 (d, *J* 2.0 Hz), while the appearance of two doublets at δ 5.43 and 5.60 with coupling constants (*J* 12.0 Hz) indicated a 2,3-*trans* stereochemistry of a flavanonol unit. Chemical shifts of the signals in the $^1$H n.m.r. spectrum of (**2**) were in line with those of the acetates of the reference compounds (+)-taxifolin (**4**) and (+)-catechin (**6**) except for resonances attributable to B-ring protons which appeared as sharp singlets at δ 7.11 and 7.24 in contrast to the expected well-defined ABC systems of the model compounds. The presence of a 1:1 mixture, however, could be excluded since the two-proton singlets (δ 7.11 and 7.24) were replaced, as expected, in a mixture containing both these phenols (**4**) and (**6**) by two spin-systems which arise from 3',4'-*ortho* disubstituted B-rings. Additionally, the acetates (**2**), (**4**) and (**6**) were differentiated by their $R_F$ values. The remarkably high relative mobility in benzene–acetone (9:1) as solvent system ($R_F$ 0.42, 0.51, and 0.46, respectively) contrasted with known large $R_F$ differences between monomeric and dimeric flavan-3-ols.[12]

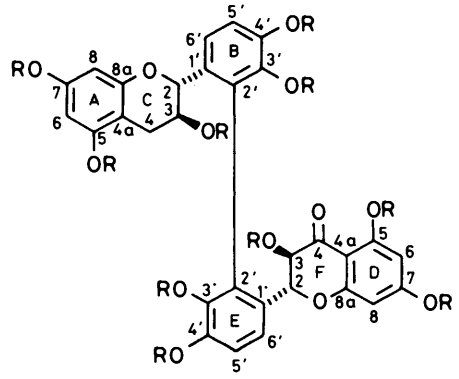
Thus, the main interest centred around the arrangement of ring B substituents and an assessment of the possible points of bonding between the aforementioned derivatives (**4**) and (**6**). $^1$H N.m.r. data indicated a possible [6',6']-interflavonoid linkage considering the two lowfield singlets at δ 7·11 and 7·24 in the $^1$H n.m.r. spectrum of (**2**) observed in a variety of solvent systems, presumably indicative of *para*-couplings. However, the magnitudes of $^1$H n.m.r. coupling constants do not provide unambiguous information as regards the placing of protons on the equivalent B-rings. Both *para* and *ortho* arrangements have to be taken in account, considering recent observations [13] on (+)-mesquitol and its oligomers where in some derivatives $J_{5',6'}$ appeared as a singlet due to their relative chemical shifts $\Delta\delta = 0$ while in others the expected coupling constant $J_{5',6'}$ *ca.* 8.5 Hz was observed.

Mass spectral analysis of the acetate (**2**) gave no decisive information regarding the molecular constitution. Thus, the e.i.m.s. displayed the ion of highest mass at *m/z* 575 (2.2%) and the f.d.m.s. at *m/z* 649 (10%) [*vs.* the expected $(M)^+$ *m/z* 1012] which cannot be accommodated in terms of sequential loss of substituents, whilst the low relative abundance of these ions and other fragments in the high mass range reflect the instability of the molecule under the conditions of ionization. The f.a.b. technique provided successful molecular weight information, showing the $(M + 1)^+$ peak at *m/z* 1013. Elemental analysis was in complete agreement with the expected formula of $C_{50}H_{44}O_{23}$ for compound (**2**).

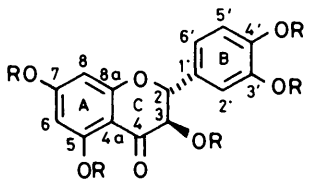
Proof of structure (**2**) was further attempted by $^{13}$C n.m.r. analysis revealing again the close structural resemblance with the monomeric constituents in question (*cf.* Table 2). Apart from the presence of expected resonances attributable to the carbons of rings B and E, residual signals compared favourably with those of (**2**). However, comparison of the B- and E-ring signals of (**2**) with the equivalents of (**4**) and (**6**) did not permit allocation of the points of attachment of the interflavonoid bond of (**2**) [14–17] on account of lack of suitable reference compounds.

However, further evidence regarding the biphenyl-type bonding between the flavan units was available from the autodegradation products (**7**)—(**10**) of the acetylated biflavonoid (**2**) which resulted during storage (*ca.* 4 weeks); behaviour which is at variance with the relative stability of most biphenyl units.

Application of thin layer chromatography (t.l.c.) resulted in the isolation of two groups of autodegradation products which



(1) R = H
(2) R = Ac



(3) R = H
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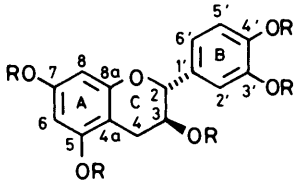
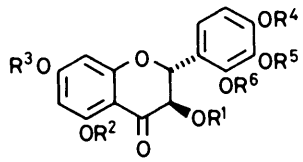
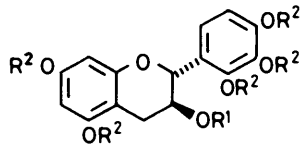


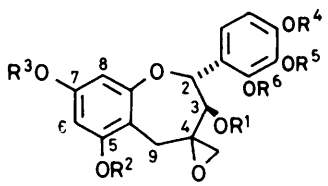
(5) R = H
(6) R = Ac



(7) $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = Ac$
(8) $R^2 = R^6 = H$, $R^1 = R^3 = R^4 = R^5 = Ac$
(9) $R^3 = H$, $R^1 = R^2 = R^4 = R^5 = R^6 = Ac$



(10) $R^1 = R^2 = Ac$
(12) $R^1 = Ac$, $R^2 = Me$



(11) $R^1 = Ac$, $R^2 = R^3 = R^4 = R^5 = R^6 = Me$

**Table 1.** $^1H$ N.m.r. data for the acetates of the biflavonoid (**2**) and the reference compounds (+)-catechin (**6**) and (+)-taxifolin (**4**) ($CDCl_3$, $SiMe_4$ as internal standard, coupling constants in Hz)

| H | (2) | (6) | (4) |
|---|---|---|---|
| 'Upper' unit | | | |
| 2-H (C) | 5.11 d (*J* 6.5) | 5.14 d (*J* 6.5) | |
| 3-H (C) | 5.20 m | 5.25 m | |
| 4-H (C) | 2.66 dd (*J* 6.5) | 2.65 dd (*J* 6.5) | |
| | 2.91 dd (*J* 5.0) | 2.86 dd (*J* 5.0) | |
| 6-H (A) | 6.60 d (*J* 2.2) | 6.59 d (*J* 2.2) | |
| 8-H (A) | 6.66 d (*J* 2.2) | 6.65 d (*J* 2.2) | |
| Ring B | 7.11 s (2 × H) | 7.16 t | |
| | | 7.20 s | |
| | | 7.25 dd | |
| 'Lower' unit | | | |
| 2-H (F) | 5.43 d (*J* 12.0) | | 5.43 d (*J* 12.0) |
| 3-H (F) | 5.60 d (*J* 12.0) | | 5.65 d (*J* 12.0) |
| 6-H (D) | 6.80 d (*J* 2.0) | | 6.79 d (*J* 2.0) |
| 8-H (D) | 6.61 d (*J* 2.0) | | 6.60 d (*J* 2.0) |
| Ring E | 7.24 s (2 × H) | | 7.25 s |
| | | | 7.29 m |
| | | | 7.39 dd |

**Table 2.** $^{13}C$ N.m.r. data for the acetates of the biflavonoid (2) and the reference compounds (**6**) and (**4**) ($CDCl_3$, $SiMe_4$ as internal standard)

| C | (2) | (6) | (4) |
|---|---|---|---|
| 'Upper' unit | | | |
| C-2 | 77.34 | 77.42 | |
| C-3 | 68.15 | 68.14 | |
| C-4 | 24.00 | 23.78 | |
| C-4a | 110.08 | 110.07 | |
| C-5 | 149.29* | 149.28* | |
| C-6 | 108.78 | 108.64 | |
| C-7 | 149.75* | 149.72* | |
| C-8 | 107.58 | 107.55 | |
| C-8a | 154.10 | 154.23 | |
| C-1' | 135.71 | 135.99 | |
| C-2' | 134.48 | 121.63 | |
| C-3' | 143.40* | 141.95 | |
| C-4' | 143.38* | 141.95 | |
| C-5' | 118.77* | 123.55 | |
| C-6' | 119.59* | 124.27 | |
| 'Lower' unit | | | |
| C-2 | 79.89 | | 80.17 |
| C-3 | 73.01 | | 72.99 |
| C-4 | 184.75 | | 184.75 |
| C-4a | 110.45 | | 110.43 |
| C-5 | 151.30 | | 151.25 |
| C-6 | 111.45 | | 111.29 |
| C-7 | 161.97 | | 162.09 |
| C-8 | 108.92 | | 108.85 |
| C-8a | 156.35 | | 156.28 |
| C-1' | 133.10 | | 133.39 |
| C-2' | 135.20 | | 122.66 |
| C-3' | 143.40* | | 141.95* |
| C-4' | 143.38* | | 142.75* |
| C-5' | 118.77* | | 123.65 |
| C-6' | 119.59* | | 125.18 |

* Assignments may be interchanged.

may be described as 'taxifolin-type' (**7**)—(**9**) and 'catechin-type' (**10**) products, as shown by $^1H$ n.m.r. spectroscopy. Similarity of the latter with (+)-catechin penta-acetate readily followed from the characteristic splitting-pattern of heterocyclic and aromatic A-ring proton resonances and from identity of their chemical shifts. Analysis of the $^1H$ n.m.r. spectra revealed the presence of six acetoxy resonances in addition to a two-proton lowfield aromatic singlet (δ 7.09) similar to that observed in the $^1H$ n.m.r. spectrum of (**2**), thus defining (**10**) as a hexa-acetate derivative of a flavan-3-ol.

The 'taxifolin-type' products (7)—(9) were similarly identified as hexa-acetate, monohydroxy-penta-acetate, and dihydroxy-tetra-acetate derivatives, based on spectroscopic and chemical studies. A notable feature of each $^1$H n.m.r. spectrum was the lowfield two-proton aromatic singlet at *ca.* δ 7·25, indicative of similar arrangement of ring B substituents in each instance. The acetyl region exhibited one aliphatic acetoxy signal, but differentiation between (7)—(9) was possible from the number of aromatic acetoxy resonances. Comparison of chemical shifts of acetoxy resonances of taxifolin penta-acetate and the hexa-acetate (7) indicated the presence of the additional acetyl group to be located on ring B, considering the well-defined AB system attributable to ring A. The free phenolic hydroxy functions in (8) and (9) could be placed on a similar basis. Lack of the typical C-5 acetoxy signal[18] (δ 2.38) in the $^1$H n.m.r. spectrum of (8) is indicative of one free phenolic hydroxy group on ring A, supported by the relative upfield position of the *meta*-coupled ring A protons (δ 6.40 and 6.33) relative to the corresponding signals of (7) (δ 6.80 and 6.61). The second hydroxy group may be placed on ring B as concluded from comparison of chemical shifts of aromatic A-ring protons and acetoxy resonances with the related product (9). The highfield two-proton aromatic singlet at δ 6.25 (ring A) of the monohydroxypenta-acetate analogue (9) is significant in indicating the position of the free hydroxy group at C-7. Conversion of (8) and (9) into the same hexa-acetate (7) on treatment with acetic anhydride–pyridine confirmed the respective presence of two and one free phenolic functions in (8) and (9).

Conjecture regarding the introduction of the acetyl groups found support in the mass spectra of (8) and (9), exhibiting the molecular ions at *m/z* 488 and 530, respectively, in complete agreement with the proposed structures, the flavonoid skeleton being substantiated by RDA fragmentation preceded by the loss of ketene and acetoxy radicals in each instance. Similar initial fragmentation sequences were evident for the 'catechin-type' analogue (10) displaying the molecular ion at *m/z* 558.

Significantly, the series of degradation products was extended by the characterization of one other dihydroflavonol (11), the product of methylene insertion reactions, and flavan-3-ol (12) analogue, respectively, obtained on methylation of the free phenolic 'biflavonoid' fraction and subsequent acetylation of the resultant methyl ethers. The products (11) and (12) assisted in confirming the above conclusions in terms of the arrangement of B-ring substituents. The structural relationships between (11) and the 'taxifolin' derivatives (7)—(9) were self-evident on the basis of $^1$H n.m.r. features. However, treatment of (11) with diazomethane resulted in the expansion of the heterocyclic ring system as evinced by methylene signals at δ 3.04 and 3.58 (AB quartet, C-ring, $J_{AB}$ 14.0 Hz) and 2.49 and 2.71 (AB quartet, oxirane ring, $J_{AB}$ 6.0 Hz) in $C_6D_6$; behaviour which is consistent with similar observations of the biflavonoids (13) and (14) (see later). Confirmation of the proposed structure (11) was also provided by mass spectral analysis [*m/z* 460 ($M^+$)]. Although chemical shifts of aromatic and heterocyclic protons of the acetates of (7) and (10) in $CDCl_3$ and $C_6D_6$ agreed well with the corresponding derivatives of (+)-ampelopsin and (+)-gallocatechin, respectively, differentiation with respect to the pyrogallol-type B-ring substitution pattern is permitted by solvent-induced chemical shifts of methoxy resonances of (11) and (12) in $C_6D_6$ showing that only one methoxy group was *ortho* to an aromatic proton in each instance,[19-23] thus eliminating also the alternative 2',4',5'-arrangement of substituents.

The $^1$H n.m.r. spectrum of (12) was significant in that it indicated duplication of resonances attributable to the aliphatic 3-OAc (δ 1.96 and 1.97) and one B-ring methoxy function (δ 3.86 and 3.87) due to steric hindrance. Temperature elevation (100 °C, $CDCl_3$) led to sharpening of the former signals and coalescence of the latter. However, broadening of the 6'-H signal at δ6.91 at increased temperatures (100 °C) coupled with persistent duplication of the 3-OAc resonance indicated severe steric inhibition of rotation even under these experimental conditions. From the foregoing collective evidence the structures of (7)—(10) were established as indicated.

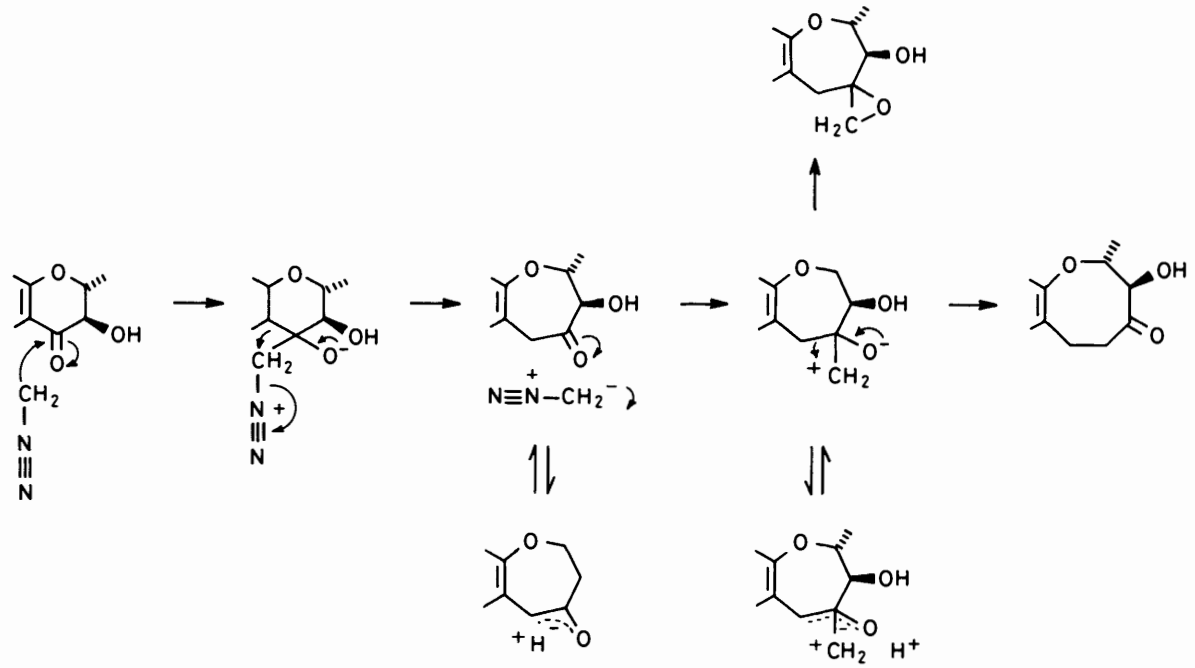
In order to provide direct evidence in terms of the arrangement of B-ring substituents and hence bonding points between the monomeric flavan units of (2) it was now essential to form a methylated derivative of (1) in order to apply the crucial n.O.e. difference spectrometric method, supported by solvent-induced shifts of methoxy functions. Thus, reaction of enriched fractions of (1) with diazomethane afforded a multicomponent mixture of products from which the novel bi-(+)-taxifolin analogues (13) and (14) were isolated as the major products by chromatographic techniques. The presence of $\Delta^7$-oxocin-4-one (13) and $\Delta^6$-oxepine-4-spiro-oxirane (14) ring systems instead of dihydropyranones was obviously due to methylene insertions[24] in (+)-taxifolin units by nucleophilic addition of diazomethane to the 4-carbonyl group (*cf.* Scheme 1). This side-reaction, similar to the expansion of the chromanone ring system of crombeone,[25] is due to the high migratory aptitude of the phloroglucinol (A-ring) unit once the well-known attack by diazomethane at the 4-carbonyl function has occurred, thus promoting 1,2-aryl migration.

Insertion of a second methylene function implies migration by a benzylic group *via* a similar 1,2-shift mechanism after further attack by diazomethane at the 4-carbonyl to give $\Delta^7$-oxocin-4-ones or cyclization to $\Delta^6$-oxepine-4-spiro-oxirane ring systems.

The suggested mechanism of formal ring expansion (*cf.* Scheme 1) leading to the enlarged eight-membered ring contrasts with the previous proposal of successive methylene additions prior to a concerted 1,3-migration by the phloroglucinol type A-ring.[26] The methyl ether acetates (13) and (14) represent the first reported examples of biflavonoids originating from sequential methylene ring-insertion reactions on addition of diazomethane.

Evidence for the structure of (13) was obtained by a combination of $^1$H n.m.r. techniques, involving extensive spin-decoupling experiments, application of n.O.e. difference spectrometry, and the advantage of solvent-induced chemical-shift effects, while mass spectrometry proved to be unsuccessful. Thus, eight methoxy and two acetoxy functions were clearly defined in the $CDCl_3$ spectrum of (13) in agreement with the expected structure. Determination of protons of the heterocyclic ring systems C and F by spin-decouplings permitted assignment of B- and E-ring resonances to constituent units. Irradiation at the frequency of 2-H of the respective C- and F-rings [δ 4.48(C) and 5.20(F)] collapsed not only the 3-H signals [δ 5.30 and 5.06 for C and F, respectively], but sharpened the lowfield aromatic singlet at δ 6.72 in the former and at δ 6.75 in the latter experiment as a result of relief from benzylic coupling. The *trans*-diaxial stereochemistry of 2-H and 3-H of C- and F-rings clearly followed from their common AB-quartets with coupling constants of $J_{2,3}$ 9.0(C) and 8.5(F) Hz, respectively. Similarly, spin-decoupling in the aromatic region confirmed the presence of two pairs of *meta*-coupled protons (*J* 2.2 Hz), represented by AB-systems at δ 5.96—6.25. These resonances were assigned to the aromatic A- and D-ring protons but with reversal in chemical shift of 8-H (upfield) and 6-H (downfield)[27] relative to corresponding shifts in the phloroglucinol ring system in flavan-3-ols. The methylene region (δ 2.50—3.45) of the $^1$H n.m.r. spectra of (13) was exceedingly complex due to the overlap of resonances of four $CH_2$-functions.

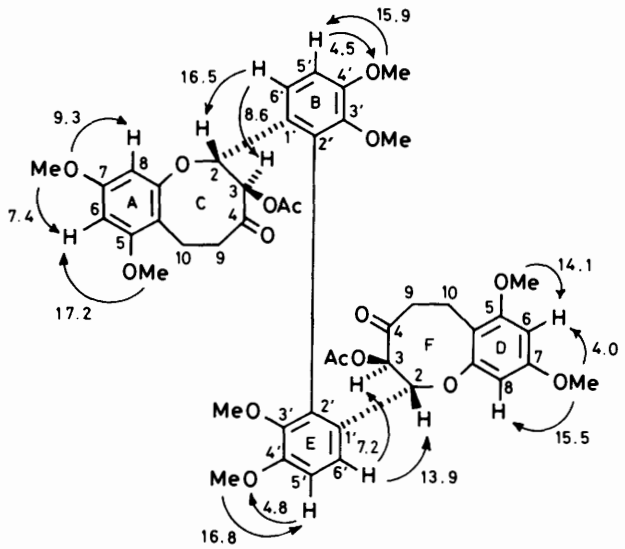
The points of bonding followed directly from n.O.e. difference experiments establishing unequivocally the structure of (13) as a [2',2'] -bi-(+)-taxifolin derivative. The n.O.e. difference spectra



**Scheme 1.** Mechanism of heterocyclic ring expansion on addition of diazomethane leading to $\Delta^7$-oxocin-4-one and $\Delta^6$-oxepine-4-spiro elements

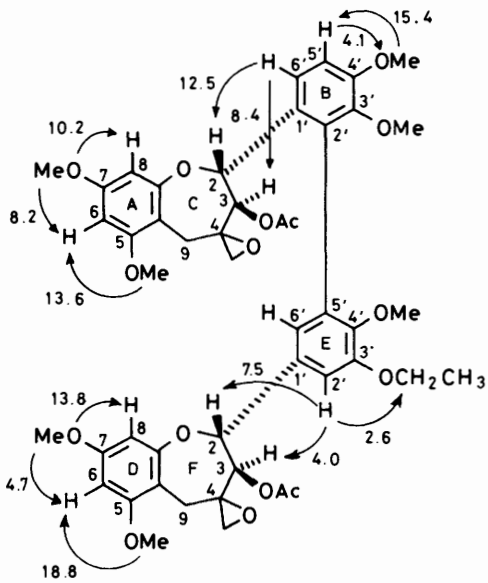
of (**13**) (*cf.* Figure 1) revealed significant interactions between 6′-H(B), 2-H and 3-H(C) on the one hand and between 6′-H(E), 2-H and 3-H(F) on the other. Association of the 5-OMe resonances with those of the 6-H, and 7-OMe with both 6-H and 8-H of the respective aromatic rings A and D indicated that A- and D-ring methoxy resonances were always located to highfield relative to those of the B- and E-rings. Finally, solvent-induced chemical shifts of methoxy resonances in $C_6D_6$ showed that two of the group of 4′- and 3′-OMe (B and E) signals remain static, relative to their shifts in $CDCl_3$, hence eliminating the alternative [6′,6′]-linkage.

Noteworthy, is the chemical-shift difference $\Delta\delta_{5'-H,6'-H}$ which proved to be zero in the B and E rings, respectively, shown by the appearance of lowfield singlets at δ 6.72 and 6.75 in the $^1$H n.m.r. spectrum of (**13**). The similarities with respect to resonances of B-ring protons between (**13**) and the related



(**13**)

**Figure 1.** Connectivities (%) established by n.O.e. difference experiments of the biflavonoid (**13**)*



(**14**)

**Figure 2.** Connectivities (%) established by n.O.e. difference experiments of the biflavonoid (**14**)*

* A and D Ring n.O.e. effects are interchangeable since there are no connectivities between them and BC and EF respectively.

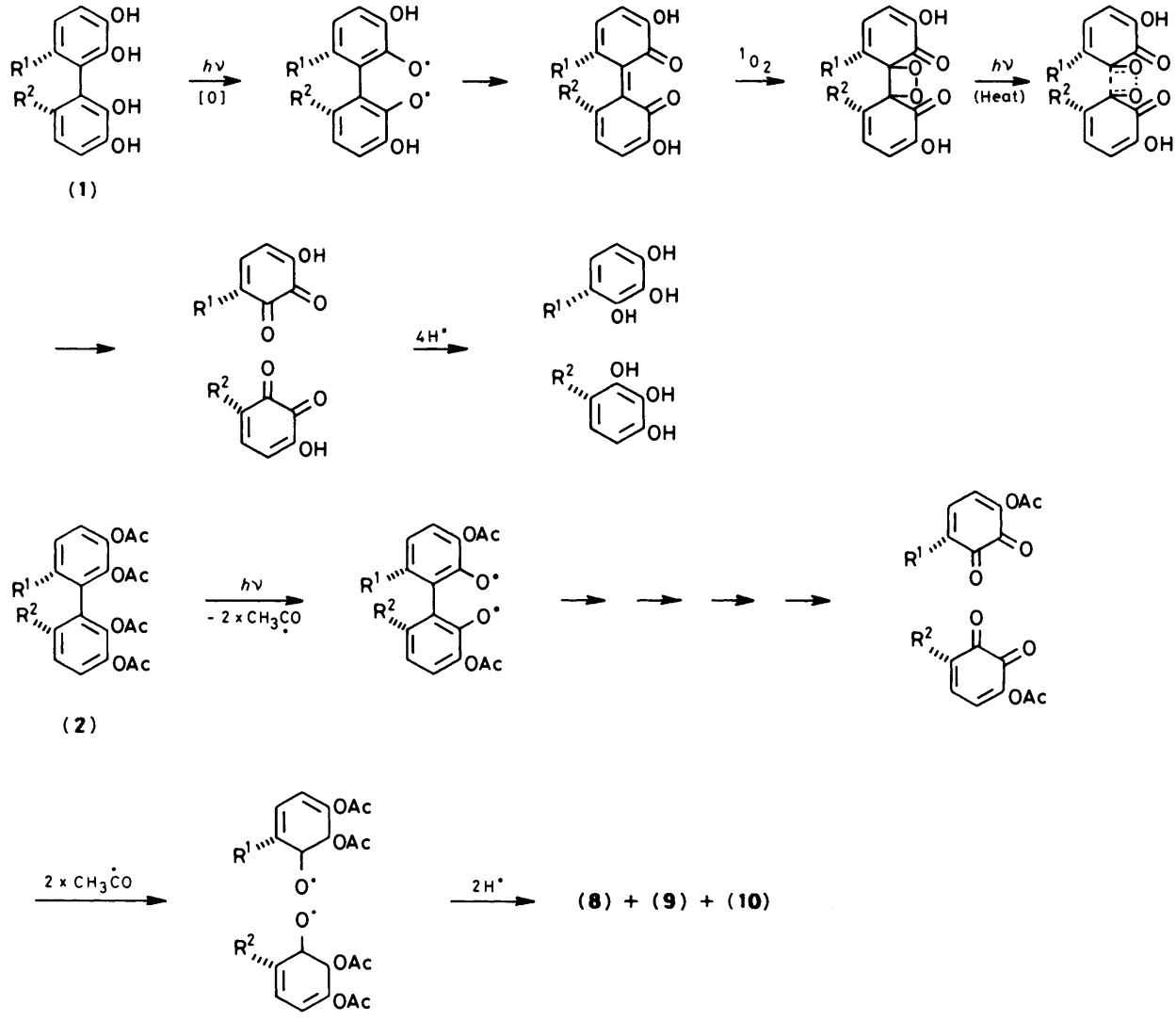
biflavonoid (**2**) provided indirect evidence for the same mode of interflavonoid linkage. The 'abnormal' coupling constants $J_{5',6'}$ of the biflavonoids (**13**) and (**2**) may, therefore, be attributed to the quite exceptionally small chemical-shift difference.

Although the possibility of n.O.e. difference spectrometry of (**2**) was precluded by the fact that the aromatic acetoxy proton resonances are too closely spaced to permit selective n.O.e. irradiation and that effects would also be expected to be small for acetates, structural elucidation of (**2**) was simplified by the characterization of the biflavonoid analogue (**13**) which served as useful reference for spectrometric comparison.

The molecular structure of the second product of methylation (**14**), which is isomeric with (**13**) but defined by the $\Delta^6$-oxepine-4-spiro-oxirane element was evident from similar spectrometric evidence. Allocation of the A, C, D, and F rings appeared feasible by comparing the $^1$H n.m.r. spectra of (**13**) and (**14**), when taken in conjunction with similar decoupling experiments and n.O.e. difference spectrometry. The latter technique similarly revealed similar strong association of 6'-H (B) with 2-H and 3-H(C). The above, supplemented by evidence of an 15.4% enhancement of the 5'-H (B) signal upon irradiation of the 4'-methoxy resonance (B) in the n.O.e. experiment (*cf.* Figure 2), indicated the same arrangement of substituents on ring B as demonstrated for (**13**).

By contrast, confirmation of the point of bonding at C-5' in ring E proved to be problematical in view of the abnormally small coupling constant (*J ca.* 1.5 Hz) in $CDCl_3$ compared with those usually associated with *meta*-coupled protons (*J* 2—2.5 Hz). This problem was overcome by the analysis of $^1$H n.m.r. spectra in $C_6D_6$ exhibiting the coupling constant of the same AB-system (*J* 2.0 Hz) approximated to those usually observed. This difference may be attributed to the relatively small chemical-shift difference ($\Delta\delta$ 0.01) in the former compared with the equivalent ($\Delta\delta$ 0.07) in the latter case. The [2',5']-interflavonoid linkage was also supported by the position of methoxy resonances considering the relative OMe shifts in $CDCl_3$ and $C_6D_6$.[19–23] Thus, it was shown by the benzene-induced methoxy experiment that the two methoxy signals, attributable to 3'-OMe (B) and 4'-OMe (E), remained 'stationary' and that no aromatic protons were therefore *ortho* to each of these functional groups. The solvent-dependent OMe shifts were accordingly consistent with the formula (**14**).

Other problems associated with structure (**14**) were the apparent presence of only seven methoxy groups; the placing of the $OCH_2CH_3$ functionality as evinced by the highfield triplet at $\delta$ 1.42 coupled with the quartet at $\delta$ 4.05; and the absence of n.O.e. effects involving 6'-H (E) and 2-H (F) on one side and 2'-H

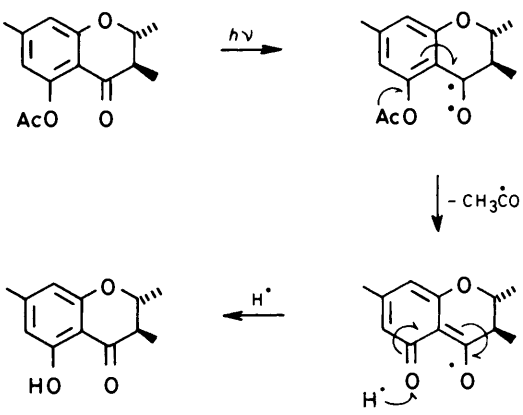



**Scheme 2.** Proposed photofragmentation of the biphenyl-type interflavonoid linkage of (**1**) and its acetate derivative (**2**)

(E) and 3'-OMe (E) on the other. Indication of the presence of the $OCH_2CH_3$ group at the 3'-position (E) was available from n.O.e. difference spectrometry, based on connectivities between 2'-H (E) and the methylene group of the ethoxy function. This may be rationalized on the assumption that the $OCH_2CH_3$ functionality was introduced before methylation (because of handling in ethanol during chromatography on Sephadex LH-20) as the result of the existence of an acidic phenolic group.

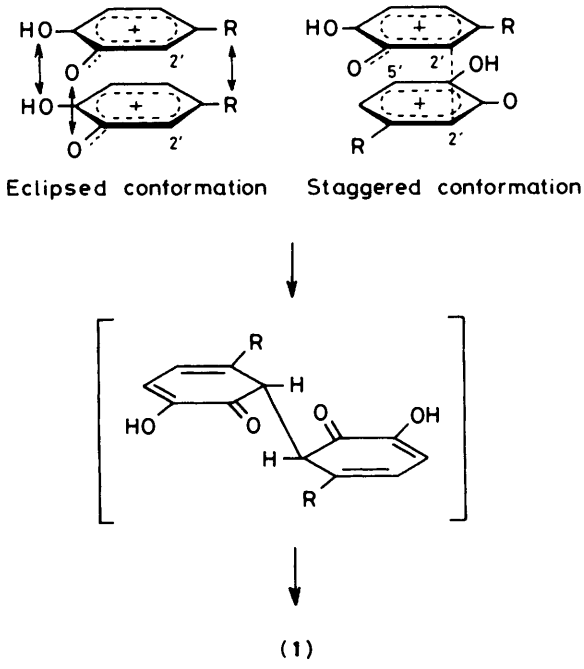
The question of immediate interest concerns the mechanism leading to the aforementioned degradation products (**7**)—(**12**) on the basis of a photochemical process involving singlet oxygen. The photofragmentation of the aryl-aryl bond plausibly involves formation of a 1,2-dioxetane as initial step, presumed to occur mechanistically *via* hydrogen abstraction from either hydroxy groups placed *ortho* to the biphenyl linkage. Hydrogen abstraction may be attributed to the presence of quinone-type species presumably due to radical oxidation by $O_2$ giving rise to *ortho,ortho'*-diphenoquinones (*cf.* Scheme 2). Opening of the postulated dioxetane ring in a concerted type of mechanism[28] results in the formation of *ortho*-benzoquinones which require formal hydrogen transfer to give the expected products (**7**) and (**10**) in their free phenolic forms. The proposed course of this photoreaction is supported by the characterization of the methyl ether acetates (**11**) and (**12**) indicative of fission of the biphenyl linkage *via* a dioxetane intermediate.

The acetate (**2**) is apparently subject to a similar photolytic process as evident from the products (**7**)—(**10**). The photochemical loss of acetyl should be a facile process leading to the equivalent *ortho,ortho'*-diphenoquinone. Preferred 'deacetylation' in the *ortho,ortho'*-positions could be attributed to resonance stabilization of the *o*-phenoxy radical. Formation of the equivalent 1,2-dioxetane by $^1O_2$ [2 + 2] cycloaddition corresponds to that of the free phenol, while a combination of hydrogen abstraction and migration/transfer of acetyl groups in the final steps leads to products (**8**)—(**10**). The 'intermolecular' acetyl transfer as reflected in the products (**8**) and (**10**) may occur from the (+)-taxifolin derivative (**8**) to the (+)-catechin analogue (**10**). Such acetyl transfer may occur either to the exicted state or from the electronically excited unit with expulsion of an acetyl radical, since one of the two fragments of fission of the intermediate 1,2-dioxetane will be in an electronically excited state. The characterization of the dihydroxy-tetra-acetoxy-dihydroflavanol (**8**) implies that the transfer involves the acetyl group at C-5, which could readily arise as result of the illustrated mechanism.




Storage of (**2**) under nitrogen significantly reduced the formation of photodegradation products, hence reflecting an oxygen-dependent mechanism as postulated. The novel (+)-catechin-(+)-taxifolin represents the only biflavonoid known hitherto to undergo this type of photolytic scission of the interflavonoid linkage.

Finally, the biosynthetic origin of the biflavonoid (**1**) requires consideration. Direct phenolic oxidative coupling of two appropriately positioned phenyl groups, similar to the formation of hydrolysable tannins[29] and biflavonoids,[30] may be regarded as the biosynthetic pathway. In general, aromatic C–C bond formation can be formulated by abstracting one hydrogen atom from the starting phenol following coupling exclusively at *ortho* and *para* positions to the hydroxy groups due to the high density of the unpaired electron at these positions.[31] On the basis of recent reports[32–35] the two radicals involved in coupling preferentially approach each other in a 'sandwich-like' manner permitting orbital overlap. Comparison between eclipsed and staggered sandwich geometries of approach, both permitting the anticipated [2',2']-coupling, favours the latter mode of approach (*cf.* Scheme 3). This may be




**Scheme 3.** 'Sandwich' approach of phenoxyl radicals. Arrows indicate steric interactions and electrostatic repulsions

rationalized on the basis of non-bonded interactions, particularly repulsion effects between the oxygen functions and steric interactions caused by the bulky 2*H*-benzopyran substituents, which are minimized in the staggered arrangement. This transition state could also give rise to [2',5']-coupling. This aryl-aryl bond formation bears some resemblance to the susceptibility to phenol coupling of (+)-mesquitol with consequent formation of recently discovered condensed tannins based on biphenyl and *o*-terphenyl.[36–38]

The low overall yield of the biflavonoids (**13**) and (**14**) from enriched fractions (15 g) leads to the surmise of a hypothetical [2',2']-bi-(+)-catechin which follows an oxidation sequence *via* (**1**) to the [2',2']-bi-(+)-taxifolin. By analogy with oxidation work on (+)-mesquitol[13] the isolation of the (+)-dihydroflavanol analogue in very low yield lends support to the suggested steps of conversion. It may be noted that the methylene function of (+)-catechin should be far more susceptible to oxidation due to the pattern of 1,3,5-oxygenation of the A-ring.

## Experimental

N.m.r. spectra were recorded at 300 MHz in $CDCl_3$ with $SiMe_4$ as internal standard. Mass spectra were obtained by electron impact with a Varian CH-5 and Varian MAT 44S Spectro System MAT 188 instrument, and c.d. data in MeOH on a Jasco J-20 spectropolarimeter. C and H analyses were performed by the Department of Organic Chemistry of this University. Preparative plates (p.l.c.) [Kieselgel $PF_{254}$ (0.5 mm)] were air-dried and used without prior activation. Methylations were performed with an excess of diazomethane, while acetylations were carried out with acetic anhydride–pyridine.

*Isolation of Phenolic Metabolites from Willow Bark.*—Plant material (2 kg, Fa. Caesar & Loretz, Hilden) was exhaustively extracted with MeOH and the combined extracts were evaporated under reduced pressure to dryness. The crude residue (87 g) was dissolved in water and the solution extracted with light petroleum and ethyl acetate. A portion of the ethyl acetate–soluble fraction (20 g) was applied to a column (3.5 × 80 cm) of Sephadex LH-20, eluted with ethanol. After the emergence of phenolic material 15-ml fractions were collected.

[2′,2′]-(+)-*Catechin*-(+)-*taxifolin* (**1**).—Fractions 53—82 (1.2 g) represented a mixture of components containing (+)-catechin (**5**) and the biflavonoid (**1**). Acetylation of the phenolic mixture (200 mg) and subsequent purification by preparative t.l.c. ($C_6H_6$—$Me_2CO$, 4:1; $R_F$ 0.49) yielded the acetate (**2**) (78 mg) (Found: C, 59.3; H, 4.45. $C_{50}H_{44}O_{23}$ requires C, 59.27; H, 4.38%); δ($CDCl_3$) 7.24 [s, 2 × H (E)], 7.11 [s, 2 × H (B)], 6.80 [d, *J* 2.0 Hz, 6-H (D)], 6.66 [d, *J* 2.2 Hz, 8-H (A)], 6.61 [d, *J* 2.0 Hz, 8-H (D)], 6.60 [d, *J* 2.2 Hz, 6-H (A)], 5.60 [d, *J* 12.0 Hz, 2-H (F)], 5.43 [d, *J* 12.0 Hz, 3-H (F)], 5.20 [m, 3-H (C)], 5.11 [d, *J* 6.5 Hz, 2-H (C)], 2.91 [dd, *J* 5.0 and 16.0 Hz, 4-$H_{eq}$(F)], 2.66 [dd, *J* 6.5 and 16.0 Hz, 4-$H_{ax}$(F)], and 2.39, 2.20 (×8), 2.02 (each s, 10 × OAc); δ[$(CD_3)_2CO$] 7.46 [s, 2 × H (E)], 7.25 [s, 2 × H (B)], 6.89 [d, *J* 2.0 Hz, 6-H (D)], 6.70 [d, *J* 2.0 Hz, 8-H (D)], 6.68 [d, *J* 2.2 Hz, 8-H (A)], 6.61 [d, *J* 2.2 Hz, 6-H (A)], 5.81 [d, *J* 12.0 Hz, 2-H (F)], 5.71 [d, *J* 12.0 Hz, 3-H (F)], 5.26 [m, 2 × H, 2-H and 3-H (C)], 2.80 [m, 2 × H, $-CH_2$ (F)], and 1.93—2.31 (m, 10 × OAc); *m/z* (e.i.) 575 (2.2%), 532 (4.2), 522 (5.4), 498 (32), 456 (62), 414 (34), 397 (37), 372 (47), 355 (25), 344 (14), 330 (28), 313 (15), 302 (15), 297 (45), 282 (13), 239 (31), 213 (25), 198 (25), 181 (14), 168 (47), 165 (23), 153 (24), 150 (33), and 139 (100); *m/z* (f.d.) 649 (10%), 572 (100), and 559 (74); *m/z* (f.a.b.) 1 013 ($M^+$ + 1, 4%), 833 (2.4), 724 (7.8), 697 (12.3), 679 (16.2), 637 (2.6), 601 (32), 559 (80), 531 (52), 489 (48), 457 (100), and 415 (96); c.d. $[\theta]_{350}$ + 2 310, $[\theta]_{330}$ + 4 250, $[\theta]_{316}$ 0, $[\theta]_{298}$ − 5 400, $[\theta]_{282}$ − 4 630, $[\theta]_{268}$ − 6 950, $[\theta]_{243}$ 0, $[\theta]_{222}$ + 19 700, $[\theta]_{217}$ + 13 130, $[\theta]_{210}$ + 48 280, and $[\theta]_{202}$ 0; $^{13}C$ n.m.r. data (*cf.* Table 2).

*Products of Photolysis related to* (+)-*Taxifolin.*—2′,3,3′,4′,5,7-*Hexa-acetoxydihydroflavonol* (**7**). δ($CDCl_3$) 7.24 [s, 2 × H (B)], 6.80 [d, *J* 2.0 Hz, 6-H (A)], 6.61 [d, *J* 2.0 Hz, 8-H (A)], 5.60 [d, *J* 12.0 Hz, 3-H (C)], 5.43 [d, *J* 12.0 Hz, 2-H (C)], 2.38, 2.31, 2.30 (×3), and 2.10 (each s, 6 × OAc); δ($C_6D_6$) 7.12 [s, 2 × H (B)], 6.61 [d, *J* 2.2 Hz, 6-H (A)], 6.52 [d, *J* 2.2 Hz, 8-H (A)], 5.53 [d, *J* 12.0 Hz, 3-H (C)], 4.66 [d, *J* 12.0 Hz, 2-H (C)], 2.12, 1.76, 1.74, 1.72 (×2), and 1.62 (each s, 6 × OAc).

3,3′,4′,7-*Tetra-acetoxy*-2′,5-*dihydroxydihydroflavonol* (**8**). δ($CDCl_3$) 7.26 [s, 2 × H (B)], 6.40 [d, *J* 2.0 Hz, 6-H (A)], 6.33 [d, *J* 2.0 Hz, 8-H(A)], 5.69 [d, *J* 12.0 Hz, 3-H (C)], 5.43 [d, *J* 12.0 Hz, 2-H (C)], 2.31 (×3), 2.13 (each s, 4 × OAc), and 1.60 (br s, 2 × OH).

2′,3,3′,4′,5-*Penta-acetoxy*-7-*hydroxydihydroflavonol* (**9**).—δ($CDCl_3$) 7.21 [s, 2 × H (B)], 6.25 [s, 2 × H (A)], 5.54 [d, *J* 12.0 Hz, 3-H (C)], 5.32 [d, 12.0 Hz, 2-H (C)], 2.37, 2.30 (×3), 2.08 (each s, 5 × OAc), and 1.63 (br s, 1 × OH).

Acetylation of compounds (**8**) and (**9**), respectively, gave the hexa-acetate (**7**) in each instance. Mass fragmentation spectra of (**8**) and (**9**) and their respective relative abundances: *m/z* 530 (—, 46 $[M]^+$), 488 (23 $[M]^+$, 24), 470 (24, 28), 446 (62, 57), 428 (39, 44), 404 (80, 95), 386 (68, 89), 362 (68, 69), 344 (88, 100), 320 (22, 18), 302 (92, 96), 250 (42, 51), 239 (35, 33), 208 (52, 80), 197 (22, 56), 181 (44, 11), 166 (91, 86), 153 (100, 99), and 139 (76, 18).

3-*Acetoxy*-6,8-*dimethoxy*-2-(2,3,4-*trimethoxyphenyl*)-2,3,4,5-*tetrahydro*-1-*benzoxepin*-4-*spiro*-*oxirane* (**11**).* δ($CDCl_3$) 6.64 [s, 2 × H (B)], 6.26 [d, *J* 2.5 Hz, 6-H (A)], 6.22 [d, *J* 2.5 Hz, 8-H (A)], 5.44 [d, *J* 9.5 Hz, 3-H (C)], 4.78 [d, *J* 9.5 Hz, 2-H (C)], 3.88, 3.86 (×2), 3.78, 3.73 (each s, 5 × OMe), 3.34 [d, *J* 16.5 Hz, 1 × H, $CH_2$(C)], 2.91 [d, *J* 5.0 Hz, 1 × H, oxirane], 2.82 [d, *J* 16.5 Hz, 1 × H, $CH_2$ (C)], 2.81 [d, *J* 5.0 Hz, 1 × H, oxirane], and 1.87 (s, 1 × OAc); δ($C_6D_6$) 6.78 [s, 2 × H (B)], 6.43 [d, *J* 2.2 Hz, 6-H (A)], 6.28 [d, *J* 2.2 Hz, 8-H (A), 5.88 [d, *J* 10.0 Hz, 3-H (C)], 5.05 [d, *J* 10.0 Hz, 2-H (C)], 3.82, 3.44 (×2), 3.25, 3.24 (each s, 5 × OMe), 3.58 [d, *J* 14.0 Hz, 1 × H, $CH_2$ (C)], 3.04 [d, *J* 14.0 Hz, 1 × H, $CH_2$ (C)], 2.71 [d, *J* 6.0 Hz, oxirane], and 2.49 [d, *J* 6.0 Hz, oxirane]; *m/z* 460 ($M^+$, 12%), 400 (61), 372 (22), 358 (20), 210 (54), 195 (23), 181 (36), 167 (100), 151 (11), and 137 (18).

*Products of Photolysis related to* (+)-*Catechin.*—2′,3,3′,4′,5,7-*Hexa-acetoxyflavan* (**10**). δ($CDCl_3$) 7.11 [s, 2 × H (B)], 6.65 [d, *J* 2.2 Hz, 8-H (A)], 6.60 [d, *J* 2.2 Hz, 6-H (A)], 5.20 [m, 3-H (C)], 5.12 [d, *J* 6.5 Hz, 2-H (C)], 2.91 [dd, *J* 5.0 and 17.0 Hz, 4-$H_{eq}$ (C)], 2.66 [dd, *J* 6.5 and 17.0 Hz, 4-$H_{ax}$ (C)], 2.28 (×5), and 2.03 (each s, 6 × OAc); δ($C_6D_6$) 7.19 [s, 2 × H (B)], 6.83 [d, *J* 2.2 Hz, 8-H (A)], 6.76 [d, *J* 2.2 Hz, 6-H (A)], 5.17 [m, 3-H (C)], 4.71 [d, *J* 7.0 Hz, 2-H (C)], 2.85 [dd, *J* 5.5 and 17.0 Hz, 4-$H_{eq}$ (C)], 2.55 [dd, *J* 7.5 and 17.0 Hz, 4-$H_{ax}$ (C)], 1.77, 1.69 (×2), and 1.66, 1.65, and 1.58 (each s, 6 × OAc); *m/z* 558 ($M^+$, 6%), 498 (42), 456 (91), 439 (25), 414 (78), 397 (37), 372 (84), 355 (35), 330 (48), 288 (52), 239 (20), 198 (21), 181 (21), 168 (37), 151 (35), and 137 (100).

3-*Acetoxy*-2′,3′,4′,5,7-*pentamethoxyflavan* (**12**). δ($CDCl_3$; 80 MHz; 30 °C) 6.91 [s, 1 × H (B)], 6.62 [s, 1 × H (B)], 6.20 [d, *J* 2.3 Hz, 8-H (A)], 6.12 [d, *J* 2.3 Hz, 6-H (A)], 5.36 [m, 3-H (C)], 5.03 [d, *J* 6.7 Hz, 2-H (C)], 2.78 [m, 2 × H, $CH_2$ (C)], 3.86, 3.85, 3.83, 3.82, 3.78 (each s, 5 × OMe), and 1.97 and 1.96 (2 × s, 1 × OAc); δ($CDCl_3$; 80 MHz; 100 °C) 6.87 [m, 1 × H (B)], 6.62 [s, 1 × H (B)], 6.18 [d, *J* 2.3 Hz, 8-H (A)], 6.10 [d, *J* 2.3 Hz, 6-H (A)], 5.31 [m, 3-H (C)], 4.94 [d, *J* 6.7 Hz, 2-H (C)], 2.81 [m, 2 × H, $CH_2$ (C)], 3.81, 3.80 (×2), 3.76, 3.75 (each s, 5 × OMe), and 1.92 and 1.91 (2 × s, 1 × OAc); δ($C_6D_6$; 300 MHz; 30 °C) 6.71 [s, 2 × H (B)], 6.43 [d, *J* 2.2 Hz, 8-H (A)], 6.17 [d, *J* 2.2 Hz, 6-H (A)], 5.61 [m, 3-H (C)], 5.17 [d, *J* 6.0 Hz, 2-H (C)], 3.80, 3.39, 3.38 (×2), 3.28 (each s, 5 × OMe), 2.90—3.10 [m, 2 × H, $CH_2$ (C)], and 1.53 (s, 1 × OAc).

*Methyl Ether Acetates of the 'Biflavonoid' Fraction.** Combined test tubes from the primary separation (5 g) were re-chromatographed on a Sephadex LH-20 column (2 × 50 cm), using ethanol as eluant. Methylation of the contents of tubes 10—17 (864 mg) afforded four fractions of methyl ethers at $R_F$ 0.50 (11 mg), 0.37 (285 mg), 0.16 (60 mg), and 0.10 (43 mg), respectively, in toluene–acetone (9:1). Acetylation of the $R_F$ 0.37 fraction followed by preparative t.l.c. in the same solvent system yielded three bands at $R_F$ 0.82 (79 mg), 0.78 (70 mg), and 0.73 (37 mg). The $R_F$ 0.73 product was subjected to secondary separations by preparative t.l.c. in dichloroethane–acetone (49:1; ×2), yielding the biflavonoids (**13**) and (**14**) at $R_F$ 0.62 (9 mg) and 0.60 (3 mg), respectively.

* For convenience, the numbering adopted for the spectroscopic results for compounds (**11**), (**13**), and (**14**) follows that of 'common' flavonoids and is illustrated in the displayed formulae; it differs from that used in systematic name of the heading.

2,2′-*Bis*(3-*acetoxy*-7,9-*dimethoxy*-4-*oxo*-3,4,5,6-*tetrahydro*-1-*benzoxocin*-2-*yl*)-5,5′,6,6′-*tetramethoxybiphenyl* (**13**). δ($CDCl_3$) 6.75 [s, 2 × H, 5′-H + 6′-H (E)], 6.72 [s, 2 × H, 5′-H + 6′-H (B)], 6.25 [d, *J* 2.2 Hz, 6-H (D)], 6.16 [d, *J* 2.2 Hz, 6-H (A)], 6.09 [d, *J* 2.2 Hz, 8-H (D)], 5.96 [d, *J* 2.2 Hz, 8-H (A)], 5.30 [d, *J* 9.0 Hz, 3-H (C)], 5.20 [d, 8.5 Hz, 2-H (F)], 5.06 [d, *J* 8.5 Hz, 3-H (F)], 4.68 [d, *J* 9.0 Hz, 2-H (C)], 3.90, 3.88, 3.86 (× 2), 3.78, 3.76, 3.68, 3.66 (each s, 8 × OMe), 2.50—3.45 [m, 8 × H, 4 × $CH_2$ (C and F)], and 2.03 and 1.88 (2 × s, 2 × OAc); δ($C_6D_6$) 6.88 [s, 2 × H, 5′-H + 6′-H (E)], 6.87 [s, 2 × H, 5′-H + 6′-H (B)], 6.30 [d, *J* 2.2 Hz, 6-H (D)], 6.26 [d, *J* 2.2 Hz, 6-H (A)], 6.18 [d, *J* 2.2 Hz, 8-H (D)], 6.11 [d, *J* 2.2 Hz, 8-H (A)], 5.58 [d, *J* 9.0 Hz, 3-H (C)], 5.50 [d, *J* 8.5 Hz, 2-H (F)], 5.41 [d, *J* 8.5 Hz, 3-H (F)], 5.07 [d, *J* 9.0 Hz, 2-H (C)], 3.85, 3.83, 3.45, 3.39, 3.21 (× 2), 3.18, 3.15 (each s, 8 × OMe), 3.20 (m, 2 × H, $CH_2$), 2.87 (m, 2 × H, $CH_2$), 2.70 (m, 2 × H, $CH_2$), 2.07 (m, 2 × H, $CH_2$), and 1.50, and 1.41 (2 × s, 2 × OAc); *m*/*z* 474 (2.6%), 460 (3), 252 (60), 211 (12), 210 (100), 195 (13), and 167 (26).

2,2′-*Bis*(3-*acetoxy*-6,8-*dimethoxy*-2,3,4,5-*tetrahydro*-1-*benzoxepine*-4-*spiro*-*oxiran*-2-*yl*)-6-*ethoxy*-5,5′,6′*trimethoxybiphenyl* (**14**). δ($CDCl_3$) 6.72 [s, 2 × H (B)], 6.61 [d, *J* 1.5 Hz, 1 × H (E)], 6.60 [d, *J* 1.5 Hz, 1 × H (E)], 6.27 [d, *J* 2.2 Hz, 6-H (D)], 6.23 [d, *J* 2.2 Hz, 6-H (A)], 6.18 [d, *J* 2.2 Hz, 8-H (D)], 6.13 [d, *J* 2.2 Hz, 8-H (A)], 5.40 [d, *J* 9.5 Hz, 3-H (C)], 5.10 [d, *J* 6.0 Hz, 2-H (F)], 5.03 [d, *J* 6.0 Hz, 3-H (F)], 4.74 [d, *J* 9.5 Hz, 2-H (C)], 4.05 [q, Σ *J* 14.0 Hz, $OCH_2CH_3$ (E)], 3.86, 3.84 (× 2), 3.80, 3.75, and 3.71 (× 2) (each s, 7 × OMe), 3.32 [d, *J* 16.0 Hz, 1 × H, $CH_2$ (C or F)], 3.11 [d, 16.0 Hz, 1 × H, $CH_2$ (C or F)], 2.95 [d, *J* 16.0 Hz, 1 × H, $CH_2$ (C or F)], 2.88 [d, *J* 5.0 Hz, 1 × H, oxirane], 2.84 [d, *J* 16.0 Hz, 1 × H, $CH_2$ (C or F)], 2.79 [d, *J* 5.0 Hz, 1 × H, oxirane], 2.48 [d, *J* 5.0 Hz, 1 × H, oxirane], 2.44 [d, *J* 5.0 Hz, 1 × H, oxirane], 1.85, and 1.70 (2 × s, 2 × OAc), and 1.42 [t, Σ *J* 14.0 Hz, $OCH_2CH_3$ (E)]; δ($C_6D_6$) 6.99 [s, 2 × H (B)], 6.83 [d, *J* 2.0 Hz, 1 × H (E)], 6.76 [d, *J* 2.0 Hz, 1 × H (E)], 6.44 [d, *J* 2.2 Hz, 6-H (D)], 6.33 [d, *J* 2.2 Hz, 6-H (A)], 6.30 [d, *J* 2.2 Hz, 8-H (D)], 6.28 [d, *J* 2.2 Hz, 8-H (A)], 5.91 [d, *J* 9.5 Hz, 3-H (C)], 5.59 [d, *J* 6.0 Hz, 2-H (F)], 5.50 [d, *J* 6.0 Hz, 3-H (F)], 5.05 [d, *J* 9.5 Hz, 2-H (C)], 4.15 [q, Σ *J* 14.0 Hz, $OCH_2CH_3$ (E)], 3.86, 3.84, 3.43, 3.25, 3.24, 3.22, and 3.21 (each s, 7 × OMe), 3.05 [d, *J* 16.0 Hz, 1 × H, $CH_2$ (C or F)], 2.70 [d, *J* 5.0 Hz, 1 × H, oxirane], 2.50 [d, *J* 5.0 Hz, 1 × H, oxirane], 2.46 [d, *J* 16.0 Hz, 1 × H, $CH_2$ (C or F)], 2.45 [d, *J* 16.0 Hz, 1 × H, $CH_2$ (C or F)], 2.18 [d, *J* 5.0 Hz, 1 × H, oxirane], 2.11 (d, *J* 5.0 Hz, 1 × H, oxirane), 2.10 [d, *J* 16.0 Hz, 1 × H, $CH_2$ (C or F)], 1.45, 1.31 (2 × s, 2 × OAc), and 1.15 [t, Σ *J* 14.0 Hz, $OCH_2CH_3$ (E)].

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