The First Natural Biflavonoid with Flavanol and Dihydroflavonol Constituent Units Coupled at the B-Ring

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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 being established by nuclear Overhauser effect difference spectroscopy of the methyl ether acetate of the bi-(+)-taxifolin analogue, which represents a product of oxidation and which undergoes methylene insertion reactions with diazomethane.

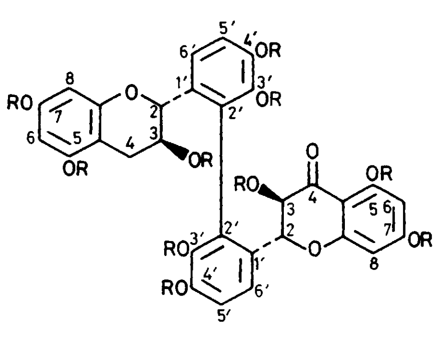
Apart from biflavanoids of black[1] and green[2] tea possessing an interflavanoid linkage between the two B-rings of the flavan units this type of natural C–C bonding is atypical, the biphenyl moiety being commonly found only in biflavonoids[3] and hydrolysable tannins.[4] Re-examination of the phenolic content of commercial willow bark (Salix spp.) has revealed the existence of a [2′,2′]-(+)-catechin-(+)-taxifolin biflavonoid, thus providing the first indication of a novel B-ring–B-ring coupled analogue of a flavano–dihydroflavonol biflavonoid amongst metabolites of this class.[5]

Evidence for the first natural biflavonoid (**1**) with different monomeric constituent units (flavanol and dihydroflavonol moieties, respectively) coupled *via* B-rings was obtained by acetylation of the appropriate fraction from chromatography on Sephadex LH-20. Isolation of the acetate (**2**) led to its characterization as a derivative of a [2′,2′]-(+)-catechin-(+)-taxifolin biflavonoid.

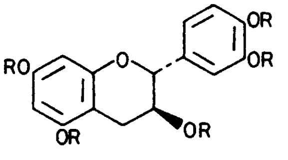
Fast atom bombardment mass spectrometry provided successful molecular weight information for (**2**), showing the $[M + 1]^+$ peak at m/z 1013, corresponding to the molecular formula $C_{50}H_{44}O_{23}$. The structure and stereochemistry were assigned on the basis of ^{1}H and ^{13}C n.m.r. spectroscopy, supported by the characterization of a range of flavan-3-ols and dihydroflavonols derived from its photo-oxidative cleavage of the biphenyl-type interflavonoid linkage. Structural elucidation of the derivative (**7**), which represents a product of oxidation of the (+)-catechin moiety and which undergoes expansion of the hererocyclic rings on addition of diazomethane, served as useful reference in terms of assessment of the points of attachment of the parent biflavonoid (**1**) through application of the nuclear Overhauser effect (n.O.e.) difference spectroscopy (*cf.* Figure 1).

The ^{1}H n.m.r. spectrum of the acetate (**2**) exhibited *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 (C-ring). These resonances were accompanied by a second aromatic spin system at δ 6.81 and 6.61 (J 2.0 Hz) originating from two *meta*-coupled protons, while the appearance of two doublets at δ 5.60 and 5.43 (with coupling constants J 12.0 Hz) indicated a 2,3-*trans* stereochemistry for the flavanol unit. Chemical shifts of the signals in the ^{1}H n.m.r. spectrum of (**2**) agreed fairly well with those of the acetates of the reference compounds (+)-catechin (**4**) and (+)-taxifolin (**6**) except for resonances attributed to B-ring protons which appeared as sharp singlets at δ 7.24 and 7.11 in contrast to the expected well-defined ABC systems of the model compounds. The two low-field aromatic singlets (δ 7.24 and 7.11) observed in a variety of solvent systems indicated a possible [6′,6′]-interflavanoid linkage. 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-ring as evidenced by recent observations on (+)-mesquitol and its oligomers.[6]

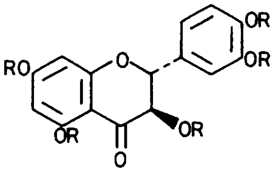
In order to provide direct evidence in terms of the arrangement of B-ring substituents and hence bonding points between the monomeric flavan units of (**2**) by means of n.O.e.



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



(3) R = H
(4) R = Ac



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

difference spectroscopy it was essential to form a methylated derivative of (**1**). However, reaction of (**1**) with diazomethane afforded the novel product (**7**). Contrary to expectations biflavonoid (**1**) did not form the desired methyl ether derivative, but indicated oxidation of the (+)-catechin part to the dihydroflavonol unit. By analogy with oxidation work on (+)-mesquitol, the isolation of the dihydroflavonol analogue in low yield lends support to the observed conversion of the (+)-catechin unit[6] and hence formation of a bi-(+)-taxifolin. It may be noted that the methylene function of (+)-catechin should be far more readily oxidised owing to the phloroglucinol pattern of 1,3,5-oxygenation of the A-ring.

The presence of the Δ^7-oxocin-4-one ring systems instead of the dihydropyranone may be rationalized on the basis of methylene insertions in (+)-taxifolin units by nucleophilic addition of diazomethane to the 4-carbonyl group.[7] This side-reaction, similar to the expansion of the chromanone ring system of crombeone,[8] may be attributed to the high migratory aptitude of the phloroglucinol A-ring once the attack by diazomethane at the 4-carbonyl function has occurred, and thus promoting 1,2-aryl migration. Insertion of a second methylene function implies migration by a benzylic group *via* a similar mechanism.

Evidence for the structure (**7**) was obtained by a combination of ^{1}H n.m.r. techniques, involving spin-decoupling experiments, application of n.O.e. difference spectroscopy, and the advantage of solvent-induced chemical shift effects, establishing unequivocally the structure of (**7**) as a [2′,2′]-bi-(+)-taxifolin derivative, but defined by the Δ^7-oxocin-4-one element (ring C and F).

The n.O.e. difference spectra of (**7**) 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. The fact that no n.O.e. effect was observed on irradiation of the 3′-methoxy resonances (B and E) may indicate the absence of protons in the vicinity of these substituents, as would also be the case in the alternative structure with [6′,6′]-bonding. Finally, solvent-induced chemical shifts of methoxy resonances in C_6D_6[9,10] showed that only two of the group of 4′- and 3′-methoxy (B and E) signals remain static relative to their chemical shifts in $CDCl_3$.

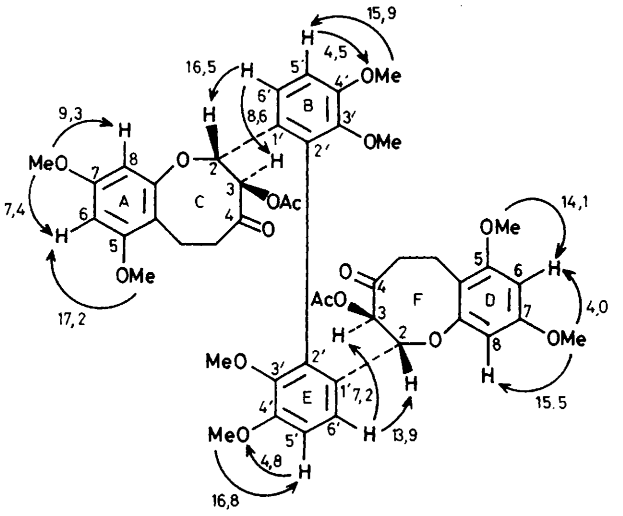


Figure 1. Connectivities (%) established by n.O.e difference experiments of the biflavanoid (**7**). A and D ring n.O.e. effects are interchangeable since there are no connectivities between them and BC and EF respectively.

Noteworthy is the chemical shift difference Δδ 5′-H, 6′-H which proved to be zero in the B and E rings, respectively, verified by the appearance of low-field singlets at δ 6.75 and 6.72 in the ^{1}H n.m.r. spectra of (**7**). The similarities with respect to resonances of B-ring protons between (**7**) and the related biflavonoid (**2**) provided indirect evidence for the same mode of linkage. The 'abnormal' coupling constant $J_{5',6'}$ of the biflavonoids (**7**) and (**2**) may therefore be attributed to the exceptionally small chemical shift difference.

The characterization of the [2′,2′]-(+)-catechin-(+)-taxifolin represents an extension of the range of natural biflavonoids, while compound (**7**) provides the first reported example of a biflavonoid originating from a sequential methylene ring insertion reaction on addition of diazomethane.

Spin-decoupling experiments and n.O.e. difference spectroscopy were carried out by Professor D. Ferreira and Dr. E. V. Brandt, University of the Orange Free State.

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