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Isolation of [4-O-4]-linked Biflavanoids from *Acacia melanoxylon*: First Examples of a New Class of Single Ether-linked Proanthocyanidin Dimers

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The isolation of the unique [4-0-4]-linked biflavanoids, [4-O-4]-bis(2,3-*cis*-3,4-*trans*-3,3′,4′,7,8-pentahydroxyflavan) (**5**) and the isomeric 2,3-*cis*-3,4-*trans*-3,3′,4′,7,8-pentahydroxyflavan-[4-O-4]-2,3-*cis*-3,4-*cis*-3,3,4′,7,8-pentahydroxyflavan (**6**), from the heartwood of *Acacia melanoxylon* further extends the heterogeneity of the interflavanoid linkage among natural proanthocyanidin dimers and oligomers.

The overwhelming predominance of carbon–carbon interflavanoid bonding between C-4 and C-6 or C-8 of flavanoid monomeric units in proanthocyanidins, almost to the exclusion of other forms of linkage, has been well established.[1—4] An additional point of linkage, although relatively uncommon, is found in the doubly linked A-type biflavanoids which in addition to the normal carbon–carbon bond also possess an ether link between C-2 and C-5 or C-7.[5—7] Proanthocyanidins linked exclusively by an ether linkage are rare, the only reported examples being the two double ether-linked 'dioxane-type' biflavonoids reported in the heartwood of *Acacia mearnsii*.[8,9] Thus the isolation of the novel ether linked [4-O-4]biflavanoids; [4-O-4]bis(2,3-*cis*-3,4-*trans*-3,3′,4′,7,8-pentahydroxyflavan) (**5**) and its configurational isomer 2,3-*cis*-3,4-*trans*-3,3′,4′,7,8-pentahydroxyflavan-[4-O-4]-2,3-*cis*-3,4-*cis*-3,3′,4′,7,8-pentahydroxyflavan (**6**) from the heartwood of *A. melanoxylon* provides the first examples of a new class of proanthocyanidin dimers linked by single ether interflavanoid bonds.

Compounds (**5**), R_F 0.10 (cellulose t.l.c., HOAc–H_2O, 6:94 v/v), 0.20 (Bu^tOH–HOAc–H_2O, 3:1:1 v/v), $[\alpha]_{589}$ +16.0° (*c* 0.16, MeOH), and (**6**), R_F 0.20 (HOAc–H_2O, 6:94 v/v), 0.30 (Bu^tOH–HOAc–H_2O, 3:1:1 v/v), $[\alpha]_{589}$ +1.0° (*c* 0.10; MeOH) were isolated from an EtOAc extract of the heartwood in approximately equal ratio by repeated chromatography on a Sephadex LH 20 column using EtOH–H_2O (1:1 v/v) as eluant.

Both compounds yielded melacacinidin (**1**)[10] on heating with HCl in EtOH, and under milder conditions (HOAc, EtOH) gave 4-*O*-ethylisomelacacidin (**4**)[10,11] (Scheme 1), which clearly shows that these compounds are more closely related to the leucomelacacidins (**1**)—(**4**) than the more common carbon–carbon linked proanthocyanidins.[3,12] Fast atom bombardment (f.a.b.) m.s. gave $(M + H)^+$ peaks at *m/z* 595 for both compounds, indicating that they have a biflavanoid constitution. This structure was further corroborated by chemical ionisation (c.i.) m.s. of their methylated and acetylated products, which were consistent with the octamethyl and the deca-acetate derivatives respectively.

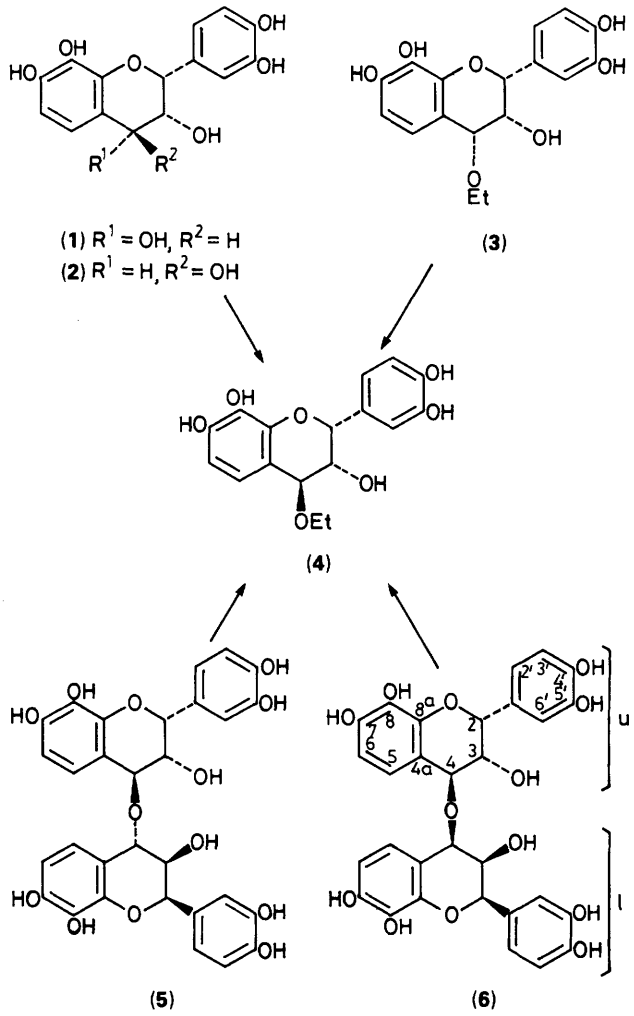
The ^{13}C n.m.r. spectrum of (**5**) showed only fifteen fully resolved carbon resonances and in conjunction with the mass spectral data clearly indicated that the compound consisted of two identical flavanoid units linked through a point of symmetry. The upfield position of the C-2 resonance (δ 76) was in accord with the substituent at C-4 being axial in orientation[10,13] and together with the observed C-3 and C-4 resonances at δ 69.9 and 75.0, respectively, was consistent with 2,3-*cis*-3,4-*trans* configuration of the heterocyclic *C*-ring as similarly observed for the concurrent isomelacacidin derivatives[10] (Table 1). This assignment was also corroborated by the ^1H n.m.r. data, which were readily identifiable with those of (**2**) and (**4**). The retention of all the carbon bonded protons in the isomelacacidin nucleus suggested that the interflavanoid linkage had to be *via* an ether bond. Although several interflavanoid ether linkages were possible, only the C-4–O–C-4 bond could satisfactorily account for the observed ^{13}C chemical shifts and the facile reaction with EtOH in the presence of HOAc to give 4-*O*-ethylisomelacacidin.[10,11] This deduction was also fully supported by considerations based on substituent shift effects where substitution with an ethyl group at the C-4 oxygen in isomelacacidin resulted in a large downfield shift of the C-4 resonance and a corresponding moderate upfield shift of the neighbouring C-4a resonance (Table 1). Comparison of the carbon resonances of the biflavanoid (**5**) with those of isomelacacidin showed the same direction and magnitude of shift effects on C-4 (Δδ +3.1 p.p.m.) and C-4a (Δδ −2.1 p.p.m.) thus confirming (**5**) to be [4-O-4]-bis(2,3-*cis*-3,4-*trans*-3,3′,4′,7,8-pentahydroxyflavan).

The presence of two dissimilar flavan units in (**6**) was readily evident from both the ^1H and ^{13}C n.m.r. spectra, which showed six distinct proton or carbon resonances in the heterocyclic region attributable to the two different pyran rings. Assignment of these signals was accomplished by 2D n.m.r. techniques using $^1H^1H$-COSY in conjunction with $^1H\{^{13}C\}$-COSY, which enabled the carbon resonances at δ 76.0 (C-4), 74.6 (C-2), and 70.0 (C-3) to be identified as belonging to one flavan unit, clearly identifiable with the isomelacacidin unit as in (**5**), and the remaining resonances at

Table 1. ^{13}C N.m.r. chemical shifts (in [$^2H_6$]acetone–H_2O) of monomeric and dimeric C-4 oxygenated 3,3',4',7,8-pentahydroxyflavans.

	C-2	C-3	C-4	C-4a	C-5	C-6	C-8
(1)	79.5	68.2	70.1	116.9	119.0	109.8	132.7
(2)	75.4	68.2	71.8	115.1	123.0	110.2	132.4
(3)	79.3	66.3	75.7	115.0	119.3	109.7	132.7
(4)	75.8	69.3	76.0	113.0	123.3	109.6	133.0
(5)	75.0	69.9	76.0	113.6	122.9	110.3	133.1
	75.0	69.9	76.0	113.6	122.9	110.3	133.1
(6)	u[a]74.6	70.0	76.0	112.9	123.2	109.6	133.3
	l[a]79.5	67.2	74.0	115.1	119.3	109.6	132.7

[a] *Symbols*: u = upper unit, and l = lower unit, as depicted in Scheme 1.



Scheme 1. Acid catalysed ethanolysis of promelacacidins.

δ 79.4 (C-2), 74.0 (C-4), and 67.2 (C-3) as those of another unit. As all the methine carbons and protons in both the aromatic and the pyran rings of the two flavan units could be accounted for by n.m.r., the linkage between the flavans again had to be *via* an ether bond. The position of this interflavanoid linkage was derived from considerations based on substituent shift effects resulting from displacing the C-4 hydroxy in (**1**) and (**2**) by an ethoxy group to give (**3**) and (**4**) respectively (see Table 1). Comparison of the carbon resonances of the biflavanoid (**6**) with those of (**1**) and (**2**) showed the same order of magnitude and direction of the shift to the C-4 ($\Delta\delta\sim$ +4 p.p.m.) and the neighbouring C-4a ($\Delta\delta\sim$ −2 p.p.m.) resonances, thus establishing the structure to be 2,3-*cis*-3,4-*trans*-3,3',4',7,8-pentahydroxyflavan-[4-O-4]-2,3-*cis*-3,4-*cis*-3,3',4',7,8-pentahydroxyflavan. This constitution is in complete accord with all other ^{13}C resonances (Table 1) and also with the observed proton chemical shifts and *J* values of the two H-4 resonances (δ 5.00, *J* 3.6 Hz and δ 4.41, *J* 3.0 Hz) attributed to the 3,4-*cis* and 3,4-*trans* configurations, respectively.[14] Further confirmation of the structure was obtained by mild ethanolysis using EtOH–HOAc, which yielded not only 4-*O*-ethylisomelacacidin as in the case of (**5**) but also melacacidin (**1**). The latter product with the 3,4-*cis* configuration being more resistant to mild ethanolysis[10,11] was generated intact.

The characterization of these two novel stereoisomeric biflavanoids extends the heterogeneity of the interflavanoid bondings which occur among natural oligomeric proanthocyanidins. The presence of these ether-linked compounds in *A. melanoxylon* in addition to the carbon–carbon bonded dimer[12] is probably an indication of the much reduced nucleophilicity of the pyrogallol *A*-ring which enables other centres to participate in interflavanoid bond formation.

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