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DIASTEREOISOMERIC LEUCOANTHOCYANIDINS FROM THE HEARTWOOD OF *ACACIA MELANOXYLON*

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Key Word Index—*Acacia melanoxylon*; Leguminosae; leucoanthocyanidins; melacacidin; isomelacacidin; 4-*O*-ethylmelacacidin; 4-*O*-ethylisomelacacidin; melacacinidin; leucomelacacinidin; quinone-methide.

Abstract—The isolation of two pairs of diastereoisomeric leucoanthocyanidins, namely (2*R*,3*R*,4*R*)-2,3-*cis*-3,4-*cis*-3,3',4,4',7,8-hexahydroxyflavan or melacacidin, (2*R*,3*R*,4*S*)-2,3-*cis*-3,4-*trans*-3,3',4,4',7,8-hexahydroxyflavan or isomelacacidin and (2*R*,3*R*,4*R*)-2,3-*cis*-3,4-*cis*-4-ethoxy-3,3',4',7,8-pentahydroxyflavan or 4-*O*-ethylmelacacidin, (2*R*,3*R*,4*S*)-2,3-*cis*-3,4-*trans*-4-ethoxy-3,3',4',7,8-pentahydroxyflavan or 4-*O*-ethylisomelacacidin is described. 4-*O*-Ethylmelacacidin is a new compound and all four leucoanthocyanidins are natural constituents of the heartwood of *Acacia melanoxylon*. Melacacinidin is the name proposed for the anthocyanidin 3,3',4',7,8-pentahydroxyflavylium and leucomelacacinidins for the corresponding leucoanthocyanidins. Quinone-methide formation is proposed to account for the difference in reactivity between the diastereoisomers.

INTRODUCTION

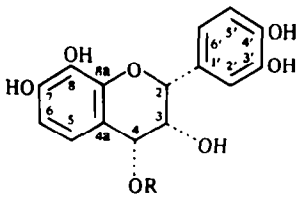
Acacia melanoxylon, more commonly known as Australian blackwood, is a medium size tree native to a large area of Australia. The wood is primarily used in furniture making. Earlier studies [1–3] of the heartwood constituents showed the flavonoids to have the 3',4',7,8-hydroxylation pattern which is characteristic of a great number of *Acacia* species as well as containing a pair of diastereoisomeric leucoanthocyanidins [4–6]. (2*R*,3*R*,4*R*)-2,3-*cis*-3,4-*cis*-3,3',4,4',7,8-Hexahydroxyflavan or malacacidin (**1**) was the first flavan-3,4-diol to be identified and its chemical structure fully characterized [4–6]. (2*R*,3*R*,4*S*)-2,3-*cis*-3,4-*trans*-3,3',4,4',7,8-Hexahydroxyflavan or isomelacacidin (**2**) was later identified in the extractives of the heartwood of both *A. excelsa* and *A. harpophylla* and shown to be the C-4 epimer of melacacidin [5]. The present report describes a re-examination of the heartwood of *A. melanoxylon* which has resulted in the isolation of a further pair of diastereoisomeric leucoanthocyanidins.

RESULTS AND DISCUSSION

Clark-Lewis and Mortimer [5] have alluded to the difficulty of separating the pair of diastereoisomers (**1** and **2**) and their resolution was facilitated by conversion of the more reactive isomelacacidin to the 4-*O*-ethylisomelacacidin (**4**). Separation of all of these diastereoisomers may now be achieved by repeated column chromatography alternating between Sephadex LH20 and MCI Gel, or high porosity polystyrene gel. The use of both LH20 and MCI gel was initially devised by Nonaka *et al.* [7] for separating various flavanoids, their gallate esters and oligomers. In this manner two additional diastereoisomers were isolated and shown to be (2*R*,3*R*,4*S*)-2,3-*cis*-3,4-*trans*-4-ethoxy-3,3',4',7,8-pentahydroxyflavan or more commonly known as 4-*O*-ethylisomelacacidin (**4**) and its epimer (2*R*,3*R*,4*R*)-2,3-*cis*-3,4-*cis*-4-ethoxy-3,3',4',7',8-pentahydroxyflavan (**3**), which therefore may be known as 4-*O*-ethylmelacacidin. Although 4-*O*-ethylisomelacacidin has been synthesized previously by solvolysis of isomelacacidin in ethanol in the presence of acetic acid, its diastereoisomer (**3**) has not been described before. All these compounds gave rise to the same anthocyanidin when heated with mineral acids.

The recognition by Clark-Lewis and Mortimer [5] of the facile reaction of isomelacacidin with ethanol under mild acid conditions prompted them to suggest that 4-*O*-ethylisomelacacidin, which they isolated from the heartwood *A. excelsa* and *A. harpophylla*, was an artefact of their work up method. The present studies show solvolysis in aqueous ethanol in the absence of acid was unlikely because none of the four leucomelacacinidins reacted when left to stand at ambient temperatures in ethanol–water (1:1) for over 3 days. However, when a small amount of acetic acid was added to these four samples and the solutions warmed on a water bath for 10 min, only 4-*O*-ethylisomelacacidin underwent solvolysis to give rise to some isomelacidin. In the presence of stronger acid (dilute HCl) and with some warming, the two 3,4-diols gave rise to some amount of 4-*O*-ethylisomelacacidin. In no instance was 4-*O*-ethylmelacacidin produced by such treatments as would have been predicted because of the stereoselectivity of such reactions whereby the products of solvolysis are predominantly 3,4-*trans* compounds. On this evidence alone, the extracted novel compound, 4-*O*-ethylmelacacidin is unlikely to be an artifact brought about by the extraction and isolation procedure used. Most importantly, both 4-*O*-ethylmelacacidin and 4-*O*-ethylisomelacacidin due to their relative high mobility were readily detected on 2D-TLC of wood extracts where only acetone and ethyl acetate were employed in their extraction.

These leuoanthocyanidins which readily undergo solvolysis in the presence of dilute HCl, are remarkably stable in comparison with leucocyanidins and leucofisetinidins. The latter mentioned compounds undergo rapid



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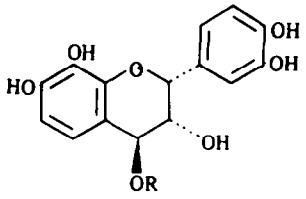

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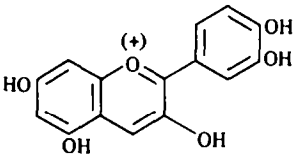

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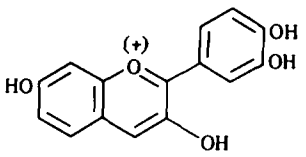

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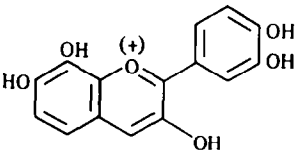

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self-condensation giving rise to oligomers in the presence of dilute acid. The presence of the hydroxyl at C-8 in the A-ring has the effect of partially negating the localization of electrons by the C-5 and C-8a oxygens in the leucomelacacinidins thus rendering them less effective than leucocyanidins and leucofisetinidins as nucleophiles.

As their names suggest, leucocyanidins and leucofisetinidins are monomeric flavans which produce cyanidin (**5**) and fisetinidin (**6**), respectively, when treated with mineral acids. The leucoanthocyanidins from *A. melanoxylon*, when similarly treated with alcoholic HCl, yield the anthocyanidin 3,3',4',7,8-pentahydroxyflavylium (**7**). Although this anthocyanidin has been one of the earliest known, it has no natural counterpart and no common name has been assigned to this pigment. In order to facilitate the description of this group of natural products as well as to be consistent with established nomenclature on this subject, it is appropriate at this point to suggest that the anthocyanidin **7** be called melacacinidin and the corresponding leucoanthocyanidins will therefore be known as leucomelacacinidins.

The distinction between melacacidin and its C-4 epimer isomelacacidin was made readily by the facile reaction of the latter compound with ethanol in the presence of acid to give 4-*O*-ethylisomelacacidin. This in turn facilitated the identify of another diastereoisomer, 4-*O*-ethylmelacacidin (**3**), which was also isolated from the heartwood extract. The ^{13}C NMR data (Table 1) of these compounds are fully in accord with their chemical structures and the value of the C-2 chemical shift enables the stereochemistry of these C-4 oxygenated flavans to be assigned. Melacacidin and 4-*O*-ethylmelacacidin, both of which possess the 2,3-*cis*-3,4-*cis* configuration, have C-2 signals at *ca* δ79.5 which is comparable to the C-2 chemical shifts of 2,3-*cis*-flavan-3-ols [8]. The C-2 chemical shifts for isomelacacidin and 4-*O*-ethylisomelacacidin are at δ75.4 and 75.9, respectively, or *ca* 4 ppm upfield from the respective diastereoisomers. The magnitude of this upfield shift is characteristic of 1,3-steric interaction or γ-gauche effect due to the axial orientation of the C-4 substituent in 3,4-*trans* configuration of these compounds [8, 9]. It follows that due to the lack of such interactions in the 3,4-*cis* compounds, the substituents at C-4 are necessarily in the equatorial conformation. Examination of the aromatic A-ring chemical shifts also showed a significant difference of the order of 4 ppm in the chemical shift of C-5 between the two pairs of epimers, with the values of the 3,4-*trans* compounds being more downfield. This difference in chemical shifts of C-5 may be steric in origin and the C-4 substituents in the 3,4-*cis*-configuration, as discussed above as being in the equatorial conformation, are therefore more or less in the same plane as the aromatic A-ring and hence exert a crowding effect on the hydrogen at C-5. The dual steric effects observed on C-2 and C-5 brought about by the proposed conformations of the substituents at C-4 are self-consistent and lend considerable support to their axial orientation in the 2,3-*cis*-3,4-*trans* compounds and equatorial orientation in 2,3-*cis*-3,4-*cis* compounds. The replacement of the hydroxyl group by an ethoxy function resulted in the anticipated downfield shift of the resonance of C-4 by *ca* 4–6 ppm [10].

IR spectroscopy has been shown to be useful in determining the stereochemistry as well as the oxidation pattern of the B-ring of the flavan monomers in various proanthocyanidins [11]. Absorption in the 795–800 cm^{-1} region has been shown to be associated with the 2,3-*cis*-configuration and thus the presence of this band in the IR

Table 1. ^{13}C NMR chemical shifts (in acetone-d_6–water) of leucomelacacinidins

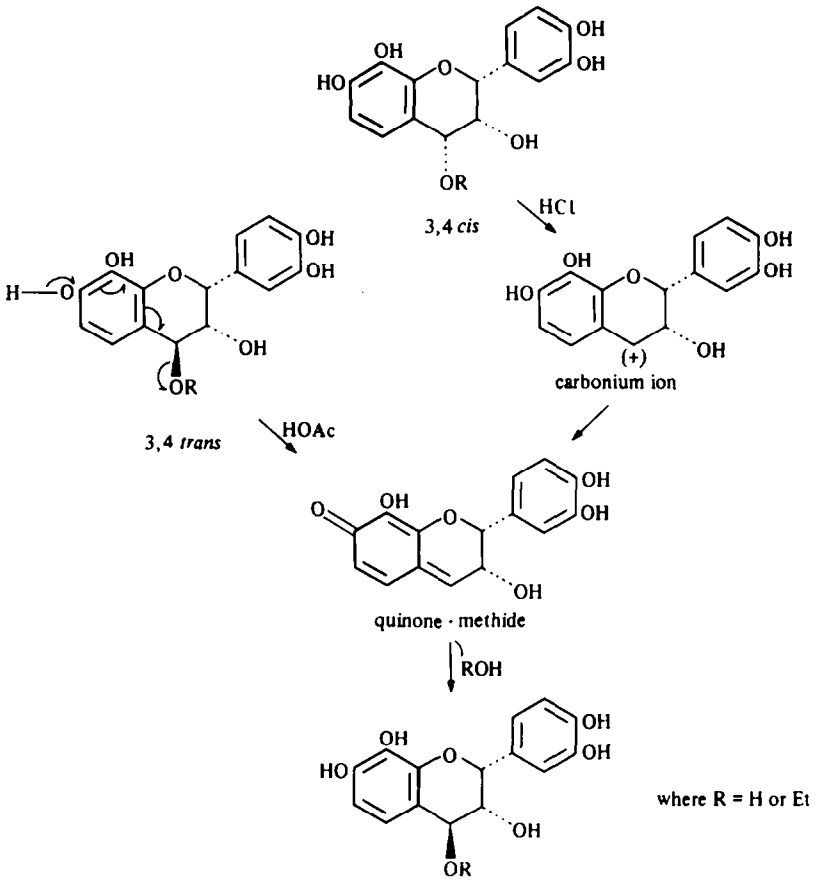
	C-2	C-3	C-4	C-4a	C-5	C-6	C-8	C-1′	C-2′	C-5′	C-6′	C-7	C-8a	C-3′	C-4′	$-CH_2-$	Me
Melacacidin (1)	79.5	68.2	70.1	116.9	119.0	109.8	132.7	131.3	116.4	115.5	119.8	145.2	145.2	145.2	145.3	—	—
Isomelacacidin (2)	75.4	68.2	71.8	115.1	123.0	110.2	132.4	131.1	116.7	115.5	119.8	144.5	145.1	145.1	145.8		
4-*O*-Ethylmelacacidin (3)	79.3	66.3	75.7	115.0	119.3	109.7	132.7	131.3	116.4	115.5	119.7	145.2	145.2	145.2	145.3	65.4	15.0
4-*O*-Ethylisomelacacidin (4)	75.8	69.3	76.0	113.0	123.3	109.6	133.0	131.1	116.5	115.6	119.7	145.2	145.2	145.2	146.1	65.0	15.0

*Assignment of chemical shifts may be interchangable between the carbons in that bracket.

spectra of 2,3-*cis*-diastereoisomeric leucomelacacinidins is therefore not unexpected. This phenomenon is apparently linked to vicinal substituents which are in the *cis* conformation in general, rather than to the nature of the substituents or the position of their substitution in the heterocyclic ring. The intensity of this 800 cm^{-1} band is relatively more pronounced in the spectra of the 2,3-*cis*-3,4-*cis* compounds than that of the 2,3-*cis*-3,4-*trans* isomers.

All four leucomelacacinidins show very similar electron impact mass spectral data. Without exception no molecular ions were observed and all gave the highest peak at *m/z* 288, a value consistent with the loss of water from the flavan-3,4-diols or loss of ethanol from the 4-*O*-ethyl derivatives. This observation suggests that the benzylic hydroxyls, rather than those attached to C-3, are more susceptible to cleavage not only during solvolysis but also during electron bombardment. The loss of ethanol or water is facilitated in the iso- or 3,4-*trans* compounds as would have been predicted from the solvolysis reaction. A comparison of the intensity of the parent ions showed that 3,4-*trans* compounds possessed significantly more intense *m/z* 288 peaks (17–19% as opposed to 4% of base ion) than those derived from the 3,4-*cis* isomers. A distinction between the flavan-3,4-diols and the 4-*O*-ethyl compounds is possible in spite of the absence of molecular ions. The peak at *m/z* 183, while absent in the 3,4-diols, is common to both the C-4-ethoxy compounds and this fragment may be rationalized as arising from a retro-Diels–Alder fission of the heterocyclic ring with subsequent hydrogen abstraction. The diols undergo similar retro-Diels–Alder reaction to give rise to the corresponding ion at *m/z* 154.

The difference in reactivity of melacacidin and isomelacacidin has been attributed to the conformational differences of the C-4 hydroxyl leading to the formation of a C-4 carbonium ion [5]. The stereoselectivity of the solvolysis reaction leading to 3,4-*trans* products has been attributed to participation of the C-3 hydroxyl leading to an epoxide intermediate with subsequent *trans* opening of the ring [5, 12]. In view of the lack of any evidence concerning anchimeric assistance by the C-3 hydroxyl [12], it is most probable that a quinone-methide intermediate is involved in this reaction. Recent studies [13, 14] on procyanidin synthesis have indicated the formation of such intermediates which resulted in stereospecific addition to C-4 to give rise to 3,4-*trans* products. This proposal has considerable support from the work of Attwood *et al.* [15] who demonstrated the generation of quinone-methides from 5- and 7-hydroxyflavan-4-ols in both acid and alkaline solutions. Furthermore the marked difference in the reactivity between melacacidin and isomelacacidin towards mild acid catalysed solvolysis also may be rationalized by the invocation of such intermediates. The C-4 hydroxyl and ethoxyl of isomelacacidin and 4-*O*-ethyl-isomelacacidin, respectively, being in the axial conformation have the C–O bonding orbital favourably exposed for overlapping with the π electrons of the aromatic A-ring thus facilitating the formation of the quinone-methide. The C-4 equatorial conformation of the substituents in melacacidin and 4-*O*-ethylmelacacidin is not favoured for such orbital overlap and hence requires a stronger acid medium to generate a carbonium ion at C-4 which then may be stabilized by formation of the quinone-methide (see Scheme 1).



Scheme 1. Proposed mechanisms for the solvolysis of leucomelacacinidins.

EXPERIMENTAL

Specific rotations were obtained at ambient temp. in EtOH. ^{13}C NMR spectra were measured in Me_2CO-d_6–H_2O (1:1) using TMS as ext. standard. IR spectra were recorded on KBr pellets (1.5–2.0 mg sample in a 13 mm pellet). TLC was on Schleicher and Schull cellulose using *t*-BuOH–HOAc–H_2O (3:1:1, solvent A) and HOAc–H_2O (3:47, solvent B).

Heartwood (1 kg) of *A. melanoxylon* was extracted twice with Me_2CO–H_2O (7:3) by soaking the chips for a minimum of 24 hr each time and the combined extracts shaken with NaCl. The resulting upper layer was concd and the residue diluted with H_2O and filtered over glass wool. The filtrate was exhaustively extracted with EtOAc, the extracts dried (Na_2SO_4) and concd to give a solid residue (14 g). The residue was subjected to CC, first on Sephadex LH20, eluting with EtOH to yield a mobile fraction which was then treated on a reverse phase (MCI gel) column using MeOH–H_2O (3:7). The latter procedure resulted in the isolation of 4-*O*-ethylisomelacacidin (1.10 g) and 4-*O*-ethylmelacacidin (0.50 g) and a mixture consisting primarily of the isomeric flavan 3,4-diols. Melacacidin (1.30 g) was isolated by treating the mixture on the reverse phase column and eluting with H_2O. The remaining mixture yielded isomelacacidin (0.15 g) when treated on Sephadex LH20 using H_2O as eluant.

(2R,3R,4R)-2,3-cis-3,4-cis-3,3',4,4',7,8-*Hexahydroxyflavan* (*melacacidin*). $[\alpha]^D$ −74° (*c* 0.08; EtOH); R_f (A) 0.40, (B) 0.40. MS *m/z* (rel. int.): 288 (4%), 260 (8), 154 (35), 153 (41), 152 (32), 150 (14), 123 (100%). IR ν_{max}^{KBr} cm^{-1}: 3250–3650, 1630, 1523, 1475, 1360, 1290, 1205, 1167, 1117, 1086, 1043, 983, 932, 863, 797, 720. The compound remained unchanged in EtOH–H_2O (1:1) at room temp. for over 3 days but yielded 4-*O*-ethylisomelacacidin on warming with 1% HCl in EtOH.

(2R,3R,4S)-2,3-cis-3,4-trans-3,3',4,4',7,8-*Hexahydroxyflavan* (*isomelacacidin*). $[\alpha]^D$ −32° (*c* 0.10; EtOH); R_f (A) 0.50; (B) 0.50 MS *m/z* (rel. int.): 288 (19), 260 (45), 154 (30), 153 (32), 152 (30), 150 (70), 139 (58), 138 (28), 137 (36), 123 (100%). IR ν_{max}^{KBr} cm^{-1}: 3150–3520, 1630, 1525, 1480, 1450, 1365, 1290, 1230, 1205, 1170, 1108, 1080, 1035, 983, 862, 805, 765, 713. The compound remained unchanged in EtOH–H_2O (1:1) at room temp. for over 3 days but readily converted to 4-*O*-ethylisomelacacidin when a small amount of HOAc was added and the mixture warmed for 15 min on a H_2O-bath.

(2R,3R,4R)-2,3-cis-3,4-cis-4-*Ethoxy*-3,3',4',7,8-*pentahydroxyflavan* (4-*O*-*ethylmelacacidin*). $[\alpha]^D$ −48° (*c* 0.05; EtOH); R_f (A) 0.70; (B) 0.60. MS *m/z* (rel. int.): 288 (4), 260 (10), 183 (8), 154 (15), 153 (17), 152 (26), 150 (13), 139 (12), 137 (6), 123 (100%). IR ν_{max}^{KBr} cm^{-1}: 3250–3500, 1625, 1522, 1473, 1350, 1290, 1210, 1168, 1120, 1065, 985, 940, 865, 797, 720. The compound remained unchanged in EtOH–H_2O (1:1) at room temp. for over 3 days. Addition of one drop of HOAc and warming on H_2O-bath for 15 min did not give rise to additional spots when examined on TLC. When dil HCl was added and the soln warmed for 15 min, some 4-*O*-ethylisomelacacidin and isomelacacidin were produced.

2R,3R,4S)-2,3-cis-3,4-trans-4-*Ethoxy*-3,3',4',7,8-*pentahydroxy*-flavan (4-O-*ethylisomelacacidin*). $[\alpha]^D - 75°$ (*c* 0.02; EtOH); R_f (A) 0.80; (B) 0.75. MS *m/z* (rel. int.): 288 (17), 260 (28), 198 (42), 183 (42), 169 (15), 168 (72), 154 (29), 153 (73), 152 (29), 150 (79), 139 (50), 137 (9), 123 (100%). IR ν_{max}^{KBr} cm^{-1}: 3250–3500, 1620, 1525, 1480, 1448, 1360, 1290, 1235, 1210, 1105, 1080, 1070, 1000, 980, 860, 800, 770, 718. The compound remained unchanged in EtOH–H_2O (1:1) at room temp. for over 3 days but when one drop of HOAc was added with warming, isomelacacidin was detected on TLC.

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