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8-*O*-METHYL- AND THE FIRST 3-*O*-METHYLFLAVAN-3,4-DIOLS FROM *ACACIA SAXATILIS*

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Key Word Index—*Acacia saxatilis*; Leguminosae; coloured heartwood constituents; flavan-3,4-diolmethyl ethers; diastereoisomers; flavonols; flavanones.

Abstract—The light purple heartwood of *Acacia saxatilis* contains (+)-2,3-*trans*-3,4-*trans*- and (+)-2,3-*trans*-3,4-*cis*-diastereoisomers of 8-methoxy-7,3',4'-trihydroxy- and 7,3',4'-trihydroxyflavan-3,4-diols as major components. Evidence was also obtained of the first 3-methyl ether of metabolites of this type, notably of (+)-8-methoxy-7,3',4'-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol. Flavonol, dihydroflavonol and flavanone analogues accompany these. The correlation between colour of *Acacia* heartwoods and structure, phenolic substitution, stereochemistry and composition of their flavonoid components is discussed.

INTRODUCTION

FOLLOWING recent studies[1–4] on the purple or purple-ringed heartwoods of *Acacia crombei*, *A. carnei* and *A. peuce*, which are hitherto unique amongst the Mimosaceae (Leguminosae; Mimosoideae) for their peltogynoid content, attention was drawn to the somewhat lighter purple of *A. saxatilis* heartwood. Attempts were made to relate the unusual colour to the structure and stereochemistry of its components, and the comparison is extended also to other *Acacia* species.

RESULTS AND DISCUSSION

A. saxatilis E. Moore represents an exceptionally rare Australian member of the genus. The leucoanthocyanidin reaction in conjunction with paper chromatography showed the presence of three anthocyanidins, namely fisetinidin chloride (pink, reddish fluorescent in UV light, R_f 0·50 in 3 N HCl-90% formic acid),[5] 8-methoxy-7,3',4'-trihydroxyflavylium chloride (wine-red, black in UV light, R_f 0·69)[6] and a highly mobile (R_f 0·71) wine-red anthocyanidin obviously bearing structural relationship with the latter, but with one free hydroxyl less.

In view of the small quantity of heartwood available from the shrub, and the known instability[7] of 7,8,3',4'-tetrahydroxyflavonoids, fractions obtained from initial chromatographic separation of the extract were methylated to give the methyl ethers of no less

[1] TINDALE, D. M. and ROUX, D. G. (1974) *Phytochemistry* **13**, 829.
[2] BRANDT, E. V. (1973) Ph.D. Thesis, University of the Orange Free State.
[3] BRANDT, E. V., FERREIRA, D. and ROUX, D. G. (1971) *Chem. Commun.* 116.
[4] BRANDT, E. V., FERREIRA, D. and ROUX, D. G. (1972) *J.C.S. Chem. Commun.* 392.
[5] ROUX, D. G. (1957) *Nature* **179**, 305.
[6] DU PREEZ, I. C. and ROUX, D. G. (1970) *J. Chem. Soc.* (*C*), 1800.
[7] FOURIE, T. G., DU PREEZ, I. C. and ROUX, D. G. (1972) *Phytochemistry* **11**, 1763.

TABLE 1. NMR SPECTRA* OF 3- AND 4-*O*-methyl derivatives, their acetates, and

(a) 2,3-*trans*-3,4-*cis*	(+)-7,8,3',4'-tetramethoxyflavan-3,4-diols 2	3	H-4	5	6	2'
3-*O*-methyl	4·73 $J_{2,3}$8·0	6·37	5·17 $J_{3,4}$3·5	2·87	3·37	~2·97
4-*O*-acetyl-3-*O*-methyl	4·85 $J_{2,3}$10·0	6·33	3·70 $J_{3,4}$3·6	2·91	3·42	~2·92
4-*O*-methyl	4·87 $J_{2,3}$9·6	5·98	5·70 $J_{3,4}$3·5	3·01	3·40	~2·97
3-*O*-acetyl-4-*O*-methyl	4·57 $J_{2,3}<1$	4·59	5·60 $J_{3,4}\leq 1$	3·01	3·40	~2·98
3,4-diol	4·95 $J_{2,3}$9·8	6·05	5·33 $J_{3,4}$3·7	2·90	3·40	~2·98
3,4-di-*O*-acetyl	4·75 $J_{2,3}$10·0	4·50	3·81 $J_{3,4}$3·1	2·95	3·40	~3·00
(b) 2,3-*trans*-3,4-*trans*						
3,4-diol	5·25 $J_{2,3}$9·5	~6·2	5·23 $J_{3,4}$8·0	2·83	3·42	~3·00
3,4-di-*O*-acetyl	4·90 $J_{2,3}$9·0	4·45	3·76 $J_{3,4}$7·0	3·08	3·38	~3·00

(c) 2,3-*trans*-3,4-*cis*	(±)-7,3',4'-trimethoxyflavan-3,4-diols† 2	3	H-4	5	6	8
3-*O*-methyl	4·82 $J_{2,3}$9·0	6·38	5·15 $J_{3,4}$3·5	2·80	3·38	3·45
4-*O*-methyl	4·93 $J_{2,3}$10·0	5·95	5·70 $J_{3,4}$3·2	2·80	3·42	3·48
(d) 2,3-*trans*-3,4-*trans*						
3-*O*-methyl	5·16 $J_{2,3}$9·8	6·39	5·13 $J_{3,4}$7·6	2·55	3·36	3·50

See Table 2 for footnotes.

than six flavan-3,4-diols (**1**–**6**; R_1=Me, R_2=H) in the ratio of 6·4:7·4:2·4:2:1·7:1 respectively. Four of these were readily recognized as the methyl ether derivatives of (+)-2,3-*trans*-3,4-*trans*- and (+)-2,3, *trans*-3,4-*cis*-diastereoisomers of the 8-methyl ether of 7,8,3',4'-tetrahydroxyflavan-3,4-diol (8-*O*-methylmelacacidins: **1**, **2**; R_1=Me) and 7,3',4'-trihydroxyflavan-3,4-diol (mollisacacidins: **5**, **6**; R=Me) by their coupling constants ($J_{2,3}$ ~ 9·5, $J_{3,4}$ ~ 7·5 and $J_{2,3}$ ~ 10·0, $J_{3,4}$ ~ 3·5 Hz respectively) and by the benzenoid AB- and ABX-spin systems respectively of the A-rings in the NMR-spectra of their methyl ethers and methyl ether diacetates (see Table 1). Considering that no 7,8,3',4'-tetrahydroxyflavylium chloride was generated during the leucoanthocyanidin reaction, the isolation of the full methyl ethers **1** and **2** (R_1=Me) is indicative of the presence of 8-methyl ethers of **1** and **2** (R_1=H), as supported by the formation of 8-methoxy-7,3',4'-trihydroxyflavylium chloride.

Surprising was the simultaneous isolation from the high R_f fraction of appreciable amounts of two (+)-7,8,3',4'-tetramethoxy-2,3-*trans*-flavan-3,4-*cis*-diol derivatives (**3** and **4**; R_1=Me, R_2=H), each of which showed a single aliphatic methoxy group to high field (τ 6·73, 6·52 resp.) in their NMR spectra. These compounds proved to be the 3- and 4-*O*-methyl derivatives (**3**, **4**; R_1=Me, R_2=H) respectively as shown by chemical shifts ($\Delta\tau$ –

of diacetates of 7,3′,4′-trimethoxy- and 7,8,3′,4′-tetramethoxyflavan-3,4-diols

–H		–OCH$_3$			–OCOCH$_3$	
5′	6′	Phenolic	3	4	3	4
3·10	~2·97	6·09 (×2), 6·11, 6·15	6·73	—	—	—
3·10	~2·92	6·07 (×2), 6·11, 6·17	6·81	—	—	7·85
3·10	~2·97	6·10 (×3), 6·17	—	6·52	—	—
3·13	~2·98	6·10 (×3), 6·13	—	6·52	8·03	—
3·12	~2·98	6·10 (×2), 6·12, 6·15	—	—	—	—
3·12	~3·00	6·10 (×3), 6·17	—	—	8·12	7·85
3·16	~3·00	6·13 (×3), 6·23	—	—	—	—
3·16	~3·00	6·12 (×4)	—	—	8·12	7·99

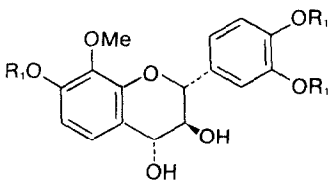
2′	–H 5′	6′	–OCH$_3$ Phenolic	3	4
~2·93	3·07	~2·93	6·07 (×2), 6·25	6·71	—
~2·97	3·16	~2·97	6·07 (×2), 6·21	—	6·47
~2·95	3·10	~2·95	6·06 (×2), 6·22	6·90	—

1·47, −1·37) of protons geminal to the residual 4- and 3-hydroxyl functions respectively on acetylation (see Table 1). The allocations also correlate with the anticipated RDA-fragments obtained by mass spectrometry of the 3- and 4-*O*-methyl ethers (**3**, **4**; R_1=Me, R_2=H) and of their respective 4- and 3-*O*-acetates (**3**, **4**; R_1=Me, R_2=Ac).

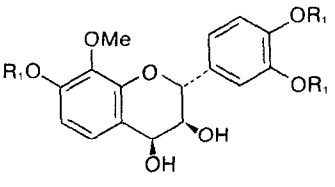
Finally, the chemical shifts of the 3- and 4-*O*-methyl resonances were compared with those of the corresponding synthetic derivatives of the same relative configuration, but based on 7,3′,4′-trimethoxy substitution. Thus, complete methylation of (±)-2,3-*trans*-fustin, readily available from *Rhus glabra*,[8] with dimethyl sulphate/anhydrous K_2CO_3 in dry acetone gave (±)-3,7,3′,4′-tetra-*O*-methyl-2,3-*trans*-fustin,[9] which on reduction with $LiAlH_4$ gave a mixture of (±)-3,7,3′,4′-tetramethoxy-2,3-*trans*-3,4-*trans*- and -2,3-*trans*-3,4-*cis*-flavan-4-ols.[10] The chemical shift of the 3-methoxy function in the latter derivative (τ 6·71) was almost identical (6·73) with that of the naturally-derived 3-methoxy analogue (**3**; R_1=Me, R_2=H). Similarly synthetic (+)-4,7,3′,4′-tetramethoxy-2,3-*trans*-3,4-*cis*-flavan-3-ol[11] and its acetate showed chemical shifts (τ 6·50, 6·52) of its 4-methoxyl function almost

[8] Roux, D. G. and Paulus, E. (1960) *Biochem. J.* **77**, 315.
[9] Van der Merwe, J. P., Ferreira, D., Brandt, E. V. and Roux, D. G. (1972) *J.C.S. Chem. Commun.* 521.
[10] Lillya, C. P., Drewes, S. E. and Roux, D. G. (1963) *Chem. Ind. (London)* 783.

identical to those (τ 6·52, 6·52) of the corresponding naturally-derived analogue (**4**; R_1=Me, R_2=H) and its acetate. Chemical shifts of the 4- and 3-*O*-acetyl methyl resonances of the acetates of the 3- and 4-*O*-methyl flavan-3,4-diols (**3**, **4**; R_1=Me, R_2=Ac) (τ 7·85, 8·03 resp.) were also representative of shifts (τ 7·85, 8·12) of the corresponding groups in (+)-3,4-*cis*-di-*O*-acetyl-7,8,3′,4′-tetramethoxy-2,3-*trans*-flavan (diacetate of **2**; R=Me). Absolute configurations of the above compounds (**1**–**6**; R_1=R_2=H) follow from the specific rotations of derivatives in comparison with the literature.



(1)



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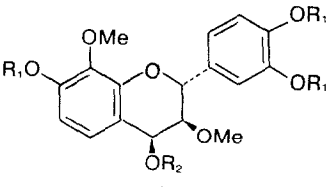

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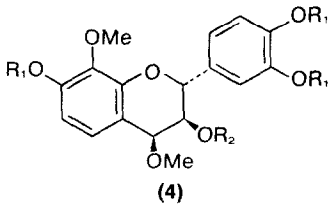

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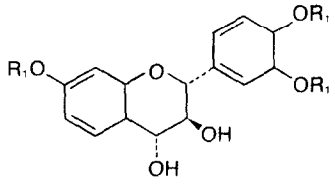
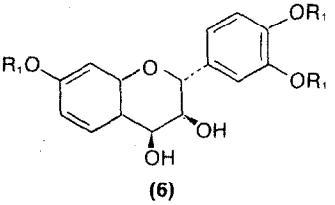


(5)



(6)

The 4-*O*-methyl flavandiol (**4**; R_1=Me, R_2=H) could represent an artefact resulting from methylation of (+)-8-*O*-methyl-7,8,3′,4′-tetrahydroxy-2,3-*trans*-flavan-3,4-*cis*-diol (**2**; R=H), since the 4-*axial*-hydroxyl exchanges with relative ease[12] for methoxy derived from the methanol-ether solution used during treatment with diazomethane. The substantial proportion of the corresponding 3-*O*-methylflavandiol (**3**; R_1=Me, R_2=H) obtained cannot, however, originate in this way (see Experimental) and its presence suggests that the parent compound (**3**; R_1=R_2=H) is formed biogenetically from the sequence α-hydroxychalcone→α-methoxychalcone→3-*O*-methyldihydroflavonol→3-*O*-methylflavandiol, two of these representing new classes of compounds (α-hydroxychalcone, 3-*O*-methyldihydroflavonols) which were recently found in *Trachylobium verrucosum*,[9] and subsequently in *Peltogyne pubescens* and *P. venosa*,[13] and also in *Acacia carnei*.[2] The relative prominence of the high R_f anthocyanidin and its mobility, which is identical to that of 3,8-dimethoxy-7,3′,4′-trihydroxy-flavylium chloride but differs from that of all other 3-*O*-alkyl derivatives of 3,7,8,3′,4′-pentahydroxy- and 8-methoxy-3,7,3′,4′-tetrahydroxy-flavylium chlorides (see Table 2 and Experimental), indicates that the 3-*O*-methylflavandiol exists naturally as the 8-methyl ether (**3**; R_1=R_2=H).

The 7,8,3′,4′-tetra-substituted and 7,3′,4′-tri-substituted flavonoid substitution patterns are also reflected in the low concentrations of associated dihydroflavonols, flavonols and flavanone. The dihydroflavonols were isolated as the methyl ethers of the 7,8,3′,4′-tetrahyd-

[11] Du Preez, I. C., Ferreira, D. and Roux, D. G. (1971) *J. Chem. Soc* (*C*), 336.
[12] Clark-Lewis, J. W., Katekar, G. F. and Mortimer, P. I. (1961) *J. Chem. Soc*. 499.
[13] Malan, E. and Roux, D. G. (1974) *Phytochemistry* In press.

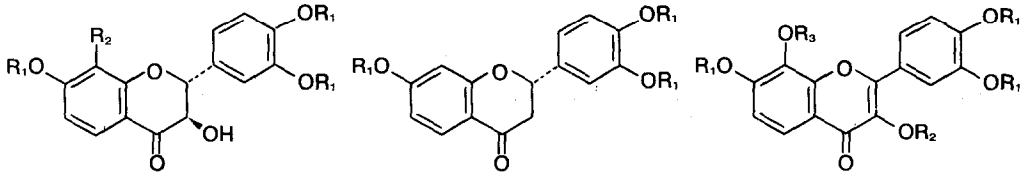
TABLE 2. R_f VALUES OF 3-*O*-ALKYL DERIVATIVES OF 3,7,8,3',4'-PENTAHYDROXY- AND 3,7,3',4'-TETRAHYDROXY-8-METHOXYFLAVYLIUM CHLORIDES†

Derivative	R_f in 3 N HCl—90% HCOOH (1:1)	
	3,7,8,3',4'-pentahydroxy-flavylium chloride and derivatives	8-methoxy-3,7,3',4'-tetrahydroxy-flavylium chloride and derivatives
Free phenol	0·35*	0·60*
3-*O*-methyl	0·53	0·71
3-*O*-ethyl	0·53	0·74
3-*O*-*n*-propyl	0·58	0·75
3-*O*-*iso*propyl	0·65	0·83
3-*O*-*n*-butyl	0·59	0·76
3-*O*-*sec*-butyl	0·68	0·86
3-*O*-*tert*-butyl	0·60	0·77

* Used as reference-values in each group.

† Notable is the gradational increase in R_f in the sequence methyl, ethyl, *n*-propyl, *n*-butyl ethers, and also the unusually high R_f values of both secondary alkyl ethers, *iso*propyl and *sec*-butyl (see ref.[27]). Increase in mobility is related to progressive loss of planarity in each series of 3-*O*-alkylanthocyanidins.

roxy-2,3-*trans* racemate[14] (**7**; R_1=Me, R_2=OMe) and 2,3-*trans*-fustin (**7**; R_1=Me, R_2=H), and the flavanone as racemic tri-*O*-methylbutin (**8**; R_1=Me). Methylation of the immobile yellow fluorescent band (R_f 0·00 in 2% acetic acid) gave 3,7,8,3',4'-pentamethoxyflavone (**9**; R_1=R_2=R_3=Me), and the high R_f 0·67 (water-satd. *sec*-butanol) of the free phenolic form indicates that the parent compound exists as 8-methoxy-3,7,3',4'-tetrahydroxyflavone. Present also was a powder-blue fluorescent compound, indicative of a 3-*O*-methylflavonol. Its position relative to the flavonol on two-dimensional chromatograms is characteristic of 3-*O*-methylflavonols[15,16] and NMR and mass spectral (M^+ 456) examination of the crude acetate indicated the presence of two aromatic methoxyl resonances. This compound is most likely 3,8-dimethoxy-7,3',4'-trihydroxy-flavone (**9**; R_1=H, R_2=R_3=Me) related to the 3-*O*-methyl-2,3-*trans*-flavan-3,4-*cis*-diol (**3**; R_1=H, R_2=Me).



*Racemates: 2R-(**7**) and 2S-(**8**) enantiomers indicated

The colour of Acacia *heartwoods.* The purple tinge of the heartwood of *A. saxatilis* is apparently due to a high proportion of 2,3-*trans*-flavan-3,4-*cis*-diols and a 3-methoxy-flavan-4-ol of the same configuration all possessing 4-*axial*-hydroxyl and 3-*axial*-proton function. From these, 3,4-*trans*-elimination of the elements of water leading to anthocyanidin formation, occurs more readily than from the 2,3-*trans*-3,4-*trans*-diastereoisomer

[14] CLARK-LEWIS, J. W. and MORTIMER, P. I. (1960) *J. Chem. Soc.* 4106.
[15] DREWES, S. E. and ILSLEY, H. A. (1969) *Phytochemistry* **8**, 1039.
[16] CLARK-LEWIS, J. W. and PORTER, L. J. (1972) *Australian J. Chem.* **25**, 1943.

where the corresponding groups are placed *equatorial* and *axial* respectively (see lit.[17]). In addition the absence of 7,8-dihydroxy function, due to natural methylation at the 8-position, provides a relatively light background for observation of the purple (see effect of 7,8-dihydroxy function below).

Other purple heartwoods encountered in a broad survey[1] of *Acacia* spp. are hitherto confined to *A. crombei*, *A. carnei* and *A. peuce*.[2] As in the case of *Peltogyne* spp. the so-called "purple-hearts" endemic to the West Indies, Venezuela, the Guianas and Northern Brazil, the intense purple is associated with the presence of peltogynols and mopanols.[18] Of these peltogynol is known to yield a violet anthocyanidin as opposed to a more reddish purple derived from mopanol,[2] and again the 2,3-*trans*-3,4-*cis*-diastereoisomers (peltogynol B and mopanol B) of each molecular species, possessing a 4-*axial*-hydroxyl, should be mainly responsible for the unusual heartwood pigment. The purple heartwoods of above *Acacia* spp. contain (+)-peltogynol and (+)-peltogynol B, both in high concentration.

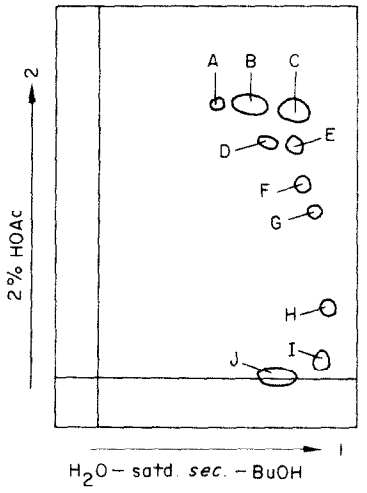


Fig. 1. Two-dimensional chromatogram of flavonoids from the heartwood of *Acacia saxatilis*. (A) (+) 3,8-dimethoxy-7,3',4'-trihydroxy-2,3-*trans*-3,4-*cis*-flavan-4-ol;* (B) (+) 8-methoxy-7,3',4'-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol; (C) (+) 8-methoxy-7,3',4'-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol; (D) (+) 7,3',4'-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol; (E) (+) 7,3',4'-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol [(+)-mollisacacidin]; (F) (±) 7,8,3',4'-tetrahydroxy-2,3-*trans*-dihydroflavonol (probably 8-methyl ether); (G) (±)-fustin; (H) (±)-butin; (I) 3,8-dimethoxy-7,3',4'-trihydroxy-flavone; (J) 3,7,8,3',4'-pentahydroxyflavone (probably 8-methyl ether). * Assignment to this position uncertain.

Oxidation undoubtedly plays a highly significant role in heartwood colour. Thus, compounds of the melacacidin type (7,8,3',4'-tetrahydroxyflavan-3,4-diols and analogues) with vicinal hydroxyls on each of the A- and B-rings give exceptionally dark brown to black heartwoods, e.g. blackwood (*A. melanoxylon*)[19] and knobwood (*A. nigrescens*)[7] due to the susceptibility of these groupings to autoxidation. Predominance of analogues of the teracacidin type (7,8,4'-trihydroxyflavan-3,4-diols and analogues) confer a much less intense light chocolate-brown due to vicinal hydroxyls on the A-ring only, e.g. *A. auriculiformis*[20] and *A. orites*.[16]

[17] CLARK-LEWIS, J. W. (1962) *Rev. Pure Appl. Chem.* **12**, 96.
[18] DREWES, S. E. and ROUX, D. G. (1967) *J. Chem. Soc.* (C) 1407.
[19] KING, F. E. and BOTTOMLEY, W. (1954) *J. Chem. Soc.* 1399.
[20] DREWES, S. E. and ROUX, D. G. (1966) *Biochem. J.* **98**, 493.

Vicinal 7,8-hydroxyls forming portion of a pyrogallol-based A-ring (as in melacacidins and teracacidins) are undoubtedly more liable to oxidation than 3',4'-vicinal hydroxyls comprising the catechol system of the B-ring. Hence heartwoods with constituents representative of resorcinol-type A-ring flavanoids (7,3',4'-trihydroxy- and 7,4'-dihydroxyflavan-3,4-diols and analogues) commonly distributed under the Botryocephaleae and Racemosae (Uninerves),[1] vary from light brown to a strong tendency towards pink (e.g. *A. mearnsii*[21]) depending on aging conditions. A photochemical effect undoubtedly plays a significant role in developing the pink colour.

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$ with TMS as internal standard on a Varian T-60 spectrometer: optical rotations were on a Bilger and Watts M-302 polarimeter using acetone-H_2O (9:1). 2-D PC was run by ascent on Whatman No. 1 (28 × 46 cm) sheets in H_2O-satd. *sec*.-BuOH and in 2% HOAc. Preparative PC was by ascent on Whatman No. 3 (46 × 57 cm) sheets in 2% HOAc. TLC was on Kieselgel PF_{254} (0·25 mm), and by preparative scale on the same substrate (1·0 mm). Plates were air-dried and unactivated, and sprayed with H_2SO_4–40% formaldehyde (40:1) (SAF-spray). The purity of each compound was established by NMR spectrometry.

Acetylation was with Ac_2O-pyridine at 40° for 5 hr, and methylation with diazomethane in MeOH–ethyl ether at −15° for 48 hr.

Extraction and preliminary separation. Drillings (623 g) of the pale purple heartwood of *A. saxatilis* S. Moore (NSW 107010) were extracted exhaustively at ambient temperatures with acetone-water (4:1) over 6 days with daily renewal of solvent. The combined extracts were evaporated to dryness under vacuum. Brown solids (44 g, 7·1%) obtained were examined by 2-D PC (Figure) and by the leucoanthocyanidin reaction in isopropanol.[22] Chromatography of the resultant anthocyanidins in 3 N HCl-90% HCO_2H (1:1)[5] indicated the presence of fisetinidin chloride [R_f 0·50, pink (visible), pink fluorescent (UV)],[5] 8-methoxy-3,7,3',4'-tetrahydroxypyrylium chloride [R_f 0·60, wine-red (visible), black (UV)][6] and a third prominent anthocyanidin [R_f 0·71, wine-red (visible), black (UV)]. The extract (42 g) was dissolved in 2·5 l acetone and applied to preparative sheets (~80 mg/sheet) and the chromatograms developed in 2% HOAc. Bands were located in UV light and with various spray reagents[23] at R_f 0·72, 0·62, 0·55, 0·11 and 0·0 (bands 1–5 respectively). These bands were cut and eluted with 20% acetone-water giving solids (6·3, 2·6, 1·4, 0·87 and 2·8 g respectively). A portion (400 mg) of band 1, R_f 0·72, was methylated with CH_2N_2 and the product separated by preparative TLC (30 plates) in 1,2-dichloroethane-Me_2CO (17:3) into four bands 1·1 (R_f 0·43), 1·2 (0·38), 1·3 (0·22) and 1·4 (0·17).

(+)-4,7,8,3',4'-*Pentamethoxy*-2,3-trans-3,4-cis-*flavan*-3-*ol* (**4**. R_1=*Me*, R_2=*H*). Band 1·1 (29 mg) gave a red colour with SAF spray and was *non-crystalline*, M^+ 376 (57%), *m/e* 316 (12), 270 (9·2), 255 (9·2), 238 (11), 231 (11), 224 (11), 223 (17), 211 (18), 209 (8·5), 208 (23), 207 (9·9), 205 (13), 198 (40), 197 (100), 196 (70), 195 (23), 194 (28), 193 (14), 191 (13), 183 (10·6), 182 (28), 181 (85), 180 (57), 179 (18), 169 (10·6), 168 (11), 167 (71), 166 (56), 165 (38), 164 (15), 163 (10·6), 161 (9·1), 159 (9·2), 157 (9·2), 155 (14), 154 (10·6), 153 (16·3), 152 (14·2), 151 (45), 150 (27), 149 (64), $[\alpha]_D^{27}$ + 14·9° (*c*. 0·5). The NMR spectrum (Table 1) is consistent with the above assignment (*cf.* refs.[6,11]).

(+)-3-*O*-*Acetyl*-4,7,8,3',4'-*pentamethoxy*-2,3-trans-3,4-cis-*flavan* (**4**. R_1=*Me*, R_2=*Ac*). Acetylation of the flavan-3-ol gave a *non-crystalline product* (25 mg), M^+ 418 (19%), $[\alpha]_D^{27}$ + 28·1° (*c*. 0·3). Found: C 63·03, H 6·31. Calc. for $C_{22}H_{26}O_8$: C 63·13, H 6·26%. The NMR spectrum (Table 1) was characteristic of the above assignment,[11] the unusual coupling constants, $J_{2,3} < 1$; $J_{3,4} \leq 1$, being due to non-bonded interaction between 2-phenyl and 3-acetoxy groups.

(+)-3,7,8,3',4'-*Pentamethoxy*-2,3-trans-3,4-cis-*flavan*-4-*ol* (**3**. R_1=*Me*, R_2=*H*). Band 1·2 (35·5 mg) gave a red with SAF spray and was *non-crystalline*, M^+ 376 (48%), *m/e* 279 (7·6), 211 (5·2), 210 (13), 208 (9·5), 198 (5·7), 197 (47), 196 (24), 195 (43), 194 (100), 193 (48), 183 (8·6), 182 (94), 181 (36), 180 (32), 179 (46), 178 (7·1), 167 (29), 166 (5·7), 165 (15), 164 (7·1), 163 (8·6), 158 (5·7), 153 (7·1), 152 (10·5), 151 (62), 152 (6·2), 149 (29), $[\alpha]_D^{27}$ + 3·1° (*c*.0·5). The NMR spectrum is consistent with the above assigment, the chemical shift of the 3-methoxyl differing significantly from that of the 4-methoxyl (Table 1).

(+)-4-O-*Acetyl*-3,7,8,3',4'-*pentamethoxy*-2,3-trans-3,4-cis-*flavan* (**3**. R_1=*Me*, R_2=*Ac*). Acetylation of the flavan-4-ol gave an *amorphous acetate*, m.p. 98°, M^+ 418 (39%), $[\alpha]_D^{27}$ +87·1° (*c*. 0·5), Found: C 63·07, H 6·36. Calc. for $C_{22}H_{26}O_8$: C 63·13, H 6·26%. The NMR spectrum is consistent with the above assignment, the chemical shift of the 4-acetoxy resonance being characteristic of this group (Table 1).

(+)-7,8,3',4'-*Tetramethoxy*-2,3-trans-*flavan*-3,4-cis-*diol* (**2**. R_1=*Me*). Band 1·3 (111 mg), red with SAF spray, crystallized from ethanol as fine needles, m.p. 166–167°, M^+ 362 (28%), *m/e* 316 (9·2), 183 (28), 182 (28), 181 (44), 180 (100), 167 (11), 166 (6·2), 165 (39), 152 (5·6), 151 (33), $[\alpha]_D^{27}$ +10·2° (*c*. 0·5). The NMR spectrum (Table 1) is consistent with the above assignment (see ref.[7]).

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[23] ROUX, D. G. and MAIHS, E. A. (1960) *J. Chromatog.* **4**, 65.

(+)-3,4-cis-*Di*-O-*acetyl*-7,8,3',4'-*tetramethoxy*-2,3-trans-*flavan*. Acetylation of the flavan-3,4-diol (40 mg) gave the diacetate which crystallized from ethanol in fine needles (35 mg), m.p. 113·5°, M^+ 446 (37%), $[\alpha]_D^{27}$ +93·8° (*c*, 0·4). The NMR spectrum (Table 1) was similarly consistent with the above assignment (see ref.[7]).

(+)-7,8,3',4'-Tetramethoxy-2,3-trans-*flavan*-3,4-trans-*diol* (**1**, R_1=*Me*). Solids from band 1·4 (97 mg) crystallized from ethanol as needles, m.p. 149°, M^+ 362 (15%), *m/e* 344 (7·1), 317 (5·0), 316 (23), 301 (6·4), 183 (24), 182 (23), 181 (41), 180 (100), 179 (5·0), 167 (10·0), 166 (5·7), 165 (37), 152 (5·0), 151 (26), $[\alpha]_D^{27}$ −2·9° (*c*, 0·5). Found: C 62·70, H 5·96. Calc. for $C_{19}H_{22}O_7$: C 62·95, H 6·12%. The NMR spectrum (Table 1) was consistent with the above assignment, reflecting *trans*-diaxial coupling of all heterocyclic protons.

(+)-3,4-trans-*Di*-O-*acetyl*-7,8,3',4'-*tetramethoxy*-2,3-trans-*flavan*. Acetylation of the flavan-3,4-diol (40 mg) gave the diacetate which crystallized from ethanol in fine needles (35 mg), m.p. 113°, M^+ 446 (95%), *m/e* 387 (9·5), 386 (28·6), 345 (21·9), 344 (95), 343 (15), 329 (35), 328 (81), 327 (100), 317 (11), 316 (49), 301 (10), 284 (8·6), 238 (17), 224 (13), 223 (34·8), 222 (43), 208 (21), 197 (6·7), 196 (44), 195 (9·5), 194 (9·5), 193 (12), 191 (14·3), 190 (21), 183 (44), 182 (48), 180 (52), 179 (20), 172 (16), 168 (21), 165 (52), 153 (7·6), 152 (15), 151 (50), $[\alpha]_D^{27}$ −15·6° (*c*, 0·55). Found: C 61·95, H 5·94. Calc. for $C_{23}H_{26}O_9$: C 61·85, H 5·87%. The NMR spectrum (Table 1) was similarly consistent with the above assignment (see refs.[6,24]). Solids (500 mg) from band 2, R_f 0·62, of the primary fractionation were methylated with CH_2N_2, and the product separated by TLC (36 plates) in 1,2-dichloroethane–Me_2CO (17:3) to give four bands, 2·1 (R_f 0·55), 2·2 (0·50), 2·3 (0·32) and 2·4 (0·23). Bands 2·1 (18·8 mg) and 2·2 (14·3 mg) proved to be mixtures giving unsatisfactory NMR spectra.

(+)-7,3',4'-*Trimethoxy*-2,3-trans-*flavan*-3,4-cis-*diol* (**6**, R_1=*Me*). Solids from band 2·3 (44·4 mg) crystallized from ethanol in fine needles, m.p. 185·3°, M^+ 332 (20%), *m/e* 281 (11), 181 (12·5), 180 (100), 165 (15), 153 (5·3), 151 (8·8), $[\alpha]_D^{22}$ +48·2° (*c*, 0·5). The NMR spectrum was consistent with the above assignment {lit.[6] m.p. 178·5°, $[\alpha]_D$ +40·3°}.

(+)-3,4-*cis*-*Di*-O-*acetyl*-7,3',4'-*trimethoxy*-2,3-trans-*flavan*. Acetylation of the flavan-3,4-diol gave an amorphous diacetate (25 mg), M^+ 416 (16·9%), $[\alpha]_D^{22}$ +128·1° (*c*, 0·4) {lit.[6] $[\alpha]_D$ +123·5°}. The NMR spectrum was consistent with the above structure.

(+)-7,3',4'-*Trimethoxy*-2,3-trans-*flavan*-3,4-trans-*diol* (**5**, R_1=*Me*). Solids from band 2·4, red with SAF spray, gave fine needles (75 mg) from ethanol, m.p. 128·8°, M^+ 332 (46%), *m/e* 285 (24), 264 (10·4), 184 (9·6), 182 (6·7), 181 (63), 180 (100), 167 (8·4), 165 (63), 153 (21), 152 (7·5), 151 (33), 149 (19), $[\alpha]_D^{22}$ −12·2° (*c*, 0·5). The NMR spectrum was consistent with the above assignment {lit.[6] m.p. 128°, $[\alpha]_D$ −9·4°}.

(+)-3,4-trans-*Di*-O-*acetyl*-7,3',4'-*trimethoxy*-2,3-trans-*flavan*. Acetylation of the flavan-3,4-diol (30 mg) gave the diacetate (25 mg) which crystallized as needles from ethanol, m.p. 102–103°, M^+ 416 (8·4%), $[\alpha]_D^{22}$ −18·3° (*c*, 0·4). The NMR spectrum was consistent with the above structure {lit.[6] m.p. 87°, 101–102°, $[\alpha]_D$ −19·6°}. Solids (1·3 g) from band 3, R_f 0·55, of the primary fractionation were purified by column chromatography (80 g Kieselgel 60) in C_6H_6–EtOAc–Me_2CO (6:4:1). The product was methylated and the methyl ethers separated by TLC (20 plates) in C_6H_6–EtOAc (4:1). Three bands 3·1 (R_f 0·47), 3·2 (0·40) and 3·3 (0·20) were located and eluted.

(±)-3',4',7-*Trimethoxyflavanone* (**8**, R_1=*Me*). Solids (23·6 mg) from band 3·1 failed to crystallize. The compound was optically inactive and the NMR spectrum was identical to the corresponding (−)-butin derivative[11] [τ 2·06(*d*, 5-H), 2·91 (m, 2'-H and 6'-H), 3·06 (*d*, 5'-H), 3·31 (*dd*, 6-H), 3·45 (*d*, 8-H), 4·53 (q, 2-H), 6·05, 6·07, 6·13 (s, 3 × OMe), 6·86–7·13 (m, CH_2)], M^+ 314.

(±)-7,3',4'-*Trimethoxy*-2,3-trans-*dihydroflavonol* [(±)-7,3',4'-*Tri*-O-*methyl*-2,3-trans-*fustin* (**7**, R_1=*Me*, R_2=*H*). Solids (72·7 mg) from band 3·2 crystallized from ethanol in needles, m.p. 142° (lit.[8] 142–3°). The compiund was optically inactive and its NMR spectrum was identical with data in the literature[24] [τ 2·13 (*d*, 5-H), 2·87 (m, 2'-H and 6'-H), 3·06 (*d*, 5'-H), 3·31 (*dd*, 6-H), 3·42 (*d*, 8-H), 4·90 (*d*, 2-H), 5·42 (*d*, 3-H), 6·08, 6·11, 6·18 (s, 3 × OMe), $J_{2,3}$ 12·2 Hz], M^+ 330.

(±)-7,8,3',4'-*Tetramethoxy*-2,3-trans-*dihydroflavonol* (**7**, R_1=*Me*, R_2=*OMe*). Solids (84·9 mg) from band 3·3 crystallized from ethanol in needles, m.p. 165° (lit.[7] 163°). The compound was optically inactive and its NMR spectrum was consistent with the above structure [τ 2·27 (*d*, 5-H), 2·70–3·20 (m, 2'-H and 6'-H), 3·03 (*d*, 5'-H), 3·27 (*d*, 6-H), 4·90 (*d*, 2-H), 5·43 (*d*, 3-H), 6·04, 6·06, (× 2), 6·13 (s, 4 × OMe), $J_{2,3}$ 12·2 Hz], M^+ 360. Solids (600 mg) from band 4, R_f 0·11, of the primary fractionation were acetylated. From the acetates only one compound (6 mg) could be separated by preparative TLC on 35 plates in C_6H_6–EtOAc–Me_2CO (3:2:1). The NMR spectrum indicated the presence of two phenolic methoxyl (τ 5·96, 6·05) and phenolic acetyl functions (τ 7·60, 7·64, 7·70), and the absence of heterocyclic protons, M^+ 456. The powder-blue colour in UV of the free phenolic form of the component, and its position relative to 3,7,8,3',4'-pentahydroxyflavonol (bright yellow fluorescence) (see Figure 1) is consistent with 3-*O*-methylflavonols.[15,16] The component is most likely 3,8-dimethoxy-7,3',4'-trihydroxyflavone. Solids (200 mg) from band 5, R_f 0·0, of the primary fractionation were methylated and the product separated by TLC on 16 plates in C_6H_6–Me_2CO (4:1). Only one band, R_f 0·45, yellow with SAF spray was removed and eluted.

3,7,8,3',4'-*Pentamethoxyflavone* (**9**, R_1=R_2=R_3=*Me*). Solids (35 mg) obtained from the above separation were crystallized from ethanol as pale yellow needles, m.p. 152° (lit.[7,25] 148°, 153°). The NMR spectrum was

[24] CLARK-LEWIS, J. W., JACKMAN, L. M. and SPOTSWOOD, T. M. (1964) *Australian J. Chem.* **17**, 632.
[25] CLARK-LEWIS, J. W. and NAIR, V. (1964) *Australian J. Chem.* **17**, 1164.

consistent with the above structure [τ 1·97 (*d*, 5-H), 2·07–2·13 (m, 2′-H and 6′H), 2·78 (*d*, 5′-H), 2·93 (*d*, 6-H), 5·95, 5·98, 6·00 (× 2), 6·08 (s, 5 × OMe)], M^+ 372.

Proof of the presence of a 3,8-dimethoxy-7,3′,4′-trihydroxyflavan-3,4-diol in the heartwood of A. saxatilis. Considering that 3-*O*-alkyl ethers of anthocyanidins result as artefacts from the reaction of flavan-3,4-diols with alcohols in the presence of HCl under anhydrous conditions (high yield)[26] and even in the presence of moisture (low yield),[26–28] and that methylation of the equivalent of the 3-hydroxyl in biflavonoids has been recorded,[29] additional proof of the presence of the 3-*O*-methylflavan-3,4-diol was provided as follows: (a) *Leucoanthocyanidin reaction of 8-substituted flavan-3,4-diols.* A mixture of (−)-melacacidin [(−)-7,8,3′,4′-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol] and (−)-isomelacacidin (4-epimer) from *A. nigrescens*,[7] and also the contents of band 1 from the preliminary separation of *A. saxatilis* [predominantly 8-*O*-methyl ethers of the (+)-2,3-*trans*-3,4-*trans* and (+)-2,3-*trans*-3,4-*cis* diastereoisomers of the above compounds (**1**, **2**; R_1=H)] were treated individually under anhydrous conditions[26] with various alcohols[27] in the presence of dissolved HCl gas to form the 3-*O*-alkyl ethers[26–28] of the corresponding flavylium chlorides in high yield in addition to the anticipated anthocyanidins[26] (Table 2).

The mobility of the unknown anthocyanidin (R_f 0·71) generated from the acetone-soluble extractives of *A. saxatilis* heartwood [or from band 1, obtained by paper chromatographic separation of the acetone-solubles in 2% acetic acid, followed by stripping with acetone–water] in isopropanol–3 N HCl (4:1) solution,[22] was identical to that of 3,8-dimethoxy-7,3′,4′-trihydroxyflavylium chloride (see Table 2), but differed significantly from that of the 3-isopropoxy-8-methoxy-7,3′,4′-trihydroxyflavylium chloride 0·83). Generation of the former was taken as indicative of the presence of the corresponding 3·8-dimethoxyflavan-4-ol (**3**: R_1=R_2=H) in the natural extract, since the use of methanol was avoided during all stages of extraction, separation and stripping of the flavan-3,4-diols, as well as during generation of the anthocyanidin. (b) *Extended methylation of flavan-3,4-diols with diazomethane.* (+)-7,8,3′,4′-Tetramethoxy-2,3-*trans*-flavan-3,4-*trans*-diol (**1**. R_1=Me) and the (+)-2,3-*trans*-3,4-*cis* diastereoisomer (**2**. R_1=Me) (100 mg each), both representing products of methylation of band 1 with CH_2N_2, were subjected to continued treatment with CH_2N_2 under the same conditions as before (48 hr at − 15° in Et_2O–MeOH). Traces of products corresponding in R_f to the 3- and 4-*O*-methyl derivatives (**3**, **4**. R_1=Me, R_2=H) from the latter (**2**. R_1=Me) and to the 4-*O*-methyl derivative only from the former (**1**. R_1=Me) could be detected [comparison in 1,2-dichloroethane-Me_2CO (17:3) on TLC] but in quantities insufficient for their isolation (< 1 mg).

Methylation of (+)-7,3′,4′-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol (**5**. R_1=H) (600 mg) with excess CH_2N_2 for a longer period 120 hr) gave the anticipated 7,3′,4′-trimethyl ether (**5**. R_1=Me) together with a low yield of its 4-methyl ether derivative (14 mg, 2·3%). The above indicates that the 3-hydroxyl group of flavan-3,4-diols is resistant to methylation, and thus that the substantial yield of the 3-methyl ether (**3**. R_1=Me, R_2=H) obtained from the acetone-soluble extract of *A. saxatilis* on methylation is unlikely to have originated from the corresponding flavan-3,4-diol (**2**. R_1=H) under the experimental conditions used.

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