Novel Flavan-3,4-diols from *Acacia cultriformis*

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Flavan-3,3′,4,4′,7-pentaols (mollisacacidins) of (2*R*,3*S*,4*R*)- and (2*R*,3*S*,4*S*)-configurations are common to the heartwoods of a number of authentic samples of *Acacia cultriformis*. These are accompanied in some instances by a stereochemically analogous pair of flavan-3,4,4′,7-tetraols (guibourtacacidins) and the 8-*O*-methyl derivatives of (2*R*,3*S*,4*R*)-flavan-3,4,4′,7,8-pentaol (teracacidins) and (2*R*,3*S*,4*S*)-flavan-3,3′,4,4′,7,8-hexaol (melacacidins). This represents the first isolation of methyl ethers of natural flavan-3,4-diols.

Complete analysis of the n.m.r. spectra of their methyl ethers and methyl ether diacetates enables comparison of the chemical shifts of benzenoid protons for the first time.

A. CULTRIFORMIS A. Cunn. ex G. Don (Phyllodineae: Section vi, Uninerves: Section v, Racemosae [1]) is a sparsely distributed phyllodinous and narrow-stemmed shrub of the northern regions of New South Wales and southern Queensland, and a member of the exceptionally large group (*ca.* 600) of Australian species of *Acacia*. These have the unusual group of 5-deoxyflavonoids common to their heartwoods.[2] *A. cultriformis* exhibits variable heartwood content [2] but with the 3′,4′,7-triol phenolic pattern prominent as in other members of the Racemosae.[2]

The compounds (+)-*trans*-2,3-*trans*-3,4- and (+)-*trans*-2,3-*cis*-3,4-flavan-3,3′,4,4′,7-pentaol, (I) and (II), were identified by their conversion into fisetinidin chloride (flavylium-3,3′,4′,7-tetraol chloride), and by chromatographic comparison of their free phenolic forms with eight known diastereoisomers.[3,4] They were separated as methyl ethers, and their relative and absolute configurations were established by comparisons of their n.m.r. spectra and the optical rotations of their methyl ethers and methyl ether diacetates.[4-7]

These flavan-3,3′,4,4′,7-pentaols are associated with stereochemically analogous (+)-flavan-3,4,4′,7-tetraols (III) and (IV). The latter are rare compounds [2] related to the guibourtacacidins present in *Guibourtia coleo-*

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[1] G. Bentham and F. Mueller, 'Flora Australiensis,' Lovell Reeve, London, 1864, p. 301.

[2] M. D. Tindale and D. G. Roux, *Phytochemistry*, 1969, **8**, 1713.

[3] S. E. Drewes and D. G. Roux, *Biochem. J.*, 1965, **94**, 482.

[4] S. E. Drewes and D. G. Roux, *Biochem. J.*, 1965, **96**, 681.

[5] J. W. Clark-Lewis and D. G. Roux, *J. Chem. Soc.*, 1959, 1402.

[6] J. W. Clark-Lewis, L. M. Jackman, and T. M. Spotswood, *Austral. J. Chem.*, 1964, **17**, 632.

[7] S. E. Drewes and D. G. Roux, *Biochem. J.*, 1964, **90**, 343.

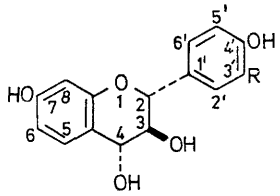
sperma [8,9] and their structures follow from the generation of flavylium-3,4′,7-triol chloride (guibourtinidin chloride). Their structures and relative configurations were confirmed by n.m.r. spectrometry in comparison with synthetic racemates [9] (Table 1), and their absolute configurations were derived by comparison of the rotations of their diacetates ($[\alpha]_D$ −18·5° and +115·0°, respectively) with those of the corresponding derivatives of the associated flavan-3,3′,4,4′,7-pentaol pair ($[\alpha]_D$ −19·6°, +123·5°). Configurational identity is accordingly established, and it follows that the (+)-*trans*-2,3-*trans*-3,4- and (+)-*trans*-2,3-*cis*-3,4-flavan-3,4,4′,7-tetraols (III) and (IV) have the absolute configurations [3,10] as illustrated. Mass spectra of the methyl ethers and methyl ether diacetates of the pairs of diastereoisomers are in close agreement with those found by Drewes.[11]

The third phenolic pattern is represented by a flavan-3,4-diol which gave an anthocyanidin ($\lambda_{max.}$ 510 nm.) of a similar dull pink as generated (520 nm.) from (−)-teracacidin [12] [(−)-*cis*-2,3-*cis*-3,4-flavan-3,4,4′,7,8-pentaol] but differed in its higher mobility on paper chromatograms (R_F 0·68 and 0·50 respectively in 3N-HCl–90% formic acid), thus indicating relative loss of hydroxy-function in the former anthocyanidin. The crystalline methyl ether, obtained by methylation with diazomethane, and its diacetate had n.m.r. spectra which showed an A_2B_2 system for the B-ring and an *ortho*-coupled AB system for the A-ring in the aromatic region. The coupling constants and chemical shifts of the heterocyclic protons were similar to those cited for synthetic (±)-*trans*-3,4-diacetoxy-4′,7,8-trimethoxy-*trans*-2,3-flavan.[6,12]

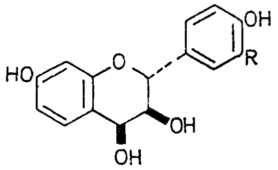
Alkali fusion of the free phenolic form under anhydrous conditions gave *p*-hydroxybenzoic acid and an acid); (−)-teracacidin, treated similarly, gives *p*-hydroxybenzoic acid and pyrogallol (the latter in low yield). The pink colour afforded with bis-diazotized benzidine spray [13,14] (similar to that given by β-resorcyclic acid) and the absence of strong reducing properties shown by spraying with ammoniacal silver nitrate [15] indicates that the unknown acid is the 2-*O*-methyl derivative of pyrogallol-4-carboxylic acid. Confirmation is provided by the pink afforded by the degradation product with ferric alum, whereas an intense blue is anticipated from the alternative 1-*O*-methylpyrogallol-4-carboxylic acid.[16] The natural compound must, therefore, be the 8-*O*-methyl derivative of *trans*-2,3-*trans*-3,4-flavan-3,4,4′,7,8-pentaol. This is confirmed by mass spectrometry of its tetra-acetate; rationalization of the fragmentation correlates with the presence of a methoxy-group on the A-ring. The n.m.r. spectrum of this acetate confirms the presence of a single methoxy-group (τ 6·17).

The rotations of the methyl ether and methyl ether diacetate ($[\alpha]_D$ −2·3°, −29·1°) are in agreement with those of the corresponding derivatives of (2*R*,3*S*,4*R*)-flavan-3,3′,4,4′,7-pentaol (I) [(+)-mollisacacidin] (−9·4°, −19·6°), indicating the same absolute configuration for the parent *trans*-2,3-*trans*-3,4, 8-*O*-methylflavan-3,4,4′,7,8-pentaol (V).

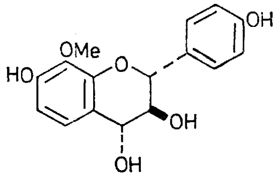
A fourth phenolic pattern is represented by a flavan-3,4-diol which gave an anthocyanidin ($\lambda_{max.}$ 535 nm.) of the same wine-red (540 nm.) as generated from (−)-melacacidin [17] [(−)-*cis*-2,3-*cis*-3,4-flavan-3,3′4,4′,7,8-hexaol], but differed as before in its higher mobility on paper chromatograms (R_F 0·61 and 0·31, respectively in 3N-HCl–90% formic acid). The tetramethyl ether from methylation with diazomethane gave a diacetate with an n.m.r. spectrum identical to that from synthetic (±)-*cis*-3,4-diacetoxy-3′,4′,7,8-tetramethoxy-*trans*-2,3-flavan.[18] The free phenol gave degradation products analogous to those derived from (V), and was shown by similar means to be the 8-*O*-methyl derivative of (+)-*trans*- 2,3-*cis*-3,4-flavan-3,3′,4,4′,7,8-hexaol (VI).



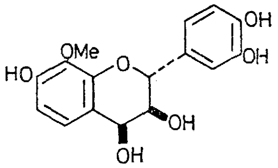
(I) R = OH
(III) R = H



(II) R = OH
(IV) R = H



(V)



(VI)

Compounds (I—VI) are associated in the approximate proportions of 2 : 2·5 : 1 : 1 : 4·2 : 1·3 in some of the heartwoods examined. In others only the mollisacacidins (I) and (II) and the 8-*O*-methyl ether of the melacacidin (VI) are associated, in the proportions of 6 : 4 : 1, while in yet a third type only mollisacacidins (I) and (II) are present. Such variation amongst authentic samples is consistent with the modern population concept of species.

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[8] D. G. Roux and G. C. de Bruyn, *Biochem. J.*, 1963, **87**, 439.

[9] H. M. Saayman and D. G. Roux, *Biochem. J.*, 1965, **96**, 36.

[10] (*a*) K. Weinges, *Annalen*, 1958, **615**, 203; (*b*) J. W. Clark-Lewis and G. F. Katekar, *J. Chem. Soc.*, 1962, 4502; (*c*) C. P. Lillya, S. E. Drewes, and D. G. Roux, *Chem. and Ind.*, 1963, 783.

[11] S. E. Drewes, *J. Chem. Soc.* (*C*), 1968, 1140.

[12] J. W. Clark-Lewis, G. F. Katekar, and P. I. Mortimer, *J. Chem. Soc.*, 1961, 499.

[13] G. Linstedt, *Acta Chem. Scand.*, 1940, **4**, 448.

[14] D. G. Roux and E. A. Maihs, *J. Chromatog.*, 1960, **4**, 65.

[15] E. C. Bate-Smith and R. G. Westall, *Biochim. Biophys. Acta*, 1950, **4**, 637.

[16] E. Pacsu, *Ber.*, 1923, **56**, 407.

[17] F. E. King and J. W. Clark-Lewis, *J. Chem. Soc.*, 1955, 3384.

[18] J. W. Clark-Lewis and L. R. Williams, *Austral. J. Chem.*, 1967, **20**, 2151.

This work represents the first isolation of (+)-*trans*-2,3-*cis*-3,4-flavan-3,3′,4,4′,7-pentaol (II) from natural sources, although a leucofisetinidin of identical relative configuration but unknown rotation is present in Rhodesian Copalwood (*Guibourtia coleosperma*) in association with (—)-*cis*-2,3-*cis*-3,4- and (—)-*cis*-2,3-*trans*-3,4-diastereoisomers.[4] The presence of (+)-*trans*-2,3-*cis*-3,4-flavan-3,4-diols is not unknown amongst *Acacia* spp.; a teracacidin analogue (flavan-3,4,4′,7,8-pentaol) is present in *A. auriculiformis*.[19] The association of (+)-*trans*-2,3-*trans*-3,4- and (+)-*trans*-2,3-*cis*-3,4-flavan-3,3′,4,4′,7-pentaols (leucofisetinidins) in the heartwood of the mopane tree (*Colophospermum mopane*) is also known.[20]

Although the presence of a mixture of *trans*-2,3-*trans*-3,4-, *trans*-2,3-*cis*-3,4-, and predominantly *cis*-2,3-*trans*-3,4-diastereoisomers of flavan-3,4,4′,7-tetraol (guibourtacacidin) in Rhodesian Copalwood (*G. coleosperma*)[8] was established by n.m.r. spectroscopy by comparison with synthetic racemates,[9] the present study also represents the first isolation of optically pure guibourtacacidin derivatives from natural sources. They are of interest since novel bimolecular leucoguibourtinidins have been isolated from the woods of the South African trees *Acacia luederitzii* var. *retinens* and *A. reficiens* sub. sp. *reficiens*.[21] Chromatographic evidence has been obtained of the association of flavan-3,3′,4,4′,7-pentaols (leucofisetinidins) and flavan-3,4,4′,7-tetraols (leucoguibourtinidins) in other *Acacia* spp. indigenous to Australia (*A. neriifolia*, *A. vestita*. *A. deanei* ssp. *deanei*).[2]

The 8-*O*-methylflavan-3,4,4′,7,8-pentaol (V) and 8-*O*-methylflavan-3,3′,4,4′,7,8-hexaol (VI) similarly represent the first isolated methoxylated flavan-3,4-diols, and are the first reported natural representatives of the teracacidin and melacacidin series with *trans*-2,3-*trans*-3,4- and *trans*-2,3-*cis*-3,4- relative configurations respectively. They have the same absolute configurations (2*R*) at the 2-position as (—)-teracacidin and (—)-melacacidin. These 8-*O*-methylflavan-3,4-diols are widely distributed amongst the heartwoods of *Acacia* spp. of Australian origin.

High-resolution n.m.r. spectrometry (100 MHz) of the flavan-3,4-diols and their diacetates permits complete analysis of spectra (Table), and hence examination of the chemical shifts of benzenoid protons over the limited range of compounds. On the A-ring the 6-proton signal shows remarkably small variation ($\Delta\tau$ 0·06) in relation to changes in both benzenoid substitution and stereochemistry. Changes in the relative position of an hydroxy-group from 4-axial (as in *trans,cis*-compounds) to 4-equatorial (*trans,trans*) results in an anticipated deshielding ($\Delta\tau$ — 0·21) of the 5-proton in the *peri*-position. However, a relatively

N.m.r. spectra (τ values; J in Hz) of flavan-3,4-diol methyl ethers and their acetates

	2	3	4	5	6	8	2′ + 6′	3′ + 5′	OMe 4′	OMe 7	OH	
Flavan-3,4-diol												
(*a*) 4′,7-dimethoxy-												
(+)-*trans*-2,3-*trans*-3,4-	5·28(d) $J_{2,3}$ *ca.* 10·0	*b*	5·26(d) $J_{3,4}$ *ca.* 8·0	2·46(d) $J_{5,6}$ 8·5	3·45(q)	3·61(d) $J_{6,8}$ 2·5	2·64(d) $J_{2',3'}$ and $J_{5',6'}$ 8·5	3·10(d)	6·21(s)	6·26(s)	6·4—7·2(m)	
(+)-*trans*-2,3-*cis*-3,4-	5·00(d) $J_{2,3}$ *ca.* 9·5	*b*	5·27(d) $J_{3,4}$ *ca.* 3·5	2·85(d)	3·42(q)	3·50(d)	2·57(d)	3·04(d)	6·18(s)	6·24(s)	6·8—7·6(m)	
(*b*) 3′,4′,7-trimethoxy-								5′	3′,4′			
(+)-*trans*-2,3-*trans*-3,4- [a]	5·22(d) $J_{2,3}$ 9·5	*b*	5·16(d) $J_{3,4}$ 8·0	2·59(d) $J_{5,6}$ 8·5	3·41(q)	3·58(d) $J_{6,8}$ 2·5	3·00(s)	3·12(d) $J_{5',6'}$ *ca.* 8·5	6·10(s)	6·25(s)	7·8—8·6(m)	
(+)-*trans*-2,3-*cis*-3,4- [c]	5·02(d) $J_{2,3}$ 9·5	*b*	5·23(d) $J_{3,4}$ 4·0		3·42(q)	3·53(d)	3·00(s)	3·11(d)	6·10(s)	6·24(s)	7·8—8·4(m)	
(*c*) 4′,7,8-trimethoxy-									4′,7,8			
(+)-*trans*-2,3-*trans*-3,4-	5·30(d) $J_{2,3}$ *ca.* 9·0	*b*	5·27(d) $J_{3,4}$ *ca.* 8·0	2·89(d) $J_{5,6}$ 9·0	3·45(d)		2·65(d) $J_{2',3'}$ and $J_{5',6'}$ 8·6	3·10(d)	6·17(s), 6·22(s)		6·4—7·2(m)	
Flavan-3,4-diacetate											OAc	
(*a*) 4′,7-dimethoxy-								3′ + 5′	4′	7	4	3
(+)-*trans*-2,3-*trans*-3,4-	4·93(d) $J_{2,3}$ *ca.* 9·2	4·47(q) ΣJ 16·4	3·73(d) $J_{3,4}$ 7·2	2·88(d) $J_{5,6}$ 9·0	3·41(q)	3·50(d) $J_{6,8}$ 2·4	2·61(d) $J_{2',3'}$ and $J_{5',6'}$ *ca.* 9·0	3·07(d)	6·18(s)	6·21(s)	7·98(s)	8·13(s)
(+)-*trans*-2,3-*cis*-3,4- [a]	4·81(d) $J_{2,3}$ 10·5	4·57(q) ΣJ 14·0	3·85 $J_{3,4}$ 3·5	2·82(d) $J_{5,6}$ 8·5	3·47(q)	3·55(d) $J_{6,8}$ 2·5	2·65(d) $J_{2',3'}$ and $J_{5',6'}$ *ca.* 9·0	3·10(d)	6·22(s)	6·27(s)	7·90(s)	8·19(s)
(*b*) 3′,4′,7-trimethoxy-								5′	3′,4′			
(+)-*trans*-2,3-*trans*-3,4-	4·95(d) $J_{2,3}$ 9·6	4·42(q) ΣJ 17·0	3·76(d) $J_{3,4}$ 7·4	2·88(d) $J_{5,6}$ 8·6	3·42(q)	3·48(d) $J_{6,8}$ 2·4	*ca.* 3·10(s)	*ca.* 3·13(d)	6·10(s)	6·21(s)	7·95(s)	8·13(s)
(±)-*trans*-2,3-*cis*-3,4- [a]	4·81(d) $J_{2,3}$ 10·5	4·52(q) ΣJ 14·0	3·84(d) $J_{3,4}$ 3·5	2·82(d) $J_{5,6}$ *ca.* 8·5	3·46(q)	3·54(d) $J_{6,8}$ *ca.* 2·5	3·05(s)	3·17(d) $J_{5',6'}$ 8·5	6·12(s)	6·24(s)	7·88(s)	8·16(s)
(*c*) 4′,7,8-trimethoxy-								3′ + 5′	4′,7,8			
(+)-*trans*-2,3-*trans*-3,4-	4·78(d) $J_{2,3}$ 8·8	4·48(q) ΣJ 15·6	3·78(d) $J_{3,4}$ 6·8	3·10(d) $J_{5,6}$ 9·0	3·40(d)		2·61(d) $J_{2',3'}$ and $J_{5',6'}$ 8·6	3·10(d)	6·13(d), 6·20(d)		8·03(s)	8·13(s)
(*d*) 4′,7-diacetoxy-8-methoxy-									8		4′,7	3,4
(+)-*trans*-2,3-*trans*-3,4-	4·73(s) $J_{2,3}$ 8·8	4·50(q) ΣJ 14·6	3·82(s) $J_{3,4}$ 6·0	3·05(d) $J_{5,6}$ 9·0	3·30(d)		2·53(d) $J_{2',3'}$ and $J_{5',6}$ 8·6	2·90(d)	6·17(s)		7·70, 7·73(s)	8·10(s)
(*e*) 3′,4′,7′,8-tetramethoxy-								5′	3′ 4′ 8	7	4	3
(+)-*trans*-2,3-*cis*-3,4- [a]	4·75(d) $J_{2,3}$ *ca.* 10·5	4·56(q) ΣJ 14·0	3·84(d) $J_{3,4}$ 3·5	3·00(d)	3·42(d) $J_{5,6}$ 8·5		3·03(s)	3·16(d) $J_{5',6'}$ *ca.* 8·5	6·10, 6·11(d)	6·16(s)	7·85(s)	8·12(s)
(±)-*trans*-2,3-*cis*-3,4-	4·77(d) $J_{2.3}$ 10·0	4·52 ΣJ 13·0	3·83(d) $J_{3,4}$ 3·0	3·00(d)	3·42(d) $J_{5,6}$ 8·5		3·04(s)	*ca.* 3·17	6·12(s) [d]	6·15(s)	7·87(s)	8·13(s)

[a] Spectra at 100 MHz. 3-H quartet overlapped by OMe signals. [c] Compound sparingly soluble in deuteriated chloroform. [d] Doublet on scale expansion.

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[19] S. E. Drewes and D. G. Roux, *Biochem. J.*, 1966, **98**, 493.

[20] S. E. Drewes and D. G. Roux, *J. Chem. Soc.* (*C*), 1967, 1407.

[21] I. C. du Preez, A. C. Rowan, and D. G. Roux, *Chem. Comm.*, 1970, 492.

small but reverse effect ($\Delta\tau$ +0·06) occurs with the corresponding diacetates. 8-Methoxylation results in an anticipated ' mesomeric effect '[22] ($\Delta\tau$ +0·18) on the 5-proton. The 8-proton signal shifts downfield consistently with the change 4*eq*-OH $\longrightarrow$ 4*ax*-OH, but the reverse pertains for 4*eq*-OAc $\longrightarrow$ 4*ax*-OAc. In the B-ring the equivalent 2′- and 6′-proton signals experience an upfield shift with 3′-methoxylation. Methoxy-protons at the 4′- and 7-positions are deshielded ($\Delta\tau$ −0·08 to −0·10) on *ortho*-methoxylation in those rings in which they are present.

Mass spectrometry of the full acetate of (V) supports unpublished findings[21] that acetates of many flavonoids give vigorous fragmentations well substantiated by metastable peaks. These lead through appropriate loss of acetic acid, acetoxy-radicals, or keten to the free phenolic ions, amongst which the location of the methoxy-function is readily established in this instance.

EXPERIMENTAL

N.m.r. spectra were obtained for solutions in deuteriochloroform (tetramethylsilane as internal standard) with Varian T-60 and HA-100 spectrometers. Mass spectra were obtained with an A.E.I. MS9 spectrometer.

The heartwoods were extracted with acetone and the extractives were handled in acetone solution during preparative paper chromatography and until methylation. Dissolution in ethanol and methanol was avoided during these stages to prevent side-reaction through formation of 4-ethers particularly of the *trans*-2,3-*cis*-3,4-flavan-3,4-diol analogues.

Drillings (218 g.) of the heartwood of *A. cultriformis* (NSW 101238: collected by the Agricultural Research Station, Bathurst, N.S.W.) were extracted exhaustively with acetone, acetone–water (9 : 1 v/v), and acetone–water (8 : 2 v/v) (successively), and the extracts were combined to give a brown solid (16·2 g.) on evaporation. Two-way chromatography of the extract on Whatman no. 1 paper with water-saturated butan-2-ol followed by 2% acetic acid showed the presence of pink areas, R_F 0·72 and 0·63, and 0·72 and 0·74, respectively, after spraying with toluene-*p*-sulphonic acid in ethanol.[23] The former spot ran concurrently with (+)-mollisacacidin (*cf.* refs. 3 and 4).

The compounds were separated by chromatography on Whatman no. 3 22·5 × 18·25 in. sheets, with 2% acetic acid as developer. Bands were cut out and eluted with acetone–water (2 : 8 v/v), yielding brown solids [1·73 g. (R_F 0·63) and 0·06 g. (0·74)].

(+)-trans-2,3-trans-3,4- *and* (+)-trans-2,3-cis-3,4-*Flavan*-3,3′,4,4′,7-*pentaols*.—Microfusions with molten potassium hydroxide under controlled conditions[24] gave, from the lower R_F (0·63) band, resorcinol and β-resorcylic and protocatechuic acids. Treatment with 3N-hydrochloric acid–propan-2-ol (1 : 4 v/v) under pressure[25] gave a pink flavylium-3,3′,4′,7-tetraol chloride (fisetinidin chloride), $\lambda_{max.}$ 525 nm., R_F 0·43 [3N-HCl–90% formic acid (1 : 1 v/v)].[26]

(+)-3′,4′,7-*Trimethoxy*-trans-2,3-*flavan*-trans-3,4-*diol*.—Solids (1·7 g.) from the lower R_F (0·63) band were methylated with excess of diazomethane in methanol–ether for 48 hr. at −15°. The methyl ethers were separated on Kieselgel PF_{254} (Merck) by preparative thin-layer (1 mm.) chromatography with ethyl methyl ketone–toluene (6 : 4 v/v), giving two bands (R_F 0·62 and 0·68) in equal concentration.

The band of lower R_F (0·62) gave a white solid (198 mg.) on elution with acetone. This crystallized readily from ethanol–water in needles, m.p. 128°, $[\alpha]_D^{20}$ −9·4° (*c* 0·8 in *sym*-tetrachloroethane), M^+ 332 (lit.,[3] m.p. 130°, $[\alpha]_D$ −9·5°).

The diacetate, prepared with pyridine–acetic anhydride, crystallized from ethanol as needles, m.p. 87°, $[\alpha]_D^{20}$ −19·6° [*c* 0·6 in acetone–water (8 : 2 v/v)], M^+ 416 (lit.,[5] m.p. 101—102°, $[\alpha]_D$ +16·5° for the enantiomer).

The 3-monoacetate, obtained by restricting the amount of anhydride[27] gave *needles* from ethanol, m.p. 176°, $[\alpha]_D^{20}$ +2·0° [*c* 0·5 in acetone–water (8 : 2 v/v)], M^+ 374.

(+)-3′,4′,7-*Trimethoxy*-trans-2,3-*flavan*-cis-3,4-*diol*.—The band of higher R_F (0·68) from the separation on Kieselgel, gave a white solid (117 mg.) which crystallized readily from ethanol as needles, m.p. 178·5°, $[\alpha]_D^{20}$ +40·3° [*c* 0·5 in acetone–water (9 : 1 v/v)], M^+ 332 {lit.,[3] m.p. (177—180°) 186—187°, $[\alpha]_D$ +42·9°}.

The diacetate was non-crystalline, $[\alpha]_D^{26}$ +123·5° [*c* 0·7 in acetone–water (8 : 2 v/v)], M^+ 416.

The compounds described were shown to have the assigned relative configurations by n.m.r. spectrometry (*cf.* Table).

(+)-trans-2,3-cis-3,4-8-O-*Methylflavan*-3,3′,4,4′,7,8-*hexaol*.—Solids from the higher R_F (0·74) band from preparative separations on cellulose develop a wine-red colour on treatment with hydrochloric acid as before. The colour ($\lambda_{max.}$ 535 nm.) was similar to that due to flavylium-3,3′,4′,7,8-pentaol chloride (540 nm.) developed from (−)-melacacidin,[10b] but the R_F value[26] (0·61) was higher than that of the latter anthocyanidin (0·31).

Whereas alkali fusion[24] of melacacidin gives protocatechuic acid and pyrogallol [R_F 0·11 and 0·08 respectively in benzene–acetic acid–water (4 : 6 : 2 v/v)], these solids give protocatechuic acid (0·11) and an unknown acid (0·25) with mobility similar to that of β-resorcylic acid (0·29), and with weak reducing properties towards ammoniacal siver nitrate.[14,15] The compound gives a red colour with bis-diazotized benzidine[13,14] and a pink with ferric alum.[14]

(+)-3′,4′,7,8-*Tetramethoxy*-trans-2,3-*flavan*-cis-3,4-*diol*.—The solids (60 mg.) were methylated with excess of diazomethane and the methyl ether was purified by preparative t.l.c. on Kieselgel PF_{254} [R_F 0·71 in ethyl methyl ketone–toluene (6 : 4 v/v)]. The product (30 mg.) crystallized from ethanol in *needles*, m.p. 173·5°, $[\alpha]_D^{22}$ −3·6° [*c* 0·6 in acetone–water (9 : 1 v/v)], M^+ 362 (lit.,[18] m.p. 178—179° for the racemate). The corresponding *trans*-2,3-*trans*-3,4 methyl ether was absent.

The diacetate was non-crystalline, $[\alpha]_D^{26}$ +79·2° [*c* 0·2 in acetone–water (8 : 2 v/v)], M^+ 446. The n.m.r. spectrum (Table) was consistent with a *trans*-2,3-*cis*-3,4-configuration

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($J_{2,3}$ *ca.* 10·5, $J_{3,4}$ *ca.* 3·5 Hz), and identical with the spectrum of the synthetic racemate,[18] m.p. 111—112°.

The penta-acetate of the natural (+)-*O*-monomethylflavan-3,3′,4,4′,7,8-hexaol was obtained by acetylation of the fraction (20 mg.) with acetic anhydride–pyridine, M^+ 530. Attempted purification on Kieselgel PF_{254} by t.l.c. led to partial deacetylation. This difficulty could not be overcome despite use of buffered (sodium acetate) as well as deactivated substrates. N.m.r. spectrometry confirmed the presence of a methoxy- (τ 6·13) and five acetoxy-groups [τ 7·67 (3H), 7·27 (6H), 8·05 (3H), and 8·11 (3H)].

(+)-trans-2,3-trans-3,4- and (+)-trans-2,3-cis-3,4-*Flavan*-3,4,4′,7-*tetraols*.—Drillings from the heartwood of a second sample of *A. cultriformis* (NSW 101494: collected by Mr. B. Scott at West Wyalong-Temora, N.S.W.) (123 g.) were extracted exhaustively with acetone–water (4 : 1 v/v) at room temperature. Two-way chromatography of the solid extractives (12·4 g.) gave four pink areas with toluene-*p*-sulphonic acid: *A*, R_F 0·72 and 0·63; *B*, 0·82 and 0·63; *C* 0·71 and 0·76; and *D*, 0·81 and 0·76. Preparative separation on Whatman no. 3 sheets in 2% acetic acid gave two bands, *AB* (1·15 g.) and *CD* (0·67 g.).

The solids from band *AB* were converted into flavylium-3,3′,4′,7-tetraol (fisetinidin), $\lambda_{max.}$ 525 nm., R_F 0·43, and flavylium-3,4′,7-triol chlorides (guibourtinidin), $\lambda_{max.}$ 485 nm., R_F 0·60, the latter with characteristic yellow colour under u.v. light.[26] The solids were methylated as before and the product (1·10 g.) was separated on Kieselgel PF_{254} in ethyl methyl ketone–toluene (4 : 6 v/v) to give four bands, at R_F 0·33 (127·5 mg.), 0·40 (95·5 mg.), 0·48 (33·5 mg.), and 0·53 (31·1 mg.). Bands at R_F 0·33 and 0·40 were the methyl ethers of (+)-*trans*-2,3-*trans*-3,4- and (+)-*trans*-2,3-*cis*-3,4-flavan-3,3′,4,4′,7-pentaols, identical with those from other samples of *A. cultriformis*.

(+)-4′,7-*Dimethoxy*-trans-2,3-*flavan*-trans-3,4-*diol*.—Solids from the R_F 0·48 band crystallized from ethanol–water in *needles*, m.p. 75°, M^+ 302 (lit.,[9] m.p. 119—120° for the racemate).

The diacetate crystallized from ethanol in *needles*, m.p. 106°, $[\alpha]_D^{26}$ −18·5° [*c* 0·4 in acetone–water (8 : 2 v/v)], M^+ 386 (lit.,[9] m.p. 135—136° for the racemate).

(+)-4′,7-*Dimethoxy*-trans-2,3-*flavan*-cis-3,4-*diol*.— Solids from the R_F 0·53 band crystallized from ethanol in *needles*, m.p. 144°, M^+ 302 (lit.,[9] m.p. 138—140° for the racemate).

The diacetate did not crystallize, $[\alpha]_D^{26}$ +115·0° [*c* 0·3 in acetone–water (8 : 2 v/v)], M^+ 386. The racemate was also non-crystalline.[9]

N.m.r. and mass spectra, in comparison with those of the synthetic racemates,[9] confirm the assigned structures and configurations (*cf.* Table).

8-O-*Methyl Derivatives of* (+)-trans-2,3-trans-3,4-*Flavan*-3,4,4′,7,8-*pentaol and* (+)-trans-2,3-cis-3,4-*Flavan*-3,3′,4,4′,7,8-*hexaol*.—Solids (0·67 g.) from the preparative band *CD* were separated on prewashed Whatman no. 3 paper in water-saturated butan-2-ol into bands *C* (R_F 0·71; 0·12 g.) and *D* (0·81; 0·39 g.). Component C proved to be (+)-*trans*-2,3-*cis*-3,4-8-*O*-methylflavan-3,3′,4,4′,7,8-hexaol, isolated from sample NSW 101238.

Component *D* gave a dull pink anthocyanidin, R_F 0·68, $\lambda_{max.}$ 510 nm., reminiscent of flavylium-3,4′,7,8-tetraol chloride, R_F 0·50, $\lambda_{max.}$ 520 nm., generated from (−)-teracacidin [12] [(−)-*cis*-2,3-*cis*-3,4-flavan-3,4,4′,7,8-pentaol]. Alkali fusion yields *p*-hydroxybenzoic acid and material identified as 2-*O*-methylpyrogallol-4-carboxylic acid by methods similar to those already described.

(+)-8-*Methoxy*-3,4,4′,7-*tetra-acetoxy*-trans-2,3-trans-3,4-*flavan*.—Solids (100 mg.) from band *D* were acetylated with acetic anhydride–pyridine at room temperature and recovered from water. The dry product was purified by separation on Kieselgel PF_{254} in benzene–acetone (7 : 1 v/v). The band at R_F 0·51 gave an amorphous *solid* (76 mg.), $[\alpha]_D^{30}$ −8·8° (*c* 3·1 in acetone), M^+ 472, which was shown to be pure by n.m.r. spectrometry, $J_{2,3}$ 9·0, $J_{3,4}$ 6·0 Hz.

(+)-4′,7,8-*Trimethoxy*-trans-2,3-*flavan*-trans-3,4-*diol*.—Solids (150 mg.) from band *D* were methylated as before. The product was purified by t.l.c. on Kieselgel PF_{254} in benzene–acetone (7 : 3 v/v), and the resulting methyl ether (R_F 0·31; 80 mg.) crystallized from ethanol in *needles*, m.p. 157°, $[\alpha]_D^{28}$ −2·3° [*c* 0·5 in acetone–water (9 : 1 v/v]. The synthetic racemate [12] had m.p. 126—127°.

The diacetate crystallized from ethanol in *needles*, m.p. 143°, $[\alpha]_D^{26}$ −29·1° [*c* 1·3 in acetone–water (8 : 2 v/v)].

N.m.r. (Table) and mass spectra confirm the assignments.

Chromatographic Examination of Heartwood of Other Samples of A. cultriformis.—Sample NSW 104257 contains components (I), (II), (V), and (VI); samples NSW 104283, 104284, 104285, 112619, and 124709 contain mainly (I), (II), and (VI); and sample NSW 104286 mainly (I) and (II).

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