A New Flavan-3,4-diol from *Acacia auriculiformis* by Paper Ionophoresis

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1. The heartwood of *A. auriculiformis* contains a typical mixture of analogues consisting of three isomeric flavan-3,4-diols, a dihydroflavonol, flavanone, flavonol and chalcone based on the 4',7,8-trihydroxyl pattern. These were resolved by preparative paper chromatography and preparative paper ionophoresis. 2. Crystalline (−)-teracacidin [(2*R*,3*R*,4*R*)-4',7,8-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol] was obtained in high (10%) yield, and a new crystalline derivative of (−)-isoteracacidin [(−)-2,3-*cis*-3,4-*trans* isomer] was isolated. The crystalline methyl ether of a new (+)-2,3-*trans*-3,4-*cis* isomer was isolated. 3. The absolute configurations of (−)-isoteracacidin (2*R*,3*R*,4*S*) and of the (+)-2,3-*trans*-3,4-*cis* isomer (2*R*,3*S*,4*S*) were tentatively assigned on the basis of nuclear-magnetic-resonance spectroscopy, paper ionophoresis and paper-chromatographic comparison with the epimerization products of (−)-teracacidin. 4. Possible reasons for the absence of polymeric leuco-anthocyanidin tannins are discussed. 5. (±)-4',7,8-Trihydroxydihydroflavonol and (±)-4',7,8-trihydroxyflavanone were isolated for the first time. 6. The bark polyphenols consist mainly of polymeric leuco-delphinidins and leuco-cyanidins which redden exceptionally rapidly to light. The mechanism of this phenomenon is discussed.

The heartwood of *Acacia auriculiformis* A. Cunn. contains a mixture of phenolic components that is characterized by the prominence of three flavan-3,4-diols but absence of significant amounts of complex leuco-anthocyanidin tannins which frequently accompany them in related *Acacia* spp. This interesting observation prompted a closer examination of the heartwood components to account for a phenomenon that could be related to certain structural factors or to the absence of enzymes.

(−)-Teracacidin[(−)-4',7,8-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol] is shown to be the predominant component, and is accompanied in high proportion by the geometric isomers (−)-isoteracacidin (2,3-*cis*-3,4-*trans*) and a new (+)-4',7,8-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol. The last-named were concurrent on paper chromatograms, but their methyl ethers were elegantly resolved by paper ionophoresis on a preparative scale.

The flavan-3,4-diols are accompanied by the usual pattern (cf. Roux & Paulus, 1960, 1961*a*,*b*, 1962; Drewes & Roux, 1963) of dihydroflavonol, flavanone, flavonol and chalcone analogues.

EXPERIMENTAL AND RESULTS

Nuclear-magnetic-resonance spectra were recorded by Dr K. Pachler, Chemical-Physics Group, C.S.I.R., Pretoria, South Africa, and by Dr J. Feeney, Varians Associates, Walton-on-Thames, Surrey, on the Varian A-60 and HA-100, spectrometers with deuterochloroform as solvent and tetramethylsilane as internal standard. Band positions are on the τ scale. Coupling constants were measured with an accuracy of $\pm 0{\cdot}2$ cyc./sec.

Compounds were dried at 100° for 2hr. under vacuum before analysis and measurement of optical rotations. C,H, methoxyl and acetyl determinations were by Dr F. Pascher and E. Pascher, Bonn, Germany. All melting points are uncorrected.

Two-dimensional chromatograms were run in water-satd. butan-2-ol and then 2% (v/v) acetic acid on sheets (18¼in. × 11¼in.) of Whatman no. 1 paper. Preparative paper chromatograms were on sheets (18¼in. × 22½in.) of Whatman no. 3 paper.

Origin of samples and distribution of species. The wood and bark of *A. auriculiformis* A. Cunn. *ex* Benth. were collected by Mr D. I. Nicholson from the plantations of the Forest Department, Sandakan, Sabah, Malaysia. Cross-sections were cut at 5 and 15ft. from a tree 40ft. high with 41in. girth at breast height. The specimen was just under 11 years old.

A. auriculiformis is indigenous to Thursday Island and other islands off the coast of Northern Australia and Queensland. The species is often confused with *A. aulacocarpa* A. Cunn., but they are quite distinct.

Two-dimensional paper chromatography and colour-reactions of bark and heartwood components

Bark components. The bark contains 19·0% of hot-water-soluble substances and 18·3% of cold-water-soluble substances. Of the latter 13·6% reacted as tannins and 4·7% as non-tannins to hide powder in the empirical Official Shake Method (Atkin & Thompson, 1937). Two-dimensional chromatograms of both hot- and cold-water extractives showed an intense streak extending from the origin to R_F 0·55 in the first direction (water-satd. butan-2-ol) with limited extension (R_F 0·26) in the second direction (2% acetic acid). These polymeric tannins were accompanied by two compounds in the lower-R_F region (0·33, 0·24 and 0·33, 0·39).

These phenolic compounds all gave an ochre with *bis*-diazotized benzidine (indicative of a phloroglucinol A-nucleus in flavans), and reduced instantaneously with ammoniacal $AgNO_3$ (pyrogallol or catechol B nuclei). The tannin mixture gave only delphinidin and cyanidin chlorides in high yield with the method of Pigman, Anderson, Fischer, Buchanan & Browning (1953), and the mixture probably contains leuco-delphinidins and leuco-cyanidins in both monomeric and polymeric form. The solid bark extract and leather tanned with it rapidly develop exceptional redness when exposed to sunlight.

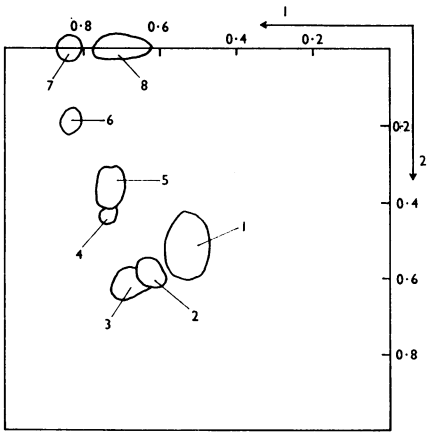


Fig. 1. Two-dimensional paper chromatography of the heartwood components of *A. auriculiformis*. Water-satd. butan-2-ol was used for the first direction and 2% (v/v) acetic acid for the second. Key to compounds: 1, (−)-teracacidin; 2, (−)-isoteracacidin; 3, (+)-4′,7,8-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol; 4, possibly a 4′,7,8-trihydroxyflavan-3-ol, from relative position and colour reactions; 5, (±)-4′,7,8-trihydroxydihydroflavonol; 6, (±)-4′,7,8-trihydroxyflavanone; 7, 2′,3′,4′,4-tetrahydroxychalcone; 8, 4′,7,8-trihydroxyflavonol. The compounds were present in the proportions 1 (10): 2+3 (5): 4 (< 1): 5 (4): 6 (1): 7 (trace): 8 (< 1). Other spots represent unknowns.

Heartwood components. Two-dimensional paper chromatograms of the methanol-soluble components of heartwood drillings showed the presence (Fig. 1) of seven major and three minor components, when sprayed with ammoniacal $AgNO_3$. These had the following values in water-saturated butan-2-ol and 2% acetic acid respectively: (−)-tetracacidin (0·54, 0·52), (−)-isoteracacidin (0·63, 0·59), (+)-4′,7,8-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol (0·66, 0·61), (±)-4′,7,8-trihydroxyflavanonol (0·73, 0·39), an unknown, probably 4′,7,8-trihydroxyflavan-3-ol (0·74, 0·45), (±)-4′,7,8-trihydroxyflavanone (0·83, 0·19), 2′,3′,4′,4-tetrahydroxychalcone (0·83, 0·0), 4′,7,8-trihydroxyflavonol (0·72, 0·0). Tannins, usually represented by streaks in the lower-R_F region, were either entirely absent or else present in low concentration.

All compounds showed instantaneous reduction with ammoniacal $AgNO_3$, indicating the presence of vicinal phenolic hydroxyl groups. With bis-diazotized benzidine spray all compounds gave a yellow colour. The leuco-anthocyanidins gave a dull pink with toluene-*p*-sulphonic acid spray, whereas the dihydroflavonol developed a brilliant yellow fluorescence when viewed under u.v. light after treatment with the same spray. The chalcone showed a characteristic intensification of yellow when exposed to ammonia vapour, and the flavonol gave a brilliant yellow fluorescence under u.v. light which is characteristic of 5-deoxyflavonols.

Extraction of the heartwood of A. auriculiformis. The drillings from the dry heartwood (816 g.) were extracted four times for successive periods of 1, 2, 4 and 8 days with similar volumes of methanol (total 3 l.) at room temperature. Evaporation of the combined methanolic extracts under vacuum left a brown powder (22·8 g.). This was stirred into 250 ml. of hot water (80°), and the mixture immediately filtered through a coarse filter (Whatman no. 541) leaving 1·3 g. of insoluble residue. The solubles were cooled, diluted to 650 ml. with ethanol and streaked on to 130 preparative sheets of Whatman no. 3 paper as before (Roux & Paulus, 1962). The chromatograms were developed by upward migration with 2% (v/v) acetic acid. Bands were cut corresponding to isoteracacidin and 4′,7,8-trihydroxy-2,3-*trans*-flavan-3,4-*cis*-diol (R_F 0·53), teracacidin (0·44), the dihydroflavonol (0·34), flavanone (0·17), flavonol and chalcone (0·02–0·0). These were eluted with 70% ethanol.

(−)-*Teracacidin* [(−)-4′,7,8-*trihydroxy*-2,3-cis-*flavan*-3,4-cis-*diol*]. Teracacidin (2·21 g.) crystallized readily from the eluates of band R_F 0·44 on concentration to small volume. These were obtained as fine buff needles in rosettes, m.p. 224–226° (browning with decomposition), on recrystallizing from water, $[\alpha]_D^{20} -73·3 \pm 0·8°$ (*c* 0·65 in ethanol) (Found: C, 62·2; H, 5·1. Calc. for $C_{15}H_{14}O_6$: C, 62·1; H, 4·9%). Micro-fusion with dry KOH (Roux, 1958) yielded pyrogallol and *p*-hydroxybenzoic acid.

Clark-Lewis & Dainis (1964) cite m.p. 225–226°, $[\alpha]_D$ −71°, for the same compound.

(−) - 4′,7,8 - *Trimethoxy* - 2,3 - cis - *flavan* - 3,4 - cis - *diol*. (−)-Teracacidin (125 mg.), in methanol, was methylated with ethereal diazomethane for 48 hr. at 0°. Clusters of fine needles (90 mg.) were obtained from methanol, m.p. 163°, $[\alpha]_D^{20} -71·0 \pm 0·7°$ (*c* 0·8 in ethanol) (Found: C, 65·3; H, 6·1. Calc. for $C_{18}H_{20}O_6$: C, 65·1; H, 6·1%). Paper ionophoresis in borate buffer under standard conditions (Drewes & Roux,

1964*b*) showed migration (+2·0cm.) characteristic of a 2,3-*cis*-3,4-*cis*-diol.

Clark-Lewis, Katekar & Mortimer (1961) cite m.p. 159°, $[\alpha]_D^{18} - 65°$.

(−)-3,4-cis-*Diacetoxy*-4′,7,8-*trimethoxy*-2,3-cis-*flavan*. The trimethyl ether of (−)-teracacidin (75mg.) was acetylated with acetic anhydride–pyridine (1:1, v/v) (0·6ml.) for 24hr. at room temperature. From water a white solid (85mg.) was obtained which failed to crystallize. Reprecipitation from water gave an amorphous white solid, m.p. 63°, $[\alpha]_D^{26} - 44·3 \pm 0·4°$ (*c* 0·6 in ethanol). Thin-layer chromatography (Kieselgel G, ¼mm. thick) with benzene–acetone (19:1, v/v) as irrigant showed only one discrete spot, R_F 0·49 (Found: C, 63·6; H, 5·6. $C_{22}H_{24}O_8$ requires: C, 63·5; H, 5·8%).

The nuclear-magnetic-resonance spectrum of the compound (Table 1) in comparison with spectra of the four possible geometrical arrangements of related flavan-3,4-diols (Drewes & Roux, 1964*a*; Saayman & Roux, 1965) showed it to be the pure 2,3-*cis*-3,4-*cis* form.

Separation of overlapping flavan-3,4-diol components by successive thin-layer chromatography and paper ionophoresis of their methyl ethers

From the bands of R_F 0·53 a brown powder (1·28g.) was obtained on elution with aq. 70% (v/v) ethanol. The product in methanol was methylated for 48hr. at −10°. The mixture was reduced in volume (1ml.) and applied as a narrow (0·5cm.) band to 21 thin-layer chromatographic plates (20cm.×20cm.) previously coated with Kieselgel G (Merck) 1mm. thick. The chromatograms were developed in ethyl acetate–chloroform (7:3, v/v) to the tops of the plates (1·5hr.). A band (R_F 0·60) giving an orange–red with H_2SO_4–aq. 40% formaldehyde (20:1, v/v) was scraped off, eluted with acetone and concentrated to dryness at 50° (water bath) to give a white residue (465mg.). This process removed brown condensed material resulting from methylation which remained on the origin and also the 4-ethyl or 4-methyl ethers which had high mobility (approx. 0·90). Examination of the residue by paper ionophoresis in borate buffer under standard conditions (Drewes & Roux, 1964*b*) indicated that it consisted of a mixture of 2,3-*cis*-3,4-*trans* (−2·1cm. migration) and 2,3-*trans*-3,4-*cis* isomers (+3·1cm.), the former predominating.

The mixture of methylated isomeric flavan-3,4-diols (30–35mg.) in ethanol (0·5ml.) was applied as a streak on a wet Whatman no. 3 (18¼in.×20½in.) sheet, previously soaked in sodium borate–boric acid buffer (pH 8·8) (Cooper & Roux, 1965). A current of 0·33mA/cm. width of paper at 150–170v was applied for 21hr. On an average, the band of the 2,3-*trans*-3,4-*cis* isomer was located (toluene-*p*-sulphonic acid spray) at +8·5cm., and the 2,3-*cis*-3,4-*trans* band at −4·0cm. The bands were cut and eluted with aq. 50% (v/v) ethanol. The combined eluates from each band from 15 successive ionophoretic runs were concentrated under reduced pressure to remove all ethanol, acidified with acetic acid and the aqueous solution was extracted exhaustively with ethyl acetate. The combined extracts of each were washed with water and concentrated to dryness at 50° (vacuum). The residues of each isomer were again subjected to preparative thin-layer chromatography under the above conditions (three plates each).

Table 1. *Nuclear-magnetic-resonance spectra of isomeric* 3,4-*diacetoxy*-4′,7,8-*trimethoxyflavans and dihydroflavonol analogue*

The solvent used was deuterochloroform, with tetramethylsilane as internal standard.

	Chemical shifts: τ values (p.p.m.)											
						H						
	Me (acetyl)		Me (methoxyl)			Heterocyclic			Benzenoid			
	3-	4-	7-	4′-	8-	2-	3-	4-	5-	6-	3′-+5′-	2′-+6′-
(+)-2,3-*trans*-3,4-*cis* (III)†	8·21	7·93	6·25	6·24	6·20	4·72	4·56	3·83	3·00	3·41	3·09	2·61
(−)-2,3-*cis*-3,4-*trans* (II)‡	8·13	7·88	6·19	6·11	6·09	4·68	4·77	4·12	2·97	3·41	3·09	2·56
(−)-2,3-*cis*-3,4-*cis* (I)‡	8·11	7·92	6·20	6·13	6·11	4·67	4·38	3·68	3·09	3·41	3·11	2·57
			Phenolic acetyls									
(±)-2,3-*trans*-Dihydroflavonol (V)‡	8·00	—	7·81		7·72	4·63	4·29	—	2·24	3·13	2·93	2·59

Spin–spin coupling constants for 2-, 3- and 4-protons (cyc./sec.)*

	$J_{2,3}$	$J_{3,4}$
(+)-2,3-*trans*-3,4-*cis*	10.2	3·1
(−)-2,3-*cis*-3,4-*trans*	1·5	2·9
(−)-2,3-*cis*-3,4-*cis*	~1·0	4·0
(±)-2,3-*trans*-Dihydroflavonol	11·9	—

* $J_{5,6}$, $J_{2',3'}$ and $J_{5',6'}$ approximate to 8·9–9·0 cyc./sec. for all compounds.
† Measured on the Varian HA-100 spectrometer.
‡ Measured on the Varian A-60 spectrometer.

Derivatives of the (+)-2,3-trans-3,4-cis-diastereoisomer of teracacidin

(+)-4′,7,8-*Trimethoxy*-2,3-trans-*flavan*-3,4-cis-*diol*. From the 2,3-*trans*-3,4-*cis* fraction in absolute ethanol, feathery needles in rosettes (30mg.), m.p. 168–170°, were obtained. Paper ionophoresis indicated a residual trace of the 2,3-*cis*-3,4-*trans* isomer, but on recrystallization from ethanol this was entirely removed, and the product (24mg.) had m.p. 179°, $[\alpha]_D^{20}+7{\cdot}0\pm1{\cdot}6°$ (*c* 0·3 in ethanol) (Found: C, 64·9; H, 6·0; OMe, 27·7. $C_{18}H_{20}O_6$ requires C, 65·1; H, 6·1; OMe, 28·0%).

The compound had a mobility (+3·1 cm.) in sodium borate buffer under standard conditions characteristic of methylated 2,3-*trans*-3,4-*cis*-diols (Drewes & Roux, 1964*b*, 1965*a*; Saayman & Roux, 1965). Similarly, its mobility in sodium borate–boric acid buffer (pH 8·8) (Cooper & Roux, 1965) on Schleicher and Schull no. 2043 (4cm. × 41cm.) paper strips was +6·7cm. at 0·31mA/cm. width, and 150v for 15hr.

(+)-3,4-cis-*Diacetoxy*-4′,7,8-*trimethoxy*-2,3-trans-*flavan*. The trimethyl ether (15mg.) was acetylated with acetic anhydride–pyridine (1:1, v/v) (0·1ml.) for 20hr. at room temperature. From water a white amorphous solid (17·9mg.) melting slowly from 52° onwards was obtained (Found: C, 64·4; H, 6·2. $C_{22}H_{24}O_8$ requires C, 63·5; H, 5·8%). The compound failed to crystallize from common solvents.

The nuclear-magnetic-resonance spectrum of the 2,3-*trans*-3,4-*cis*-diacetate (Table 1), when compared with previous work on related flavan-3,4-diols of similar relative configuration (Drewes & Roux, 1964*a*, 1965*a*; Clark-Lewis, Jackman & Williams, 1962; Saayman & Roux, 1965), confirmed the 2,3-*trans*-3,4-*cis* assignment.

New derivatives of isoteracacidin

(−)-4′,7,8-*Trimethoxy*-2,3-cis-*flavan*-3,4-trans-*diol*. After separation by preparative paper ionophoresis and thin-layer chromatography the 2,3-*cis*-3,4-*trans* fraction from methylation with diazomethane yielded a white solid (180mg.) which did not crystallize, m.p. 68–78°, $[\alpha]_D^{20}-40{\cdot}3\pm0{\cdot}9°$ (*c* 0·7 in ethanol) (Found: C, 65·2; H, 6·2; OMe, 27·7. $C_{18}H_{20}O_6$ requires: C, 65·1; H, 6·1; OMe, 28·0%).

The compound had the mobilities −1·7cm. in sodium borate buffer (Drewes & Roux, 1964*b*), and −2·1cm. in sodium borate–boric acid (Cooper & Roux, 1965).

(−)-3,4-trans-*Diacetoxy*-4′,7,8-*trimethoxy*-2,3-cis-*flavan*. The amorphous trimethyl ether (52mg.) was acetylated with acetic anhydride–pyridine as above, giving a white solid (58mg.) from water. This crystallized readily from ethanol, m.p. 149–150° (Found: C, 64·0; H, 6·0; OMe, 22·0; $CO{\cdot}CH_3$, 21·5. $C_{22}H_{24}O_8$ requires C, 63·5; H, 5·8; OMe, 22·4; $CO{\cdot}CH_3$, 20·7%).

The nuclear-magnetic-resonance spectrum of the diacetate (Table 1) was similar to those of flavan-3,4-diols of the same 2,3-*cis*-3,4-*trans* configuration (see above). The presence of a trace of the corresponding 2,3-*trans*-3,4-*trans* isomer was indicated by a weak acetyl signal at τ 8·02 p.p.m.

(±)-4′,7,8-*Trihydroxy*-2,3-trans-*dihydroflavonol*. The compound (157mg.) crystallized from aqueous solution of the eluates of band R_F 0·34 after concentration to small (2ml.) volume. Recrystallization from water gave colourless needles, m.p. 220–221° with browning, $[\alpha]_D^{20}+3{\cdot}0\pm0{\cdot}6°$ (*c* 0·7 in ethanol) (Found: C, 62·0; H, 4·2. $C_{15}H_{12}O_6$ requires C, 62·5; H, 4·2%). This low rotation suggested that the compound was racemic, and this was confirmed by hydrogenation over platinum oxide (Adams catalyst) which afforded enantiomeric 4′,7,8-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diols [cf. Drewes & Roux (1964*a*), Roux & Paulus (1960) and Freudenberg & Weinges (1959) for the analagous hydrogenations of (+)- and (−)-fustins] as shown by two-dimensional paper chromatography. The dihydroflavonol gives a dark purple with Mg–HCl, and affords the flavonol analogue on heating with mineral acid (chromatographic evidence).

(±)-3,4′,7,8-*Tetracetoxy*-2,3-trans-*dihydroflavonol*. The dihydroflavonol (43mg.) was acetylated at room temperature with 0·4ml. of acetic anhydride and 0·2ml. of pyridine. A white solid (50mg.) was recovered from water. This crystallized from ethanol in clusters of prisms (39mg.), m.p. 159–160° (Found: C, 60·0; H, 4·8. $C_{23}H_{20}O_{10}$ requires C, 60·5; H, 4·4%).

The 2,3-*trans* configuration of the racemate is evident from the large coupling constants of the 2- and 3- protons [$J_{2,3}$ 11·9 cyc./sec. (Table 1)].

(±)-4′,7,8-*Trihydroxyflavanone*. After the formation of a brown sludge, the compound crystallized from the aqueous concentrate of the eluates of band R_F 0·17. Recrystallization from water gave needles (21mg.), m.p. 104–105°. The compound gave a deep blue with Zn–HCl reagent, typical of flavanones.

(±)-4′,7,8-*Triacetoxyflavanone*. The flavanone (16mg.) was acetylated as above. Long needles (15mg.), m.p. 165–166°, were obtained from ethanol, $[\alpha]_D^{26}-2{\cdot}9\pm0{\cdot}8°$ (*c* 0·3 in ethanol) (Found: C, 63·0; H, 4·6. $C_{21}H_{18}O_8$ requires C, 63·3; H, 4·6%).

4′,7,8-*Trihydroxyflavonol*. The eluates of band R_F 0·02 were concentrated and dissolved in methanol (80ml.). The methanolic solution was streaked on to 16 sheets of Whatman no. 3 paper, and the chromatograms were developed downward with aq. acetic acid (13:30, v/v). Four bands were located as follows: R_F 0·48, yellow fluorescent under u.v. light; 0·57, yellow, intensified when exposed to ammonia vapour; 0·65, bright-yellow fluorescent under u.v. light, and 0·74, dark under u.v. light, becoming yellow when exposed to ammonia vapour.

The band R_F 0·48 gave the crude flavonol (200mg.) on stripping. After recrystallizing twice from ethanol–water (3:1, v/v) pale-yellow needles were obtained, m.p. 310° (decomp.) (Found: C, 63·0; H, 4·1. Calc. for $C_{15}H_{10}O_6$: C, 62·9; H, 3·5%).

Clark-Lewis & Dainis (1964) found m.p. 310–319° for the same compound from *A. sparsiflora*, whereas Kostanecki & Schreiber (1905) found m.p. 319° for the synthetic compound.

3,4′,7,8-*Tetra-acetoxyflavone*. The flavonol (20mg.) was acetylated as before, yielding buff needles (18mg.) from ethanol, m.p. 180–181° after sintering at 165–170° (Found: C, 61·0; H, 4·4. Calc. for $C_{23}H_{18}O_{10}$: C, 60·8; H, 4·0%). Kostanecki & Schreiber (1905) found m.p. 175° for the synthetic compound, and Clark-Lewis & Dainis (1964) found 170–175° for the same compound derived from *A. sparsiflora*.

2′,3′,4′,4,-*Tetrahydroxychalcone*. The yellow band R_F 0·57 from the above separation intensified to a deep yellow when exposed to ammonia vapour, a reaction typical of chalcones. The solids obtained from eluates of the band refused to crystallize from ethanol–water, and after further purification by chromatography in water-

saturated butan-2-ol had $\lambda_{max.}$ 284 and 357 mμ. The quantity available was too small for further work.

Examination of related Acacia *spp. for the presence of* 2,3-trans-3,4-cis-*flavan*-3,4-*diols*

The heartwood extract of *A. intertexta* Sieb. was found to contain (−)-melacacidin [(−)-3′,4′,7,8-tetrahydroxy-2,3-*cis*-flavan-3,4-*cis*-diol] by two-dimensional paper chromatography. Its geometrical isomers (see below), as well as its dihydroflavonol, flavanone, flavonol and chalcone analogues were also present. Bands corresponding to isomelacacidin were cut from preparative chromatograms run in 2% acetic acid. Solids obtained from the eluates of these bands were methylated, and the product was examined by paper ionophoresis under standard conditions (Drewes & Roux, 1964*b*). The presence of 2,3-*cis*-3,4-*trans* and 2,3-*trans*-3,4-*cis* diastereoisomers of (−)-melacacidin was established in this way. The flavonoid mixture from the heartwood of *A. intertexta* is an exact parallel to the pattern present in *A. auriculiformis* except for its added degree of (3′-) hydroxylation.

The 2,3-*trans*-3,4-*cis* diastereoisomer of (−)-melacacidin and isomelacacidin, was shown to be absent from *A. melanoxylon* R. Br. heartwood by similar means.

Epimerization of (−)-*teracacidin*

Teracacidin (5 mg.) in water (5 ml.) was autoclaved for 2 hr. at 15 lb./in.^{2} (Drewes & Roux, 1964*d*, 1965*a*) and the product examined by two-dimensional chromatography. Only two reducing areas were visible with ammoniacal $AgNO_3$ spray, the starting material (R_F 0·54, 0·52) and compounds of higher mobility (0·68, 0·59).

The same product, examined by two-dimensional chromatography with water-satd. phenol (40 cm. migration) and then 2% (v/v) acetic acid (approx. 50 cm. migration), showed the presence of four diastereoisomers: *cis–cis* (0·60, 0·44), *trans–trans* (0·65, 0·49), *cis–trans* (0·62, 0·54) and *trans–cis* (0·68, 0·54). The *cis–trans* and *trans–cis* isomers were recognized by comparison with the corresponding natural isomers from *A. auriculiformis* and the *trans–trans* isomer with the catalytic (Adams catalyst) hydrogenation products of racemic 4′,7,8-trihydroxy-2,3-*trans*-dihydroflavonol from the same source. The various isomers were present in the following proportions after 2 hr.: *cis–cis* (55%), *cis–trans* (12%), *trans–trans* (8%) and *trans–cis* (25%) (cf. Drewes & Roux, 1965*c*).

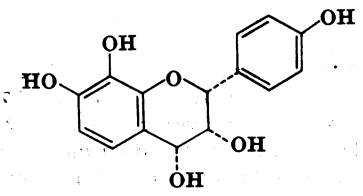
DISCUSSION

The heartwood of *A. auriculiformis* contains flavan-3,4-diols, dihydroflavonol, flavanone, flavonol and chalcone analogues based on the 4′,7,8-trisubstituted pattern of phenolic hydroxylation. This represents the first established occurrence of a wide group of analogues with this pattern, but the association parallels others with different patterns among the Leguminosae, e.g. *A. mearnsii* bark (3′,4′,5′,7-tetrahydroxy and 3′,4′,7-trihydroxy) and heartwood (3′,4′,7-trihydroxy) (Roux & Paulus, 1960, 1961*a*,*b*; Drewes & Roux, 1963) and the heartwoods of *A. melanoxylon* (3′,4′,7,8-tetrahydroxy) (Clark-Lewis & Mortimer, 1960), *A. dealbata*, *A. decurrens*, *A. pycnantha* (3′,4′,7-trihydroxy) (Roux, Maihs & Paulus, 1961) and *Robinia pseudacacia* (3′,4′,7-trihydroxy and 3′,4′,5′,7-tetrahydroxy) (Roux & Paulus, 1962). The characteristic distribution pattern of these classes of flavonoids (cf. Fig. 1) on two-dimensional chromatograms may now be readily recognized.

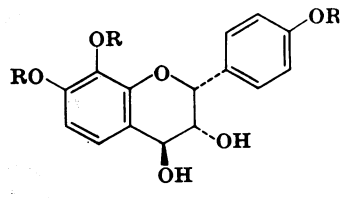
However, the polyphenolic mixture from *A. auriculiformis* differs from those above, through the presence of no less than three diastereoisomeric flavan-3,4-diols, representing (−)-2,3-*cis*-3,4-*cis*, (−)-2,3-*cis*-3,4-*trans* and (+)-2,3-*trans*-3,4-*cis* configurations. Of these (−)-teracacidin (I) (*cis–cis*) is readily separated by preparative paper chromatography, but the remaining isomers overlap and may be separated after methylation by paper ionophoresis on a preparative scale. Thus isolation of the crystalline methyl ether of the (+)-*trans–cis* isomer (III, R = CH_3) represents the first conclusive evidence of the natural presence of a flavan-3,4-diol of this configuration (III, R = H) (Drewes & Roux, 1965*d*), although a leuco-fisetinidin (3′,4′,7-trihydroxyflavan-3,4-diol) and an isomer of guibourtacacidin (4′,7-dihydroxyflavan-3,4-diol) of the same configuration was previously shown to be present in *Guibourtia coleosperma* (Drewes & Roux, 1964*e*, 1965*b*; Saayman & Roux, 1965) by paper chromatography and ionophoretic methods, and a similar isomer of (−)-melacacidin (3′,4′,7,8-tetrahydroxyflavan-3,4-diol) apparently exists in *A. intertexta*, but is absent from *A. melanoxylon*.

A crystalline trimethyl ether diacetate of (−)-isoteracacidin (2,3-*cis*-3,4-*trans* isomer) (II, R = H) was prepared for the first time, thus unambiguously demonstrating its presence in the flavan-3,4-diol mixture. (−)-Teracacidin (2,3-*cis*-3,4-*cis*) was readily obtained from *A. auriculiformis* in crystalline form, as opposed to difficulties experienced in its previous isolation in amorphous form from *A. orites* (formerly identified in error as *A. intertexta*) (Clark-Lewis *et al.* 1961; Clark-Lewis & Dainis, 1964). This compound was crystallized only recently by Clark-Lewis & Dainis (1964) from *A. sparsiflora*, but not without emphasis on the difficulties experienced. *A. auriculiformis* therefore constitutes an exceptionally rich (10% yield on extract weight) source of crystalline (−)-teracacidin (I), whereas the mixture of 2,3-*trans*-3,4-*cis* (III, R = H) and 2,3-*cis*-3,4-*trans* (II, R = H) isomers were obtained in lower (5%) yield in the crude form. These flavan-3,4-diols predominate amongst the flavonoid components.

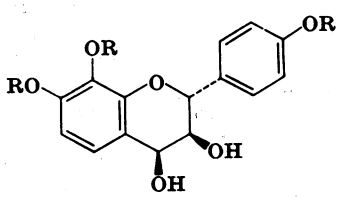
The relative configurations of the flavan-3,4-diols were confirmed (teracacidin) and assigned (isoteracacidin and *trans–cis* isomer) by comparison of their ionophoretic mobilities and nuclear-magnetic-resonance spectra (spin–spin splitting patterns of



(I)



(II)



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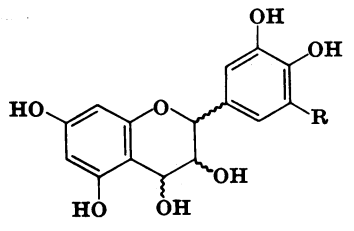
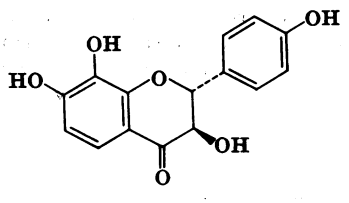
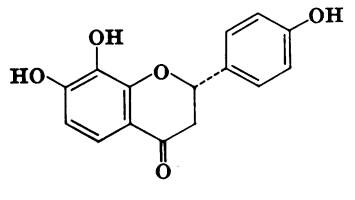


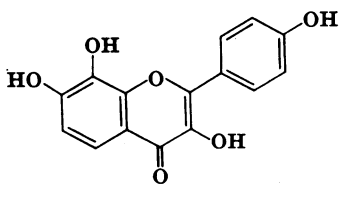
(IV)



(V)



(VI)



(VII)

3-, 4- and 5-protons) (Table 1) with those of other flavan-3,4-diols of similar geometrical arrangements (Clark-Lewis *et al.* 1962; Drewes & Roux, 1964*a*,*b*, 1965*a*; Saayman & Roux, 1965).

Presuming a half-chair conformation for the heterocyclic ring and a 2(*eq*) arrangement for the 2-phenyl group, the coupling constants of the 2(*ax*)-, 3(*eq*)- and 3(*eq*)-,4(*ax*)- protons of 2,3-*cis*-3,4-*cis*-(−)-teracacidin ($J_{2,3} \sim 1{\cdot}0$cyc./sec. and $J_{3,4}$ 4·0cyc./sec.) correlate well with their dihedral angles of approx. 70° and 45°, as observed from Dreiding models, on the basis of the Karplus equation (Conroy, 1959). Similar correlations were possible for 2,3-*cis*-3,4-*trans*- (−)-isoteracacidin ($J_{2,3}$ 1·5cyc./sec. and $J_{3,4}$ 2·9cyc./sec.) with dihedral angles of approx. 70° and 60° for a 2(*ax*),3(*eq*),4(*eq*) arrangement of protons and for the (+)-2,3-*trans*-3,4-*cis* isomer ($J_{2,3}$ 10·0cyc./sec. and $J_{3,4}$ 3·4cyc./sec.) with dihedral angles approx. 170° and 50° for a 2(*ax*),3(*ax*),4(*eq*) arrangement of protons.

The absolute configuration of (−)-teracacidin (2*R*,3*R*,4*R*) (I) is known (Clark-Lewis & Katekar, 1962), and that of (−)-isoteracacidin (2*R*,3*R*,4*S*) (II, R = H) is now confirmed from its relative configuration, and from its formation by selective epimerization of (−)-teracacidin at C-4 (Clark-Lewis *et al.* 1961). The methyl ether of the new 2,3-*trans*-3,4-*cis* isomer has a (+)-rotation, and by analogy with the epimerization products of

(+)-mollisacacidin (Drewes & Roux, 1964*d*, 1965*a*), most likely represents the 2*R* form and therefore the 2*R*,3*S*,4*S* (III, R = H) configuration. A leuco-fisetinidin of similar configuration (paper-chromatographic evidence) is present in *G. coleosperma* (Drewes & Roux, 1964*e*, 1965*b*).

The 4′,7,8-trihydroxyl substitution in derivatives of the isomeric flavan-3,4-diols and their dihydroflavonol analogue is confirmed from their nuclear-magnetic-resonance spectra by analysis of the spin–spin splitting pattern of benzenoid protons. Thus the A_2B_2 system of the B-ring results in the typical overlap of equivalent protons (2′,6′ and 3′,5′) to give doublets which show mainly strong *ortho*-coupling ($J_{2',3'} = J_{5',6'} \approx 8{\cdot}9$–$9{\cdot}0$ cyc./sec.), whereas the 5- and 6-protons form an AB system which appear as *ortho*-coupled doublets ($J_{5,6}$ 8·9–9·0 cyc./sec.) unsplit by further *meta*- or *para*- coupling. Notable is the strong deshielding of the 5-proton in *peri*-position to the 4-carbonyl in the dihydroflavonol (cf. Table 1), as well as the paramagnetic shift of the 5-proton in (−)-isoteracacidin (4-*axial* acetoxy group) compared with (−)-teracacidin (4-*equatorial* acetoxy group) ($\Delta\tau$ 0·10 p.p.m.). This effect has been noticed in all previous work (Drewes & Roux, 1964*a*, 1965*a*; Saayman & Roux, 1965) and may be correlated with some factor of their stereochemistry at C-4.

The dihydroflavonol (V) and flavanone (VI) analogues of (−)-teracacidin have now been isolated for the first time. Both compounds are partially racemized, the (+)- and (−)-forms respectively predominating in accordance with their usual occurrence in Nature. The large coupling constant ($J_{2,3}$ 11·9 cyc./sec.) of the 4′,7,8-trihydroxydihydroflavonol indicates a diaxial arrangement of the 2- and 3-protons with a dihedral angle approaching 180° (Conroy, 1959), and therefore a 2,3-*trans* arrangement of substituents. No exception has yet been found to the 2,3-*trans* arrangement of natural dihydroflavonols, and in *A. auriculiformis* heartwood this contrasts with the predominantly 2,3-*cis* arrangement of the associated flavan-3,4-diols [(−)-teracacidin, isoteracacidin]. The flavonol (VII) analogue has been isolated from natural sources for the second time only, having recently been obtained from *A. sparsiflora* (Clark-Lewis & Dainis, 1964) in association with (−)-teracacidin and isoteracacidin.

Considering the predominance of the 4′,7,8-trihydroxy-3,4-diols in *A. auriculiformis*, the exceptional absence of tannins (related flavan-3,4-diol polymers) in the heartwood is of interest, since it contrasts with the predominance of tannins based on other phenolic patterns in *G. coleosperma* and also with their prominence in *A. mearnsii* and closely related species, and in *R. pseudacacia*. However, the presence of tannins, although in low relative proportion in the heartwoods of *A. melanoxylon* and *A. intertexta* that contain the related (same pyrogallol-derived *A. nucleus*) 3′,4′,7,8-tetrahydroxyflavan-3,4-diols [(−)-melacacidin and isomelacacidin], suggests that factors other than the reactivity of the flavan-3,4-diol nucleus (e.g. blocking of the reactive 8-position by hydroxylation, or affecting the reactivity of the benzylic 4-hydroxyl) might be responsible for the lack of biochemical condensation of the flavan-3,4-diols in *A. auriculiformis*. The absence of tannins should, perhaps, rather be sought in the absence in the wood of those enzymic systems which promote condensation or polymerization.

The bark of *A. auriculiformis* contains a preponderance of polymeric leuco-delphinidin (3′,4′,5′,5,7-pentahydroxyl pattern) (IV, R = OH) and leuco-cyanidin (3′,4′,5,7-tetrahydroxyl) (IV, R = H) tannins associated with traces of the corresponding flavan-3,4-diols. These differ completely from phenolic heartwood components which are based on a single and different (4′,7,8-trihydroxyl) pattern. The leuco-delphinidin and leuco-cyanidin bark tannins redden exceptionally rapidly when exposed to sunlight in the solid form, in solution, or when combined with collagen in leather. The exceptional reddening is attributed to the greater electron release from the 7-hydroxyl groups of 5,7-disubstituted tannins, than from 7,8-dihydroxyl (melacacidin and teracacidin isomers and polymers) or 7-hydroxyl (leuco-robinetinidin and leuco-fisetinidin isomers and polymers) tannins (cf. Hammet δ-constants; Clark-Lewis, 1962). The electron effect of the 5-hydroxyl reinforces that of the 7-hydroxyl leading to enhanced reactivity of the benzylic 4-hydroxyl and the facile formation of a chromophore (7-keto anhydrobase of the flavylium structure) (Drewes & Roux, 1964*c*; Roux & Drewes, 1965).

Epimerization of (−)-teracacidin by autoclaving (cf. Drewes & Roux, 1965*a*) gives rise to three new geometrical isomers as with (−)-leuco-fisetinidin (Drewes & Roux, 1964*e*, 1965*b*). These may be resolved with water-saturated phenol and then 2% acetic acid on paper chromatograms (cf. Drewes & Roux, 1965*c*). The natural (−)-isoteracacidin from *A. auriculiformis* was found to be concurrent with the (−)-isoteracacidin (2,3-*cis*-3,4-*trans*) epimerization product, whereas the natural (+)-2,3-*trans*-3,4-*cis* isomer migrated to a position slightly in advance of the corresponding (−)-epimerization product, as expected. This chromatographic evidence confirms the above assignments.

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