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3',4',7,8-TETRAHYDROXYFLAVONOIDS FROM THE HEARTWOOD OF *ACACIA NIGRESCENS* AND THEIR CONVERSION PRODUCTS

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Abstract—Nigrescin, the first optically active 2-hydroxy-2-benzylcoumaranone, and a (+)-2,3-*trans*-flavan-3,4-*cis*-diol, represent new members of the natural 3',4',7,8-tetrahydroxyflavonoid group. These accompany chlacone, flavanone, flavonol and dihydroflavonol analogues, the flavan-3,4-diols (−)-melacacidin and (−)-isomelacacidin, and protocatechuic acid in the black heartwood of *Acacia nigrescens*. Polyflavonoid tannins are significantly absent. Synthetic nigrescin tetramethyl ether together with its flavonol analogue is obtained from (±)-3',4',7,8-tetra-*O*-methyldihydroflavonol by brief treatment with alkali. Reduction of the flavanone tetramethyl ether with $LiAlH_4$ affords new flavan-4α-ol and flavan-4β-ol analogues. Treatment of 2R:3R:4R-flavan-3,3',4,4',7,8-hexaol [(−)-melacacidin:2,3-*cis*-3,4-*cis*], or its methyl ether with thioglycollic acid yields, after methylation, the anticipated methyl (−)-(2,3-*cis*-3,4-*trans*-3-hydroxy-3',4',7,8-tetramethoxyflavan-4-ylthio) acetate, together with optically pure 2*S*-flavanone and 2*R*:3*R*-dihydroflavanol (2,3-*trans*) analogues for the first time. The same reaction applied to (±)-2,3,-*trans*-[4-^{2}H]-3',4',7,8-tetramethoxyflavan-3,4-*trans*-diol does not give the flavanone analogue. The relative chemical shifts of 8-methoxy proton resonances of flavan-3,4-diol, flavanone, coumaranone and flavone analogues obtained by progressive addition of C_6D_6 to $CDCl_3$ solutions are of diagnostic value in polyflavanoid chemistry.

INTRODUCTION

Acacia nigrescens Oliv. (previously *A. pallens* Rolfe) (Leguminosae), more generally known as knoppiesdoring (Afrikaans) or knobwood is one of the indigenous trees of the Kruger National Game Reserve.[1] It derives its vernacular name from the rough black bark which is often covered with raised knobs terminating in sharp spines. The tree attains a height of up to 17 m and a stem diameter of 20–50 cm. Its habitat is mainly sub-tropical to tropical with a distribution covering parts of the Transvaal, Zululand, Swaziland, Mozambique, Botswana, Malawi and Tanzania.

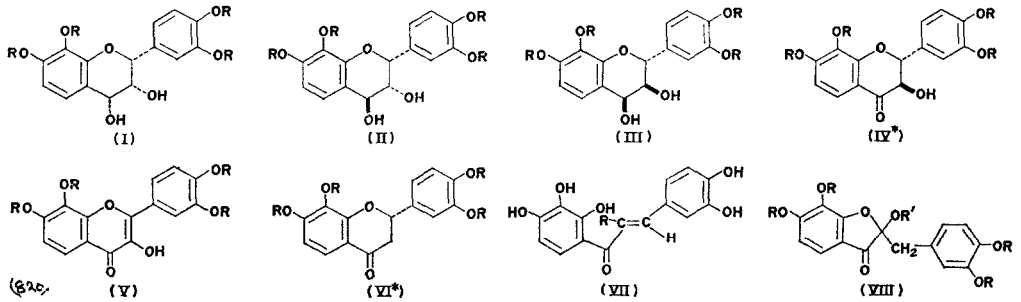
Flavonoids of the dense brown–black heartwood are exlusively based on the 3',4',7,8-tetrahydroxyl pattern, and are thus closely similar to those found in a number of Australian *Acacia* spp.[2] The present study was aimed at isolating new members of this group of compounds of which (−)-melacacidin and (−)-iso-melacacidin[3] are the best-known representatives; studying their conversion products and the biogenetic implications of these conversions; and finally studying the chemical shifts of their 8-methoxyl resonances as a key to the interflavanoid linkages in polyflavonols and polyflavanones.

RESULTS AND DISCUSSION

Complete analysis of the phenolic heartwood components from *A. nigrescens* has shown, that with the exception of protocatechuic acid, all compounds are 3',4',7,8-tetrahydroxyflavonoids. Protocatechuic acid, accordingly, represents their B-ring substitution pattern.

[1] L. E. W. CODD, *Trees and Shrubs of the Kruger National Park*, p. 47, Department of Agriculture Botanical Survey, Memoir No. 26, Government Printer, Pretoria (1951).
[2] M. D. TINDALE and D. G. ROUX, *Phytochem.* **8**, 1713 (1969).
[3] J. W. CLARK-LEWIS and P. I. MORTIMER, *J. Chem. Soc.* 4106 (1960).

These flavonoids may be more strictly subdivided into flavanoids represented by 2,3-*cis*-3,4-*cis*[(−)-melacacidin, I, R = H], 2,3-*cis*-3,4-*trans*[(−)-isomelacacidin, II, R = H], and (+)-2,3-*trans*-3,4-*cis*-flavandiols (III, R = H), and into flavonoids represented by (±)-dihydroflavonol (IV, R = H), flavonol (V, R = H), (±)-flavanone (VI, R = H), chalcone (VIII, R = H) and 2-hydroxy-2-benzylcoumaran-3-one (VIII, R = R′ = H) analogues.



* Racemates, with only one enantiomer of each illustrated: 2R:3R (dihydroflavonol) and 2S (flavanone).

Amongst the former group, the (+)-2,3-*trans*-flavan-3,4-*cis*-diol (III, R = H) represents a new natural compound of this class. The mixture of three flavan-3,4-diol diastereoisomers (I–III, R = H) is stereochemically analogous to that based on the 4′,7,8-trihydroxy pattern [(−)-teracacidin analogues] previously isolated from *A. auriculiformis*.[4] The optically pure flavan-3,4-diol diastereoisomers of *A. nigrescens* are related (2R) at C-2, having 2R:3R:4R (*cis–cis*), 2R:3R:4S (*cis–trans*) and 2R:3S:4S (*trans–cis*) absolute configurations (I–III). Notable for its absence in *A. nigrescens* is the 3′,4′,7,8-tetrahydroxyflavan-3-ol (catechin) analogue.

By contrast, the fully representative group of flavonoids is characterized not only by the completely racemized dihydroflavonol and flavanone analogues, but by a new and optically active 2-hydroxy-2-benzylcoumaran-3-one (VIII, R = R′ = H), named nigrescin. Structural proof of nigrescin was provided by conversion of the racemic 3′,4′,7,8-tetramethoxy-2,3-*trans*-dihydroflavonol (IV, R = Me) into the 2-hydroxy-2-benzyl-3′,4′,7,8-tetramethoxycoumaran-3-one (VIII, R = Me, R′ = H) by brief treatment with alkali, according to the method of Chopin and Bouillant.[5] Remarkably, in this instance, the 3′,4′,7,8-tetramethoxyflavonol (V, R = Me) is also formed, presumably by oxidative conversion under alkaline conditions, instead of the anticipated 3-benzalcoumaran-2-one resulting from a benzylic acid rearrangement.[6]

Nigrescin, a 2,3′,4′,6,7-pentahydroxy-2-benzylcoumaran-3-one (VIII, R = R′ = H) is the fifth natural compound of this class, others being alphitonin (2,3′,4,4′,6-pentahydroxy) from the heartwood of *Alphitonia excelsa*,[7] 2,3′,6-trihydroxy-4′-methoxy and 2,3′,4′,6-tetrahydroxy derivatives from the heartwood of the quebracho tree (*Schinopsis balansae* and *S. lorentzii*)[8] and maesopsin (2,4,4′,6-tetrahydroxy) from the heartwood of *Maesopsis eminii*[9] and *Phyllogeiton zeyheri*.[10] Nigrescin is the first of these to exhibit optical activity

[4] S. E. DREWES and D. G. ROUX, *Biochem. J.* **98**, 493 (1966).
[5] M. J. CHOPIN and M. L. BOUILLANT, *Compt. Rend.* **254**, 3699 (1962).
[6] J. GRIPENBERG, *Acta Chem. Scand.* **7**, 1323 (1953).
[7] A. J. BIRCH, E. RITCHIE and R. N. SPEAKE, *J. Chem. Soc.* 3593 (1960).
[8] H. G. C. KING, T. WHITE and R. B. HUGHES, *J. Chem. Soc.* 3234 (1961).
[9] N. F. JANES, F. E. KING and J. W. W. MORGAN, *Chem. & Ind.* 346 (1961).
[10] F. DU R. VOLSTEEDT and D. G. ROUX, *Tetrahedron Letters* 1674 (1971); H. A. CANDY and J. M. MCGARRY, *J. S. African Chem. Inst.* **24**, 159 (1971).

as evidenced by its CD-curve (Fig. 1). However, no analogies exist at present which permit interpretation of the positive Cotton-effect in terms of its absolute configuration at C-2.

The optical activity of nigrescin (and of all three flavan-3,4-diols) contrasts remarkably with the completely racemized forms of the dihydroflavonol (IV, R = H) and flavanone (VI, R = H) analogues, although partial racemization amongst related (5-deoxy) natural dihydroflavonols and flavanones is well known. Complete racemization in the wood of *A. nigrescens* implies inversion at both C-2 and C-3 for the dihydroflavonol, and at C-2 for the flavanone. This could occur through α-hydroxychalcone (VII, R = OH) and chalcone (VII, R = H) intermediates respectively. Evidence of the latter is found in the presence of a trace of the chalcone (VII, R = H) while the former represents an intermediate leading to 2-hydroxy-2-benzylcoumaranone (VIII, R = R′ = H) biosynthesis. The optical activity of nigrescin suggests greater stability of its five-membered heterocyclic ring, than in the flavanone six-membered system.

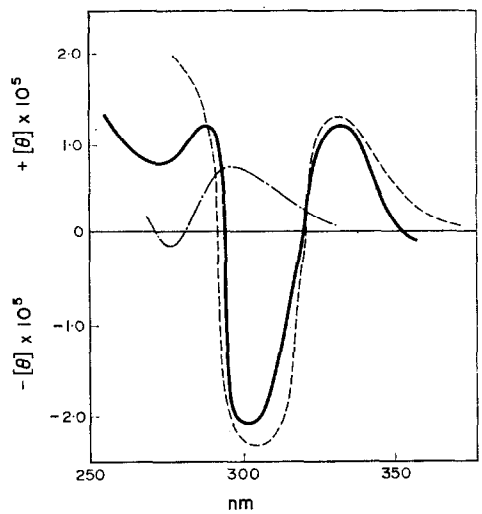


FIG. 1. CD-CURVES OF 3′,4′,7,8-TETRAMETHOXYFLAVONOID ANALOGUES IN METHANOL.
– – – – (+)-3′,4′,7,8-Tetramethoxy-2,3-*trans*-dihydroflavonol; ——— (−)-3′,4′,7,8-Tetramethoxyflavanone; —.—.—. (+)-2,3′,4′,6,7-Pentamethoxy-2-benzylcoumaranone.

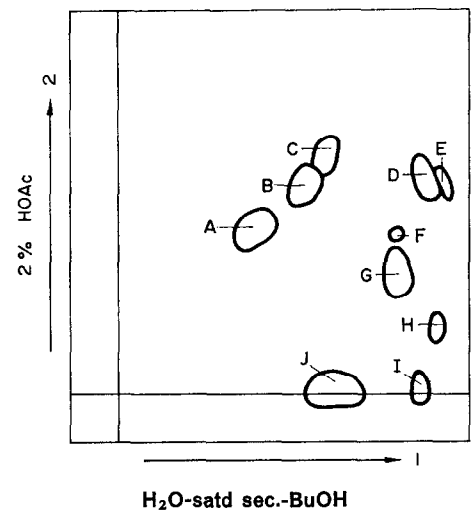


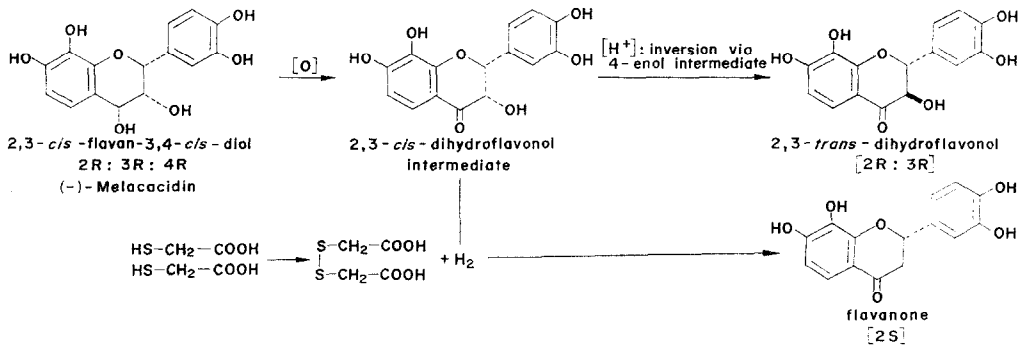
FIG. 2. TWO-DIMENSIONAL CHROMATOGRAM OF 3′,4′,7,8-TETRAHYDROXYFLAVONOIDS FROM THE HEARTWOOD OF *A. nigrescens*.
(A) (−)-melacacidin; (B) (−)-isomelacacidin; (C) (+)-2,3-*trans*-flavan-3,4-*cis*-diol; (D) 2,3′,4′,6,7-pentahydroxy-2-benzylcoumaranone; (E) unknown (F) protocatechuic acid; (G) (±)-2,3-*trans*-dihydroflavonol; (H) (±)-flavanone; (I) chalcone; (J) flavonol.

The similarity in hydroxylation pattern of the flavonoids suggests that they may arise from a common precursor, perhaps the chalcone (VII, R = H) or perhaps the α-hydroxychalcone (VII, R = OH). However, in *A. nigrescens* as in almost all *Acacia* spp. hitherto examined, chalcones are present in such negligible proportions that they are considered to be isomerization-products of flavanones, while flavan-3,4-diols followed by dihydroflavonols inevitably predominate.

Flavonoid interconversions occur with relative ease; thus, (−)-2,3-*cis*-3,4-*cis*-melacacidin (I, R = H) is converted into the (+)-2,3-*trans*-dihydroflavonol (IV, R = H, 2R:3R), and the latter loses its 3-hydroxyl function to give the optically active flavanone

(VI, R = H, 2S) in the presence of aqueous thioglycollic acid. This reaction occurs with equal facility with (+)-2,3-*trans*-3,4-*trans*-flavan-3,4-diols,[11,12] but the final step to the flavanone, in what is obviously a sequence, is inhibited when deuterium replaces a proton at C-4 in flavan-3,4-diol starting-material.

Problems have been encountered[11] in rationalizing the mechanism of these interesting conversions, and an alternative, now considered to be in some ways more likely, is offered. Conversion of the (−)-2,3-*cis*-flavan-3,4-*cis*-diol to the 2,3-*cis*-dihydroflavonol remains an autoxidative step, limited by the presence of thioglycollic acid which possesses anti-oxidant properties.[13,14] Complete inversion at C-3 of the 2,3-*cis*-dihydroflavonol to the (+)-2,3-*trans*-dihydroflavonol results from protonation (see Scheme 1) and the greater thermodynamic stability[15] of the product. Final reduction of the latter to the flavanone could result from a reductive reaction with thioglycollic acid. Such relative ease of reduction, and also of oxidation,[16] of the 3-position of flavanones suggests that these reactions might find biosynthetic parallels. However, the non-formation of the flavanone analogue from the 4-deuteriated compound, (±)-3′,4′,7,8-tetramethoxy-[4-^{2}H]-2,3-*trans*-flavan-3,4-*trans*-diol see ref. 11), cannot be explained on the basis of the above mechanism.



SCHEME 1. SUGGESTED MECHANISM OF CONVERSION OF (−)-MELACACIDIN INTO 2,3-*trans*-DIHYDROFLAVONOL AND FLAVANONE ANALOGUES IN AQUEOUS THIOGLYCOLLIC ACID.

Tannins are absent from the heartwood of *A. nigrescens* and also generally from other wood species which contain either melacacidin (3′,4′,7,8-tetrahydroxy) or teracacidin (4′,7,8-trihydroxy) analogues, whereas other 5-deoxyflavan-3,4-diols, e.g. guibourtacacidins (4′,7-dihydroxy),[17] leucofisetinidins (3′,4′,7-trihydroxy)[18] and leucorborinetinidins (3′,4′,5′,7-tetrahydroxy)[19] are known to be associated with self-condensed polyflavanoids. This reflects either the absence of tannin-forming enzymes in specific instances, or more likely, that 8-hydroxylation of 7-hydroxyflavan-3,4-diols, while increasing the general reactivity of the A-ring towards electrophilic substitution, removes the nucleophilic focal points at C-6 and C-8 which are usually involved in interflavanoid bond formation.[20] Alternatively

[11] I. C. DU PREEZ, T. G. FOURIE and D. G. ROUX, *Chem. Commun.* 333 (1971).
[12] I. C. DU PREEZ, D. FERREIRA and D. G. ROUX, *J. Chem. Soc.* C, 336 (1971).
[13] A. WATANABI, M. TERAO and T. SAKASHITA, *Ann. Rept. Takeda Research Lab.* **11**, 124 (1952).
[14] E. SCHEULLER, U.S. Pat. No. 2610941; in *Chem. Abs.* **47**, 1094i (1953).
[15] J. W. CLARK-LEWIS, R. W. JEMISON and V. NAIR, *Austral. J. Chem.* **21**, 3015 (1968).
[16] T. R. SESHADRI, *Tetrahedron* **6**, 169 (1959); V. B. MAHESH and T. R. SESHADRI, *J. Chem. Soc.* 2503 (1955).
[17] H. M. SAAYMAN and D. G. ROUX, *Biochem. J.* **96**, 36 (1965).
[18] S. E. DREWES and D. G. ROUX, *Biochem. J.* **96**, 681 (1965); S. E. DREWES, D. G. ROUX, S. H. EGGERS and J. FEENEY, *J. Chem. Soc.* C, 1217 (1967).
[19] D. G. ROUX and E. PAULUS, *Biochem. J.* **82**, 324 (1962).
[20] D. FERREIRA, H. K. L. HUNDT and D. G. ROUX, *Chem. Commun.* 1257 (1971).

8-hydroxylation might counteract electron-release from the 7-hydroxyl group, thus reducing the tendency of 7,8-dihydroxyflavanoids to form 4-carbonium ions or quinone methide intermediates which are considered essential for self-condensation. Melacacidin and teracacidin diasteroisomers are accordingly regarded as less significant candidates for tannin-formation, than their 7-hydroxy or 5,7-dihydroxy counterparts.

Examination of the chemical shifts of methoxy proton resonances of melacacidin analogues with progressive addition of C_6D_6 to their $CDCl_3$ solutions, shows that for the flavan-3,4-diol methyl ethers the 8-methoxy group remains almost stationary as anticipated, while other methoxy groups undergo pronounced shifts upfield ($\Delta\tau$ + 0·22 — 0·57) (Table 1). Flavanone and flavone analogues, on the other hand, exhibit chemical shifts of all methoxy proton resonances, with those attributed to 8-methoxyls ($\Delta\tau$ + 0·08 — 0·15)

TABLE 1. CHEMICAL SHIFTS OF METHOXY PROTON RESONANCES IN $CDCl_3$, $CDCl_3$:C_6D_6 (1:3) AND C_6D_6 SOLUTIONS

3′,4′,7,8-Tetramethoxy derivative	Methoxy τ values					
	$CDCl_3$			C_6D_6		
	3′,4′,7	8		3′,4′,7	8	
(−)-2,3-*cis*-flavan-3,4-*cis*-diol	6·18 (9H)	6·18		6·42, 6·45 (6H)*	6·15*	
(−)-2,3-*cis*-flavan-3,4-*trans*-diacetoxy	6·08 (6H), 6·10	6·10		6·50, 6·55,6·62	6·08	
(+)-2,3-*trans*-flavan-3,4-*cis*-diacetoxy	6·11 (9H)	6·21		6·56, 6·61 (6H)	6·21	
			3			3
3-methoxyflavone	5·94 (6H), 6·00	6·00	6·07	6·30, 6·45, 6·56*	6·10*	6·18*
(±)-flavanone	6·04 (6H), 6·07	6·07		6·55, 6·60, 6·62	6·22	
(±)-2,3-*trans*-dihydroflavonol	6·07 (6H), 6·12	6·07		6·22, 6·50, 6·58*	6·15*	
	3′,4′,6	7	3	3′,4′,6	7	2
(+)-2-benzylcoumaranone	6·04, 6·08, 6·22	6·22	6·70	6·69, 6·85 (6H)	6·19	6·54

* All chemical shifts in $CDCl_3$:C_6D_6 (1:3, v/v) due to low solubility.

less pronounced than the remainder ($\Delta\tau$ + 0·10 — 0·62). An apparent exception is the 2-hydroxy-2-benzylcoumaranone (VIII, R = R′ = Me) where the 8-methoxy resonance remains almost stationary. Shifts of the 8-methoxy groups of flavanones and flavones which have no protons in *ortho* positions are attributable[21] to the electron-withdrawing properties of the 4-carbonyl groups. The assessment of these shifts is of diagnostic value when establishing the position of interflavanoid links in polyflavanol (tannin) and polyflavanone chemistry by NMR spectrometry.

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$ with TMS as internal standard: optical rotations on a Hilger and Watts M-412 polarimeter using acetone–water (9:1) unless otherwise specified, and CD-curves on a JASCO ORD/CD-5 spectropolarimeter. Two-dimensional paper chromatograms were run by ascent on Whatman No. 1 (28 × 46 cm) sheets in water-satd. *sec*-BuOH and in 2% HOAc. Preparative-scale paper chromatography was by ascent on Whatman No. 3 (46×57 cm) sheets in 2% or 20% HOAc, or by descent in water-satd. *sec*-BuOH on pre-washed sheets (distilled water). TLC was on Kieselgel PF_{254} (0·25 mm), and on a 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). The purity of compounds was established by NMR spectrometry, and their identity by conversion.

Extraction and preliminary separation. Drillings (2·45 kg) of the black heartwood were extracted (at ambient temperatures) successively with three volumes of 80% acetone–water over a period of 6 days, and the combined extracts evaporated to dryness under vacuum. Brown solids (390 g, 17·4%) resulted and

[21] J. H. BOWIE, J. RONAYNE and D. H. WILLIAMS, *J. Chem. Soc.* **B**, 785 (1966).

showed the presence of ten components on two-dimensional paper chromatograms (Fig. 2), and the complete absence of tannins. Partitioning of the extract between EtOAc and water (10 plates:100 ml per phase) led to separation into the less mobile flavan-3,4-diols (components A–C, located mainly in the upper phases of plates 1–3) and the remaining compounds (D–J, mainly in both phases of plates 7–9).

Flavan-3,4-diol components. Solids (18 g) representative of the former were dissolved in acetone and applied to 180 preparative cellulose sheets at 100 mg per sheet. The chromatograms were developed in 2% HOAc. Isomelacacidin and the 2,3-*trans*-flavan-3,4-*cis*-diol isomer migrate as a single band (R_f 0·59), well separated from melacacidin (0·48). The bands were cut, eluted in 20% acetone–water and the eluants taken to dryness under vacuum yielding melacacidin (1·37 g, 7·6%) and the above mixture of flavan-3,4-diols (1·08 g, 6·0%).

(−)-2,3-cis-3′,4′,7,8-*Tetramethoxyflavan*-3,4-cis-*diol* [(−)-*melacacidin tetramethyl ether*]. The pure amorphous (−)-melacacidin afforded colour reactions, alkali degradation products (pyrogallol and protocatechuic acid) and an anthyolocyanidin[22] (hot HCl–*iso*-PrOH) consistent with its structure. The tetramethyl ether, m.p. 143°, $[\alpha]_D^{22}$ −92° (*c*, 0·6 in EtOH), M^+ 362 (lit.[3] m.p. 144–145°, $[\alpha]_D^{25}$ −83·5°) was obtained as needles from ethanol. The crystalline diacetate was obtained similarly, m.p. 185°, $[\alpha]_D^{25}$ −33·4° (*c*, 0·2 in EtOH), M^+ 446 (lit.[3] m.p. 191–192°, $[\alpha]_D^{25}$ −39·5°). The NMR and mass fragmentation spectra were consistent with the above structures and identical to those in the literature,[23,24]

Paper-ionophoretic separation of methylated flavan-3,4-diols. The dark brown powder from band R_f 0·59 was methylated with excess CH_2N_2 for 48 hr at −10°. The methyl ethers could not be separated by TLC in six solvents, and the mixture was accordingly resolved into the *cis–trans*-diol (137 mg) and the *trans–cis* diol (20 mg) by paper ionophoresis in borate buffer.[25,26]

(−)-2,3-cis-3′,4′,7,8-*Tetramethoxyflavan*-3,4-trans-*diol* [(−)-*isomelacacidin tetramethyl ether*]. The compound from paper ionophoresis was further purified by TLC in benzene–acetone (6:4) (R_f 0·41). The pure tetramethyl ether (69 mg) is a non-crystalline solid, $[\alpha]_D^{26}$ −59° (*c*, 0·7, M^+362. Its diacetate is also a non-crystalline solid, $[\alpha]_D^{26}$ +15·5° (*c*, 0·5), M^+446. The NMR and mass fragmentation spectra were consistent with the above structures, [23,24,27]

(+)-2,3-trans-3′,4′,7,8-*Tetramethoxyflavan*-3,4-cis-*diol.* The diol from paper ionophoresis was pure, crystallizing in *needles* from ethanol, m.p. 162°, $[\alpha]_D^{25}$ −6·0° (*c*, 0·4), M^+446. (Found: C, 61·7; H, 5·9. Calc. for $C_{23}H_{26}O_9$:C, 61·9; H, 5·8%.) The NMR spectrum of the diacetate was consistent with a 2,3-trans-3,4-*cis* configuration ($J_{2,3}$ 10·0, $J_{3,4}$ 4·3 Hz) and the above structure. The mass fragmentation spectrum was identical to those of the above compounds, differing only in the relative abundance of peaks.[27]

Flavanone, flavone and 2-hydroxy-2-benzylcoumaranone components. Mobile components (20 g) from plates 7–9 of the partition separation, dissolved in acetone-water were applied to 200 Whatman No. 3 paper sheets (100 mg/sheet) and the chromatograms developed in 2% HOAc. The dihydroflavonol (R_f 0·37) and flavanone (0·22) were obtained in pure condition after the first separation. The benzylcoumaranone (0·63) required separation from a second components by downward separation in water-satd. *sec*-BuOH (R_f 0·66, 0·75 resp.). The chalcone and flavonol remained on the origin and were eluted with 70% EtOH; taken to dryness, and separated in 20% HOAc. The bands R_f 0·29 (chalcone) and 0·09 (flavonol) were eluted as before. Protocatechuic acid (R_f 0·45 in 2% HOAc) was obtained in pure form after downward separation in water-satd. *sec*-BuOH (R_f 0·84) as before.

(+)-2-*Benzyl*-2,3′,4′,6,7-*pentamethoxycoumaran*-3-*one.* The 2-benzyl-2,3′,4′,6,7-pentahydroxycoumaran-3 one (450 mg) was methylated with excess CH_2N_2 at −10° for 48 hr. The pentamethyl ether was purified by TLC in petroleum (b.p. 67·5–69·5°)–acetone (8:2) by three successive developments of the same plates, followed by TLC in benzene–acetone (8:2), R_f 0·53. The compound develops a characteristic purple colour changing to light brown, on spraying with H_2SO_4–formaldehyde. The pentamethyl ether crystallized from EtOH in colourless *needles*, m.p. 116°, ν_{max} 1715 cm^{-1}, $[\alpha]_D^{27}$ +4·0° (*c*, 0·5), M^+374. (Found: C, 64·1; H. 6·0. Calc. for $C_{20}H_{22}O_7$: C, 64·1; H, 5·9%.) The optical activity was confirmed by its CD-curve (Fig. 1). The NMR and mass spectra were consistent with the above structure.[27]

(±)-3′,4′,7,8-*Tetramethoxy*-2,3,trans-*dihydroflavonol.* The free phenol (200 mg) was methylated with CH_2N_2 and purified by TLC of the methyl ether in petroleum–acetone (3:2). The band (R_f 0·72) gave a yellow colour with H_2SO_4-formaldehyde. The compound (39 mg) crystallized from EtOH, m.p. 163°, $[\alpha]_D^{24}$ 0·0° (*c*, 0·3), M^+360 (lit. [28] m.p. 166°). The NMR and MS are identical with those in the literature.[23,24]

(±)-3′,4′,7,8-*Tetramethoxyflavanone.* The flavanone (200 mg) was methylated and the product purified

[22] D. G. ROUX, *Nature, Lond.* **179**, 305 (1957).
[23] J. W. CLARK-LEWIS, *Austral. J. Chem.* **21**, 2059 (1968).
[24] S. E. DREWES, *J. Chem. Soc.* **C**, 1140 (1968); J. W. CLARK-LEWIS, *Austral. J. Chem.* **21**, 3025 (1968).
[25] S. E. DREWES and D. G. ROUX, *Biochem. J.* **92**, 555 (1964).
[26] D. R. COOPER and D. G. ROUX, *J. Chromatogr.* **17**, 396 (1965).
[27] T. G. FOURIE, *Omskakelingsreaksies van die Analoë van (−)-Melacacidien uit* Acacia nigrescens, M.Sc. Thesis, University of the Orange Free State (1971).
[28] J. W. CLARK-LEWIS and G. F. KATEKAR, *J. Chem. Soc.* 4502 (1962).

by TLC in benzene–acetone (4:1). The tetramethyl ether (R_f 0·41, orange-yellow with H_2SO_4–formaldehyde) crystallizes from EtOH (59 mg), m.p. 138°, $[\alpha]_D^{24}$ 0·0° (*c*, 0·4), M^+344 (lit.[29] m.p. 141°). The NMR and MS are consistent with the above structure.

3,3′,4′,7,8-*Pentamethoxyflavone*. The flavonol (400 mg) is methylated with CH_2N_2 and the product purified by TLC in benzene–acetone (7:1), R_f 0·61. It crystallizes from EtOH in fine needles, m.p. 148° M^+372 (lit.[29] m.p. 153°).

Protocatechuic acid. The protocatechuic acid (300 mg) was methylated as above, and separated by TLC in petroleum (b.p. 67·5–69·5°)–acetone (3:1). The ester (R_f 0·49, 203 mg) crystallizes from EtOH in needles, m.p., and m.m.p. with an authentic speciment (Koch-Light) 59° (lit.[30] m.p. 60°). Considerable amounts of protocatechuic acid were occluded by the flavonol and chalcone on the origin.

Reaction of (−)-*melacacidin and its tetramethyl ether with mercaptoacetic* (*thioglycollic*) *acid*. The above reaction was carried out by two methods: (i) without, and (ii) with pressure.

Method (i) (*without pressure*). (−)-Melacacidin tetramethyl ether (200 mg) was dissolved in dioxane (20 ml), water (8 ml) and mercaptoacetic acid (70 mg), and the mixture heated on a waterbath for 1 hr. The neutral and acidic fractions were separated by the bicarbonate technique. The acid fraction was methylated with CH_2N_2 and a *solid* (3 mg), R_f 0·42, isolated after separation by TLC with benzene–acetone. The neutral fraction separated, as above, into two bands, R_f 0·56, [flavone tetramethyl ether (23 mg)] and R_f 0·39 [dihydroflavonol tetramethyl ether (34 mg)].

Method (ii) (*with pressure*). (−)-Melacacidin (free phenol, 300 mg) was heated for 30 min under pressure (119°, 15 lbs/in^2). The reaction gave identical products after methylation excepting that the acid component, R_f 0·42, was available in higher yield (29 mg).

(−)-3′,4′,7,8-*Tetramethoxyflavone*. The compound develops a yellow colour with H_2SO_4–formaldehyde, and crystallizes from EtOH in fine *needles*, m.p. 133°, $[\alpha]_D^{25}$ −43·7° (*c*, 0·8), CD-curve (Fig. 1), M^+344. The NMR spectrum and MS fragmentation were identical with those of the natural racemate.

(+)-3′,4′,7,8-*Tetramethoxy*-2,3-trans-*dihydroflavonol*. The compound affords a yellow colour with H_2SO_4–formaldehyde, and crystallizes from ethanol in *needles*, m.p. 163°, $[\alpha]_D^{25}$ +12·0° (*c*, 0·2), CD-curve (Fig. 1), M^+360. The NMR spectrum and MS fragmentation were identical to those of the natural racemate.

Methyl [(−)-2,3-cis-3,4-trans-3-*hydroxy*-7,8,3′,4′,-*tetramethoxyflavan*-4-*ylthio*]*acetate*. The compound is an *amorphous solid*, $[\alpha]_D^{22}$ −86·0° (*c*, 0·2), M^+450. Acetylation with Ac_2O–pyridine gave an *amorphous acetate*, $[\alpha]_D^{26}$ −23·0° (*c*, 0·3), M^+492. The NMR spectra and mass spectral fragmentations are consistent with the above structures.[27]

Reduction of (±)-3′,4′,7,8-*tetramethoxyflavone with* $LiAlH_4$ *to flavan*-4-*ols*. The compound (186 mg) was reduced with $LiAlH_4$ in tetrahydrofuran.[30] The mixture of two isomers obtained (166 mg) is separated by TLC using two successive developments with MEK–toluene (3:7). Two bands (R_f 0·54, 63 mg and 0·48, 28 mg), both giving a purple colour with H_2SO_4–formaldehyde, were recovered.

(±)-3′,4′,7,8-*Tetramethoxyflavan*-4β-*ol*. The compound (R_f 0·54) crystallizes from EtOH in *needles*, m.p. 114°, M^+346. The monoacetate is an *amorphous solid*, M^+388.

(±)-3′,4′,7,8-*Tetramethoxyflavan*-4α-*ol*. The compound (R_f 0·48) is an *amorphous solid*, M^+346. The monoacetate crystallizes from EtOH in *needles*, m.p. 119°, M^+388. Physical constants from the NMR spectra of the flavan-4-ols and their monoacetates, and also their MS fragmentations are consistent with the above structures.[24,27]

Reduction of (±)-3′,4′,7,8-*tetramethoxy*-2,3-trans-*dihydroflavonol with* $LiAlD_4$. The compound (270 mg) was reduced under the same conditions as used above but with $LiAlD_4$. The product was resolved into two bands (R_f 0·14, 0·19) after two successive developments in MEK–toluene (3:7). Both compounds develop a red colour with H_2SO_4–formaldehyde.

(±)-2,3-trans-[4-^{2}H]-3′,4′,7,8-*Tetramethoxyflavan*-3,4-trans-*diol*. The compound (149 mg, R_f 0·14) crystallized from EtOH in *needles*, m.p. 117°, M^+363. The diacetate crystallized from EtOH in *needles*, m.p. 127°. Both compounds showed an AB heterocyclic proton system with $J_{2,3}$ 9·8 Hz (diol) and 9·0 Hz (diacetate), confirming deuteriation at C-4.

(±)-2,3-trans-[4-^{2}H]-3′,4′,7,8-*Tetramethoxyflavan*-3,4-cis-*diol*. The compound (15 mg, R_f 0·19) crystallized from EtOH in *needles*, m.p. 187°, M^+363. The 3,4-*trans* and 3,4-*cis*-forms were differentiated by their paper ionophoretic mobility in borate buffer[25,26] and their relative mobilities on TLC are the same as ordinary flavan-3,4-diol methyl ethers. Mass spectra are consistent with the location of deuterium at C-4. Reaction of the 4-deuteriated *trans–trans*-diol with mercaptoacetic acid under the identical conditions applied to (−)-melacacidin, gave only two products, the flavan-4-ylthio-acetate and the dihydroflavonol analogue. The course of the reaction therefore differs from that with (+)-2,3-*trans*-3,4-*trans*-mollisacacidin methyl ether where the flavanone analogue also results.[11]

[29] J. W. Clark-Lewis and V. Nair, *Austral. J. Chem.* **17**, 1164 (1964).
[30] K. U. Matsmoto, *Chem. Ber.* **11**, 128 (1878).
[31] R. Bognar and M. Rakosi, *Chem. & Ind.* 188 (1956).

Conversion of (±)-3′,4′,7,8-*tetramethoxydihydroflavonol to* 2-*benzyl*-2-*hydroxy*-3′,4′,6,7-*tetramethoxy-coumaran*-3-*one*. The reaction was according to the method of Chopin and Bouillant.[5] The compound (160 mg) was heated for 3 min on a waterbath at 95° in 15% methanolic KOH. The reaction mixture was cooled rapidly, acidified with 3N H_2SO_4, extracted with EtOAc and dried. After vacuum evaporation the products, R_f 0·39, 0·48, separated on TLC in toluene–EtOAc (1:1).

(±)-2-*Benzyl*-2-*hydroxy*-3′,4′,6,7-*tetramethoxycoumaran*-3-one. The compound (R_f 0·39, 49·4 mg) gave a light purple with H_2SO_4–formaldehyde and crystallizes from EtOH in *plates*, m.p. 126°. The NMR and MS confirmed the structural assignement.[27]

3-*Hydroxy*-3′,4′,7,8-*tetramethoxyflavone*. The product (R_f 0·48, 18 mg) gives a yellow colour with H_2SO_4 formaldehyde, and crystallizes from ethanol in fine *needles*, m.p. 209°, M^+358. The NMR and MS were consistent with this structural assignment.

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