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Trimeric proteracacinidins and a (6→6)-bis-leucoteracacinidin from *Acacia galpinii* and *Acacia caffra*☆

Linette Bennie[a], Johan Coetzee[a], Elfranco Malan[a], Desmond Slade[b], Jannie P.J. Marais[b], Daneel Ferreira[b,*]

[a]*Department of Chemistry, University of the Free State, PO Box 339, Bloemfontein, 9300 South Africa*
[b]*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

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Abstract

The rare series of trimeric proteracacinidins is extended by identification of the first analogs with exclusive C–C interflavanyl bonds, i.e. epioritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol,oritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol, and epioritin-(4β→6)-epioritin-(4β→6)-epioritin-4α-ol. These compounds are accompanied by the bis-leucoteracacinidin, epioritin-4α-ol-(6→6)-epioritin-4β-ol, the first naturally occurring bis-flavan-3,4-diol.

Keywords: *Acacia galpinii*; *Acacia caffra*; Leguminosae; Trimeric proteracacinidins; Proanthocyanidins; Bis-flavan-3,4-diol

1. Introduction

Recent screening of the heartwood extracts of *Acacia galpinii* and *Acacia caffra* has revealed a considerable structural diversity as far as the presence of proteracacinidin- and promelacacinidin-type pro- and leucoanthocyanidins are concerned. These proanthocyanidins comprise (4β→6)-linked proteracacinidin and promelacacinidin dimers (Malan and Sireeparsad, 1995; Malan et al., 1997; Bennie et al., 2002b), dimeric proteracacinidins possessing the rare (4→5) interflavanyl linkages (Malan, 1995; Bennie et al., 2002a), unique doubly-linked proteracacinidin dimers (Malan et al., 1994; Bennie et al., 2001b), ether linked dimeric proteracacinidins and promelacacinidins (Coetzee et al., 1998a, 1998b; Bennie et al., 2000), and the first triflavanoids possessing both ether and carbon–carbon interflavanyl bonds (Bennie et al., 2001a). Such a diversity presumably arises from the absence of flavan-3-ols with *m*-oxygenated A-rings, hence permitting the emergence of alternative nucleophilic centers as participants in interflavanyl bond formation. Here we report the structures of the first trimeric proteracacinidins with exclusive C-C interflavanyl linkages, epioritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol **3**, oritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol **5**, and epioritin-(4β→6)-epioritin-(4β→6)-epioritin-4α-ol **7**, as well as the first naturally occurring bis-leucoanthocyanidin with a biphenyl linkage, epioritin-4α-ol-(6→6)-epioritin-4β-ol **1** from the heartwoods of *A. caffra* and *A. galpinii*.

2. Results and discussion

In addition to the variety of di- and tri-meric pro- and leuco-anthocyanidins referred to in the Introduction, the methanol extracts of the heartwoods of *A. caffra* and *A. galpinii* also contain the first trimeric proteracacinidins with exclusive C–C interflavanyl bonds, i.e. epioritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol **3**, and oritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol **5**. These compounds are accompanied by epioritin-(4β→6)-epioritin-(4β→6)-epioritin-4α-ol **7**, in *A. caffra*, and in *A. galpinii* by the unique bis-leucoanthocyanidin with a biphenyl linkage, epioritin-4α-ol-(6→6)-epioritin-4β-ol **1**. Owing to the complexity of the respective polyphenolic mixtures, compounds **1**, **3**, **5** and **7** were purified and identified as their permethylaryl ether acetate derivatives **2**, **4**, **6** and **8**.

☆ Part 37 in the series 'Oligomeric flavanoids'. Part 36 (Bennie et al., 2002)

* Corresponding author. Tel.: +1-662-915-1572; fax: +1-662-915-7062.
E-mail address: dferreir@olemiss.edu (D. Ferreira).

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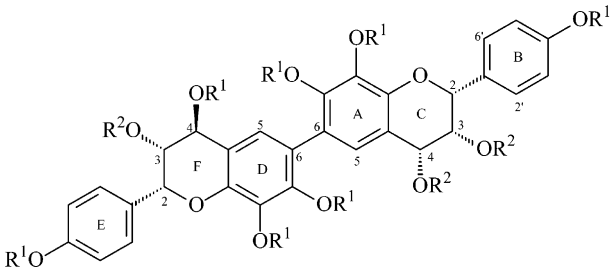

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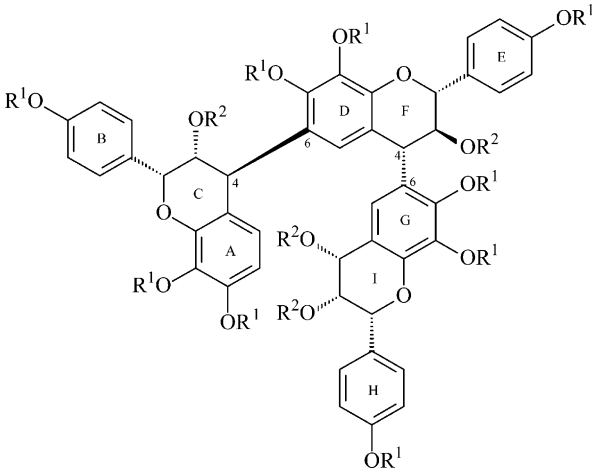

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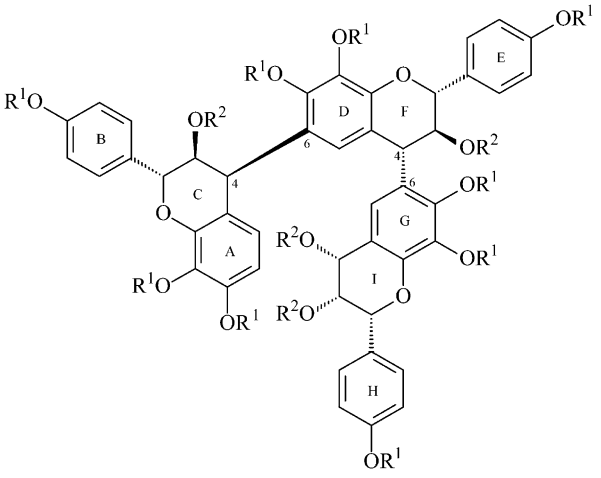

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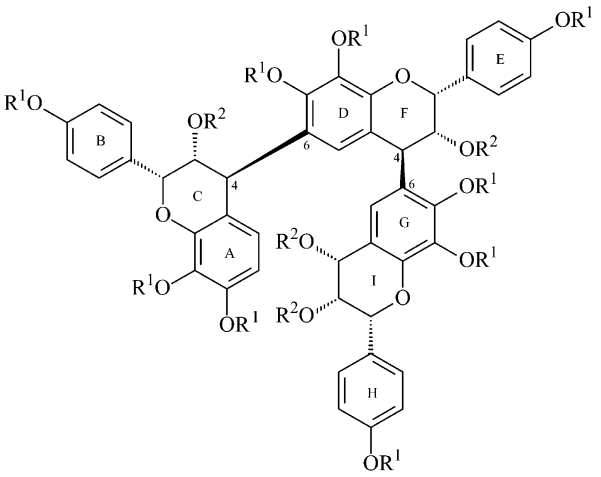

7 $R^1 = R^2 = H$
8 $R^1 = Me$, $R^2 = Ac$

The ^1H NMR spectra of the epioritin-4α-ol-(6→6)-epioritin-4β-ol derivative **2** were recorded in both C_6D_6 and $CDCl_3$ (Table 1). Owing to the superior resolution of signals, the spectral data in the former solvent were used for structure elucidation. Two aromatic AA′BB′-spin systems, two aromatic singlets and two AMX-systems for protons of the heterocyclic rings, reflected the dimeric nature of the compound. Differentiation of the spin systems and the connectivities between aromatic and heterocyclic protons were effected with 2D COSY experiments. The presence of six aromatic *O*-methyl resonances, a single benzylic *O*-methyl resonance, three *O*-acetyl signals, the two aromatic singlets, as well as FAB-MS data (m/z 802 = $C_{43}H_{46}O_{15}$) strongly suggested a biphenyl-type linkage of two leucoteracacinidin moieties. The benzylic *O*-methyl signal, the presence of three instead of the four *O*-acetyl resonances normally required for a bis-flavan-3,4-diol as well as the chemical shift differences of the two 4-H resonances (δ 4.37 and 6.53), suggested that 4-OH(F) was solvolyzed during derivatization with diazomethane in methanol.[1] Strong NOE association of both 3-H(F) and 5-H(D) with the aliphatic *O*-methyl group not only confirmed its location at 4-C(F) but also differentiated the spin systems of the C- and F-ring protons, and the 5-H(A) (δ 7.36) and 5-H(D) (δ 7.13) resonances. $^4J_{HH}$ coupling between the respective 2- and 2′,6′-protons, typically smaller than 1.0 Hz, permitted differentiation of the B- and E-ring spin systems. The C-6(A)→C-6(D) biphenyl interflavanyl linkage was confirmed by NOE associations of 5-H(A) with both 5-H(D) and 7-OMe(D), and of 5-H(D) with both 5-H(A) and 7-OMe(A). This also indicated free

[1] H NMR of the crude fraction containing compound **1** showed the absence of *O*-methyl derivatives.

Table 1
^{1}H NMR (300 MHz, 296 K) spectral data of bis-flavan-3,4-diol **2** and trimeric derivatives **4**, **6** and **8**. Splitting patterns and *J*-values are in parentheses

Ring	H	**2**—C_6D_6	**4**—$(CD_3)_2CO$	**6**—$(CD_3)_2CO$	**8**—$(CD_3)_2CO$
A	5	7.36 (*br. s*, 1.0)	6.47 (*d*, 9.0)	6.33 (*d*, 9.0)	6.71 (*d*, 9.0)
	6	–	6.64 (*d*, 9.0)	6.55 (*d*, 9.0)	6.64 (*d*, 9.0)
B	2,6	7.38 (*d*, 9.0)	7.29 (*d*, 9.0)	7.30 (*d*, 9.0)	7.27 (*d*, 9.0)
	3,5	6.89 (*d*, 9.0)	6.91 (*d*, 9.0)	6.96 (*d*, 9.0)	6.86 (*d*, 9.0)
C	2	4.96 (*br. s*, 1.0)	5.13 (*br. s*, 1.5)	5.25 (*d*, *br. s*, 6.5)	5.00 (*br. s*, 1.5)
	3	5.97 (*dd*, 1.0, 4.5)	5.34 (*dd*, 1.5, 3.0)	5.39 (*dd*, 6.5, 4.5)	5.32 (*dd*, 1.5, 3.0)
	4	6.53 (*d*, 4.5)	4.37 (*d*, 3.0)	4.56 (*d*, 4.5)	4.43 (*d*, 3.0)
D	5	7.13 (s)	5.80 (*br. s*)	6.08 (*br. s*)	6.22 (*br. s*)
E	2,6	7.57 (*d*, 9.0)	7.50 (*d*, 9.0)	7.51 (*d*, 9.0)	7.31 (*d*, 9.0)
	3,5	6.92 (*d*, 9.0)	6.97 (*d*, 9.0)	6.97 (*d*, 9.0)	6.98 (*d*, 9.0)
F	2	5.72 (*br. s*, 1.5)	5.18 (*d*, 9.5)	5.17 (*d*, 9.5)	5.30 (*d*, 1.5)
	3	5.74 (*dd*, 1.5, 3.0)	5.63 (*dd*, 9.5, 9.5)	5.71 (*dd*, 9.5, 9.5)	5.35 (*dd*, 1.5, 3.0)
	4	4.37 (*d*, 3.0)	4.65 (*d*, 9.5)	4.65 (*br. d*, 9.5)	4.33 (*d*, 3.0)
G	5	–	6.64 (*br. s*)	6.75 (*br. s*)	6.44 (*br. s*)
H	2,6	–	7.55 (*d*, 9.0)	7.53 (*d*, 9.0)	7.49 (*d*, 9.0)
	3,5	–	7.00 (*d*, 9.0)	7.00 (*d*, 9.0)	6.97 (*d*, 9.0)
I	2	–	5.61 (*br. s*, 1.0)	5.64 (*br. s*, 1.0)	5.69 (*br. s*, 1.0)
	3	–	5.53 (*dd*, 1.0, 4.5)	5.53 (*dd*, 1.0, 4.5)	5.59 (*dd*, 1.0, 4.5)
	4	–	6.14 (*d*, 4.5)	6.19 (*d*, 4.5)	6.33 (*d*, 4.5)
	OMe	3.37 (×2), 3.43, 3.82, 3.93, 3.97, 4.04 (each *s*)	3.97, 3.89, 3.88, 3.84, 3.83, 3.79, 3.76, 3.73, 3.65 (each *s*)	3.87, 3.84, 3.83, 3.82, 3.80, 3.75 (×2), 3.71, 3.69 (each *s*)	4.00, 3.98, 3.87, 3.85, 3.84, 3.82, 3.79, 3.76, 3.75 (each *s*)
	OAc	1.49, 1.59, 1.86 (each *s*)	1.98, 1.92, 1.84, 1.60 (each *s*)	2.00, 1.90, 1.87, 1.63 (each *s*)	2.19, 1.95, 1.86, 1.85 (each *s*)

rotation about the interflavanyl bond and hence the absence of atropisomers.

The coupling constants of the C- ($^3J_{2,3}=1.0$; $^3J_{3,4}=4.5$ Hz) and F-ring ($^3J_{2,3}=1.5$; $^3J_{3,4}=3.0$ Hz) protons were reminiscent of 2,3-*cis*-3,4-*cis* and 2,3-*cis*-3,4-*trans* relative configuration, respectively. These assignments were confirmed by NOE association between 2- (δ 4.96) and 4-H(C) (δ 6.53) for the all-*cis* configured C-ring, and by the association between 2-H(F) (δ 5.72) and 4-OMe(F) (δ 3.43) for the 2,3-*cis*-3,4-*trans* F-ring.

Following the acquisition of ^{1}H NMR data in $CDCl_3$, we subjected the same sample to ^{13}C NMR analysis. Prolonged exposure to $CDCl_3$ eventually led to extensive decomposition of the acid-sensitive leucoteracacinidin structural moieties, which precluded the acquisition of an optical rotation and both ^{13}C NMR and circular dichroic data. The indicated (2*R*,3*R*,4*R*) and (2*R*,3*R*,4*S*) absolute configuration at the stereocenters of the C- and F-rings, respectively, is thus tentative and based on coupling constants of the heterocyclic protons, and on the assumption that the co-occurring flavan-3,4-diols, epioritin-4α- and 4β-ol (Malan, 1995; Malan and Sireeparsad, 1995), served as biogenetic precursors to this unique bis-flavan-3,4-diol. Its interflavanyl biaryl bond most likely formed via a one-electron phenol oxidative coupling process.

FAB-MS analysis of the permethylaryl ether acetate derivatives **4**, **6** and **8** of compounds **3**, **5** and **7** indicated molecular formulas of $C_{62}H_{64}O_{20}$ (*m*/*z* 1128) for all three compounds. When taken in conjunction with the nine *O*-methyl and four *O*-acetyl resonances in their ^{1}H NMR spectra (Table 1), these formulas confirmed the trimeric nature of all three analogs. Since the ^{1}H NMR spectra in $CDCl_3$, conspicuously free of the effects of dynamic rotational isomerism about the interflavanyl bonds, showed poor resolution of the protons of the heterocyclic rings, the spectral data in $(CD_3)_2CO$ were used for structure elucidation. The spin systems and the connectivities between aromatic and heterocyclic protons were affected with the 2D COSY experiments, while ^{13}C resonances were assigned by HMQC and HMBC experiments. In addition to the nine *O*-methyl and four *O*-acetyl resonances, the ^{1}H NMR spectrum of each compound also displayed two one-proton aromatic singlets, one aromatic AB- and three AA′BB′-spin systems, as well as three AMX-spin systems for protons of the heterocyclic rings. The data suggested trimeric structures comprising derivatized 7,8,4′-trihydroxyflavan-3-ol constituent units linked via two carbon-carbon interflavanyl bonds. One of the AMX-spin systems in the ^{1}H NMR spectra of all three compounds comprised a conspicuously deshielded 4-H resonance (δ 6.14, 6.19 and 6.33 for **4**, **6** and **8**, respectively) that was indicative of a 4-*O*-acetyl substituent and hence a flavan-3,4-diol "terminal" GHI-unit. Pronounced $^4J_{HH}$ coupling, supported by both 2D COSY and three-bond H-C correlations in the HMBC spectra, of the two aromatic singlets and one of the doublets of the aromatic AB-system with the respective heterocyclic 4-protons was used to identify the A- and C-ring, the D- and F-ring, as well as the G- and I-ring systems in derivatives

4, **6** and **8**. NOE associations of 5-H(G) with both 4-H(I) and 4-H(F) confirmed the C-4(F)→C-6(G) interflavanyl linkage. The C-4(C)→C-6(D) interflavanyl bond was similarly confirmed by NOE association of 5-H(D) with both 4-H(C) and 4-H(F) in all three analogs. Differentiation of the three aromatic AA′BB′-systems was effected via $^4J_{HH}$ coupling between the respective 2- and 2′,6′-protons of each spin system, and confirmed by both 2D COSY and three-bond H-C correlations.

The relative configurations of all three derivatives **4**, **6** and **8** were evident from the ^{1}HMR coupling constants of the heterocyclic protons (Table 1). Those derivatives with 2,3-*cis*-3,4-*trans* constituent units (C-ring of **4** and **8**, F-ring of **8**) exhibited $^3J_{2,3}=1.5$; $^3J_{3,4}=3.0$ Hz coupling constants; those with 2,3-*trans*-3,4-*trans* configured moieties (F-rings of **4** and **6**) exhibited $^3J_{2,3}=^3J_{3,4}=9.5$ Hz; the C-ring of compound **6** with a 2,3-*trans*-3,4-*cis* flavanyl unit had $^3J_{2,3}=6.5$; $^3J_{3,4}=4.5$ Hz; and analogs with 2,3-*cis*-3,4-*cis* configured units (I-rings of **4**, **6** and **8**) exhibited $^3J_{2,3}=1.0$ and $^3J_{3,4}=4.5$ Hz couplings (Bennie et al., 2001a,b, 2002b). Both 2,3-*trans*-3,4-*trans* and 2,3-*cis*-3,4-*cis* relative configurations were confirmed by NOE associations between 2- and 4-H, indicating their *cis*-cofacial arrangement in the respective F- and I-rings of e.g. derivatives **4**, **6** and **8**. 2,3-*Cis*-3,4-*trans* and 2,3-*trans*-3,4-*cis* relative stereochemistry of the heterocyclic rings, *e.g.* the C-rings in derivatives **4** and **6**, respectively, were similarly confirmed by NOE association of 2-H(C) with 5-H(D). In these analogs the NOE associations between 2- and 4-H were conspicuously absent.

The protons of the 2,3-*trans*-3,4-*cis* C-ring of **6** exhibited "abnormal" coupling constants ($^3J_{2,3}=6.5$; $^3J_{3,4}=4.5$ Hz). Previously we documented and explained similar coupling constants for a 2,4-biaryl-6-(2-benzopyranyl)-chromane (Malan et al., 1990) and (4→6)-coupled proteracacinidins (Bennie et al., 2002b) with 2,3-*trans*-3,4-*cis* stereochemistry. Such a small $^3J_{2,3}$ value presumably reflects substantial contributions of A-conformers towards the A-/E-conformational itinerary (Porter et al., 1986) of the C-ring. The dynamic conformational equilibrium would then reduce the average dihedral angle of 2- and 3-H(C), but not of 3- and 4-H(C), and hence the observed $^3J_{2,3}$ of 6.5 Hz.

The chemical shifts of the C-2 resonances (C- and F-rings) in the ^{13}C NMR spectra (Table 2) of derivatives **4**, **6** and **8** fully supported the relative configurations that was established via ^{1}H NMR coupling constants. Those analogs possessing 2,4-*trans* configured heterocyclic rings (C-rings of **4**, **6** and **8**; F-ring of **8**) displayed shielded 2-C resonances (ca. 4–7 ppm) compared to the chemical shifts of the same carbons in derivatives with 2,4-*cis* configuration (F-ring of **4** and **6**) due to the *γ-gauche* effect (Fletcher et al., 1977). The chemical shift of 2-C(C) (*δ* 76.02) in derivative **6** was notably similar to those of 4α-substituted *ent*-oritin moieties (*δ* 76.18–76.66) in a series of (4,6)-coupled proteracacinidins (Bennie et al., 2002b), albeit in different solvents. A similar congruence of 2-C(I) resonances was also evident when their chemical shifts in **4**, **6** and **8** (*δ* 77.31, 77.30 and 77.61, respectively) were compared with the 2-C(F) signals (*δ* 77.26–77.62) of 2,3-*cis*-3,4-*cis* proteracacinidin-type "terminal" DEF-moieties of the dimeric (4,6)-proteracacinidins (Bennie et al., 2002b). Similar to some of the dimeric analogs, we could also not observe the 4-C(F) resonances of compounds **4** and **6**, presumably due to the long relaxation times of these carbons on the NMR time scale (Bennie et al., 2002b).

Trimeric derivatives **4**, **6** and **8** display high-amplitude negative and positive Cotton effects (CEs) in the 280–290 and 230–250 nm regions, respectively, of their CD spectra. Negative CEs in the 280–290 nm region (^1L_b transitions) of the CD spectra of flavan-3-ols are consistently indicative of 2*R* absolute configuration (Korver and Wilkins, 1971; Van Rensburg et al., 1999). The observed negative CEs for the ^1L_b transitions of **4**, **6** and **8** may thus indicate *R* absolute configuration at the 2-C stereocenters of all three flavan-3-ol constituent units. Positive CEs near 240 nm in the CD spectra of dimeric proanthocyanidins usually indicate 4β-orientation of the DEF-flavanyl units (Van der Westhuizen et al., 1981). It is, however, unclear how the sign of the CE is influenced by the presence of both 4α- and 4β-flavanyl units at the trimeric level. The indicated absolute configurations, i.e. 2*R*,3*R*,4*R*(C): 2*R*,3*S*,4*S*(F): 2*R*,3*R*,4*R*(I) of **4**, 2*R*,3*S*,4*R*(C): 2*R*,3*S*,4*S*(F): 2*R*,3*R*,4*R*(I) of **6**, and 2*R*,3*R*,4*R*(C): 2*R*,3*R*,4*R*(F): 2*R*,3*R*,4*R*(I) of **8** are thus tentative and await confirmation *via* synthesis. We were thus far unable to synthesize this class of naturally occurring trimeric proteracacinidins from the co-occur-

Table 2
^{13}C NMR peaks (δ_C) of protonated carbons of trimeric proteracacinidin derivatives **4**, **6** and **8** in $(CD_3)_2CO$

Ring	C	**4**	**6**	**8**
A	5	125.08	124.05	125.18
	6	106.18	105.55	106.15
B	2,6	128.06	127.81	128.09 or 128.16
	3,5	113.90 or 114.08	114.06 or 114.28	113.90 or 114.01
C	2	73.44	76.02	73.67
	3	71.94	71.18	72.48
	4	40.74	35.27	41.32
D	5	125.56	125.76 or 125.84	126.27
E	2,6	129.41	129.44	128.09 or 128.16
	3,5	113.90 or 114.08	114.06 or 114.28	113.90 or 114.01
F	2	80.96	80.86	73.80
	3	72.07	71.71	72.04
	4	–	–	41.32
G	5	122.00	125.76 or 125.84	122.38
H	2,6	128.22	128.22	128.09 or 128.16
	3,5	113.90 or 114.08	113.89	113.90 or 114.01
I	2	77.31	77.30	77.61
	3	67.14	66.84	67.39
	4	66.81	67.16	67.72

ring and likely biogenetic flavan-3,4-diol precursors (Malan, 1995; Malan and Sireeparsad, 1995).

Our identification of the bis-flavan-3,4-diol **1** and the trimeric proteracacinidins **3**, **5** and **7** from the heartwoods of *A. galpinii* and *A. caffra* further demonstrates the diversity of their phenolic metabolic pools. The structures of the trimeric analogs also demonstrate the abundance of oligomeric proanthocyanidins with 7,8-dihydroxy-2,3-*cis*-3,4-*cis*-flavan-3,4-diol 'terminal' units (see references in Section 1), presumably resulting from the stability of such units towards the formation of electrophilic centers at 4-C of these moieties (Coetzee et al., 1999).

3. Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 300 and a Bruker Avance DRX 500 spectrometer, respectively, for solvents as indicated, with Me_4Si as internal standard. FAB mass spectra were recorded on a VG-70E instrument with a VG 11-250J data system and an iontech saddlefield FAB gun. CD spectra were recorded on a Jasco J-715 spectropolarimeter for solutions in MeOH. TLC was performed on precoated Merck plastic sheets (silica gel 60 PF_{254}, 0.25 mm) and the plates were sprayed with H_2SO_4–HCHO (40:1, v/v) after development. Preparative plates (PLC) [20×22 cm, Kieselgel PF_{254} (1.0 mm)] were air dried and used without prior activation. Methylations were performed with an excess of CH_2N_2 in MeOH–Et_2O for 48 h at −15 °C while acetylations were conducted in Ac_2O–pyridine at ambient temperature. Evaporations were done under reduced pressure at ambient temperature in a rotary evaporator.

3.1. Isolation of phenolic compounds

The extraction of the heartwoods of *A. galpinii* and *A. caffra* and column separations to give fractions A–UU and A–Z, respectively, were detailed in Parts 28 (Coetzee et al., 1998a) and 32 (Bennie et al., 2000).

3.2. Epioritin-4α-ol-(6→6)-epioritin-4β-ol hepta-O-methylether triacetate 2

Methylation of a portion (100 mg) of fraction U from *A. galpinii* followed by PLC in benzene-Me_2CO (4:1, v/v, ×2) gave five bands at R_f 0.65 (20 mg) (Bennie et al., 2002a), 0.42 (8 mg) (Bennie et al., 2001b), 0.35 (18 mg), 0.26 (9 mg) (Bennie et al., 2001b), and 0.11 (6 mg). The R_f 0.11 band still comprised a mixture and was not further investigated. Acetylation of the R_f 0.35 band and purification by PLC in benzene-Me_2CO (9:1, v/v) afforded the title bis-flavan-3,4-diol derivative **2** as a white amorphous solid (R_f 0.53, 14 mg). (Found: M^+, 802.2834. $C_{43}H_{46}O_{15}$ requires M^+, 802.2836); δ_H (Table 1).

3.3. Epioritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol nona-O-methylether tetra-acetate 4

Methylation of a portion (100 mg) of fraction X from *A. galpinii* and subsequent PLC in benzene–Me_2CO (4:1, v/v, ×2) afforded three bands at R_f 0.41 (15 mg), 0.30 (7 mg), and 0.20 (8 mg). The R_f 0.30 and 0.20 bands comprised mixtures and were not further investigated. Acetylation and purification of the R_f 0.41 band by PLC in hexanes–benzene–Me_2CO–MeOH (43:42:10:5, v/v, ×2) gave the proteracacinidin derivative **4** as a white amorphous solid (R_f 0.60, 9 mg). (Found: M^+, 1128.3990. $C_{62}H_{64}O_{20}$ requires M^+, 1128.3991); δ_H (Table 1); δ_C (protonated carbons) (Table 2) 19.93, 19.99, 20.25, 20.29 (4×COCH_3), 55.01, 55.06 (×2), 56.13, 60.41, 60.52, 60.62, 60.97, 61.17 (9×OCH_3), 115.52 (10-A), 128.45 (6-D), 129.78, 129.95, 130.40 (1-B, 1-E, 1-H), 137.58, 141.16 (×2) (8-A, 8-D, 8-G), 147.78, 148.22, 148.99 (9-A, 9-D, 9-G), 150.30, 152.44, 152.76 (7-A, 7-D, 7-G), 159.88, 160.18, 160.43 (4-B, 4-E, 4-H), 168.34, 169.12, 170.17, 170.59 (4×COCH_3); CD $[\theta]_{283.9}$ −11.1, $[\theta]_{244}$ +91.5.

3.4. Oritin-(4β→6)-oritin-(4α→6)-epioritin-4α-ol nona-O-methylether tetra-acetate 6

A portion (100 mg) of fraction Y from *A. galpinii* was methylated and the resulting mixture resolved by PLC in benzene–Me_2CO (4:1, v/v, ×4) into two bands at R_f 0.56 (16 mg) and 0.52 (9 mg). The latter band still contained several compounds and was not further investigated. Acetylation and purification of the R_f 0.56 band by PLC in benzene–Me_2CO (19:1, v/v, ×4) afforded the title compound as a white amorphous solid (R_f 0.58, 10 mg). (Found: M^+, 1128.3393. $C_{62}H_{64}O_{20}$ requires M^+, 1128.3991); δ_H (Table 1); δ_C (protonated carbons) (Table 2) 19.94, 20.02, 20.29, 20.47 (4×COCH_3), 55.05 (×4), 56.05, 60.19, 60.48 (×2), 60.82 (9×OCH_3), 114.42, 116.94 (10-A, 10-G or 10-D), 121.79 (5-G), 128.06 (6-D), 129.75, 129.97, 130.93 (1-B, 1-E, 1-H), 137.32 (8-A), 147.49, 148.40 (9-A, 9-D, 9-G), 151.27, 152.58 (7-A, 7-D, 7-G), 160.09, 160.19, 160.42 (4-B, 4-E, 4-H), 168.39, 169.68, 170.11, 170.60 (4×COCH_3); CD $[\theta]_{284.9}$ −18.5, $[\theta]_{246.1}$ +75.2.

3.5. Epioritin-(4β→6)-epioritin-(4β→6)-epioritin-4α-ol nona-O-methylether tetra-acetate 8

Methylation of a portion (200 mg) of fraction HH from *A. caffra* followed by PLC in benzene–Me_2CO–EtOAc (7:2:1, v/v, ×2) afforded five bands at R_f 0.68 (14.4 mg) (Bennie et al., 2001a), 0.63 (21.2 mg), 0.51 (19.7 mg), 0.47 (20.7 mg) (Bennie et al., 2002b), and 0.39 (19.2 mg) (Bennie et al., 2002b). The R_f 0.51 band still contained several compounds in low concentrations and was not further investigated. Acetylation of the R_f 0.63

band and PLC purification in benzene–Me_2CO (9:1, v/v) gave the trimeric proteracacinidin derivative **8** as a white amorphous solid (R_f 0.47, 8.3 mg). (Found: M^+, 1128.3991. $C_{62}H_{64}O_{20}$ requires M^+, 1128.3991); δ_H (Table 1); δ_C (protonated carbons) (Table 2) 20.12, 20.25 (×2), 20.62 (4×CO*C*H_3), 54.99 (×3), 55.93, 60.27, 60.51, 60.70, 61.01, 61.09 (9×O*C*H_3), 115.28, 115.52 (10-A, 10-G), 128.78 (6-D), 129.68, 130.17, 130.43 (1-B, 1-E, 1-H), 137.63, 141.08, 141.46 (8-A, 8-D, 8-G), 148.65, 149.25 (9-A, 9-D, 9-G), 150.61, 151.31, 152.78 (7-A, 7-D, 7-G), 159.95 (×2), 160.19 (4-B, 4-E, 4-H), 169.14 (x2), 169.88, 170.20 (4×CO*C*H_3); CD $[\theta]_{282.5}$ −22.5, $[\theta]_{247.3}$ +26.6.

Methylation of a portion (200 mg) of fraction KK from *A. caffra* and subsequent purification by PLC in benzene–Me_2CO–EtOAc (7:2:1, v/v, ×2) gave two bands at R_f 0.44 (28.4 mg) and 0.29 (22.1 mg). Acetylation of the former band and purification by PLC in benzene–Me_2CO (9:1, v/v, ×2) afforded derivative **4** (R_f 0.77, 16 mg), identical to the compound from *A. galpinii*. Acetylation of the R_f 0.29 band and PLC in benzene–Me_2CO (9:1, v/v, ×2) similarly gave the trimeric proteracacinidin derivative **6** (R_f 0.72, 2.7 mg), identical to the compound from *A. galpinii*.

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