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Structure and synthesis of ether-linked proteracacinidin and promelacacinidin proanthocyanidins from *Acacia caffra*☆

Linette Bennie[a], Elfranco Malan[a, 1], Johan Coetzee[a, 1], Daneel Ferreira[b,*]

[a]*Department of Chemistry, University of the Orange Free State, P.O. 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

Two new ether-linked proanthocyanidins, epioritin-(4β → 3)-epioritin-4β-ol and epimesquitol-(4β → 4)-epioritin-4β-ol, were isolated from the heartwood of *Acacia caffra*. Their structures and absolute configurations were established by spectroscopic methods and syntheses. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Acacia caffra*; Leguminosae; Proteracacinidins; Promelacacinidins; Proanthocyanidins; Synthesis

1. Introduction

The natural occurrence of carbon–carbon interflavanoid linked dimeric proanthocyanidins is well documented (Hemingway, 1989; Porter, 1994). The dioxane-type biflavanoids from *Acacia mearnsii* (Drewes & Ilsley, 1969) were unique in possessing a double ether linkage. Their identification was followed by the discovery of A-type biflavanoids with one of the two interflavanyl bonds and an ether linkage (Mayer, Goll, von Arndt & Mannschreck, 1966; Jacques, Haslam, Bedford & Greatbanks, 1974). Recently, a proteracacinidin analogue with a (4β → 7) ether linkage as well as a $C_5 \rightarrow C_6$ bond between the A- and D-rings was reported from *A. caffra* (Malan, Sireeparsad, Burger & Ferreira, 1994).

The first single ether-linked (C_4–O–C_4) dimeric promelacacinidins were identified in *A. melanoxylon* (Foo, 1989) and were followed by similar bis-teracacinidins (Coetzee, Malan & Ferreira, 1998b), and the first (C_4–O–C_3) ether-linked bis-teracacinidins (Coetzee, Malan & Ferreira, 1998a) from *A. galpinii*. We now report the structure and synthesis of the new epioritin-(4β → 3)-epioritin-4β-ol **2** and epimesquitol-(4β → 4)-epioritin-4β-ol **10**, the first biflavanoid comprising both leucomelacacinidin and leucoteracacinidin constituent units, from *A. caffra*.

2. Results and discussion

The novel proanthocyanidins **2** and **10** were isolated from the MeOH extract of the heartwood of *A. caffra* and were accompanied by *ent*-oritin-(4α → 4)-epioritin-4α-ol, epioritin-(4β → 4)-epioritin-4α-ol, epioritin-(4β → 3)-epioritin-4α-ol and epioritin-(4β → 3)-oritin-4α-ol previously isolated from *A. galpinii* (Coetzee et al., 1998a, 1998b). Owing to the complexity of the phenolic mixture, the proanthocyanidins **2** and **10** were purified and identified as the hexa- and hepta-methyl ether diacetate derivatives **3** and **11**, respectively.

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* Corresponding author. Tel.: +1-91-601-232-1572; fax: +1-91-601-232-7062.

E-mail address: dferreir@olemiss.edu (D. Ferreira).

[1] Correspondence may also be addressed to Malan or Coetzee.

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The structures and relative configurations were established by ^{1}H-NMR data (Table 1) of derivatives **3** and **11**. The presence of two AB- and two AA′BB′ spin systems in the spectrum of **3**, and two AB-, an ABX- and an AA′BB′ system in the spectrum of **11** for the aromatic protons, as well as two AMX-systems for the heterocyclic protons suggested the dimeric nature of the two compounds.

2D COSY experiments differentiated the spin systems and showed the connectivities between aromatic and heterocyclic protons. The presence of six *O*-methyl- and two *O*-acetyl resonances for **3**, and seven *O*-methyl- and two *O*-acetyl signals for **11** in conjunction with FABMS data indicating molecular ions of *m*/*z* 730 and *m*/*z* 760, were reminiscent of molecular formulae $C_{40}H_{42}O_{13}$ and $C_{41}H_{44}O_{14}$, respectively. The above information suggested ether-type interflavanyl linkages for both compounds.

In oligomeric proanthocyanidin derivatives with C–C interflavanyl linkages, 4-H(C) of the ABC chain extender unit is shielded (1.32–1.82 ppm) relative to the chemical shift of the same proton in the permethylaryl ether 3,4-di-*O*-acetyl derivative of the flavan-3,4-diol precursor (Steynberg, Steynberg, Brandt, Ferreira & Hemingway, 1997; Young, Brandt, Young, Ferreira & Roux, 1986; Malan & Sireeparsad, 1995). Application of this observation indicated a C_4–O–C_3 ether bond for **3** ($CDCl_3$) with a shielding of 1.52 ppm for 4-H(C) (δ 4.38) and 0.99 ppm for 3-H(F) (δ 4.25). The same principle was applied to compound **11** (C_6D_6), where 4-H(C) (δ 5.40) and 4-H(F) (δ 5.42) displayed shielding of 1.09 and 0.64 ppm, respectively. These shielding phenomena were in accordance with the results reported for the promelacacinidins from *A. melanoxylon* (Foo, 1989), hence, indicating a C_4–O–C_4 interflavanyl bond for derivative **11**.

The coupling constants of the two pairs of heterocyclic systems [$J_{2,3}(C) = J_{2,3}(F) = 1.5$ Hz; $J_{3,4}(C) = J_{3,4}(F) = 3.0$ Hz for both **3** and **11**] indicated 2,3-*cis*-3,4-*trans* relative configurations for the C- and F-rings of compounds **3** and **11**, respectively (Coetzee et al., 1998a, 1998b; Malan & Sireeparsad, 1995; Malan, 1995). The conspicuous absence of NOE associations between 2- and 4-H of the C- and F-rings in both derivatives could be interpreted as confirmation of the 2,4-*trans* relative configuration for the heterocyclic systems.

Phase sensitive NOESY experiments of **3** showed important associations of 3-H(F) with 3- and 4-H(C) and of 4-H(F) with 5-H(A) and 5-H(D). These associations were verified from Dreiding models and the 3-H(C) association with 2′,6′-H(E) is only possible in the event of a C_4–O–C_3 coupling. The same experiment on derivative **11** showed associations of 4-H(C) with 5-H(D), 4-H(F) with 4-H(C), 5-H(A) and 5-H(D) with 5-H(A), and the 3-H(C) association with 5-H(D) which is only possible for a C_4–O–C_4 interflavanyl coupling.

In designing a synthetic sequence towards ether-linked proanthocyanidins of types **2** and **10**, cognizance had to be taken of the acid-lability of the C-4 benzylic ether functionality (Foo, 1989) and of the previous focus on the synthesis of this class of compounds as permethylaryl ether derivatives (Coetzee et al., 1998a, 1998b). The synthesis of the proteracacinidin **2**

Table 1
^{1}H-NMR (300 MHz, 296 K) data of compounds **2**, **3**, **4**, **11**[a]

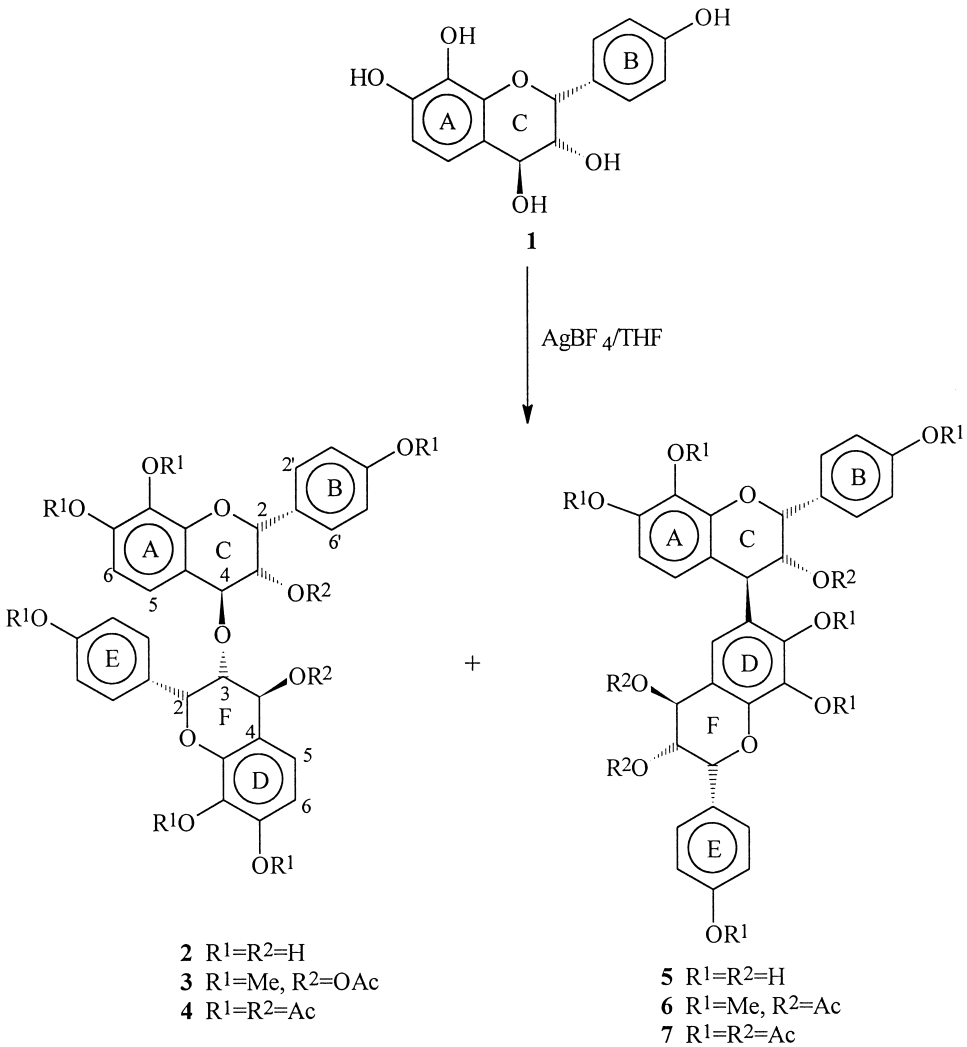
Ring	H	**2** (Acetone-d_6)	**3** ($CDCl_3$)	**4** (Acetone-d_6)	**11** (C_6D_6)
A	5	6.93 (*d*, 8.5)	6.76 (*d*, 9.0)	7.12 (*d*, 8.5)	7.56 (*d*, 9.0)
	6	6.50 (*d*, 8.5)	6.53 (*d*, 9.0)	6.78 (*d*, 8.5)	6.57 (*d*, 9.0)
B	2′,6′	7.41 (*d*, 8.5)	6.87 (*d*, 9.0)	7.11 (*d*, 8.5)	
	3′,5′	6.82 (*d*, 8.5)	6.82 (*d*, 9.0)	6.99 (*d*, 8.5)	
	2′				7.34 (*d*, 2.0)
	5′				6.74 (*d*, 8.5)
	6′				7.30 (*dd*, 2.0, 8.5)
C	2	5.25 (*s*, 1.0)	4.64 (*br.s*, 1.5)	4.68 (*br.s*, 1.5)	5.82 (*br.s*, 1.5)
	3	3.89 (*dd*, 1.0, 3.5)	4.42 (*dd*, 1.5, 3.0)	4.57 (*dd*, 1.5, 3.0)	6.04 (dd,1.5,3.0)
	4	4.65 (*d*, 3.5)	4.38 (*d*, 3.0)	4.66 (*d*, 3.0)	5.40 (*d*, 3.0)
D	5	6.50 (*d*, 8.5)	7.22 (*d*, 9.0)	7.52 (*d*, 8.5)	7.60 (*d*, 9.0)
	6	6.40 (*d*, 8.5)	6.63 (*d*, 9.0)	6.92 (*d*, 8.5)	6.58 (*d*, 9.0)
E	2′,6′	7.40 (*d*, 8.5)	7.56 (*d*, 9.0)	7.71 (*d*, 8.5)	7.67 (*d*, 9.0)
	3′,5′	6.68 (*d*, 8.5)	6.97 (*d*, 9.0)	7.29 (*d*, 8.5)	6.95 (*d*, 9.0)
F	2	5.44 (*s*, 1.0)	5.32 (*br.s*, 1.5)	5.48 (*s*, 1.5)	5.82 (*br.s*, 1.5)
	3	5.84 (*dd*, 1.0, 4.0)	4.25 (*dd*, 1.5, 3.0)	4.51 (*dd*, 1.5, 3.0)	6.00 (*dd*, 1.5, 3.0)
	4	5.46 (*d*, 4.0)	6.17 (*d*, 3.0)	6.27 (*d*, 3.0)	5.42 (*d*, 3.0)
OMe			3.99, 3.98, 3.95, 3.94, 3.93, 3.80		4.01, 4.00, 3.59, 3.49, 3.48, 3.47, 3.39
OAc			2.20, 1.83	2.32, 2.28 (×2), 2.26, 2.21, 2.19, 2.12, 1.76	1.55, 1.54

[a] Splitting patterns and *J* values (Hz) are given in parentheses.

was, thus, attempted by using silver tetrafluoroborate ($AgBF_4$) to activate the free phenolic epioritin-4β-ol **1** towards self-condensation. Treatment of epioritin-4β-ol **1** with $AgBF_4$ in THF at 0°C (Scheme 1) gave a complex mixture which had to be acetylated to enable separation. Compounds **2** and **5** were, therefore, purified and identified as their per-*O*-acetyl derivatives **4** and **7**. The C–C-linked analogue **7** had ^{1}H-NMR and CD data identical to those previously reported (Malan & Sireeparsad, 1995). The ether-linked derivative **4** was subjected to mild alkaline hydrolysis to obtain the free phenolic proteracacinidin **2** (^{1}H-NMR, Table 1). The latter was methylated and acetylated to obtain the hexa-*O*-methyl-di-*O*-acetyl derivative **3** with ^{1}H-NMR and CD spectra identical to those of the same derivative of the natural product, hence, confirming the 2*R*,3*R*,4*S*(C):2*R*,3*R*,4*S*(F) absolute configuration of the novel C_4–O–C_3 linked proteracacinidin **2**.

The stereochemical course of the coupling step is explicable in terms of a neighboring group mechanism triggered by interaction of the Lewis acid and the near-axial C-4 hydroxyl group of the flavan-3,4-diol **1** (Coetzee et al., 1998a, 1998b). We could not detect any of the anticipated C_4–O–C_4 isomer, probably as a result of the low overall yields. The proteracacinidin **2** is apparently stable towards racemization at either C-2 or C-4 of both the C- and F-rings under the mild hydrolytic conditions (1% KOH in MeOH at ca. 20°C) used for its generation from the peracetate **4**. In the absence of $AgBF_4$, epioritin-4β-ol **1** did not undergo self-condensation, hence, indicating that the Lewis-acid is a prerequisite for product formation.

In a second approach (Scheme 2), epimesquitol-4α-ol **8** was converted into the 4β-benzylsulfanyl derivative **9** via an adapted thiolytic procedure (Hemingway, Karchesy, McGraw & Wielesek, 1983). Compound **9** was then activated by the thiophilic Lewis-acid, $AgBF_4$ (Steynberg, Nel, van Rensburg, Bezuidenhoudt & Fer-



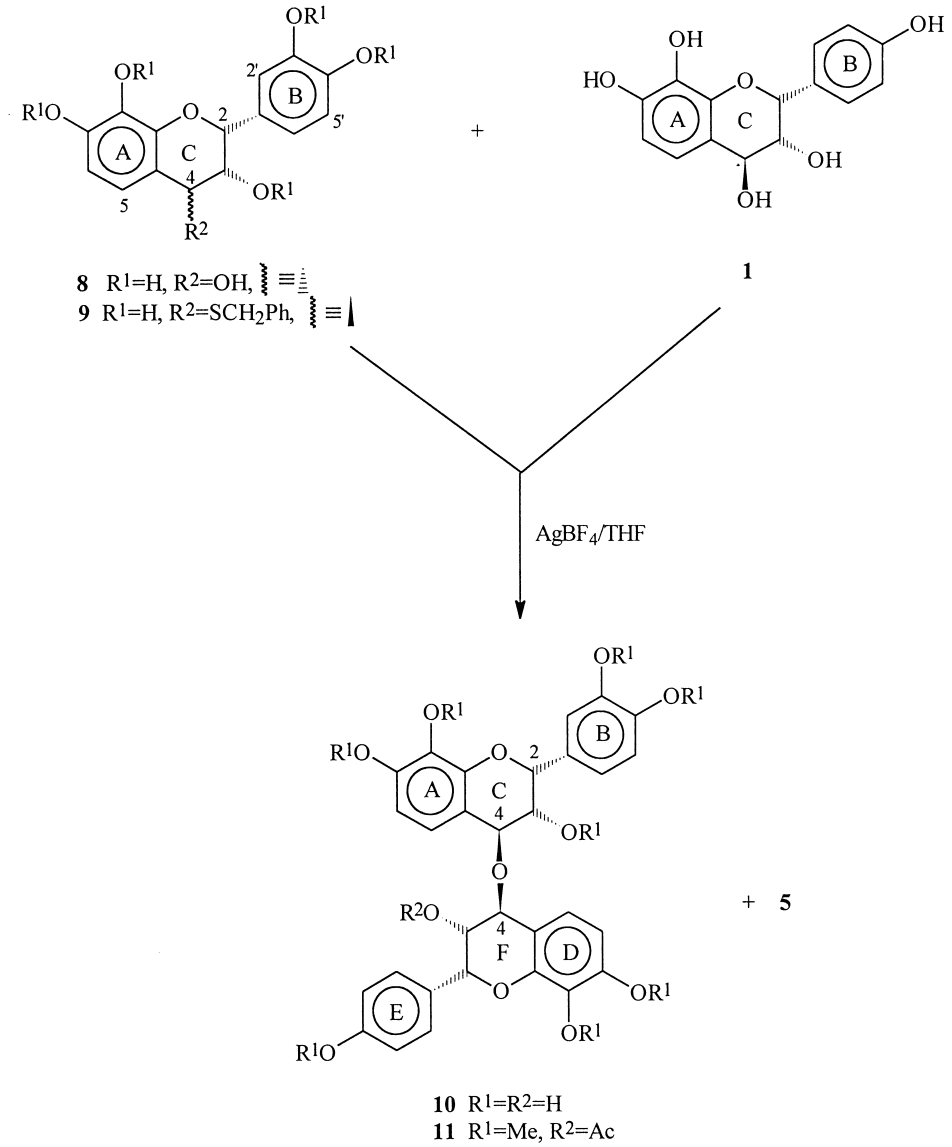
2 R^1=R^2=H
3 R^1=Me, R^2=OAc
4 R^1=R^2=Ac

5 R^1=R^2=H
6 R^1=Me, R^2=Ac
7 R^1=R^2=Ac

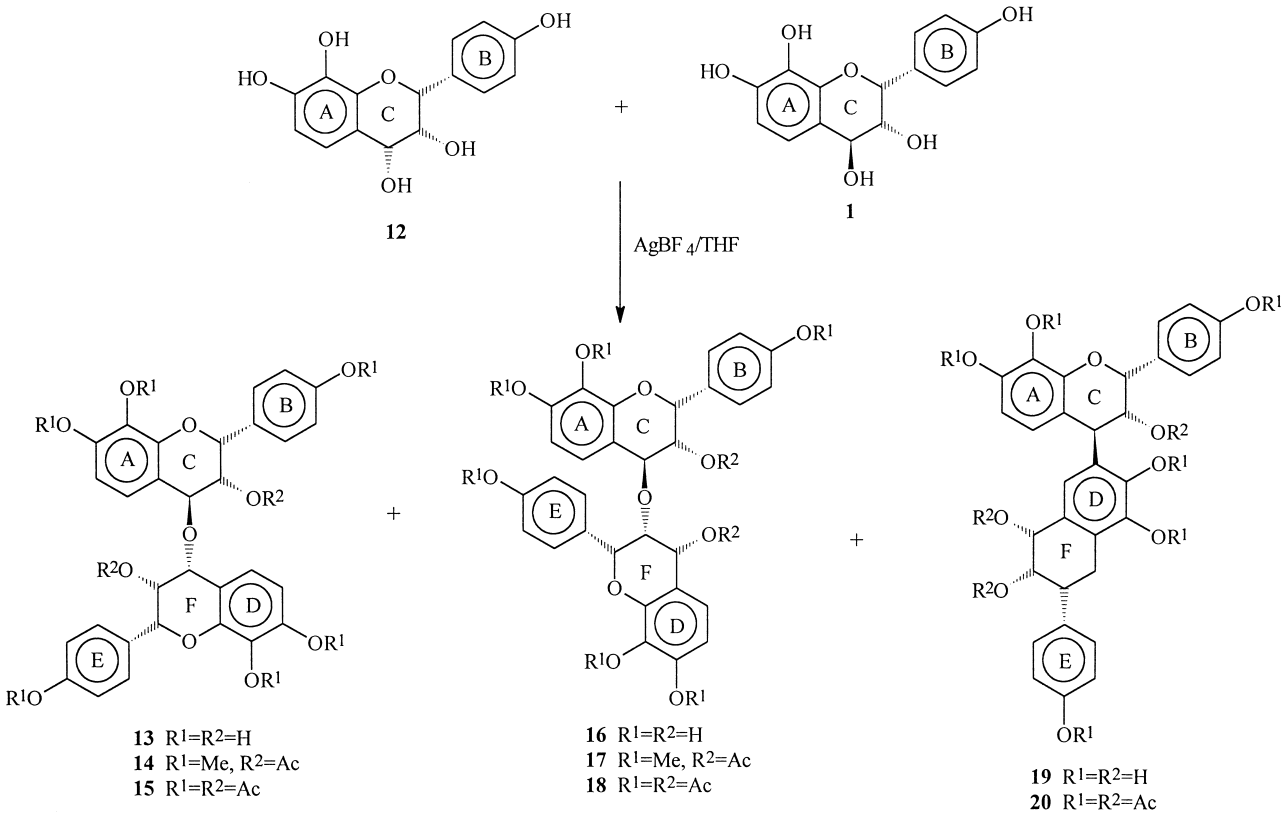
Scheme 1. Synthesis of proteracacinidins **2** and **5**.

reira, 1998). When the thioether **9**/$AgBF_4$ complex was treated with epioritin-4β-ol **1**, the latter compound acted as ambient nucleophile to give both the C_4–O–C_4 and C–C coupled analogues **10** (4.1%) and **5** (2.1%), respectively. Activation of the near-axial C_4–S bond of **9** by $AgBF_4$ once again triggers a neighboring group mechanism (Coetzee et al., 1998a, 1998b), hence, explaining the stereoselective formation of compounds **5** and **10**. Owing to the complexity of the mixture, dimers **5** and **10** were identified as their methyl ether acetate derivatives **6** and **11**, the additional chromatographic steps offered by derivatization being a pre-requisite for sample purity. The permethylaryl tri-*O*-acetyl derivative **6** showed ^{1}H-NMR and CD data identical to those previously reported (Malan & Sireeparsad, 1995). The hepta-*O*-methyldi-*O*-acetyl derivative **11** of the ether-linked analogue **10** was identical to the same derivative of the natural product **11** by comparison of ^{1}H-NMR (Table 1) and CD data. It thus possesses 2*R*,3*R*,4*S*(C):2*R*,3*R*,4*S*(F) absolute configuration.

Pursuing our studies on the synthesis of free phenolic proanthocyanidins, the $AgBF_4$ catalyzed process was applied to a mixture of the epioritin-4β- and 4α-ols **1** and **12** (Scheme 3) with a view to form epioritin-(4β → 4)-epioritin-4α-ol **13** and epioritin-(4β → 3)-epioritin-4α-ol **16**, previously synthesized as permethylaryl ether diacetates **14** and **17** (Coetzee et al., 1998a, 1998b). Both the ether-linked compounds **13** and **16** indeed formed and were purified and identified as the octa-*O*-acetyl derivatives **15** (9.1%) and **18** (7.8%) (Table 2). They were accompanied by the C–C-linked



Scheme 2. Synthesis of the 'mixed' promelacacinidin/proteracacinidin **10**.



Scheme 3. Synthesis of ether-linked proteracacinidins **13** and **16** and the C–C coupled analogue **19**.

compound, epioritin-(4β → 6)-epioritin-4α-ol **19** (Malan & Sireeparsad, 1995), identified as the per-*O*-acetyl derivative **20** (7.3%). Derivatives **15** and **18** were subjected to mild alkaline hydrolysis (1% KOH in MeOH) yielding for the first time the free phenolic proanthocyanidins **13** and **16**, respectively (^{1}H-NMR, Table 2). Subsequent methylation and acetylation gave the hexa-*O*-methylether diacetates **14** and **17** with ^{1}H-

Table 2
^{1}H-NMR (300 MHz, 296 K) data of compounds **13**, **15**, **16**, **18**[a]

Ring	H	**13** (Acetone-d_6)	**15** ($CDCl_3$)	**16** (Acetone-d_6)	**18** ($CDCl_3$)
A	5	6.66 (*d*, 8.5)	7.28 (*d*, 8.5)	6.96 (*d*, 8.5)	6.92 (*d*, 8.5)
	6	6.45 (*d*, 8.5)	6.91 (*d*, 8.5)	6.57 (*d*, 8.5)	6.72 (*d*, 8.5)
B	2′,6′	7.39 (*d*, 8.5)	7.51 (*d*, 8.5)	7.39 (*d*, 8.5)	7.08 (*d*, 8.5)
	3′,5′	6.83 (*d*, 8.5)	7.11 (*d*, 8.5)	6.84 (*d*, 8.5)	7.01 (*d*, 8.5)
	2′				
	5′				
	6′				
C	2	5.49 (*s*, 1.0)	5.43 (*br.s*, 1.5)	5.37 (*s*, 1.0)	4.89 (*br.s*, 1.5)
	3	5.85 (*dd*, 1.0, 4.0)	5.28 (*dd*, 1.5, 3.0)	5.23 (*dd*, 1.0, 3.5)	4.72 (*dd*, 1.5, 3.0)
	4	5.47 (*d*, 4.0)	4.75 (*d*, 3.0)	4.70 (*d*, 3.5)	4.08 (*d*, 3.0)
D	5	6.96 (*d*, 8.5)	7.16 (*d*, 8.5)	6.66 (*d*, 8.5)	7.11 (*d*, 8.5)
	6	6.57 (*d*, 8.57)	6.81 (*d*, 8.5)	6.45 (*d*, 8.5)	6.85 (*d*, 8.5)
E	2′,6′	7.37 (*d*, 8.5)	7.47 (*d*, 8.5)	7.37 (*d*, 8.5)	7.61 (*d*, 8.5)
	3′,5′	6.86 (*d*, 8.5)	7.14 (*d*, 8.5)	6.86 (*d*, 8.5)	7.18 (*d*, 8.5)
F	2	5.37 (*s*, 1.0)	5.47 (*br.s*, 1.0)	5.49 (*s*, 1.0)	5.45 (*br.s*, 1.0)
	3	5.23 (*dd*, 1.0, 3.5)	5.88 (*dd*, 1.0, 4.0)	5.86 (*dd*, 1.0, 4.0)	4.69 (*dd*, 1.0, 4.0)
	4	5.70 (*d*, 3.5)	5.39 (*d*, 4.0)	5.47 (*d*, 4.0)	6.32 (*d*, 4.0)
OMe					
OAc			2.35, 2.33, 2.32, 2.32, 2.30, 2.28, 1.95, 1.86		2.32, 2.31, 2.31, 2.30, 2.28, 2.24, 1.99, 1.74

[a] Splitting patterns and *J* values (Hz) are given in parentheses.

NMR and CD data identical to those previously reported (Coetzee et al., 1998a, 1998b). Formation of the ether-linked analogues **13** and **16** is explicable in terms of activation of the more reactive axial C-4 hydroxyl group of epioritin-4β-ol **1** (Clark-Lewis & Williams, 1967), epioritin-4α-ol **12** with its remarkable stable equatorial C-4 hydroxyl group (Clark-Lewis & Williams, 1967; Coetzee, Malan & Ferreira, 1999) then serving as the ambient nucleophile.

Although the yields of the ether-linked proanthocyanidins **2**, **10**, **13** and **16** were low, the $AgBF_4$ catalyzed process of activation of either the flavan-3,4-diol or its C-4 benzylsulfanyl derivative has merit, since it produced these unique ethers in free phenolic form for the first time.

3. Experimental

^{1}H-NMR spectra were recorded on a Bruker AM-300 spectrometer for solutions 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. 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. Column chromatography was done on Sephadex LH-20 in various columns, solvent systems and flow rates (to be specified in each instance). Methylations were performed with an excess of diazomethane in MeOH–Et_2O over a period of 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, and freeze drying of aqueous solutions on a Virtis 12 SL freezemobile.

3.1. General procedure for the alkaline hydrolysis

The compound was dissolved in a 1% KOH in MeOH solution (2 ml) under an Ar-atmosphere at room temperature and stirred for 10 min. The reaction was quenched by the addition of H_2O (10 ml) and the pH was adjusted to 6.5 (10% HCl) before it was extracted with EtOAc (3 × 10 ml). The combined layers were dried (Na_2SO_4) and the EtOAc was evaporated under reduced pressure at ambient temperature.

3.2. Isolation of phenolic compounds

Drillings (4.3 kg) from the heartwood of *A. caffra* were repeatedly extracted with MeOH (3 × 2.5 l) for 24 h periods at room temperature (23°C). The extract was concentrated by evaporating the MeOH under vacuum at 35°C. The concentrate was dissolved in H_2O and freeze dried to give a pale brown powder (447 g).

A portion (20.5 g) of the extract was separated on a Sephadex LH-20/EtOH column (6 × 180 cm) with a flow rate of 1 ml/min, 32 min fractions. The first 1.5 l of EtOH was discarded and the fractions of two columns (41 g) were combined as follows: A (tubes 33–41, 175 mg), B (42–48, 131 mg), C (65–69, 299 mg), D (71–77, 389 mg), E (81–87, 218 mg), F (89–101, 652 mg), G (102–109, 810 mg), H (113–125, 366 mg), I (129–134, 1.332 g), J (153–161, 1.057 g), K (163–175, 789 mg), L (177–191, 544 mg), M (192–208, 1.161 g), N (209–221, 1.834 g), O (222–235, 926 mg), P (239–276, 2.291 g), Q (277–307, 769 mg), R (309–315, 1.138 g), S (317–331, 728 mg), T (333–354, 756 mg), U (355–372, 573 mg), V (373–389, 319 mg), W (390–465, 840 mg), X (467–503, 839 mg), Y (504–514, 308 mg), Z (515–535, 219 mg), AA (537–555, 188 mg), BB (557–576, 407 mg), CC (577–633, 952 mg), DD (637–659, 290 mg), EE (660–670, 482 mg), FF (671–764, 909 mg), GG (765–813, 896 mg), HH (814–904, 830 mg), II (905–948, 476 mg), JJ (949–998, 679 mg), KK (999–1043, 674 mg), LL (1044–1115, 754 mg), MM (1116–1139, 207 mg), NN (1140–1230, 683 mg), OO (1231–1259, 505 mg), PP (1260–1300, 531 mg), QQ (1309–1130, 261 mg), RR (1131–1389, 345 mg), SS (1390–1429, 189 mg), TT (1430–1440, 117 mg), UU (1441–1480, 158 mg), column residue (812 mg).

3.3. *Epioritin-(4β → 3)-epioritin-4*β-ol hexa-*O*-*methylether diacetate* **3**

Methylation of a portion (200 mg) of fraction S followed by PLC in benzene–Me_2CO–EtOAc (7:2:1, v/v) afforded five bands at R_f 0.43 (17.4 mg), 0.34 (13.3 mg), 0.31 (22.5 mg), 0.21 (21.1 mg) and 0.13 (14.1 mg). Acetylation of the R_f 0.31 band (22.5 mg) followed by PLC in $CHCl_3$–Et_2O (49:1, ×3, v/v) gave compound **3** (R_f 0.33, 9.7 mg) as a *white amorphous solid*. (Found: M^+, 730.2702. $C_{40}H_{42}O_{13}$ requires M, 730.2704); δ_H (Table 1); CD $[\theta]_{274.8}$ −1492, $[\theta]_{273.5}$ −5886 and $[\theta]_{221.8}$ 2182. The remaining bands contain related proanthocyanidin-type compounds which will be dealt with elsewhere.

3.4. *Epimesquitol-(4β → 4)-epioritin-4*β-ol hepta-*O*-*methylether diacetate* **11**

A portion (200 mg) of fraction BB was methylated and the mixture separated by PLC in benzene–Me_2CO–EtOAc (7:2:1, ×2, v/v) to give six bands at R_f 0.72 (11.8 mg), 0.67 (5.1 mg), 0.58 (10.3 mg), 0.49 (13.2 mg), 0.38 (5.9 mg) and 0.28 (2.3 mg). Acetylation of the R_f 0.38 band followed by PLC in $CHCl_3$–Et_2O

(49:1, ×2, v/v) afforded compound **11** (R_f 0.48, 2.1 mg) as a *white amorphous solid*. (Found: M^+, 760.2731. $C_{41}H_{44}O_{14}$ requires M, 760.2731); δ_H (Table 1); CD $[\theta]_{2820}$ 790.2, $[\theta]_{243.9}$ −72,410, $[\theta]_{233.1}$ 4251 and $[\theta]_{219.4}$ 1788. The quantities and complexity of the remaining bands did not merit further investigation.

3.5. Synthesis of proteracacinidin derivatives 2–4

Epioritin-4β-ol **1** (75 mg) was dissolved in dry THF (10 ml). $AgBF_4$ (0.8 eq.) was added under N_2 and the mixture was stirred at 0°C. After 90 min, the reaction was quenched by the addition of H_2O (50 ml) and the mixture was extracted with EtOAc (6 × 30 ml). The solvent was removed under vacuum and separation of the mixture by PLC in benzene–Me_2CO–MeOH (6:3:1, ×2, v/v) gave three main bands at R_f 0.72 (3.8 mg), 0.64 (6.5 mg) and 0.54 (13.1 mg). Acetylation of the R_f 0.54 band followed by an aqueous workup and subsequent PLC in benzene–Me_2CO (9:2, ×2, v/v) gave two bands at R_f 0.40 (4.2 mg) and 0.28 (3.8 mg).

3.6. Epioritin-(4β → 6)-epioritin-4β-ol nona-acetate 7

The R_f 0.40 band was identified as the nona-acetate derivative **7** with physical data identical to those in the literature (Malan & Sireeparsad, 1995).

3.7. Epioritin-(4β → 3)-epioritin-4β-ol octa-acetate 4

The R_f 0.28 band yielded the octa-acetate derivative of the C_4–O–C_3-linked compound **4** as a *white amorphous solid*. δ_H (Table 1). FABMS, M^+, 898.2322. $C_{46}H_{42}O_{19}$ requires M^+ 898.2321.

3.8. Epioritin-(4β → 3)-epioritin-4β-ol 2

Alkaline hydrolysis of derivative **4** yielded the free phenolic compound **2** as a *light brown amorphous solid*. (Found: M^+, 562.1474. $C_{30}H_{26}O_{11}$ requires M, 562.1475); δ_H (Table 1).

3.9. Epioritin-(4β → 3)-epioritin-4β-ol hexa-O-methylether diacetate 3

Subsequent methylation and acetylation of the free phenolic compound **2** yielded a compound with ^{1}H-NMR, CD and MS data identical to those of the natural product derivative **3**.

3.10. Synthesis of (2R,3S,4S)-2,3-cis-3,4-trans-3,3′,4′,7,8-pentahydroxy-4-benzylsulfanylflavan 9

Epimesquitol-4α-ol **8** (1.0 g) was dissolved in EtOH (100 ml) and an excess of toluene-α-thiol (3 ml) and HOAc (4 ml) were added, while the solution was purged with Ar gas. The solution was transferred to a Ar-purged vial and sealed. After 24 h at 100°C in a steam bath, the solvent was removed under a stream of N_2. The residue was transferred into a container with H_2O (100 ml) and the excess toluene-α-thiol was removed by washing with hexane (2 × 50 ml). The H_2O-layer was extracted with Et_2O (5 × 20 ml). The Et_2O was removed under a stream of N_2 and the oil was applied to a Sephadex LH-20 column (4 × 150 cm) with the use of a small amount of EtOH. The column was eluted with $CHCl_3$–EtOH (4:1), at a flow rate of 1 ml/min and 16 ml fractions were collected. The fractions were combined as follows: fraction A (tubes 58–72, 100 mg), fraction B (tubes 73–170, 3.01 g), fraction C (tubes 181–229, 208 mg).

Fraction C comprised the remaining epimesquitol-4α-ol **8**. Fraction B yielded the title compound **9** as a white amorphous powder. δ_H (Acetone-d_6) 7.48–7.25 (5H, *m*, D-ring), 7.35 (2H, *d*, 9.0, H-2′,6′), 6.83 (2H, *d*, 9.0, H-3′,5′), 6.53 (1H, *d*, 9.0, 9.0, H-5), 6.433 (1H, *d*, 9.0, H-6), 5.33 (1H, *br.s*, 1.0, H-2), 4.07 (1H, *dd*, 1.0, 3.0, H-3), 4.04 (1H, *d*, 14.0, –CH_2–), 3.95 (1H, *d*, 3.0, H-4), 3.93 (1H, *d*, 14.0, –CH_2–).

3.11. Synthesis of epimesquitol-(4β → 4)-epioritin-4β-ol hepta-O-methylether diacetate 11

Epimesquitol-4β-thiobenzylether **9** (100 mg) and epioritin-4β-ol **1** (141 mg, 2.0 eq.) were dissolved in dry THF (20 ml) at 0°C and $AgBF_4$ (118 mg, 2.5 eq.) was added in one portion. After the reaction was stirred under N_2 for 90 min at 0°C, it was quenched with H_2O (80 ml) and extracted with EtOAc (6 × 50 ml). The solution was dried (Na_2SO_4), the solvent was evaporated and the resulting mixture methylated and then acetylated. PLC in benzene–Me_2CO (9:1, v/v) afforded three bands at R_f 0.62 (46.9 mg), 0.55 (23.5 mg) and 0.39 (22.6 mg). The R_f 0.62 band gave epioritin-4β-ol tri-*O*-methylether diacetate. After further PLC in $CHCl_3$–hexane–Me_2CO (45:3:2, v/v), the R_f 0.55 band afforded epioritin-(4β → 6)-epioritin-4β-ol hexa-*O*-methylether triacetate **6** (R_f 0.60, 8.7 mg) with physical data identical to those in the literature (Malan & Sireeparsad, 1995).

3.12. Epimesquitol-(4β → 4)-epioritin-4β-ol hepta-O-methylether diacetate 11

Further PLC separation in $CHCl_3$–hexane–Me_2CO (45:3:2, v/v) of the initial R_f 0.39 band gave compound **11** (R_f 0.58, 7.2 mg) as a *light brown amorphous solid* with ^{1}H-NMR, CD and MS data identical to those of the natural product derivative **11**.

3.13. Synthesis of dimeric ethers **13** and **16**

Epioritin-4α-ol **12** (160 mg) and epioritin-4β-ol **1** (80 mg) were dissolved in dry THF (20 ml) at 0°C and $AgBF_4$ (134 mg, 2.5 eq.) was added. The mixture was stirred for 90 min at 0°C under N_2 before H_2O (100 ml) was added. The mixture was extracted with Et_2O (6 × 50 ml) and the combined ether layers dried (Na_2SO_4) before evaporation under reduced pressure. PLC separation in benzene–Me_2CO–MeOH (6:3:1, ×2, v/v) gave four bands at R_f 0.75 (88.6 mg), 0.73 (36.9 mg), 0.66 (17.0 mg) and 0.55 (20.9 mg). The R_f 0.75 and 0.73 bands yielded remaining starting materials epioritin-4α-ol **12** and epioritin-4β-ol **1**, respectively.

3.14. Epioritin-(4β → 3)-epioritin-4α-ol octa-acetate **18**

Acetylation of the R_f 0.66 band followed by PLC in benzene–Me_2CO (9:1, ×2, v/v) afforded derivative **18** (R_f 0.30, 10.5 mg). δ_H (Table 2).

3.15. Epioritin-(4β → 3)-epioritin-4α-ol **16**

Alkaline hydrolysis of derivative **18** yielded the free phenolic compound **16** as a *light brown amorphous solid*. (Found: M^+, 562.1477. $C_{30}H_{26}O_{11}$ requires M, 562.1475); δ_H (Table 2).

3.16. Epioritin-(4β → 3)-epioritin-4α-ol hexa-*O*-methylether diacetate **17**

Subsequent methylation and acetylation of free phenolic compound **16** yielded derivative **17** with physical data identical to those in the literature (Coetzee et al., 1998a).

3.17. Epioritin-(4β → 6)-epioritin-4α-ol nona-acetate **20**

Acetylation of the initial R_f 0.55 band followed by PLC in benzene–Me_2CO (99:1, ×2, v/v) gave two bands at R_f 0.47 (10.7 mg) and 0.35 (8.4 mg). The 0.47 band yielded derivative **20** with ^{1}H-NMR, CD and MS data identical to those in the literature (Malan & Sireeparsad, 1995).

3.18. Epioritin-(4β → 4)-epioritin-4α-ol octa-acetate **15**

The R_f 0.35 band was subjected to further PLC in hexane–benzene–Me_2CO–MeOH (43:42:10:5, ×3, v/v) and gave one prominent band at R_f 0.38 (6.1 mg) which was identified as the per-*O*-acetyl derivative **15**. δ_H (Table 2).

3.19. Epioritin-(4β → 4)-epioritin-4α-ol **13**

Alkaline hydrolysis of derivative **15** afforded the free phenolic compound **13** as a *light brown amorphous solid*. (Found: M^+, 562.1478. $C_{30}H_{26}O_{11}$ requires M, 562.1475); δ_H (Table 2).

3.20. Epioritin-(4β → 4)-epioritin-4α-ol hexa-*O*-methylether diacetate **14**

Subsequent methylation and acetylation of free phenolic compound **13** gave a derivative with ^{1}H-NMR, CD and MS data identical to those in the literature (Coetzee et al., 1998b).

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