Identification of Two Novel Promelacacinidin Dimers from *Acacia nigrescens*

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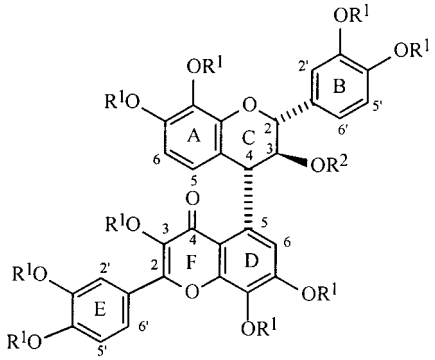
Two new flavanol−flavonol C_4(C) to C_5(D) linked promelacacinidin dimers were isolated from the heartwood of *Acacia nigrescens*. Their structures were elucidated by extensive ^{1}H and ^{13}C NMR spectroscopic studies, and the main conformational features are discussed.

The wood of *Acacia nigrescens* (Leguminosae) (Knobthorn) is dark brown, strong, tough, and close-grained. Due to its resistance to wood-decaying fungi and wood-borers, it is used commercially to make furniture, for mine roofprops, and for fencing posts. Its leaves and pods are valuable fodder[2] for a variety of browsers such as elephants and giraffe. Previous studies[3,4] on this tree led to the isolation of a variety of 3′,4′,7,8-tetrahydroxyflavonoids, but no dimeric flavonoids were found. As part of our ongoing research[5−7] for the occurrence of oligomeric flavonoids having a pyrogallol A-ring component, we investigated the MeOH extract of the heartwood of *A. nigrescens*. We herein describe the isolation and structural elucidation of two new promelacacinidins, mesquitol-(4α→5)-3,3′,4′,7,8-pentahydroxyflavonone (**1**) and epimesquitol-(4*β*→5)-3,3′,4′,7,8-pentahydroxyflavonone (**3**).

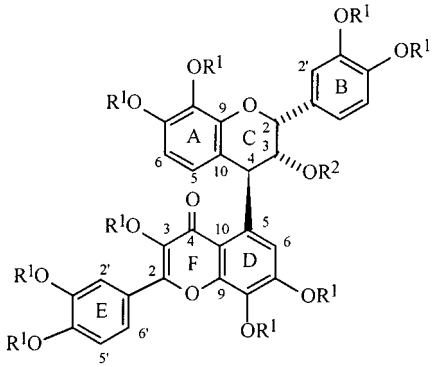
Due to the complexity of the phenolic fraction in which the proanthocyanidins **1** and **3** were found and the presence of tetrameric and higher polimeric compounds, they were purified and identified as the nonamethyl ether acetate derivatives **2** and **4**, respectively. Detailed ^{1}H, ^{13}C, and 2D experiments (^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC) were utilized for the structural elucidation.

^{1}H NMR data (Table 1) of compounds **2** and **4** were used to establish the structures and relative configurations. The presence of nine *O*-methyl and one *O*-acetyl proton signal, together with two AB and two ABX proton spin systems in the spectra of **2** and **4**, suggested the dimeric nature of the two derivatives. The FABMS analysis indicated molecular ions of *m*/*z* 758 for both compounds and confirmed their dimeric nature. Only one AMX system (C-ring) observed in the ^{1}H NMR spectra for both compounds, along with a very deshielded pair of E-ring 2′,6′-protons at *δ* 7.88/7.92 and 7.89/7.93 (Table 1), respectively, indicated the presence of a conjugated carbonyl in the bottom moiety (F-ring). This was indeed supported by the ^{13}C NMR appearance of δ_C signals at 176.7 and 176.0 (Table 2) for both **2** and **4**. This information suggested a flavonol terminal unit. Contrary to what was observed for proanthocyanidin derivatives with C−C interflavanyl linkages where the 4-H(C) of the top unit is shielded (1.32−1.82 ppm) relative to the same proton in the permethylaryl ether 3,4-di-*O*-acetyl derivative of the flavan-3,4-diol precursor,[5,8] the 4-H(C) in both **2** and **4** was deshielded to *δ* 6.66 and 6.15 respectively, because of the nearby carbonyl at 4-C(F).

NOESY experiments of **2** and **4** showed associations between 2-H(C) and 2′-H(B), 6′-H(B) and from 4-H(C) to 5-H(A) (Table 1), which facilitated the identification of the systems (A- and B-rings) belonging to the ABC units.



1 R^1=R^2=H
2 R^1=Me, R^2=Ac



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

Important is the observation that 2′-H(E) is associated with both the 3-OMe(F) and 3′-OMe(E).

HMBC data for compounds **2** and **4** showed coupling from the 4-H(C) to 4-C(F, $^4J_{CH}$), to 5-C(D, $^2J_{CH}$) and to 6-C(D, $^3J_{CH}$); from the 6-H(D) to 7- and 8-C(D, $^2J_{CH}$, $^3J_{CH}$), 4-C(F, $^4J_{CH}$), and 10-C(D, $^3J_{CH}$); and from 2′-H(E) to 3-C(F, $^4J_{CH}$). COSY experiment showed coupling between 4-H(C) and 6-H(D, $^4J_{HH}$). The above information supported the flavanol−flavonol structures as well as the 4-C(C) to 5-C(D) linkage between the units. The ^{13}C analysis confirmed the nine *O*-methyl and one *O*-acetyl groups as well as the suggested carbon structures **2** and **4** (Table 2). The chemical shifts for the 4-C(C) at δ_C 42.3 (Table 2) and 42.6 for **2** and **4** are in accordance with a phenyl substituent[9] at the 4-carbon of the top unit (ABC) of a dimer and confirmed this carbon as a linkage point.

The coupling constants of the heterocyclic systems in the ^{1}H spectrum [$J_{2,3}$(C) = 8.0 Hz; $J_{3,4}$(C) = 8.0 Hz] for **2** and [$J_{2,3}$(C) = 1.5 Hz; $J_{3,4}$(C) = 2.5 Hz] for **4** are reminiscent of a 2,3-*trans*−3,4-*trans* and 2,3-*cis*−3,4-*trans* relative stereochemistry[4,5] for the respective C-rings. The assigned

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Table 1. ^{1}H NMR Peaks (δ_H) of Compounds **2** and **4** at 300 MHz (296 K) (splitting patterns and *J* values (Hz) are given in parentheses)

ring	proton	**2**-$CDCl_3$	**4**-$CDCl_3$
A	5	6.45(d,9.0)	6.77(d,9.0)
	6	6.49(d,9.0)	6.63(d,9.0)
B	2′	6.90(d,2.0)	6.94(d,2.0)
	5′	6.79(d,9.0)	6.78(d,9.0)
	6′	7.00(dd,2.0,9.0)	6.82(dd,2.0,9.0
C	2	5.29(d,8.0)	5.08(d,1.0)
	3	5.74(dd,8.0,8.0)	5.48(dd,1.0,2.0)
	4	6.66(d,8.0)	6.15(d,2.0)
D	5		
	6	6.59(s)	6.51(s)
E	2′	7.88(d,2.0)	7.89(d,2.0)
	5′	7.05(d,9.0)	7.05(d,9.0)
	6′	7.92(dd,2.0,9.0)	7.93(dd,2.0,9.0)
OMe		3.76,3.81,3.86, 3.87,3.92,3.94, 3.98,4.00(2)	3.75,3.85(2),3.87, 3.93,4.00(2),4.03, 4.04
OAc		1.76	1.96

Table 2. ^{13}C Assignment (δ) for Compounds **2** and **4** ($CDCl_3$, 296 K)

ring	carbon	**2**	**4**
A/C	2	79.8	73.8
	3	74.2	72.2
	4	42.3	42.6
	5	124.7	125.8
	6	105.9	105.9
	7	152.4	149.1
	8	137.4	137.2
	9	148.6	149.4
	10	118.7	115.3
B	1′	130.1	130.3
	2′	110.4	110.2
	3′	149.1	152.6
	4′	148.8	149.0
	5′	111.0	111.0
	6′	119.9	119.4
D/F	2	153.3	153.2
	3	141.0	141.4
	4	176.7	176.0
	5	138.9	140.9
	6	111.4	112.7
	7	155.5	155.2
	8	135.9	136.2
	9	150.6	153.2
	10	117.7	116.6
E	1′	123.8	123.8
	2′	111.4	111.3
	3′	149.2	149.4
	4′	151.5	151.4
	5′	111.4	111.4
	6′	122.4	122.4
-O−CH_3		56.2(x2),56.3, 56.4,56.5,56.6, 60.3,61.4,61.8	56.1,56.2,56.3, 56.4,56.5,56.7, 60.3, 61.4,61.8
−C(=O)−CH_3		21.2	21.4
−C(=O)−CH_3		169.9	168.9

relative stereochemistry in conjunction with a negative Cotton effect of $[\theta]_{245.2}$ −8.765 × 10^3 for **2** and a positive Cotton effect of $[\theta]_{235.9}$ = 1.528 × 10^4 for **4** are indicative of 4α- and 4*β*-configurations[10,11] at the 4-C positions, respectively. From the above results it was possible to assign a 2*R*,3*S*,4*S*(C-ring) and a 2*S*,3*R*,4*R*(C-ring) absolute configuration[11] to the top (ABC) units of compounds **2** and **4**. A noteworthy observation is that all the proton resonances were sharp at the probe temperature (296 K), which is indicative of the free rotation around the interflavanyl bond.[12]

Notwithstanding the identical DEF units in both derivatives **2** and **4**, the different configurations at the stereogenic centers of the top moieties (ABC unit) resulted in somewhat different preferred conformations (Figures 1 and 2). The diagnostic NOESY interaction between 3-H(C) and 6-H(D) in the case of **2** prompted the building of a Dreiding model of the dimer, which was complemented by computer modeling (see Experimental Section for details) which resulted in the structure of the preferred conformation as depicted in Figure 1. The distance between 3-H(C) and 6-H(D) was measured at 2.595 Å, and that between 4-H(C) and the carbonyl at 4-C(F) was measured at 2.165 Å. The energy of this lowest energy conformer was calculated at 240.82 kcal/mol. The bottom unit (DEF) is perpendicular to the plane of the top unit (ABC), but from Figure 1 the bottom moiety is below the plane of the top unit.

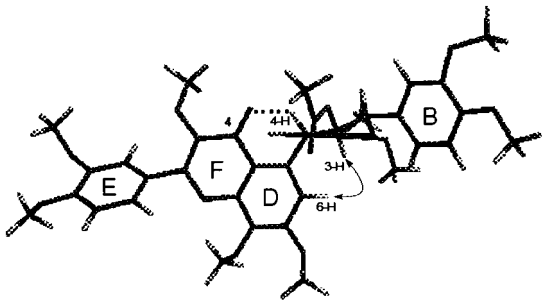

Figure 1. Preferred conformation of **2** with NOESY interactions (solid arrow) and 4-H(C) to carbonyl distance (dotted line).

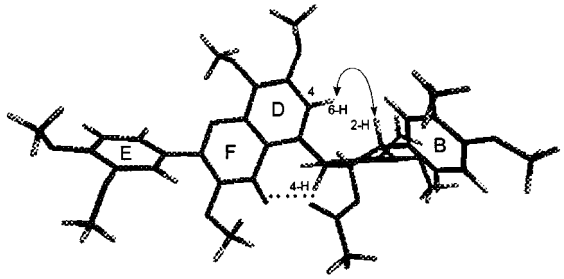

Figure 2. Preferred conformation of **4** with NOESY interaction (solid arrow) and 4-H(C) to carbonyl distance (dotted line).

NOESY interaction between 2-H(C) and 6-H(D) prompted modeling of compound **4** (Figure 2), and a distance of 2.261 Å was measured between these protons. The distance between 4-H(C) and the carbonyl functional group was measured at 2.117 Å. The calculated energy of this conformer amounted to 241.52 kcal/mol. The DEF moiety is perpendicular and at right angles to the plane of the ABC unit with the D-ring above the plane and the E- and F-rings in the plane of the top unit. The small differences in the calculated relative energy values of the two conformers (Figures 1 and 2) are insignificant, although there is a difference in the absolute stereochemistry of the two dimers **2** and **4**.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DPX 300 spectrometer with Me_4Si as internal standard. Electron impact mass spectroscopy (EIMS) data were recorded on a VG-70E instrument. CD data was obtained in methanol as solvent on a Jasco J-710 spectropolarimeter. TLC was performed on precoated Merck plastic sheets (Si 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 120 × 4 cm columns, at a flow rate of 30 mL/h using ethanol as eluent. Flash column chromatography (FCC) was carried

out in a glass column (54 × 6.5 cm) charged with Merck Kieselgel 60 (230−400 mesh) using benzene−Me_2CO as eluent at a flow rate of 60 mL/min. Acetylations were conducted in Ac_2O−pyridine at 50 °C for 24 h. Phenolic-specific methylations were carried out with diazomethane at −15 °C. 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. The PC Spartan Pro Mechanics Program (PC/X86) 6.0.6 was used to do the calculations and construct the low-energy conformers as depicted in Figures 1 and 2.

Chemical Methods for Derivatization. (a) Methylation with Diazomethane. Methylations were performed with an excess of diazomethane prepared by the reaction of potassium hydroxide [(5 g) in a 95% (v/v) ethanol solution] with *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide (15 g) in ether and distilled directly into the previously prepared reaction mixture [200 mg dry phenolic material dissolved in methanol (50 mL) and cooled to −10 °C]. After about 48 h at −15 °C the excess diazomethane and solvent were evaporated at room temperature.

(b) Acetylation Dry phenolic material was dissolved in the minimum volume of pyridine, and twice the amount of acetic anhydride was added. After 8 h at ambient temperature the reaction was terminated by addition of ice, and the excess pyridine was removed by washing out with cold water.

Plant Material. The trunk of *A. nigrescens* was collected near Ellisras, Northern Province, South Africa, and identified by Pricilla Swartz from the National Botanical Research Institute in Pretoria (voucher reference National Herbarium Pretoria 3446000/113).

Extraction and Isolation. Drillings (11.3 kg) from the heartwood of *A. nigrescens* were first extracted with $(CH_3)_2$-CO (3 × 3.0 L) for 24 h periods at room temperature (25 °C). The dried drillings were extracted with MeOH (3 × 3.0 L) under the same conditions. Subsequently, the solid residue was obtained by evaporating the MeOH under vacuum at 40 °C (315 g). An enriched extract was obtained by repeated FCC of 7 × 6 g of the MeOH extract, using Merck Kieselgel 60 as stationary phase and benzene−Me_2CO (8:2, v/v) as eluent. The following combinations were obtained: A (tubes 21−25, 9.09 g), B (53−143, 14.82 g), and C (144−360, 6.72 g). The enriched combination C (6.72 g) was separated on Sephadex LH-20 using EtOH as eluent, resulting in the following combinations: A1 up to A17 (3.582 g) comprised the monomeric flavonoids as reported previously (Fourie et al., 1972); A18 to A22 (named A20) were combined (1.153 g). Prior to methylation 20 mg of the combined fraction was dissolved in acetone-d_6 and subjected to ^{1}H NMR screening for possible naturally occurring methoxyl groups, but none were present. After methylation fraction A20 was subjected to FCC separation using benzene−Me_2CO (9:1, v/v) as eluent at a flow rate of 60 mL/min. The following 22 fractions were collected and combined with the use of TLC to monitor the fractions A20/1 to A20/22, from which most of the fractions comprised polymeric material except fractions A20/6 (68 mg, R_f 0.43−0.58), A20/10 (41 mg, R_f 0.31−0.43), A20/16 (35 mg, R_f 0.20−0.35), which were run in benzene−Me_2CO (8:2 × 2, v/v), and fraction A20/21 (104 mg, R_f 0.1−0.26, benzene−Me_2CO 8:2 × 3, v/v). All four fractions were acetylated and subsequently purified by TLC as reported with the specific compounds isolated.

Mesquitol-(4α→5)-melanoxetin Nona-*O*-methyl Ether Acetate (2). The derivatized fraction A20/6 was separated on TLC using C_6H_6−Me_2CO (8:2 × 2, v/v) as eluting solvent. The band at R_f 0.56 (14.1 mg) yielded the title compound as a yellowish amorphous solid: ^{1}H and ^{13}C data, in Tables 1 and 2; CD $[\theta]_{233.0}$ −1.253 × 10^3, $[\theta]_{245.2}$ −8.765 × 10^3, $[\theta]_{274.6}$ 9.748 × 10^3; HRFABMS *m/z* 758.2564 (calcd for $C_{41}H_{42}O_{14}$ 758.2566).

Epi-mesquitol-(4β→5)-melanoxetin Nona-*O*-methyl Ether Acetate (4). Separation of the derivatized fraction A20/10 on TLC in a C_6H_6−Me_2CO (8:2 × 2, v/v) mixture yielded the title compound as a yellow-brown amorphous solid from a band with R_f 0.50 (17 mg): ^{1}H and ^{13}C data, in Tables 1 and 2; CD $[\theta]_{232.4}$ 1.146 × 10^3, $[\theta]_{241.6}$ 1.528 × 10^4; $[\theta]_{268.7}$ −2.032 × 10^4; HRFABMS *m/z* 758.2567 (calcd for $C_{41}H_{42}O_{14}$ 758.2566).

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