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7-*O*-METHYLAPIGENINIDIN, AN ANTHOCYANIDIN FROM *SORGHUM CAUDATUM*

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Key Word Index—*Sorghum caudatum*; Poaceae; 7-*O*-methylapigeninidin; 3-deoxyanthocyanidins.

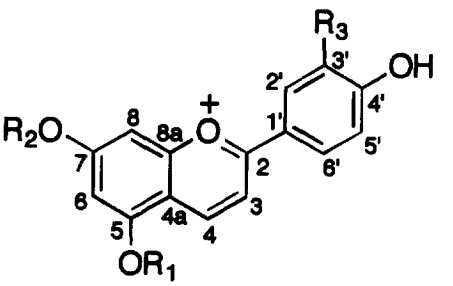
Abstract—A new anthocyanidin was isolated from the grains of *Sorghum caudatum* and identified as 7-*O*-methylapigeninidin on the basis of spectral data. This pigment was found in low concentration both in grains and in leaf sheaths.

INTRODUCTION

3-Deoxyanthocyanidins extracted from the grains of *Sorghum caudatum* [L.] Moench (Poaceae) are known for the yellow or orange coloration they show in acidic solutions. Previous studies on the grains and the leaf sheaths have shown the presence of apigeninidin and luteolinidin derivatives [1–5]. In order to complete the identification of the anthocyanin content, we describe here the isolation and the structure elucidation of a new *O*-methylated 3-deoxyanthocyanidin.

RESULTS AND DISCUSSION

Sorghum grains were extracted with ethanol under acidic conditions. The anthocyanins screening of the crude extract was achieved by HPLC with photodiode array detection; four anthocyanins **1**–**4** were detected on the basis of their UV-VIS spectra and relative retention times. The identity of the major constituent apigeninidin **3** was confirmed by co-elution with an authentic sample. Peaks 1 and 2 were tentatively attributed to the luteolinidin derivatives that were previously identified [1, 2]. The slowest pigment **4** was isolated from the crude extract as the TFA salt after chromatography successively on Sephadex LH-20 and on RP-18 silica gel. Its UV-VIS spectrum recorded in methanol with 0.1% HCl showed absorption maxima at 278.6 and 476.4 nm (Table 1), as observed for other



1 : R_1 = Glc, R_2 = H, R_3 = OH
2 : R_1 = R_2 = H, R_3 = OH
3 : R_1 = R_2 = R_3 = H
4 : R_1 = R_3 = H, R_2 = Me

3-deoxyanthocyanidins [6]; no aluminium-induced shift was observed. The SIMS showed a strong $[M]^+$ ion at *m/z* 269 consistent with the $C_{16}H_{13}O_4$ molecular formula. The ^1H and ^{13}C NMR spectral data (Table 2) were closely related to those of apigeninidin [7, 8], except for a singlet at 4.08 ppm due to an aromatic *O*-methyl group. The position of this group was unambiguously located at C-7 as DIFFNOEs were observed between the methyl protons and both the H-8 and H-6 protons. Moreover, the NOE enhancement of the H-8 signal was greater than that of the H-6 signal, suggesting that the methyl group was more oriented to H-8. **4** was therefore identified as 7-*O*-methylapigeninidin. It was not an artifact of the isolation method since it could be identified in the crude extracts

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Table 1. Chromatographic and spectral data of **4**

TLC ($R_f \times 100$)*					UV-VIS spectrum §	
BAW	BuHCl	EFW	EAFW	AHW	$\lambda_{max\ UV}$ nm (log ε)	$\lambda_{max\ VIS}$ nm (logε)
76†		44†	77†		279	476
62‡	45‡			29‡	(4.50)	(4.81)

*Solvent systems: BAW = 1-butanol–HOAc–H_2O (4:1:5) upper phase), BuHCl = 1-butanol–2 N HCl (1:1 upper phase); EFW = EtOAc–HCO_2H–2 N HCl (85:9:6); EAFW = EtOAc–HOAc–HCO_2H–H_2O (100:11:11:26); AHW = HOAc–HCl conc.–H_2O (15:3:82).

Adsorbents: † silica gel 60 (Merck) and ‡ microcrystalline cellulose F (Merck).

§ In MeOH with 0.1% HCl.

Table 2. ^1H and ^{13}C NMR data of **4**, in CD_3OD–TFA-d_1 (5:1)

	^1H NMR δ (ppm) J (Hz)	^{13}C NMR δ (ppm)
2		172.8
3	8.09, *d* (8.8)	111.8
4	9.14, *dd* (0.8, 8.8)	150.2
4a		114.1
5		160.3
6	6.75, *d* (2.1)	103.1
7		173.9
8	7.16, *d* (0.8, 2.1)	94.3
8a		160.2
1′		122.5
2′ and 6′	8.33, *dd* (1.9, 7.1)	133.9
3′ and 5′	7.10, *dd* (1.9, 7.1)	118.9
4′		168.2
-O-Me	4.08, *s*	58.0

prepared without methanol. The R_f value of **4** in standard TLC conditions are listed in Table 1; using these chromatographic conditions, low amounts of **4** were also detected in a crude extract of the leaf sheaths of the plant.

EXPERIMENTAL

Samples. grains and sheats leafs of *Sorghum caudatum* [L.] Moench (Poaceae), variety Monome Kaya, were collected in 1995 at the Experimental Station of INERA, Kamboinsé, Burkina Faso. A voucher specimen of the plant has been deposited at this Station. The grains were harvested at post maturity and freeze-dried immediately after collection.

Spectrometric data. ^1H and ^{13}C NMR spectra were recorded at respectively 600 and 62.89 MHz with TMS as int. standard. SIMS were recorded on a autospec Micromass spectrometer (matrix: glycerol–HCl, 1:1)

Analytical HPLC. HPLC was carried out following the method described in ref. [9] with a Nova PakTM C_{18} column (150 × 3.9 mm; 4 μm). The elution conditions were modified according to the polarity of the anthocyanins under investigation using a linear gradient solvent system from 30 to 60% of **B** (MeOH–H_2O–HCO_2H, 75:24.5:0.5) in **A** (H_2O–HCO_2H, 60:1) for 20 min; the final conditions of elution were kept isocratically for 10 min.

Anthocyanin isolation. 400 g of the freeze-dried grains were extracted twice by maceration with 500 ml of 1% TFA–EtOH for 48 hr at 5°. After filtration, the solution was concd to dryness under vacuum at 30°. The residue was then chromatographed on a Sephadex LH-20 column with MeOH–H_2O–TFA (30:70:0.3 then 35:65:0.3) as eluents. The frs containing **4** were pooled, concd and the final purification was achieved by chromatography on a RP-18 silica gel column with MeOH–H_2O–TFA (45:55:0.3) as eluent. The fr. containing the unknown anthocyanidin was concd and freeze-dried to yield 25 mg of **4**.

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