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PROACACIBERIN, A CYANOGENIC GLYCOSIDE FROM *ACACIA SIEBERIANA* VAR. *WOODII*

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Key Word Index—*Acacia sieberiana* var. *woodii*; Mimosoideae; Natal Camelthorn; pods; cyanogenic glycoside; acaciberin; proacaciberin.

Abstract—A new cyanogenic glycoside isolated from pods of *Acacia sieberiana* var. *woodii* has been shown by chemical and spectroscopic methods to be (2*S*)-2-[(6-*O*-α-L-arabinopyranosyl-β-D-glucopyranosyl)oxy]-3-methylbut-3-enenitrile. Acid-catalysed hydrolysis of the glycoside afforded arabinose and proacacipetalin, and base-catalysed double-bond migration gave 2-[(6-*O*-α-L-arabinopyranosyl-β-D-glucopyranosyl)oxy]-3-methylbut-2-enenitrile.

INTRODUCTION

Steyn and Rimington reported the presence of an aliphatic, cyanogenic glucoside in the bipinate leaves of African species of *Acacia* [1], unlike the phyllodes of Australian species which contain sambunigrin, an aromatic cyanogenic glucoside, thus pointing out the phytochemical difference between the two groups of *Acacia*.

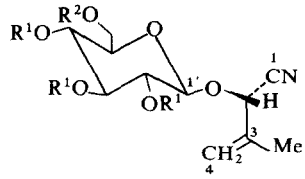
As part of a study of African plants used as food or fodder, we have investigated pods from *Acacia sieberiana* DC var. *woodii* (Burtt Davy) Keay and Brenan (Natal Camelthorn). Previously the main cyanogenic glycoside, proacacipetalin (**1a**), has been isolated [2, 3] accounting for 80–98% of the total cyanide content [4], and a dihydro derivative (**2**) has also been detected [5]. However, the presence of further cyanogenic constituents has been reported [4]. Thus paper chromatographic screening of ethanolic extracts of mature as well as immature pods disclosed 7 spots [R_f = 0.91, 0.83, 0.71 (proacacipetalin), 0.52, 0.46 (possibly **2**), 0.32, 0.23 (S_3, temp = 25°)], which could all be visualized by a modified procedure of the method of Butler and Butler [6]. The seeds were also tested but were found to be acyanogenic.

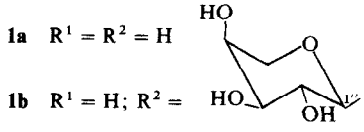
The present paper reports the isolation and structure elucidation of a compound having R_f = 0.32 in system S3.

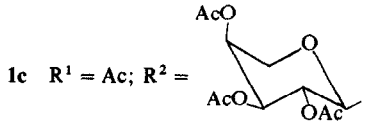
Table 1. ^{1}H NMR data for the compounds **1a–c**, **3a** and **3b**. Chemical shifts (δ) of aglycone and anomeric hydrogens

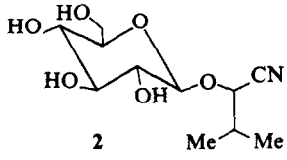
Compound	Me	Olefinic	H-2	Anomeric
1a*,§	1.90	5.40, 5.17	5.40	
1a†,§	1.88	5.42, 5.30	5.42	
1b*,‖	1.90	5.38, 5.15	5.38	4.52 (*d*, *J* = 7.6)
				4.27 (*d*, *J* = 6.7)
1b†	1.88	5.41, 5.27	5.41	4.69 (*d*, *J* = 7.5)
				4.37 (*d*, *J* = 6.8)
1c‡,¶	1.85			4.44 (*d*, *J* = 6.4)
3a*,§	1.89, 1.95			n.a.
3b*	1.90, 1.95			n.a.

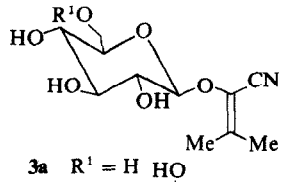
* CD_3OD (TMS (δ = 0) as internal standard).
† D_2O (MeOH (δ = 3.34) as internal standard).
‡ $CDCl_3$ (TMS (δ = 0) as internal standard).
§ Taken from ref. [3].
‖ Double resonance experiment with irradiation at δ 1.90 sharpened the signal at δ 5.15.
¶ Only one anomeric proton could be assigned.
n.a. = not assigned.











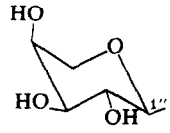


Table 2. ^{13}C NMR data for the compounds **1a–c**

Compound	Chemical shifts (δ) of carbons						
	1	3	4	5	1′	1″	Other carbons
1a*	118.2	137.8	118.7	18.4	101.1		77.0 76.5 73.5 71.1 70.3 61.4
1b*	118.1	137.7	118.6	18.4	101.0	104.4	76.3 76.0 73.4 73.0 71.5 71.0 70.1 68.9 66.9
1b†	117.9	139.6	116.9	18.7	102.2	105.6	77.9 77.3 74.6 74.3 72.5 71.5 70.6 70.0 69.5 66.8
1c‡	115.9	136.5	117.2	18.0	100.6	98.2	73.5 72.6 70.8 69.9 69.5 69.1 68.9 67.5 67.4 62.9

* D_2O (MeOH (δ = 49.73) as internal standard).
† CD_3OD (TMS (δ = 0) as internal standard).
‡ $CDCl_3$ (TMS (δ = 0) as internal standard).

Table 3. ^{13}C NMR data for the compounds **3a** and **3b**

Compound	Chemical shifts (δ) of carbons				
	1–3	Me	C-1′	C-1″	Other carbons
3a*	140.7 124.4 115.5	20.8, 18.2	104.1		78.7 78.3 75.0 71.5 62.8
3b	141.3 124.2 115.4	20.6, 18.0	104.9	104.1	78.0 77.5 74.8 74.1 72.5 71.7 69.5 69.2 66.2

CD_3OD (TMS (δ = 0) as internal standard).
* Taken from ref. [3].

RESULTS AND DISCUSSION

Chromatography of pod extracts on silica gel yielded a fraction which showed the presence of only one cyanogenic compound at $R_f = 0.32$. By further chromatography on silanized silica gel (RP2), a cyanogenic glycoside was separated from other compounds in this fraction. Apart from the δ 5–3 region, the ^{1}H NMR spectrum of the glycoside was very similar to that of proacacipetalin (Table 1), indicating a common aglycone moiety. The location of two doublets at δ 4.52 ($J = 7.6$ Hz) and 4.28 ($J = 6.7$ Hz), respectively, corresponding to signals from two anomeric protons, proved the sugar moiety to be a disaccharide. The presence of 16 signals in the proton-decoupled ^{13}C NMR spectrum of the compound (Table 2) suggested the two monosaccharides to be a pentose and a hexose, respectively. These observations were supported by determination of the [M + 1] ion as m/z 392 by field desorption mass spectrometry. After treating the glycoside with β-glucosidase (almond) two saccharides were detected in the reaction mixture. On TLC (S1, S2, S5) the products co-chromatographed with arabinose and glucose respectively, and they also gave the same colour response with naphthoresorcinol. Hydrolysis of the glycoside in 2 N trifluoroacetic acid at 80° for 2 days afforded, almost exclusively, arabinose and proacacipetalin. This result proves the order of the monosaccharides to be arabinose–glucose–aglycone and the absolute configuration of the asymmetric centre of the aglycone to be *S*, the same as that of proacacipetalin [7].

Comparison of the ^{13}C NMR data of the glycoside with those of authentic proacacipetalin and literature data for methyl α-D-arabinopyranoside [8] indicated the saccharide part of the molecule to be 6-*O*-α-arabinopyranosyl-β-glucopyranosyloxy. The D-form of the glucose moiety was shown by the finding that the glucose isolated after β-glucosidase-catalysed hydrolysis of the glycoside reacted with glucose oxidase. The L-form of the arabinose moiety was indicated by the ability of β-glucosidase to catalyse the hydrolysis of the natural product [9]. The L-configuration was proved according to the method of Leontein *et al.* [10]. Thus the GC properties of the peracetylated glycoside prepared from authentic L-arabinose and (+)-2-octanol were compared to those of the same peracetylated glycoside of the arabinose obtained by hydrolysis of the natural product. The chromatographic properties of the two products were identical, but different from those of the peracetylated glycoside of (+)-2-octanol and D-arabinose. As the natural product was not obtained in a crystalline state, the hexaacetate (**1c**) was prepared in order to characterize a crystalline derivative.

By analogy with proacacipetalin, a double-bond migration was found to take place by leaving a solution of the natural product in saturated aqueous triethylamine for 20 hr. The product was characterized by its ^{13}C and ^{1}H NMR spectra (Tables 1 and 3).

Based on these findings we suggest the structure **1b** for the natural product, the structure **1c** for the hexaacetate and the structure **3b** for the product obtained after triethylamine-catalyzed double-bond migration. In order to emphasize the structural resemblance to proacacipetalin and acacipetalin (**3a**), we suggest the name proacaciberin for the natural product **1b** and acaciberin for the isomer **3b**. The natural product is a glycoside of vicianose, which was first found in vicianine isolated from *Vicia angustifolia* Roth [11] and later from some *Davallia* species [12]. Hitherto, vicianine has been the only known cyanogenic glycoside of vicianose.

Nahrstedt has pointed out [13] that in the Rosaceae, cyanogenic glycosides containing a disaccharide moiety are found only in the generative organs. Also proacaciberin as well as the recently reported cyanogenic glycosides of gentiobiose, linustatin and neolinustatin isolated from linseed [14] have been found in the fruits.

EXPERIMENTAL

Pods of *A. sieberiana* var. *woodii* were obtained from Dr. P. J. Robbertse (Department of General Botany, University of Pretoria), who confirmed their identity by comparison with voucher specimens.

Isolation. Dried and powdered pods (1 kg) were extracted with 10 vols of boiling 96% EtOH. After concn to 25% of original vol., the extract was left for precipitation at 5° overnight. The procedure was repeated for the supernatant, and the soln obtained was evapd to leave 110 g of oily residue. The cyanogenic glycoside was isolated by column chromatography (CC) [Si gel 60, MeOH–$CHCl_3$ (1:5)] followed by CC [cellulose Avicel, 2-butanone–Me_2CO–H_2O (15:5:3)] and finally by CC [silanized Si gel 60, Merck (RP-2), H_2O–MeOH (19:1)].

Identification: TLC and PC solvents. (S1) BuOH–C_6H_5N–C_6H_5Me–H_2O (5:3:3:1, upper phase), (S2) Me_2CO–$CHCl_3$–H_2O (17:2:1), (S3) 2-butanone–Me_2CO–H_2O (15:5:3), (S4) $CHCl_3$–MeOH (2:1), (S5) EtOAc–HOAc–H_2O (3:3:1), (S6) H_2O–MeOH (9:1). Adsorbents: Si gel 60 (S1, S2, S3, S4), cellulose (S5) and silanized Si gel (Merck RP-2) (S6). PC (S3), Whatman No. 3. Detection: Sandwich method [6] using picrate impregnated TLC plates (Polygram ionex-25-SB-Ac, MN) instead of picrate paper, spraying with 0.1% naphthoresorcinol [15] and aminohippuric acid [16]. ^{1}H NMR spectroscopy: FT spectra 89.6 and 270 MHz. ^{13}C NMR spectroscopy: FT spectra 22.5 and 67.889 MHz. All spectra at 295 K.

Acetate of proacaciberin was prepared in pyridine–Ac_2O (1:1), mp 164.5° (corr.), (MeOH–H_2O), $[\alpha]_D^{20}$ − 24.5, $[\alpha]_{578}^{20}$ −25.7, $[\alpha]_{546}^{20}$ −28.0, $[\alpha]_{436}^{20}$ −48.1 and $[\alpha]_{365}^{20}$ −74.8 (*c* 0.48, $CHCl_3$), IR (KBr) cm^{-1}: 2980–2882 w, 1750 s, 1372 m, 1245 s, 1220 s. (Found: C, 52.10; H, 5.98; N, 2.10. $C_{28}H_{37}O_{15}N$ requires: C, 52.25; H, 5.79; N, 2.18%).

Sugar moiety. A soln of glycoside (15 mg) in 2 N aq. TFA was left at 80° for 2 days. After concn, proacacipetalin and L-arabinose could be isolated by prep. TLC (S2, Si gel). The identity of the isolated proacacipetalin was shown by ^{1}H, ^{13}C NMR comparison, and identical TLC (S1 and S3, Si gel; S5, cellulose) behaviour to those of an authentic sample. The identity of the isolated arabinose was established by TLC (S1, cellulose, Si gel; S2, Si gel; S5, cellulose) comparison using an authentic sample. The L-configuration was shown by the method of ref. [10], GC: column SE-54 WCOT, 20 m × 0.35 mm, temp. 50–225° at 5°/min, splitless inlet. The D-configuration of the glucose isolated by prep. TLC (S_2, Si gel) after β-glucosidase-catalyzed hydrolysis (almond, Sigma G8625, 0.1% soln) was established as an aq. soln reacted with Tes tape (Lilly). No reaction was observed with solns of proacaciberin, proacacipetalin or arabinose.

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