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STRUCTURAL ELUCIDATION AND PARTIAL SYNTHESIS OF 3-HYDROXYHETERODENDRIN, A CYANOGENIC GLUCOSIDE FROM *ACACIA SIEBERIANA* VAR. *WOODII*

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Key Word Index—*Acacia sieberiana* var. *woodii*; Mimosoideae; Natal camelthorn; cyanogenic glycosides; 3-hydroxyheterodendrin; proacacipetalin; heterodendrin; oxymercuration–demercuration.

Abstract—A new cyanogenic glucoside, isolated from pods of *Acacia sieberiana* var. *woodii*, was shown to be (2*R*)-2-(β-D-glucopyranosyloxy)-3-hydroxy-3-methylbutanenitrile by spectroscopic and chemical methods. The absolute configuration of this glucoside was correlated with that of proacacipetalin by oxymercuration of the latter, followed by borohydride reduction of the product.

INTRODUCTION

Leaves and pods of *Acacia sieberiana* DC. var. *woodii* (Burtt Davy) Keay and Brenan (Natal camelthorn), which in some parts of Africa are used as fodder, contain proacacipetalin (**1a**) [1, 2] as the major cyanogenic constituent. Moreover, the presence of a dihydro derivative in leaves and young stems was reported [3]. However, chromatographic screening indicated the presence of a number of minor cyanogenic constituents in extracts of pods [4]. Recently we described the isolation of one of these compounds, proacaciberin (**1b**), from immature pods, freed from seeds, of *A. sieberiana* var. *woodii* [5]. In this paper we report the structural elucidation and a partial synthesis of 3-hydroxyheterodendrin, a new cyanogenic glucoside isolated from the same source.

RESULTS AND DISCUSSION

Fractionation of the ethanolic extract of the plant material, immature pods freed from the non-cyanogenic seeds, by column chromatography (CC) on Si gel, followed by reverse-phase CC (RP-2), yielded a crude sample containing a cyanogenic constituent, which on PC (S1, 25°) had R_f 0.45. Investigation of this isolate by reverse-phase HPLC revealed the presence of a number of constituents. Final purification of the cyanogenic compound was achieved by repeated, reverse-phase (RP-18) preparative HPLC.

Striking similarities were found between the ^{13}C NMR chemical shift values of six signals in the carbohydrate regions of the spectra of **1a** and the new compound (Table 1), which suggest the latter to be a β-glucoside. From the chemical shift values of the remaining five peaks, the aglucone constitution shown in **2a** could be proposed. The conclusions drawn from the ^{13}C NMR spectrum were confirmed by ^{1}H NMR (Table 2) and field desorption mass spectrometry (*m/z* 278, $M^+ + 1$) data. The isolated glucoside was readily hydrolysed by β-glucosidase, and the monosaccharide released was shown to be D-glucose by co-chromatography with the authentic compound, colour reaction with naphthoresorcinol and reaction with D-glucose oxidase.

A synthesis of (2*R*)-2-(β-D-glucopyranosyloxy)-3-hydroxy-3-methylbutanenitrile was performed by oxymercuration of **1a**, followed by sodium borohydride

Table 1. ^{13}C NMR data of compounds **1a** and **2a**

Compound	Chemical shift (δ)									
	C-1	C-2	C-3	C-4	Me	C-1′	C-2′	C-3′, C-5′	C-4′	C-6′
1a*	118.2	71.1†	137.8	118.7	18.4	101.1	73.5	76.5, 77.0	70.3	61.4
2a*	118.2	75.2†	72.2		24.9 25.3	101.3	73.5	76.3, 77.0	70.3	61.4
2a‡	§	74.2	71.3		24.8 25.4	101.1	73.6	76.7, 77.0	70.6	62.0

*In D_2O [MeOH (δ 49.7) as internal standard] at 67.9 MHz.
†Assigned by selective decoupling of the proton at C-2, and the off-resonance technique.
‡In CD_3CN [nitrile group (δ 117.7) as standard] at 22.5 MHz.
§Overlapped by solvent signal.

Table 2. ^{1}H NMR data of compounds **2a** and **2b**

Compound	Chemical shift (δ)		
	Me	H-2	Anomeric (H-1′)
2a*	1.35 (*s*)†	4.78 (*s*)	‡
2a§	1.29 (*s*)†	4.55 (*s*)	4.50 (*d*, ^3J = 7.3 Hz)
2a‖	1.55 (*s*), 1.57 (*s*)	5.21 (*s*)	5.32 (*d*, ^3J = 7.6 Hz)
2b¶	1.35 (*br s*)	4.39 (*s*)	4.77 (*d*, ^3J = 7.9 Hz)

*In D_2O [MeOH (δ 3.34) as internal standard]; at 89.6 MHz the remaining protons of the sugar moiety were found at δ 3.3–3.9.

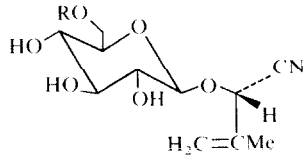
†Appeared as two singlets separated by 4 Hz (D_2O) and 2 Hz (CD_3CN) at 270 MHz.

‡Overlapped by solvent signal.

§In CD_3CN (TMS internal standard) at 89.6 MHz.

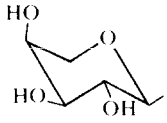
‖In C_5D_5N (TMS internal standard) at 89.6 MHz.

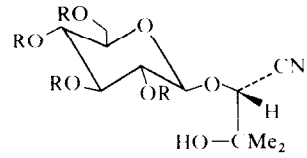
¶In $CDCl_3$ (TMS internal standard) at 270 MHz; remaining protons of the tetra-*O*-glucopyranose moiety: δ 3.78 (H-5′), 4.18 (H-6′A), 4.25 (H-6′B), 5.07 (H-2′), 5.11 (H-4′), 5.27 (H-3′), [$^3J_{2,3}$ = 9.7 Hz, $^3J_{3,4}$ = 9.5 Hz, $^3J_{4,5}$ = 10.1 Hz, $^3J_{5,6A}$ = 2.3 Hz, $^3J_{5,6B}$ = 4.9 Hz, $^2J_{6A,6B}$ = 12.4 Hz (numerical values)], acetate groups at δ 2.0–2.1.



1a R = H

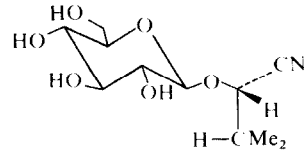
1b R =





2a R = H

2b R = MeCO



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reduction of the product [6–8]. The synthetic and the naturally occurring glucoside had identical chromatographic behaviour on TLC (S1, S2 and S3, Si gel) and HPLC (RP-18, S4) as well as superimposable ^{1}H NMR and ^{13}C NMR spectra. As a number of epimeric pairs of cyanogenic glycosides with opposite configuration of the cyanohydrin carbons can be distinguished by ^{1}H NMR and ^{13}C NMR spectroscopy, the aglucones of the natural and the synthetic glucoside were concluded to possess the same absolute configuration. Thus, the (2*R*) configuration of **2a** follows from the (2*S*) configuration of **1a**. Examples of distinguishable pairs of glucosides are proacacipetalin–epiproacacipetalin ([2]; J. W. Jaroszewski and M. G. Ettlinger, unpublished results) and heterodendrin–epiheterodendrin [9, 10].

Since the natural product was not obtained in a crystalline state, an acetate was prepared in order to characterize a crystalline derivative. According to elemental analysis and ^{1}H NMR and ^{13}C NMR spectroscopic data, a tetra-acetate was formed. The acetylation caused the signals of 5 protons to shift towards lower field. Thus the hydroxyl groups of the sugar moiety had been acetylated, whereas no reaction had occurred at the tertiary alcohol group of the aglucone. The ^{1}H NMR spectrum of the tetra-acetate was fully solved using the MIMER computer program [11] (Table 2).

On the basis of the above observations the new glucoside isolated in this work was assigned structure **2a** and the tetra-acetate structure **2b**. Since **2a** can be regarded as a derivative of heterodendrin (**3**) [(2*S*)-2-(β-D-

glucopyranosyloxy)-3-methylbutanenitrile] [12, 13], we suggest the name 3-hydroxyheterodendrin for the glucoside.

EXPERIMENTAL

General. Pods of *A. sieberiana* var. *woodii* were identified and supplied by Dr. P. J. Robbertse (Department of General Botany, University of Pretoria).

Chromatography. CC Si gel; Merck 7734; silanized Si gel (RP-2), Merck 7719. TLC: Si gel; precoated plates, Merck; microcrystalline cellulose, precoated plates, Merck. PC: Whatman No 3. HPLC: silanized Si gel (RP-18, 8 μm, Spherisorb S GP ODS, Phase Separations Ltd); refractive index detection. The efficiency of the HPLC column (250 × 8 mm; flow 2.7 ml/min), expressed as the number of theoretical plates measured with proacacipetalin when eluted by S5, was 2100. Solvents: S1, MeCOEt–Me_2CO–H_2O (15:5:3); S2, Me_2CO–$CHCl_3$–H_2O (17:2:1); S3, *n*-BuOH–pyridine–$C_6H_5CH_3$–H_2O (5:3:3:1, upper phase); S4, H_2O–MeCN (97:3); S5, H_2O–MeOH (17:3); S6, MeOH–$CHCl_3$ (1:5); S7, H_2O–MeOH (19:1); S8, EtOAc–HOAc–H_2O (3:3:1); S9, MeOH–$CHCl_3$ (1:4). Detection on chromatograms: modified sandwich method [5], spraying with naphthoresorcinol reagent [14].

Isolation of glucoside. The EtOH extract of 1 kg of the plant material was prepared as described before [5]. The cyanogenic glucoside was isolated as a syrup by CC on Si gel (S6), followed by CC on silanized Si gel (S7), and repeated prep. HPLC (S4, S5). Yield 5 mg.

Sugar moiety. A soln of the glucoside (1 mg) in 0.25 ml of 0.1% aq. emulsin (Sigma G 8625) was left overnight at room temp. The saccharide formed on hydrolysis co-chromatographed with authentic D-glucose on TLC (S2, Si gel; S3, Si gel and cellulose; S8, cellulose), and gave the same colour response after spraying with naphthoresorcinol reagent. After being isolated by prep. TLC (S2, Si gel), the saccharide gave a positive test for D-glucose with D-glucose oxidase (TES-tape, Eli Lilly).

Tetra-acetate of 3-hydroxyheterodendrin. The glucoside was acetylated with pyridine–Ac_2O (1:1) for 3 hr at room temp; yield 41%, mp 133.5–134.0° [corr.; recryst. C_6H_{12}–$C_6H_5CH_3$ (2:3)] (found: C, 51.16; H, 6.17; N, 3.08. $C_{19}H_{27}O_{11}N$ requires: C, 51.23; H, 6.11; N, 3.14%); $[\alpha]_D^{20}$ −44.2°, $[\alpha]_{578}^{20}$ −47.1°, $[\alpha]_{546}^{20}$ −52.1°, $[\alpha]_{436}^{20}$ −91.8°, $[\alpha]_{365}^{20}$ −139.4° (*c* 0.5, $CHCl_3$); IR (KBr): ν_{max} 3550–3450 (*m*), 2980–2930 (*w*), 1750 (*s*), 1380–1370 (*m*), 1230 (*s*), 1090 (*m*), 1070 (*m*), 1035 (*m*) cm^{-1}; ^{13}C NMR ($CDCl_3$, TMS): δ 24.7, 25.0, 61.5, 68.1, 70.7, 71.5, 72.2, 72.4, 74.7, 99.0, 115.8, acetate carbons at *ca* 20.5 and 169.3–170.4.

Synthesis of 3-hydroxyheterodendrin. To 0.25 g of **1a**, dissolved in 5 ml of H_2O, 2 g of $Hg(OAc)_2$ in H_2O–THF (50 ml, 3:2) was added. After stirring for 15 min at room temp., the soln was treated with 0.3 g of $NaBH_4$ in 40 ml 0.2 M Pi buffer (pH 7.9), and the stirring continued for a further 15 min. The soln was diluted with EtOH (600 ml), filtered, evaporated and the product isolated by CC on Si gel (S9). Yield 50%.

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