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2-β-D-GLUCOPYRANOSYLOXY-2-METHYLPROPANOL IN *ACACIA SIEBERANA* VAR. *WOODII*

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Key Word Index—*Acacia sieberana* var. *woodii*; Mimosoideae; glycoside; novel diol glucoside; 2-methyl-1, 2-propanediol aglycone; dihydric alcohol; glycol; diol lipids.

Abstract—A new diol glucoside, 2-β-D-glucopyranosyloxy-2-methylpropanol, the first reported naturally occurring monoglucoside of an aliphatic dihydric alcohol, was isolated from pods of *Acacia sieberana* var. *woodii*. Structure elucidation was based on ^1H and ^{13}C NMR spectroscopy, and enzymatic analyses. The compound was hydrolysed very slowly by almond β-glucosidase, but cleaved by a β-glucuronidase enzyme complex from *Helix pomatia*.

INTRODUCTION

A number of naturally occurring neutral lipids may be regarded as derivatives of simple aliphatic dihydric alcohols, e.g. ethylene glycol, propane diols and straight chain butane diols [1–6]. Some glycosides of acylated dihydric alcohols have also been found in plants. Thus, 2-*O*-β-D-galactopyranosyloxyethanol esterified at the aglycone with palmitic, oleic or stearic acid have been isolated from ripening corn seeds [7], and the presence of an analogous glucoside in immature corn and wheat seeds has been reported [7, 8]. In contrast, glycosides of aliphatic diols possessing a free hydroxyl group at the aglycone have not been reported from a natural source. A few representatives of glycosides of free dihydric alcohols, however, have been synthesized [7, 9]. We now describe the structure elucidation of a glucoside of 2-methyl-1, 2-propanediol (**1**), isolated from immature pods of *Acacia sieberana* var. *woodii*. This is the first report of a natural source of this aglycone.

RESULTS AND DISCUSSION

Fractionation of the ethanolic extract of the plant material (immature pods freed from seeds) by CC on Si gel, followed by reverse-phase CC (RP-2), yielded a crude sample containing the diol glucoside together with a number of cyanogenic constituents, mainly 3-hydroxyheterodendrin [10]. The diol glucoside was obtained in a pure state after repeated reverse-phase (RP-18) HPLC of this mixture.

In the proton decoupled ^{13}C NMR spectrum (Table 1) the location of five of the nine signals strongly suggested the compound to be a β-glucoside. This was supported by the presence of a signal at δ 4.64 (*d*, $J = 7.6$ Hz, see Experimental) in the ^1H NMR spectrum, which could be assigned to the anomeric proton. The unusual high field resonance (δ 97.3) of the anomeric carbon could be explained by assuming the glucopyranosyloxy moiety to be attached to either a tertiary carbon [11] or a carbonyl group [12]. The absence of a carbonyl band in the IR spectrum, as

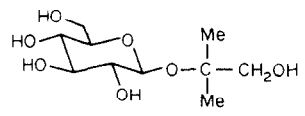
Table 1. ^{13}C NMR data of glucoside (**1**) and 2-methyl-1, 2-propanediol (**2**)

Compound*	Chemical shift (δ)								
	1	2	Me	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
1†	69.0	79.8	23.2	97.3	74.0	76.5	70.5	76.5	61.6
	(+, *t*)	(*s*)	(*q*)	(*d*)	(+, *d*)	(+, *d*)	(+, *d*)	(*d*)	(+, *t*)
2‡	70.5	72.1	25.3	—	—	—	—	—	—

*In D_2O [MeOH (δ 49.7) as int. standard] at 67.9 MHz.

†+ indicates a shift of 6.5–12.0 Hz toward higher field if the spectrum is recorded in H_2O instead of D_2O proving the carbon to be attached to a free hydroxyl group [16, 17].

‡Observed glucosylation shifts for carbons 1, 2 and Me are −1.5, +7.7 and −2.1; Tori, K. *et al.* [10] observed for tert. butyl alcohol (in pyridine-d_5): −2.9, +7.2.



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well as a resonance signal in the carbonyl region of the ^{13}C NMR spectrum, and the location of the signal due to the anomeric proton in the ^1H NMR spectrum, excluded the possibility of an acyl glucoside. The very slow hydrolysis afforded by β-glucosidase (almond) supported the possibility of a sterically hindered glucoside. In the presence of a crude enzyme complex prepared from the gastric juice of *Helix pomatia*, commercially available as β-glucuronidase or sulfatase (Sigma), hydrolysis afforded glucose and 2-methyl-1, 2-propanediol as shown by cochromatography with authentic samples on TLC, and in the case of the diol, also by GC of the TMSi derivatives. Enzyme preparations from *H. pomatia* have previously been shown to catalyse the hydrolysis of sterically hindered glycosides [13, 14]. The D-configuration of the glucose was proved by the ability of the isolated sugar to react with D-glucose oxidase. Field desorption mass spectrometry confirmed the suggested MW (*m/z* 259, $[M+Li]^+$). In the electron impact mass spectrum a prominent peak at *m/z* 221 corresponding to the fragment obtained by the loss of the CH_2OH group confirmed glucosylation at the tertiary hydroxyl group. A similar fragmentation is found in the electron impact mass spectrum of 2-methyl-1, 2-propanediol [15]. Based on these findings we suggest the structure of 2-β-D-glucopyranosyloxy-2-methylpropanol (**1**) for the new natural product.

EXPERIMENTAL

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

Chromatography. Si gel, Merck 7734; Si gel (RP-2), Merck 7719. TLC: Si gel, precoated plates, Merck; microcrystalline cellulose, precoated plates, Merck. HPLC: silanized Si gel, (Spherisorb S-GP-ODS, 8 μm, Phase Separations Ltd); RI detection. The efficiency of the HPLC column (250 × 8 mm; flow rate 2.7 ml/min), expressed as the number of theoretical plates measured with proacacipetalin [10] when eluted by S3, was 2100. Solvents: S1, MeOH–$CHCl_3$ (1:5); S2, H_2O–MeOH (19:1); S3, H_2O–MeOH (17:3); S4, H_2O–MeCN (97:3); S5, Me_2CO–$CHCl_3$–H_2O (17:2:1); S6, EtOAc–HOAc–H_2O (3:3:1).

Isolation of glucoside. The EtOH extract of 1 kg of the plant material was prepared as previously described [16]. The glucoside was isolated as a syrup by CC on Si gel (S1), followed by CC on silanized Si gel (S2), and repeated HPLC (S3, S4). Yield *ca* 15 mg.

Identification of glucoside. TLC (S5, Si gel), spraying with naphthoresorcinol (gray). IR (1 mg in MeOH evaporated on 300 mg KBr): ν_{max} cm^{-1} 3640–3040 (s), 2980–2840 (w), 1480–1350 (w), 1290–1215 (w), 1140–1000 (m). HPLC (S4), adjusted relative [3-hydroxyheterodendrin (16.7 ml) equals 1] retention vol. 0.80. ^1H NMR [D_2O, MeOH ($\delta = 3.34$) as int. standard]: δ 1.23 (6H, *s*), 4.64 (1H, *d*, $J = 7.6$ Hz), 3.1–4.0 (including a singlet at 3.51, other carbohydrate and aglycone protons). MS (EI) 70 eV *m/z* (rel. int.): 235 $[M-OH]^+$ (0.3), 221 $[M-CH_2OH]^+$ (1.1), 163 $[C_6H_{11}O_5]$ (8.5), 73 $[C_4H_9O]$ (100).

Hydrolysis. β-Glucosidase. A soln of the glucoside (0.5 mg) in 0.25 ml 1% emulsin (Pi buffer 0.2 M, pH 5) was left for 24 hr at 37°. *β-Glucuronidase.* A soln of the glucoside (0.75 mg) in 0.2 ml β-glucuronidase (Sigma G-0876; soln in H_2O, resulting pH 5.5, 6000 Fishman Units per ml) was left overnight at room temp. The formed products cochromatographed on TLC with authentic 2-methyl-1, 2-propanediol (S5, Si gel), and D-glucose (S5, Si gel; S6, cellulose) respectively, and gave the same colour responses after spraying with alkaline $KMnO_4$ (the diol), naphthoresorcinol (glucose) and aminohippuric acid (glucose) reagents. Isolation by prep. TLC (S5, Si gel) afforded a saccharide, which gave a positive test for D-glucose with D-glucose oxidase (Clinistix, Ames-Company). After evaporation of the hydrolysis mixture, 50 μl BSA–$CHCl_3$ (1:5) was added to obtain the TMSi derivative of the diol. This cochromatographed (GC) with the TMSi derivative of authentic 2-methyl-1, 2-propanediol.

GC analysis. 4.5% GE-XE-60 on Chromosorb G-AW-DMCS, 2 m × 4 mm; FID, temp. 135°; N_2, 30 ml/min.

Preparation of authentic 2-methyl-1, 2-propanediol. Prepared by the reduction of ethyl-2-hydroxyisobutyrate (33 mmol) with $LiAlH_4$ (23 mmol) in cold Et_2O. Distil bp_{18} 81–82°.

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