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1-β-VICIANOSYL-(*S*)-2-METHYLBUTYRATE, A 1-*O*-ACYLGLYCOSIDE FROM *ACACIA SIEBERANA* VAR. *WOODII**

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Key Word Index—*Acacia sieberana* var. *woodii*; Mimosoideae; acylglycoside; vicianose; ^{13}C NMR: proacaciberin; acaciberin; proacacipetalin; epiproacacipetalin; acacipetalin; HPLC.

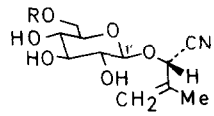
Abstract—A 1-*O*-acylglycoside, isolated from pods of *Acacia sieberana* var. *woodii*, was shown to be 1-(6-*O*-α-L-arabinopyranosyl-β-D-glucopyranosyl)-(*S*)-2-methylbutyrate by chemical and spectroscopic methods. In the assignments of the signals in the ^{13}C NMR spectrum, advantage was taken of isotope-induced chemical shift changes and off-resonance decouplings at different frequencies. CD established the absolute configuration of the aglycone.

INTRODUCTION

Apart from derivatives of various hydroxycinnamic acids [1], some tannins and metabolites of xenobiotics applied to plants [2], reports of 1-*O*-acylglycosides isolated from plants are very few. A number of interesting properties have, however, been ascribed to these compounds. Thus, the remarkable sweetness of the leaves of *Stevia rebaudiana* (Asteraceae) has been correlated with the content of stevioside, a 1-glucopyranosyl ester of a pentacyclic diterpene acid [3–5] and the antibiotic substances, Tuliposide A and B found in *Tulipa gesneriana* (Liliaceae), were shown to be 1-β-D-glucopyranosyl esters of 2-methylene-4-hydroxy- and (*S*)-2-methylene-3,4-dihydroxybutanoic acid [6]. Furthermore, anomerically pure synthetic 1-*O*-lauroyl-α- and β-D-glucopyranoses are reported to exhibit growth inhibiting effects on *Avena* coleoptiles [7] and 1-*O*-myristoyl-α-D-glucopyranose on Ehrlich Ascites carcinoma [8]. The present paper describes the isolation and structural elucidation of a 1-*O*-acylglycoside isolated from immature seed-free pods of *Acacia sieberana* DC. var. *woodii* (Burtt Davy) Keay and Brenan.

RESULTS AND DISCUSSION

Fractionation of the ethanolic extract of the plant material by CC on Si gel and cellulose gave a crude sample of the new acyanogenic acylglycoside and a number of cyanogenic glycosides, mainly proacaciberin [9]. Reversed phase CC of this mixture gave proacaciberin and a pure sample of the new glycoside. Both acid and β-glucosidase catalysed hydrolysis of the acylglycoside gave rise to glucose and arabinose. Identical GC properties of the peracetylated (+)-2-octanol glycosides prepared from the isolated monosaccharides and of the same derivatives prepared from authentic D-glucose and L-arabinose, respectively, proved the absolute configurations of the monosaccharides [9, 10]. The formation of these monosaccharides suggested the new glycoside was a derivative of vicianose. Comparison of the proton decoupled ^{13}C NMR spectra of the glycoside and of proacaciberin (**1b**) strongly supported this idea (Table 1). Only two pronounced differences were observed in the δ 60–110 regions. Firstly, in the spectrum of the new glycoside no signals were observed in this region, apart from those originating from the vicianose moiety, and secondly the signal due to one of the anomeric carbons was



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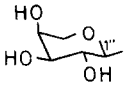
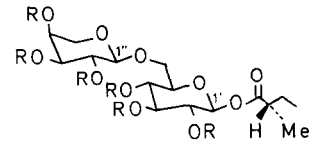
1b R = 



2a R = H

2b R = Ac

*A preliminary report on this work was given at the International Research Congress on Natural Products as Medicinal Agents, Strasbourg, 1980 [20].

Table 1. ^{13}C NMR data for compounds **1a**, **1b** and **2a** [89.6 MHz, D_2O, MeOH (δ 49.73) as int. standard]

Carbon	1a*	1b†	2a
1	118.2	118.2	179.0
2	71.1(*s*)‡	71.0(*s*)	41.6
3	137.8	137.7	27.0
4	118.7	118.6	11.5
Me	18.4	18.4	16.4
1′	101.1	101.1	94.7
2′	73.5(*d*)	73.4(+, *d*)	72.8(+)
3′	77.0(*d*)	76.3(+, *d*)	76.3(+)
4′	70.3(*d*)	70.1(+, *d*)	69.8(+)
5′	76.5(*d*)	76.0(*d*)	76.8
6′	61.4(*t*)	68.9(*t*)	68.6
1″	—	104.4	104.2
2″	—	73.0(+, *d*)	73.1(+)
3″	—	71.5(+, *d*)	71.5(+)
4″	—	68.9(+, *d*)	69.1(+)
5″	—	66.9(*t*)	67.0

*See also ref. [19].

†Incremental variation of the decoupler frequency proved the observed residual splittings (in the glucose moiety) to be proportional to the decoupler frequency offset, from resonance of the protons directly attached to the carbon.

‡In the parentheses are indicated: Multiplicity of signals in spectra selectively decoupled by irradiation at δ 5.4 (cyanhydrin proton resonance). A + indicates a shift of 6.5–12.0 Hz towards 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 [14].

found at high field (δ 94.7). A high field resonance of an anomeric carbon indicates the aglycone to be a tertiary alcohol [11] or a carboxylic acid [12]. A signal at δ 179 in the ^{13}C NMR spectrum, a carbonyl band at 1745 cm^{-1} in the IR spectrum, a positive reaction with hydroxylamine–iron(III) chloride reagent [13], and a resonance signal at δ 5.44 (*d*, $J = 7.6$ Hz) due to one of the anomeric protons, all proved the natural product to be an acyl glycoside. The magnitude of the aforementioned coupling constant and the β-glucosidase-catalysed hydrolysis established the natural product as a β-derivative of vicianose.

The ^{13}C (Table 1) as well as the ^1H NMR spectrum (see Experimental) and a peak at *m/z* 397 $[M + 1]^+$ in the field desorption mass spectrum, suggested the aglycone to be 2-methylbutyric acid. This was further confirmed by comparison of the GC-properties of the 2-octanol and menthol esters prepared by transesterification of the natural product with those of the corresponding esters of authentic 2-methylbutyric acid. The absolute configuration of the aglycone was established as *S*, since the same Cotton effect CD curve was obtained for both the aglycone and (*S*)-2-methylbutyric acid.

As the natural product was not obtained in a crystalline state, the hexa-acetate was prepared in order to characterize a crystalline derivative.

Based on the aforementioned results, we suggest the structure 1 - β - vicianosyl - (*S*) - 2 - methylbutyrate (**2a**) for the new acyl glycoside and the structure **2b** for the hexa-acetate.

The isolation of proacacipetalin (**1a**), proacaciberin and the acyl-glycoside made an assignment of the ^{13}C NMR signals due to the vicianose moiety possible. The interpretations (Table 1) are based on a comparison of the three spectra, on literature values for the spectrum of methyl - α - D - arabinopyranoside, on deuterium isotope shift experiments [14] to designate the hydroxylated carbons and on off-resonance experiments with incremental variation of the decoupling frequency [15].

EXPERIMENTAL

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

Chromatography. Detection on chromatograms: spraying with naphthoresorcinol-, aminohippuric acid- and hydroxylamine–$FeCl_3$ reagents.

Isolation of the glycoside. The ethanolic extract from 1 kg plant material was prepared as described before [9]. The acylglycoside was isolated as a syrup by CC on Si gel with MeOH–$CHCl_3$ (1:5), followed by CC on cellulose with MeCOEt–Me_2CO–H_2O (15:5:3) and CC on silanized Si gel with H_2O–MeOH (19:1). Yield 80 mg.

Identification of the glycoside. TLC [Si gel, *n*-BuOH–pyridine–toluene–H_2O (5:3:3:1, upper phase); silanized Si gel (RP-2), H_2O–MeOH (9:1)], spraying with naphthoresorcinol (deep blue) and hydroxylamine–$FeCl_3$ (light brown); IR (1 mg in MeOH evaporated on 300 mg KBr): ν_{max} cm^{-1} 3650–3100(*s*), 2980–2840(*m*), 1745(*s*), 1670–1600(*w*), 1480–1130(*m*), 1130–1030(*s*); Smell of 2-methylbutyric acid when hydrolysed with β-glucosidase; HPLC (8 μM Spherisorb S GP ODS (250 × 8 mm), H_2O–MeOH (17:3, 2.7 ml/min), RI detector, 2100 theoretical plates with proacacipetalin), adjusted relative retention vols. (proacacipetalin 11.1 ml equal to 1) of the 1-*O*-acylglycoside and of other glycosides: 3-hydroxyheterodendrin, 0.27; epiproacacipetalin, 0.91; acacipetalin, 1.39; proacaciberin, 1.41; acaciberin, 1.90; 1-*O*-acylglycoside, 2.83; ^1H NMR (CD_3OD, TMS): δ 0.93 (*t*, $J = 7.6$ Hz), 1.16 (*d*, $J = 7.0$ Hz), 1.52 (*m*), 1.71 (*m*), 2.48 (*m*), 3.36–4.09 (carbohydrate protons), 4.28 (*d*, $J = 6.4$ Hz) and 5.44 (*d*, $J = 7.6$ Hz).

Aglycone. Transesterification from glycoside to (+)-2-octanol and menthol respectively. (a) (*2-Octanol*). 4 mg glycoside heated for 2 hr at 80° with 50 μl (+)-2-octanol (HCl satd.) in a screw cap vial. Excess reagent was removed with a current of N_2. 200 μl CH_2Cl_2 and 50 μl trifluoroacetic anhydride were added to the residue, and the mixture kept at room temp. for 30 min. After removal of the excess reagent with N_2, the residue was dissolved in 200 μl CH_2Cl_2. (b) (*Menthol*): 5 mg glycoside, 30 mg menthol and 200 μl HCl satd. C_6H_6 were heated for 2 hr at 80° in a screw cap vial.

Preparation of authentic esters. Esters were prepared by reaction of the alcohols with the acid chloride, obtained by the method of Baxter *et al.* [16].

GLC. Column, 4.5% GE XE-60 on Chromosorb G-AW-DMCS (6 feet × 4 mm); detector, FID; temp. 130°; N_2 30 ml/min.

Absolute configuration. A soln of glycoside (30 mg) in 1 ml 0.1% aq. emulsin (Sigma G 8625) was left overnight at room temp., satd. NaCl soln (1 ml) was added, the mixture centrifuged, and the supernatant extracted with three 1 ml portions of CH_2Cl_2. The combined organic phases were concd to give the aglycone. Yield was determined by ^1H NMR in $CDCl_3$. (*S*)-2-Methylbutyric acid was prepared after Weisenborn *et al.* [17]. CD spectra (MeOH): $\Delta\epsilon$ at 200–220 nm = 0.03 M^{-1} cm^{-1} (room temp.), (see ref. [18]).

Sugar moiety. Solns of the acylglycoside, 1 mg in 0.25 ml 0.1% aq. emulsin (Sigma G8625, room temp.) and 1 mg in 0.25 ml 2 N aq. TFA (80°) were left overnight. The saccharides formed on hydrolysis co-chromatographed with authentic D-glucose and L-arabinose on TLC (Si gel, Me_2CO–$CHCl_3$–H_2O (17:2:1); microcrystalline cellulose EtOAc–HOAc–H_2O (3:3:1); Si gel and microcrystalline cellulose *n*-BuOH–pyridine–toluene–H_2O (5:3:3:1, upper phase)] and gave the same colour response on spraying with naphthoresorcinol reagent. The L- and D-configurations, respectively, were shown by the method of Leontein *et al.* For further details, see refs. [9, 10]. On hydrolysis with TFA, the formation of a more hydrophilic reducing sugar (possibly vicianose) could be observed on TLC [microcrystalline cellulose, EtOAc–HOAc–H_2O (3:3:1), aminohippuric acid].

Acetate of the glycoside. The glycoside was acetylated with pyridine–Ac_2O (1:1) overnight, mp 147–148.5° [corr. (recryst. MeOH–H_2O, 2:1)] (found: C, 51.50; H, 6.20; N, 0.17. $C_{28}H_{40}O_{17}$ requires: C, 51.83; H, 6.22; N, 0.00%). $[\alpha]_D^{20}$ + 8.5° ± 0.5°, $[\alpha]_{578}^{20}$ + 8.5° ± 0.5°, $[\alpha]_{540}^{20}$ + 11.2° ± 0.5°, $[\alpha]_{436}^{20}$ + 18.7° ± 0.5°, $[\alpha]_{365}^{20}$ + 29.2° ± 0.5° ($CHCl_3$; *c* 0.46); IR $\nu_{max}^{KBr}cm^{-1}$: 2970–2880(*w*), 1750(*s*), 1372(*m*), 1250–1212(*s*); ^1H NMR ($CDCl_3$, TMS): δ 0.91 (*t*, *J* = 7.6 Hz), 1.14 (*d*, *J* = 7.0 Hz), 1.48(*m*), 1.65(*m*), 2.39(*m*), sugar protons at 3.43–4.04 and 4.96–5.29, anomer protons at 4.47 (*J* = 6.7 Hz) and 5.70 (*J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, TMS): δ 11.4, 16.4, 26.2, 40.8, 62.7, 67.0, 67.4, 68.6, 68.9, 69.9, 70.3, 72.9, 74.2, 91.4, 100.5, 174.4, acetate carbons at *ca* 20.7 and 169.3.

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