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MONOTERPENE GLUCOSIDES FROM *BERCHEMIA RACEMOSA*

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Key Word Index—*Berchemia racemosa*; Rhamnaceae; monoterpene glucoside; bornanediol glucoside; angelicoidenol glucoside; isoarborinol; 2D-NMR

Abstract—Two monoterpene glycosides isolated from the stem of *Berchemia racemosa*, have been characterized as (+)-angelicoidenol-2-*O*-*β*-D-glucopyranoside and (−)-angelicoidenol-2-*O*-*β*-D-glucopyranoside on the basis of chemical and spectral evidence. Isoarborinol was also isolated from the plant.

INTRODUCTION

From the methanol extract of the stem of *Berchemia racemosa* Sieb. et Zucc., we isolated 2,6-dimethoxybenzoquinone as the physiologically active constituent which inhibits histamine release from rat mast cells induced by compound 48/80 and by Concanavalin A [1]. Recently, we also isolated two new aromatic glycosides, methoxyhydroquinone-4-*O*-*β*-D-glucopyranoside (tachioside) and syringic acid *β*-D-glucopyranosyl ester, along with three known glycosides, nudiposide, (−)-secoisolariciresinol-9′-*O*-*β*-D-glucopyranoside and methoxyhydroquinone-1-*O*-*β*-D-glucopyranoside (isotachioside) from the butanol-soluble fraction of the methanol extract [2]. We now report on the isolation of the triterpene, isoarborinol (**1**) from the hexane extract, and two new glucosides (**2** and **3**) from the butanol-soluble fraction of the methanol extract.

RESULTS AND DISCUSSION

Compound **1** was identified as isoarborinol by direct comparison of its acetate (**1a**) with an authentic sample. The physical data of the oxidation product, arborinone (**1b**) also matched the literature values. The ^{13}C NMR data of **1a** and **1b** also supported the structures.

Compound **2**, $C_{16}H_{28}O_7$, $[M+Na]^+$ *m/z* 355.1737 (calc. 3.55.1733) gave the pentaacetate, $C_{26}H_{38}O_{12}$, $[M]^+$ *m/z* 542. Methanolysis followed by GC showed the presence of glucose (converted to its TMS ether for GC). The ^{13}C NMR spectrum (C_5D_5N) of **2** showed the signals of six *β*-glucopyranosyl carbons, three methyl groups, two- CH_2 groups, one –CH group, two quarternary carbons, and two- –CH–OH groups. There were no signals for C=C groups (Table 1).

The three methyl groups gave three singlet peaks in the ^{1}H NMR spectrum. These data suggested that the aglycone moiety of **2** ($C_{10}H_{18}O_2$) was a bicyclic monoterpenediol. The chemical shifts of the aglycone moiety were very close to the reported value of angelicoidenol (**4**) [3] except for those of C-2 and C-3. The chemical shift differences ($\Delta\delta = \delta_{glucoside} - \delta_{alcohol}$) of these carbons being +10.2 and −1.4, respectively.

Table 1. ^{13}C NMR data and glucosylation shift ($\Delta\delta$ in parentheses) of compounds **2** and **3** (C_5D_5N, 67.5 MHz)

C	4*	2 (2–4)	3 (3–4)
1	50.8	50.9 (+0.1)	50.4 (−0.4)
2	75.0	85.2 (+10.2)	82.9 (+7.9)
3	37.1	35.8 (−1.4)	34.2 (−2.9)
4	53.7	53.4 (−0.3)	53.4 (−0.3)
5	75.0	74.8 (−0.2)	74.9 (−0.1)
6	39.6	40.1 (+0.5)	40.1 (+0.5)
7	47.9	47.6 (−0.3)	48.1 (+0.2)
8	21.8	21.3 (−0.5)	21.4 (−0.4)
9	20.2	20.2 (0.0)	20.3 (+0.1)
10	13.5	13.9 (+0.3)	13.6 (+0.1)
G1		106.2 (+7.4)†	103.6 (+4.8)†
G2		75.5	75.3
G3		78.6	78.7
G4		71.7	71.8
G5		78.2	78.3
G6		62.9	62.9

*Data taken from ref. [3].
†Difference from free *β*-D-glucose.

In the ^{13}C–^{1}H COSY spectrum, all of the protons were correlated to the appropriate carbon atoms. The ^{1}H–^{1}H COSY spectrum revealed the vicinal relationship of H-2*β* (*δ* 4.18) and H-3*β* (*δ* 2.38, *ddd*, *J* = 5, 10 and 14 Hz) with H-3*α*(*δ* 1.48, *dd*, *J* = 3, 14 Hz); H-3*β* also correlated to H-4*α* (*δ* 1.91, *d*, *J* = 5 Hz). The other correlation was observed for H-5*α* (*δ* 4.23) and H-6*β*(*δ* 1.74, *br d*, *J* = 13 Hz) with H-6*α* (*δ* 2.99, *dd*, *J* = 8 and 13 Hz). Weak but significant coupling was observed between H-6*β* and H-2*β*. The fact that H-4 coupled with only one proton can be explained by the 90° dihedral angles between H-4*α* and H-3*α*, and H-4*α* and H-5. All NMR data satisfy the anticipated bornan- 2,5-diol skeleton.

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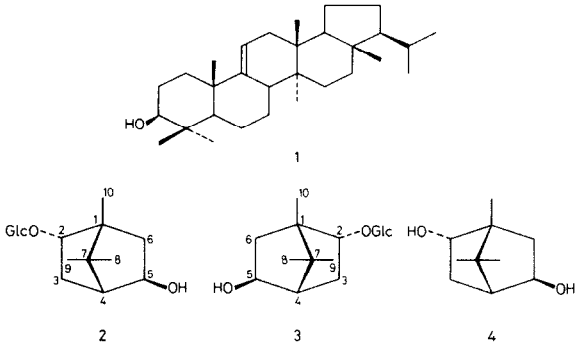
Table 2. ^{1}H NMR spectral data of compounds **2**–**4** (C_5D_5N-TMS, 270 MHz for **2** and **3**, 90 MHz for **4***)

H	4*	2	3
2β	4.50 *ddd*	4.18 *br d*	4.38 *br d*
(exo)	(9.5, 3.5, 2)	n.d.†	(9)
3α	1.15 *dd*	1.48 *dd*	1.43 *dd*
(endo)	(13.5, 3.5)	(14, 3)	(13.5, 3.5)
3β	2.20–2.50 *ddd*	2.38 *ddd*	2.31 *ddd*
(exo)	(13.5, 9.5, 5)	(14, 10, 5)	(14, 9, 5)
4α	1.95 *d* (5)	1.91 *d* (5)	1.96 *d* (5)
5α	4.19 *dd*	*ca* 4.23	4.30 *dd*
(endo)	(8.5, 3)		(8, 3)
6α	2.8–3.05 *dd*	2.99 *dd*	2.95 *dd*
(endo)	(13, 8.5)	(13, 8)	(13, 8)
6β	1.72 *dd*	1.74 *br d*	1.72 *br d*
(exo)	(13, 3)	(13)	(13)
8 (Me)	1.40 *s*‡	1.39 *s*	1.40 *s*
9 (Me)	0.90 *s*‡	0.85 *s*	0.84 *s*
10 (Me)	1.05 *s*‡	1.20 *s*	1.12 *s*
1′	Glucosyl moiety	4.93 *d* (8)	4.90 *d* (8)
2′		4.03 *dd* (10, 8)	4.01 *dd* (9, 8)
3′		4.24 n.d.†	4.25 n.d.
4′		4.26 n.d.	4.27 n.d.
5′		3.95 *ddd*	3.95 *ddd*
		(10, 5, 3)	(9, 5, 3)
6′		4.40 *dd* (12, 5)	4.40 *dd* (12, 5)
6″		4.53 *dd* (12, 3)	4.54 *dd* (12, 3)

Coupling constants in Hz are in parentheses.
*Data taken from ref. [3].
†Not determined due to overlapping of the signals.
‡Assignment interchanged by our C–H COSY results.



Compound **3**, $C_{16}H_{28}O_7$, $[M+Na]^+$ *m/z* 355.1740 (calc. 355.1733) gave rise to very similar ^{13}C and ^{1}H NMR spectra to those **2** (Tables 1 and 2). Methanolysis of **3** followed by GC showed the presence of glucose (as its TMS ether). There findings suggested that **3** was a stereoisomer of **2**.

In order to establish the position of the glucosyl residue and the absolute structure, the ^{13}C NMR glucosylation shift was considered. In the case of β-D-glucopyranosides of secondary alcohols, the substitution induced shift values of carbon signals caused by glucosylation (glucosylation shift) depend on the absolute configuration of the alcohol. The shift value ($\Delta\delta = \delta_{glucoside} - \delta_{alcohol}$) of an α-carbon is *ca* 6–8 ppm for an achiral alcohol and a chiral (*R*)-alcohol, and *ca* 10 ppm for a chiral (*S*)-alcohol. Also, the two β-carbons of the aglycone and the anomeric carbon reflect the absolute stereochemistry [4, 5].

The glucosylation shifts of both compound indicated that the glucosylated position was restricted to the C-2 hydroxyl group, and that the absolute configuration of this position is *S* for **2** [by reference to the reported value of dammaranediol (**5**)] and *R* for 3 [by analogy with ent-dammaranediol (**6**)*] (Table 1 and Fig. 1) [4].

Although the aglycones of both compounds were not obtained, they should be a pair of enantiomers. This was confirmed by K!yne's rule of glycosylation [6]. The calculated specific optical rotation of **2** and **3** from the values of **4** and methyl β-D-glucoside were −11.0° and −27.5°, respectively, which are in good accordance with the observed values, −12.6° and −26.3°, respectively. Thus, the structures of two glucosides, **2** and **3** were elucidated to be 2-β-D-glucopyranosyl-(+)-angelicoidenol and 2-β-D-glucopyranosyl-(−)-angelicoidenol, respectively. It is to be noted that two glucosides with enantiomeric aglycones exist in the same plant.

EXPERIMENTAL

Mp: uncorr; ^{1}H NMR and ^{13}C NMR: 270 and 67.5 MHz, respectively, except when otherwise stated; MS: 75 eV.

Plant material. Berchemia racemosa Sieb. et Zucc. was collected in the vicinity of Taishaku-kyo, Hiroshima Prefecture, Japan. A specimen is deposited at the Herbarium of Experimental Station of Medicinal Plants, Hiroshima University School of Medicine.

Extraction and isolation. Dried stem of *B. racemosa* (2.0 kg) were crushed and extracted with *n*-hexane followed by MeOH. The MeOH extract was suspended in H_2O, and extracted successively with *n*-hexane, Et_2O, EtOAc, BuOH and H_2O. From a

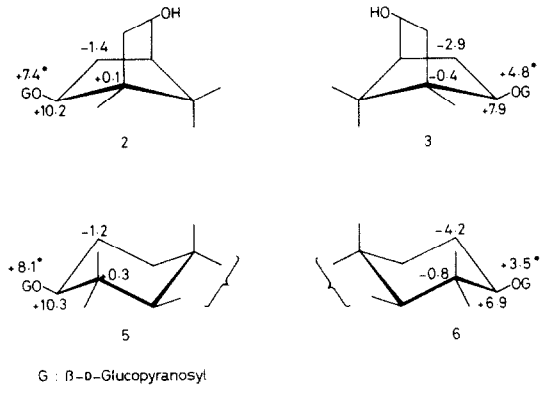


Fig. 1. ^{13}C NMR glucosylation shifts ($\Delta\delta$) on hydroxyl-bearing carbons, anomeric carbons and carbon atoms adjacent to hydroxyl-bearing carbons for the determination of absolute configuration of aglycones.

*Actual ^{13}C NMR data came from β-L-glucoside of **5** [4].

portion (4.1 g) of the combined hexane extract (10.9 g), isoarborinol (**1**) (180 mg) was isolated by silica gel CC (C_6H_6 upto C_6H_6 with 0.5% Me_2CO).

The BuOH-soluble fraction (20.2 g) was chromatographed on highly porous polymer, Diaion HP-20, developed with H_2O containing the 10, 20, 30, . . . 90% MeOH and 100% MeOH). The 50% MeOH eluent was subjected to silica gel CC ($CHCl_3$–MeOH–H_2O) and DCC ($CHCl_3$–MeOH–H_2O, 5:6:4) followed by DCC ($CHCl_3$–MeOH–H_2O–propan-1-ol, 5:6:4:1) again to give a mixture of **2** and **3**. This was subjected to Sephadex LH-20 CC (MeOH), prep. HPLC on RP-18 (MeOH–H_2O) and silica gel CC (EtOAc–EtOH–H_2O) to afford compounds **2** (54 mg), and **3** (27 mg).

Isoarborinol (**1**). Colourless needles from C_6H_6 mp 308–310°, lit. [7] mp 295–296°. MS *m/z* (rel. int.): 426 $[M]^+$ (20), 411 (30), 393 (6), 259 (20), 43 (100); IR ν_{max}^{KBr} cm^{-1}: 3230, 2930, 2860, 1470, 1385, 1375, 1031.

Isoarborinol acetate (**1a**). Acetylation of **1** (40 mg) in C_5H_5N–Ac_2O (5 ml each) at 55° for 5 hr afforded **1a** (32 mg), mp 290–292°, lit [7] 287–288°; MS *m/z*: 468, 443, 393, 301, 255, 241, 229; ^1H NMR ($CDCl_3$): δ0.75 (3H, *s*), 0.76 (3H, *s*), 0.80 (3H, *s*), 0.83 (3H, *d*, $J = 6.6$ Hz), 0.86 (3H, *s*), 0.89 (3H, *s*), 0.90 (3H, *d*, $J = 6.6$ Hz), 1.05 (3H, *s*), 4.50 (1H, *m*), 5.23 (1H, *d*, $J = 6.1$ Hz); identical with authentic spectrum. ^{13}C NMR (in $CDCl_3$, 25 MHz): δ14.0, 15.3, 16.8, 17.0, 21.4, 22.1, 23.0, 28.2 ×2 (9 × CH_3), 20.2, 21.4, 24.2, 26.6, 28.2, 29.7, 35.7, 36.0 × 2 (9 × CH_2), 30.8, 41.0, 52.1, 52.5, 59.7 (5 × CH), 36.8, 38.1, 38.2, 39.5 42.9 (5 × *q*C), 81.0 (CH-O), 114.6 (CH=C), 148.5 (CH=C<), 170.8 (−COOMe).

Arborinone (**1b**). Oxidation of **1** (80mg) with 102 mg CrO_3 in H_2O (10 ml), AcOH (2 ml) and C_6H_6 (40 ml) for 3 hr afforded arborinone (**1b**) (60 mg), mp 219–221°, lit [7], 214–214.5°; IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 2860, 1700, 1655, 1270, 1000; ^{13}C NMR (in $CDCl_3$, 25 MHz): δ14.0, 15.2, 17.0, 21.6, 22.0 ×3, 25.5 (8 × Me), 20.1, 22.5, 23.0, 26.2, 28.2, 29.6, 34.8, 35.9, 36.6 (9 × CH_2), 30.8, 41.1, 51.9, 53.2, 59.6 (5 × CH), 35.9, 38.1, 39.3, 42.8, 47.6 (5 × *q*C), 115.6 (CH=C), 147.4 (C=C<), 214.9 (C=O).

(+)-*Angelicoidenol*-2-O-β-D-glucopyranoside (**2**). Amorphous powder, $[\alpha]_D^{20} - 12.6°$ MeOH; *c* 0.65). FABMS (DMSO, glycerol + NaI) *m/z* 355.1737 $[M+Na]^+$ (calc. 355.1733, $C_{16}H_{28}O_7$ Na); IR ν_{max}^{KBr} cm^{-1}: 3600–3100 (OH), 2900, 1450, 1385, 1360, 1285, 1230, 1160, 1075, 1025; ^1H NMR and ^{13}C NMR; see Tables 1 and 2.

(−)-*Angelicoidenol*-2-O-β-D-*glucopyranoside* (**3**). Amorphous powder, $[\alpha]_D^{20} - 26.3°$ (MeOH; *c* 1.04). FABMS (DMSO, glycerol NaI) *m/z* 355.1740 $[M+Na]^+$ (calc. 355.1733, $C_{16}H_{28}O_7$ Na); ^1H NMR and ^{13}C NMR; see Tables 1 and 2.

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