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SAPONINS FROM *BARRINGTONIA ACUTANGULA*

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Key Word Index—*Barringtonia acutangula*; Lecythidaceae; barringtosides A, B and C; barringtogenol C; glucuronide saponin; acylsaponin.

Abstract—Three monodesmosidic glucuronide saponins of barringtogenol C, named barringtosides A, B and C have been isolated as their methyl esters from the dried seeds of *Barringtonia acutangula*. On the basis of chemical and spectral evidence, the structures of these new saponins were elucidated to be as follows: barringtoside A, 3-*O*-*β*-D-xylopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl barringtogenol C; barringtoside B, 3-*O*-*β*-D-xylopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-21-*O*-tigloyl-28-*O*-isobutyryl barringtogenol C; barringtoside C, 3-*O*-*α*-L-arabinopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl barringtogenol C.

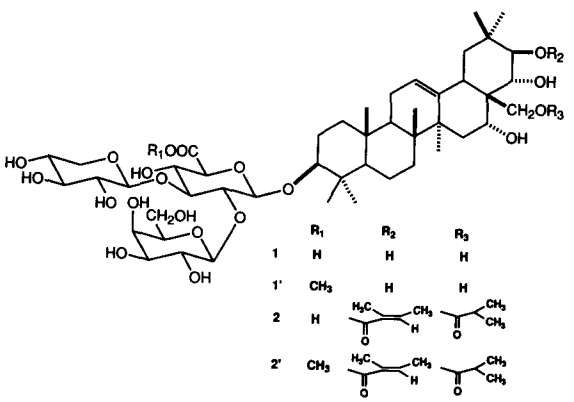
INTRODUCTION

In a previous paper, we reported the isolation and structure of triterpenoid glucoside from the dried seeds of *Barringtonia acutangula* [1]. Further examination of the polar fraction led to the isolation of saponins named barringtosides A, B and C, as their methyl esters. In the present paper, we report the isolation and structural elucidation of these three new saponins.

RESULTS AND DISCUSSION

The dried seeds of *B. acutangula* (2.5 kg) were extracted successively with ether and methanol. Preliminary investigation of the ^{1}H NMR spectrum of the methanol extract indicated that the saponins had no methoxy group. The methanol extract was esterified with diazomethane and further purified by column chromatography on silica gel and HPLC separation, leading to the isolation of methyl esters of three new compounds, named barringtosides A (**1**), B (**2**) and C (**3**). ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMBC, TOCSY and ROESY led to determination of the complete structures of **1′**–**3′**, inclusive of the sequence of the sugar moieties and the position of attachment of the sugar chains to the aglycone.

Methyl ester (**1′**) of barringtoside A, was obtained as needles and deduced to have the formula $C_{48}H_{78}O_{20}$ as the result of the quasi-molecular ion at *m/z* 973 $[M-H]^-$ in the negative FAB-mass spectrum and carbon counts in the ^{13}C NMR spectrum. Acid hydrolysis of the $NaBH_4$ reduction product (**5**) of **1′**, afforded barringtogenol C (**4**) [2, 3] as the genin, and galactose, glucose and xylose in a ratio of 1:1:1 as sugar components [4]. The ^{1}H, ^{13}C and HMQC NMR spectra of **1′** indicated the presence of one



β-galactopyranosyl unit [H-1′: *δ*5.56 (*d*, *J* = 8.0 Hz, C-1′: *δ*104.6], one *β*-glucuronopyranosyl unit [H-1′: *δ*4.98 (*d*, *J* = 8.0 Hz), C-1′: *δ*105.5] and one *β*-xylopyranosyl unit [H-1′: *δ*5.33 (*d*, *J* = 7.8 Hz), C-1′: *δ*104.9]. A ^{13}C NMR spectral comparison of **1′** with **4** revealed a glycosylation shift [5] (+11.5 ppm) at the C-3 signal (*δ*89.6) of the aglycone moiety, indicating that the sugar moiety was located only at position C-3 in the aglycone. Furthermore, evaluation of chemical shifts of each sugar, referring to those reported NMR data for methyl galactoside, methyl glucuronide and methyl xyloside, allowed identification of one terminal galactopyranosyl and xylopyranosyl and of a 2,3-disubstituted glucuronopyranosyl unit. In a HMBC experiment on **1′**, the methine carbon (C-2′ of methyl glucuronate) at *δ*79.5 gave a cross-peak with the anomeric proton (galactose) at *δ*5.56, and the methine carbon (C-3′ of methyl glucuronate) at *δ*85.4 showed a cross-peak with the anomeric proton (xylose) at

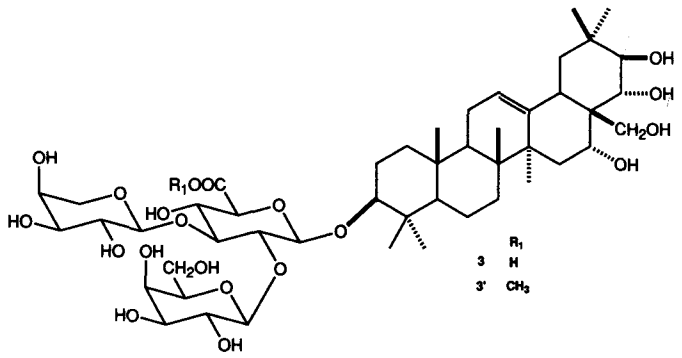
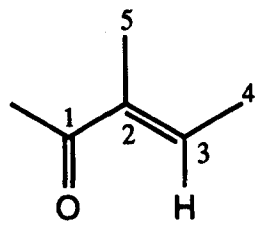


Table 1. ^{13}C NMR spectral data for compounds 1′–3′ and 4 (in pyridine-d_5, δ values)

C	1′*	2′*	3′*	4†	1′*	2′*	3′*
1	38.8	38.8	39.0	39.2	3-*O*-GlcA		
2	26.6	26.6	26.7	28.2	1′ 105.5	105.4	105.5
3	89.6	89.6	89.9	78.1	2′ 79.5	79.5	79.5
4	39.6	39.6	39.7	39.4	3′ 85.4	85.4	85.6
5	55.8	55.7	55.9	55.9	4′ 71.3	71.3	71.4
6	18.5	18.4	18.6	18.8	5′ 76.3	76.3	76.5
7	33.2	33.1	33.3	33.3	6′ 170.1	170.1	170.3
8	40.0	40.0	40.2	40.1	Me 52.2	52.2	52.4
9	47.0	46.9	47.1	47.2	Gal		
10	36.8	36.7	36.9	37.3	1′ 104.6	104.7	104.6
11	23.8	23.9	24.0	23.9	2′ 73.8	73.8	73.8
12	123.1	123.0	123.1	123.0	3′ 75.3	75.3	75.4
13	143.9	142.8	144.1	144.0	4′ 69.7	69.7	69.8
14	42.0	41.8	42.1	42.1	5′ 76.7	76.7	76.8
15	34.3	34.8	34.4	34.3	6′ 61.5	61.5	61.7
16	67.9	67.6	68.0	67.9	Xyl or Ara		
17	47.3	47.3	47.5	47.4	1′ 104.9	104.9	105.1
18	41.1	40.5	41.3	41.3	2′ 75.2	75.2	73.0
19	48.2	47.3	48.4	48.3	3′ 78.5	78.5	74.8
20	36.4	36.3	36.6	36.4	4′ 70.8	70.8	69.9
21	78.7	81.5	78.8	78.7	5′ 67.3	67.3	67.8
22	77.1	71.0	77.2	77.3	Tigloyl		
23	28.0	27.9	28.1	28.8	1 168.4		
24	16.7	16.7	16.9	16.6	2 129.9		
25	15.7	15.7	15.8	15.9	3 136.2		
26	16.9	17.0	17.1	17.1	4 14.1		
27	27.4	27.4	27.5	27.4	5 12.4		
28	68.2	66.0	68.2	68.4	Isobutyryl		
29	30.6	29.8	30.7	30.6	1 176.4		
30	19.5	20.2	19.6	19.5	2 34.5		
					3 19.0		
					4 19.3		



tigloyl

*150 MHz.
†50 MHz.

δ5.33, establishing the existence of a galactopyranosyl group at position C-2′ and a xylopyranosyl group at position C-3′ of the glucuronopyranosyl unit. Hence, the structure of 1′ was established to be the methyl ester of barringtogenol C-3-*O*-β-D-xylopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranoside.

Methyl ester (2′) of barringtoside B, was obtained as needles and had the formula $C_{57}H_{90}O_{22}$ from the quasi-molecular ion at *m/z* 1125 $[M-H]^-$ in the FAB mass spectrum. The ^{1}H and ^{13}C NMR spectral data of 1′ indicated the presence of one β-galactopyranosyl unit [H-1′: δ5.56 (*d*, J=7.8 Hz), C-1′: δ104.7], one β-glucuronopyranosyl unit [H−1′: δ4.98 (*d*, J=8.0 Hz), C-1′: δ105.4] and one β-xylopyranosyl unit [H-1′: δ5.33 (*d*, J=7.8 Hz), C-1′: δ104.9], besides one tigloyl unit [δ1.59 (3H, *d*, J =7.0 Hz), 1.86 (3H, *s*), 7.04 (1H, *q*, J=7.0 Hz)] and one *iso*butyryl unit [δ1.14 and 1.86 (each 3H, *d*, J=7.0 Hz), 2.57 (1H, *sept*, J=7.0 Hz)]. A ^{13}C NMR spectral com-

parison of **2** with that of **1** showed that the same sugar unit was also fixed to the C-3 position, and varied structurally from **1'** only in its D and E rings. By ^{1}H NMR comparison of **2** with **4**, two acylation shifts were observed at the C-21 position [+1.88 ppm (H-21)] and the C-28 position [+0.32 and 0.56 ppm (H_2-28)] indicating that in **2'**, the *O*-21 and *O*-28 should be acylated. In an HMBC experiment on **2'**, the ester carbon signal (tiglic acid) at δ 168.4 gave a cross-peak with the methine proton (H-21) at δ6.50, establishing the existence of a tigloyl group at C-21. Therefore, the *iso*butyryl group should be linked to the C-28 position. Compound **2'** was thus revealed to be the methyl ester of barringtogenol C-21-*O*-tigloyl-28-*O*-isobutyryl-3-*O*-β-D-xylopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranoside methyl ester.

Methyl ester (**3'**) of barringtoside C, obtained as needles, had the same molecular formula as **1'**. The ^{1}H and ^{13}C NMR spectra indicated the presence of one β-galactopyranosyl unit [H-1': δ5.53 (*d*, *J* = 7.7 Hz, C-1': δ104.6], one β-glucuronopyranosyl unit [H-1': δ4.97 (*d*, *J* = 7.5 Hz), C-1': δ105.5] and one α-arabinopyranosyl unit [H-1': δ5.25 (*d*, *J* = 7.3 Hz), C-1': δ104.9]. A ^{13}C NMR spectral comparison of **3'** with **1'** showed that the sugar unit was also fixed to the C-3 position, although the sugar unit was different. In an HMBC experiment on **3'**, the methine carbon (C-2' of methyl glucuronate) at δ79.5 gave a cross-peak with the anomeric proton (galactose) at δ5.53, and the methine carbon (C-3' of methyl glucuronate) at δ85.6 showed a cross-peak with the anomeric proton (arabinose) at δ5.25, establishing the existence of a galactopyranosyl group at C-2' and a arabinopyranosyl group at C-3' of the glucuronopyranosyl unit. Therefore, compound **3'** was the methyl ester of barringtogenol C-3-*O*-α-L-arabinopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranoside.

EXPERIMENTAL

Mps are uncorr. NMR spectra were recorded in pyridine-d_5 soln using TMS as int. standard. NMR expts included ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMBC, TOCSY and ROESY. Coupling constants (*J* values) are given in Hz. FAB-MS: Xe gun, 10 kV, triethylene glycol as matrix. For CC, Kieselgel 60 (230–400 mesh, Merck), and for TLC, silica gel 60F-254 (Merck) were used.

Extraction and isolation of compounds **1–3**. Dried seeds (2.5 kg) of *B. acutangula* Gaerth were successively extracted with petrol (60–80°) and then with MeOH in a percolator. The MeOH extract on removal of solvent under red. pres. afforded a viscous dark brown mass (30 g). A portion of the extract (10 g) in MeOH was esterified with CH_2N_2–Et_2O to afford crude Me esters. Chromatography of these on silica gel eluting with $CHCl_3$–MeOH (9:1) afforded fr. I and further elution with $CHCl_3$–MeOH (4:1) yielded fr. II (0.5 g) and fr. III (0.8 g). After repeated CC over silica gel using $CHCl_3$–MeOH (4:1), frs II' (0.2 g) and III' (0.31 g) were further purified by prep. HPLC (YMC, ODS, 25–35% MeCN) to afford barringtoside C (**3'**, 15 mg) from fr. II', barringtoside A (**1'**, 120 mg) and barringtoside B (**2'**, 25 mg) from fr. III', respectively.

Barringtoside A (**1'**). Needles, mp 258–260°. $[\alpha]_D$ −1.0° (MeOH; *c* 4.0). FAB-MS: *m/z* 973 $[M-H]^-$, 841 $[M-H-Xyl]^-$, 489 [genin − H]$^-$. ^{1}H NMR (600 MHz, pyridine-d_5): δ0.82 (3H, *s*, H_3-25), 0.88 (3H, *s*, H_3-26), 1.11 (3H, *s*, H_3-24), 1.29 (3H, *s*, H_3-23), 1.33 (3H, *s*, H_3-29), 1.38 (3H, *s*, H_3-30) and 1.84 (3H, *s*, H_3-27), 3.28 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-3), 3.73 (3H, *s*, COOMe), 3.71, 4.0 (each 1H, *d*, *J* = 10.0 Hz, H-28), 4.62 (1H, *d*, *J* = 10.0 Hz, H-21), 4.79 (1H, *d*, *J* = 10.0 Hz, H-22), 5.02 (1H, *m*, H-16), 5.38 (1H, *m*, H-12), 4.98 (1H, *d*, *J* = 8.0 Hz, H-1' of GlcA), 5.33 (1H, *d*, *J* = 7.8 Hz, H-1' of Xyl), 5.56 (1H, *d*, *J* = 8.0 Hz, H-1' of Gal). ^{13}C NMR: Table 1.

Barringtoside B (**2'**). Amorphous powder. $[\alpha]_D$ +12.6° (MeOH; *c* 0.9). FAB-MS: *m/z* 1125 $[M-H]^-$. ^{1}H NMR (600 MHz, pyridine-d_5): δ0.83 (3H, *s*, H_3-25), 1.0 (3H, *s*, H_3-26), 1.11 (3H, *s*, H_3-24), 1.31 (3H, *s*, H_3-23), 1.13 (3H, *s*, H_3-29), 1.35 (3H, *s*, H_3-30) and 1.83 (3H, *s*, H_3-27), 3.30 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-3), 3.73 (3H, *s*, COOMe), 4.27, 4.32 (each 1H, *d*, *J* = 10.0 Hz, H_2-28), 4.53 (1H, *d*, *J* = 9.8 Hz, H-22), 4.74 (1H, *m*, H-16), 5.49 (1H, *m*, H-12), 6.50 (1H, *d*, *J* = 9.8 Hz, H-21), 4.98 (1H, *d*, *J* = 8.0 Hz, H-1' of GlcA), 5.33 (1H, *d*, *J* = 7.8 Hz, H'-1 of Xyl), 5.56 (1H, *d*, *J* = 7.8 Hz, H-1' of Gal). ^{13}C NMR: Table 1.

Barringtoside C (**3'**). Needles, mp 240–242°. $[\alpha]_D$ +15.1° (MeOH; *c* 2.0). FAB-MS: *m/z* 973 $[M-H]^-$. ^{1}H NMR (600 MHz, pyridine-d_5): δ0.82 (3H, *s*, H_3-25), 0.89 (3H, *s*, H_3-26), 1.11 (3H, *s*, H_3-24), 1.29 (3H, *s*, H_3-23), 1.33 (3H, *s*, H_3-29), 1.38 (3H, *s*, H_3-30) and 1.85 (3H, *s*, H_3-27), 3.28 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-3), 3.70, 4.00 (each 1H, *d*, *J* = 10.0 Hz, H_2-28), 3.73 (3H, *s*, COOMe), 4.62 (1H, *d*, *J* = 10.0 Hz, H-21), 4.80 (1H, *d*, *J* = 10.0 Hz, H-22), 5.05 (1H, *m*, H-16), 5.38 (1H, *m*, H-12), 4.97 (1H, *d*, *J* = 7.5 Hz, H-1' of GlcA), 5.25 (1H, *d*, *J* = 7.3 Hz, H-1' of Ara), 5.53 (1H, *d*, *J* = 7.7 Hz, H-1' of Gal). ^{13}C NMR: Table 1.

$NaBH_4$ *reduction of* **1'**. Compound **1'** (30 mg) was dissolved in MeOH (3 ml), $NaBH_4$ (10 mg) was added and the reaction mixt. stirred for 6 hr. After neutralization with HOAc and evapn of solvent, the residue was purified by HPLC (ODS, 18% MeCN) to give a reduction product **5** (25 mg). FAB-MS: *m/z* 945 $[M-H]^-$. ^{1}H NMR spectrum did not show a signal of the carbomethoxy proton assigned to the methyl ester of the glucuronopyranosyl group.

Acid hydrolysis of **5**. A soln of compound **5** (25 mg) in 5% H_2SO_4–dioxane (1:1) was heated at 100° for 2 hr. The reaction mixt. was diluted with H_2O and extracted with $CHCl_3$. The organic layer was concd to dryness to yield barringtogenol C (**4**, 10 mg). Needles, mp 266–268°. $[\alpha]_D$ +15.4° (MeOH; *c* 0.9). EI-MS: *m/z* 490 $[M]^+$. ^{1}H NMR (200 MHz, pyridine-d_5): δ0.97, 0.97, 1.06, 1.24, 1.33, 1.39, 1.85 (all *s*, Me × 3), 3.48 (1H, *dd*, *J* = 9.0, 5.5 Hz, H-3), 3.73, 4.03 (each 1H, *d*, *J* = 10.0 Hz, H_2-28), 4.62 (1H, *d*, *J* = 10.0 Hz, H-21), 4.79 (1H, *d*, *J* = 10.0 Hz, H-22), 5.03 (1H, *m*, H-16), 5.45 (1H, *m*, H-12). ^{13}C NMR: Table 1. The aq. layer was neutralized with Amberlite IR-45 and concd *in vacuo* to dryness. The mole ratio and D (L) configuration of each sugar was determined by using RI detection and chiral detection by HPLC (Shodex RSpak DC-613,

75% MeCN, 1 ml min^{-1}, 70°) by comparison with authentic sugars (10 mmol each of D-Glc, D-Xyl and D-Gal). These sugars gave the following peaks: D-(+)-Xyl; 5.75 min, D-(+)-Glc; 7.38 min, D-(+)-Gal; 8.0 min.

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