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TRITERPENOID SAPONINS FROM *BERNEUXIA THEBETICA*

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Key Word Index—*Berneuxia thibetica*; Diapensiaceae; triterpenoid saponins; berneuxia saponins A, B, C; desacyl jegosaponin.

Abstract—Four triterpenoid saponins were isolated from *Berneuxia thibetica*. On the basis of chemical and spectroscopic evidence, three new saponins, berneuxia saponins A, B and C, were elucidated as 21-tigloylbarringtogenol C-3β-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)[β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside], 28-tigloylbarringtogenol C-3β-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)[β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranoside] and 16α-28-dihydroxyolean-12-en-21-one-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)[β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranoside]. The known saponin was desacyl jegosaponin. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Berneuxia thibetica Decne, which is widespread in the southwest of China, is used as a Chinese folk medicine for curing coughs due to pathogenic wind-cold factors, overstrain, asthma and dyspea, and wounds [1].We have reported sterols, triterpenes and flavones from this plant [2–4]. We now report the triterpenoid saponins isolated from the leaves.

RESULTS AND DISCUSSION

The crude saponin fractions were subjected to repeated CC on silica gel and silanised silica gel, affording saponins **1**, **2**, **3** and **4**. The yields were 0.008%, 0.006%, 0.01% and 0.004% of the dry leaves, respectively.

On mineral acid hydrolysis, saponin **1** yielded the aglycone **1a**, which was identified as 21-tigloylbarringtogenol C by comparison with an authentic sample. This aglycone was also isolated from the ethyl acetate extract of this plant. Four kinds of sugars, glucuronic acid, glucose, galactose and rhamnose, were detected by PC in the aqueous fraction after the removal of the aglycone. The EI-mass spectrum of its acetate showed fragment ions at *m/z* 273 [terminal rhamnose$(Ac)_3]^+$, 331 [terminal glucose $(Ac)_4]^+$ and 561 [rhamnose $(Ac)_3$ galactose$(Ac)_3]^+$. The FAB-mass spectrum showed the molecular ion at *m/z* 1219 $[M+H]^+$. The negative FAB-mass spectrum showed the fragment ions at *m/z* 1217 $[M-H]^-$, 1071 [M − rhamnose]$^-$, 1055 [M-glucose]$^-$ and 909 [M − rhamnose − galactose]$^-$. The ^{13}C NMR spectrum indicated the presence of four monosaccharide units. On hydrolysis with acid (1 M HCl), **1** gave prosapogenin **1b**. The hydrolysis of **1b** gave 21-tigloylbarringtogenol C as the aglycone, and D-glucuronic acid and D-glucose as the sugar components. The EI-mass spectrum of the acetate and Me ester of **1b** showed fragment ions at 331 [terminal glucose$(Ac)_4]^+$, 605 [glucose$(Ac)_4$ glucuronic acid-OMe $(Ac)_2]^+$. The ^{13}C NMR spectrum indicated the presence of two monosaccharide units. The glycosylation shift (82.4 ppm) indicated that the β-D-glucuronic acid was 2-*O*-glycosylated [5]. Therefore, the structure of **1b** was 21-tigloylbarringtogenol C-3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranoside. Comparison of the ^{13}C NMR signals of **1** with those reported by Calis *et al.* [6] showed that the sugar moieties were the same. The downfield shift of C-2 of glucuronic acid in **1b** (δ78.6 of **1** to δ82.4 of **1b**) may be caused by 3-*O*-glycosylation of β-D-glucuronic acid. On the basis of above, saponin **1** was elucidated as 21-tigloylbarringtogenol C-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β - D - galactopyranosyl(1 → 3) - [β - D - glucopyranosyl (1 → 2)-β-D-glucuronopyranoside], named berneuxia saponin A.

Saponin **2** yielded the aglycone **2a** by mineral acid hydrolysis. Compound **2a** was identified as 28-tigloylbarringtogenol C by comparison of the ^1H NMR signals with those reported [7]. Four sugars, glu-

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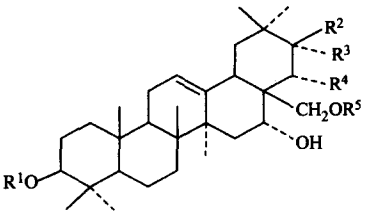

	R^1	R^2	R^3	R^4	R^5
1	rha(1→2)gal(1→3)[glc(1→2)glu]	tigloyl	H	OH	H
1a	H	tigloyl	H	OH	H
1b	glc(1→2)glu	tigloyl	H	OH	H
2	rha(1→2)gal(1→3)[glc(1→2)glu]	OH	H	OH	tigloyl
2a	H	OH	H	OH	tigloyl
3	rha(1→2)gal(1→3)[glc(1→2)glu]	$R^2, R^3 = O$		H	H
3a	H	$R^2, R^3 = O$		H	H
4	rha(1→2)gal(1→3)[glc(1→2)glu]	OH	H	OH	H

curonic acid, glucose, galactose and rhamnose, were detected by PC in the aqueons fraction after removal of the aglycone. The FAB-mass spectrum showed the molecular ion at *m/z* 1219 $[M+H]^+$. The negative FAB-mass spectrum showed the fragment ions at *m/z* 1217 $[M-H]^-$, 1071 [M − rhamnose]$^-$, 1055 [M − glucose]$^-$ and 909 [M − rhamnose − galactose]$^-$. The ^{13}C NMR spectrum of **2** resembled that of **1**, indicating they were only different in the substitution position of tigloyl group. By comparison of ^{13}C NMR signals due to aglycone of **1** with those of **2**, the obvious difference of chemical shifts occurred at C-16, C-21, C-22, C-28, C-29, indicating that C-28 was substituted by the tigloyl group. Therefore, saponin **2** were identified as 28-tigloylbarringtogenol C-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)-[β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranoside], named berneuxia saponin B.

On mineral acid hydrolysis, saponin **3** yielded the aglycone **3a**. There were 30 signals in ^{13}C NMR spectrum of **3a**. The EI-mass spectrum showed the molecular ion at *m/z* 472 $[M]^+$. According to the EI-mass spectrum and ^{13}C NMR spectrum, **3a** was identified as 3β,16α,28-trihydroxylolean-12-en-21-one, whose EI-mass spectrum and ^1H NMR spectrum were identical to those of armillarigenin from *Jacquinia armillaris* [8]. Four sugars, glucuronic acid, glucose, galactose and rhamnose, were detected by PC in the aqueous fraction after removal of the aglycone. The negative FAB-mass spectrum showed the fragment ions at *m/z* 1117 $[M-H]^-$, 971 [M − rhamnose]$^-$, 955 [M − glucose]$^-$ and 809 [M − rhamnose − galactose]$^-$. By comparison of the ^{13}C NMR signals due to the sugar moieties of **3** with those of **1**, the sugar moieties were the same. On the basis of above evidence saponin **3** was elucidated as armillarigenin-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)[β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranoside], named berneuxia saponin C.

Hydrolysis of **4** yielded **4a** as the aglycone and glucuronic acid, glucose, galactose and rhamnose as sugars. The EI-mass spectrum of its acetate showed fragment ions at *m/z* 273 [terminal rhamnose$(Ac)_3]^+$, 331 [terminal glucose $(Ac)_4]^+$ and 561 [rhamnose$(Ac)_3$ galactose$(Ac)_3]^+$. The negative FAB-mass spectrum showed the fragment ions at *m/z* 1135 $[M-H]^-$, 989 [M − rhamnose]$^-$, 973 [M − glucose]$^-$ and 827 [M − rhamnose − galactose]$^-$. Compound **4a** had the same R_f as the alkaline hydrolysate of **1a** on silica HPTLC with different solvent systems. ^{13}C NMR signals due to the aglycone were in accord with barringtogenol C except for the position of C-3 [9]. By comparison, of the ^{13}C NMR signals due to sugar moieties of **4** with those of **1**, the sugar moieties were the same. On the basis of above, **4** was elucidated as barringtogenol C-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)-[β-D-glucopyranosyl (1 → 2)-β-D-glucuronopyranoside], which was the same as desacyl jegosaponin from *Styrax japonica* [10].

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at 300 MHz in pyridine-d_5 and $CDCl_3$ using TMS as int. standard. EI-MS was measured at 40 eV accelerating voltage after acetylation. FAB-MS was measured with VG ZAB mass spectrometer. Optical rotations were measured with PE241 automatic recording spectropolarimeter.

Table 1. ^{13}C NMR shifts of compounds **1–4** and their prosapogenins (δ, ppm)

Carbon	**1***	**1a†**	**1b***	**2***	**2a†**	**3***	**3a†**	**4***
1	38.6	38.4	38.8	39.2	38.6	38.7	38.8	38.4
2	26.0	26.5	26.5	26.7	26.4	27.5	27.2	25.9
3	90.2	78.5	89.2	90.8	79.2	90.2	78.9	90.1
4	39.4	38.4	38.8	40.1	38.8	39.5	38.7	39.2
5	55.4	54.9	55.7	56.1	55.2	55.7	55.3	55.4
6	18.1	18.0	18.3	18.9	18.2	18.1	18.3	18.1
7	32.8	32.4	33.0	33.5	32.8	33.0	32.5	32.7
8	39.7	39.4	39.5	40.4	39.7	39.5	40.3	39.6
9	46.6	46.3	46.9	47.3	47.0	48.1	47.1	46.5
10	36.4	36.6	36.7	37.1	37.9	36.7	36.9	36.3
11	23.5	23.2	23.8	24.3	23.7	23.8	23.5	23.4
12	123.7	123.3	122.7	123.8	124.4	123.9	124.1	122.8
13	143.1	141.5	143.5	143.8	141.1	143.1	140.8	143.4
14	41.4	41.0	41.8	42.2	41.3	42.0	41.5	41.5
15	33.8	32.9	34.2	35.1	33.7	34.3	34.4	33.8
16	67.4	68.8	67.8	68.5	68.3	75.6	74.0	67.8
17	47.7	46.3	47.7	47.2	46.7	46.7	46.5	46.8
18	40.1	40.3	40.3	41.3	40.5	42.0	42.5	40.6
19	47.3	46.7	48.0	48.6	47.1	46.9	47.6	47.7
20	36.0	35.4	36.3	36.8	36.9	44.7	44.6	35.9
21	81.4	80.8	81.8	78.6	78.9	216.4	209.8	77.7
22	75.2	75.8	75.3	77.4	75.7	40.5	40.3	75.8
23	27.4	28.7	28.0	28.4	28.9	27.8	28.0	27.5
24	16.2	15.2	16.6	17.1	15.6	16.6	15.8	16.2
25	15.4	15.2	15.6	16.0	15.2	15.6	15.6	15.2
26	16.6	16.2	16.9	17.5	16.9	17.0	17.3	16.5
27	26.9	26.5	27.3	28.2	27.2	26.1	26.4	27.0
28	65.9	66.6	65.9	67.3	68.1	65.2	69.5	67.3
29	29.6	27.6	29.8	31.0	28.0	27.8	27.8	30.1
30	20.1	19.3	20.3	19.8	20.7	25.9	23.8	19.0
1′	168.4	169.4	168.5	168.4	167.9			
2′	129.4	128.4	129.8	129.5	128.7			
3′	135.9	137.5	136.1	137.7	138.0			
4′	12.0	11.7	12.4	12.7	12.2			
5′	14.1	14.0	14.1	14.7	14.2			
glu-1	104.9		105.7	105.5		104.9		104.6
2	78.6		82.4	79.3		78.9		78.6
3	81.1		77.8	82.1		81.7		81.3
4	71.9		73.0	71.9		72.5		72.1
5	75.2		76.8	76.2		75.7		75.3
6	172.2		170.3	176.9		175.3		175.1
			52.0					
glc-1	101.4		101.6	102.4		101.7		101.5
2	73.1		72.6	74.1		72.9		73.2
3	77.6		78.1	78.6		78.1		77.7
4	70.8		71.5	71.8		71.2		71.3
5	76.5		77.4	76.6		76.8		75.8
6	62.0		62.6	62.8		62.0		61.7
gal-1	101.9			102.8		102.0		101.9
2	77.8			79.0		78.1		78.1
3	75.6			76.2		76.1		75.3
4	70.8			71.8		71.2		71.3
5	76.5			77.4		76.8		76.7
6	63.1			64.0		63.5		63.1
rha-1	100.1			101.2		100.7		100.4
2	72.1			72.9		72.5		72.1
3	71.8			72.9		72.5		72.1
4	73.2			74.2		73.6		73.2
5	69.2			70.1		69.6		69.3
6	17.6			18.6		18.1		17.7

* pyridine-d_5;
† $CDCl_3$.

Plant material

Leaves of *Berneuxia thibetica* Decne. were collected in Xichang of Sichuan Province, China, and identified by Prof. R. N. Zhao. A specimen is deposited in the Herbarium of the Chengdu Institute of Biology, Chinese Academy of Sciences.

Extraction and isolation of saponins

Dry leaves (1.8 kg) were extracted with 95% EtOH. After removal of solvent by evapn, the combined extracts (180 g) were suspended in H_2O, extracted with petrol, EtOAc and *n*-BuOH successively. The *n*-BuOH part (45 g) were dissolved with MeOH, precipitated with Et_2O to obtain crude saponins (40 g). The crude saponin fr. was chromatographed on a silica gel column, eluted with $CHCl_3$–MeOH–H_2O to yield frs 1–46. Fr. 26 (1.12 g) was purified by silanised silica gel 60 to give saponins **1** (150 mg) and **2** (106 mg). Fr. 43 was purified by silanised silica gel 60 to give saponin **3** (202 mg). Fr. 46 was recrystallized to give saponin **4** (80 mg). Compound **1**, $C_{59}H_{94}O_{26}$, $[\alpha]_D^{13}$ −12.2° (MeOH, *c* 1.1). EI-MS *m/z*: 273 [terminal rhamnose(Ac)$_3$]$^+$, 561[rhamnose(Ac)$_3$galactose(Ac)$_3$]$^+$, 331[terminal glucose(Ac)$_3$]$^+$. FAB-MS *m/z*: 1219 [M+1]$^+$ ($C_{59}H_{94}O_{26}$+H). Negative FAB-MS *m/z*: 1217 [M−1]$^-$ ($C_{59}H_{94}O_{26}$−H), 1071 [M−rhamnose]$^-$, 1055 [M−glucose]$^-$, 909 [M−rhamnose−galactose]$^-$. Compound **1a** was identified as 21-tigloylbarringtogenol C by comparison with an authentic sample. Compound **2**, $C_{59}H_{94}O_{26}$, $[\alpha]_D^{26}$ −15.6° (MeOH, *c* 1.0). Negative FAB-MS *m/z*: 1217 [M-1]$^-$ ($C_{59}H_{94}O_{26}$−H), 1071 [M−rhamnose]$^-$, 1055 [M−glucose]$^-$, 909 [M−rhamnose−galactose]$^-$. Compound **2a**, white powder. ^{1}H NMR ($CDCl_3$): δ 6.89 (1H, *q*, *J* = 6.4 Hz, 3′-H), 5.35 (1H, *br s*, 12-H), 4.31 (1H, *br s*, 16-H), 4.09 (1H, *d*, *J* = 12.3 Hz, H-28), 3.84 (1H, *d*, *J* = 10.5 Hz, 21α-H), 3.75 (1H, *d*, *J* = 12.7 Hz, H-28), 3.70 (1H, *d*, *J* = 10.5 Hz, 22β-H), 3.23 (1H, *m*, 3α-H). Compound **3**, $C_{54}H_{86}O_{24}$, $[\alpha]_D^{13}$ −6.8° (MeOH, *c* 0.75). FAB-MS *m/z*: 1141 [M+Na]$^+$ ($C_{54}H_{86}O_{24}$+H). Negative FAB-MS *m/z*: 1117 [M-1]$^-$ ($C_{54}H_{86}O_{24}$−H), 971 [M−rhamnose]$^-$, 955 [M−glucose]$^-$, 809 [M−rhamnose−galactose]$^-$. Compound **3a**, colorless needles, mp 296–298 (MeOH) (lit. 299–301° [9]). EI-MS *m/z*: 472 [M]$^+$, 454, 436, 424, 264(a), 233, 215, 208(b), 190. ^{1}H NMR ($CDCl_3$): δ 5.44 (1H, *t*, *J* = 3.3 Hz, 12-H), 3.85 (1H, *m*, 16-H), 3.28 (1H, *m*, 3-H), 3.25 (1H, *d*, *J* = 5.0 Hz, 28-H), 3.21 (1H, *d*, *J* = 5 Hz, 28-H), 2.52 (2H, *d*, *J* = 6 Hz, 22-H), 1.32, 1.25, 1.14, 1.07, 1.00, 0.93, 0.79 (each 3H, *s*). Compound **4**, $C_{54}H_{88}O_{25}$, white powder. EI-MS *m/z*: 273 [terminal rhamnose(Ac)$_3$]$^+$, 561 [rhamnose(Ac)$_3$ galactose(Ac)$_3$]$^+$, 331 [terminal glucose(Ac)$_4$]$^+$. FAB-MS *m/z*: 1159 [M+Na]$^+$ ($C_{54}H_{88}O_{25}$+Na). Negative FAB-MS *m/z*: 1135 [M-1]$^-$ ($C_{54}H_{88}O_{25}$−H), 989 [M−rhamnose]$^-$, 973 [M−glucose]$^-$, 827 [M−rhamnose−galactose]$^-$.

Acid hydrolysis of the saponins and identification of the resulting monosaccharide

Saponin **1** (100 mg) was dissolved in MeOH and heated with 1M HCl/MeOH for 50 min. The soln was evaporated below 30°. The residue was chromatographed on silica gel to obtain **1b** (13 mg). Other frs were further hydrolysed with 5% H_2SO_4. The reaction mixture was diluted in H_2O and extracted with $CHCl_3$. Compound **1a** was isolated from the $CHCl_3$ layer. The aq. layer was neutralized with $Ba(OH)_2$ and concentrated, then subjected to PC analysis with authentic samples, Developing solvent BuOH–AcOH–H_2O (4:1:5) (upper layer), detection reagent: aniline−phthalate. Each saponin of **2–4** (20 mg) was heated with 5% H_2SO_4. The sugars were detected as above. The aglycones were obtained from $CHCl_3$ layer.

Acetylation of saponins

To each saponin (5 mg) was added Ac_2O–pyridine (1:1) (0.5 ml) in a microtube. After standing at room temp for 48 h, the soln was evapd to dryness and then subjected to EIMS analysis.

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