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TWO TRITERPENOID SAPONINS FROM *PTEROCEPHALUS BRETSCHNEIDRI*

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Key Word Index—*Pterocephalus bretschneidri*; Dipsacaceae; roots; triterpenoid saponins; bretschnoside A and B.

Abstract—Two new triterpenoid saponins, named bretschnosides A and B, were isolated from the roots of *Pterocephalus bretschneidri*. On the basis of chemical degradation and spectroscopic evidence, the structures of bretschnosides A and B were shown to be 3-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside 4 and 3-*O*-α-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside 6, respectively.

INTRODUCTION

There are only two species of the genus *Pterocephalus* growing in the southwest of China [1]. *Pterocephalus bretschneidri* (bat.) Pritz, a Chinese folk medicine, has long been used to treat rheumatism, influenza, fever etc. [2]. Triterpenoid saponins have been isolated from other genera of Dipsacaceae, such as *Cephalaria* [3], *Scabiosa* [4] and *Triplostegia* [5] and recently, W. G. Ma and coworkers reported seven new oleanane-type saponins, triplosides A–G, from *Triplostegia grandiflora* [5, 6]. We now report the isolation and structure elucidation of bretschnosides A and B.

RESULTS AND DISCUSSION

An ethanolic extract of the roots of *P. bretschneidri* gave two saponins, bretschnoside A (**4**) and B (**6**) in yields of 0.12 and 0.022%. On mineral acid hydrolysis, the two saponins yielded a common aglycone. On the basis of the ^{13}C NMR spectra and direct TLC comparison with an authentic sample, it was shown to be identical with oleanolic acid (**8**). A comparison of the ^{13}C NMR signals due to aglycone moieties with those of reported oleanane-type saponins revealed that the two saponins were bisdesmosides of **8** with glycosyl linkages at both the 3-hydroxy group and 28-carboxyl group.

Bretschnoside A (**4**) was hydrolysed with 1 M KOH to afford prosapogenin Ax (**5**). Compound **5** gave D-xylose and L-rhamnose in a ratio of 1:1 as sugar components following acid hydrolysis. Its negative FAB mass spectrum exhibited a molecular ion peak at *m/z* 1012 $[M]^-$ ($C_{52}H_{84}O_{19}$) and fragment ions at *m/z* 866 $[M-Rha]^-$, 734 $[M-Rha-Xyl]^-$, 588 $[M-2Rha-Xyl]^-$ and 456 $[M-2Rha-2Xyl]^-$, suggesting that the linear sugar sequence of **5** is aglycone-Xyl-Rha-Xyl-Rha. Its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were almost identical with those of triploside G which was isolated from *Triplostegia grandiflora* (Dipsacaceae) [6]. Thus, the structure of **5** was confirmed as oleanolic acid 3-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranoside.

On comparison of the ^{13}C NMR spectrum of bretschnoside A (**4**) with that of **3** [7], the β-D-gentiobiose proved to be attached to the 28-carboxyl group of prosapogenin Ax (**5**). Consequently, the structure of bretschnoside A (**4**) was established as 3-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

Bretschnoside B(**6**) provided prosapogenin Bx (**7**) on saponification. Compound **7** gave D-xylose, L-rhamnose and D-glucose in a ratio of 2:1:1 as sugar components. The formula of **7** was concluded from the negative ion at *m/z* 1028 $\{[M]^- (C_{52}H_{84}O_{20})\}$ and the fragment ions at *m/z* 866 $[M-Glc]^-$, 734 $[M-Glc-Xyl]^-$, 588 $[M-Glc-Xyl-Rha]^-$ and 456 $[M-Glc-2Xyl-Rha]^-$ indicated that the linear sugar sequence of **7** is aglycone-Xyl-Rha-Xyl-Glc.

Partial acid hydrolysis of **7** afforded two prosapogenins, Bx-1(**1**) and Bx-2(**2**). On acid hydrolysis, compound **1** gave D-xylose and L-rhamnose as sugar components. The negative ion FAB mass spectrum of **1** exhibited a molecular ion peak at *m/z* 734 $[M]^-$ ($C_{41}H_{66}O_{11}$), and fragment ions at *m/z* 588 $[M-Rha]^-$ and 456 $[M-Rha-Xyl]^-$. Its ^{1}H NMR spectrum had two anomeric proton signals

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Table 1. ^{13}C NMR spectral data of aglycone moieties (in pyridine-d_5)

C	1	2	4	5	3	6	7	8
1	39.0	39.0	39.1	39.1	38.8	39.1	39.0	39.0
2	26.9	27.0	27.0	27.0	26.6	27.0	27.0	23.9
3	88.6	88.6	88.7	88.6	89.6	88.6	88.6	78.2
4	39.8	39.8	39.9	40.0	39.6	39.7	39.8	39.8
5	56.2	56.2	56.3	56.2	55.8	56.2	56.2	55.9
6	18.8	18.7	18.7	18.7	18.5	18.7	18.7	18.9
7	33.3	33.4	33.3	33.3	33.1	33.2	33.3	33.4
8	39.6	39.7	39.7	39.7	39.9	40.0	39.6	39.5
9	48.1	48.1	48.1	48.2	48.0	48.2	48.1	48.2
10	37.1	37.1	37.2	37.2	36.9	37.1	37.1	37.5
11	23.8	23.9	23.9	23.8	23.7	23.8	23.9	23.9
12	122.6	122.6	122.2	122.9	122.8	122.9	122.6	122.6
13	144.9	144.9	145.6	144.3	144.1	144.2	144.9	145.0
14	42.2	42.2	42.4	42.2	42.1	42.2	42.2	42.3
15	28.1	28.2	28.2	28.2	27.9	28.2	28.2	28.1
16	23.8	23.9	23.8	23.8	23.7	23.8	23.9	23.5
17	46.7	46.7	48.1	47.1	47.0	46.6	46.7	46.8
18	42.0	42.0	42.1	42.2	41.7	41.8	41.9	42.1
19	46.5	46.5	47.1	46.4	46.3	47.1	47.0	46.6
20	31.0	31.0	31.2	30.9	30.8	30.8	30.9	31.0
21	34.3	34.3	34.6	34.1	34.0	34.3	34.3	34.5
22	33.3	33.4	33.6	33.3	32.5	33.3	33.3	33.4
23	28.4	28.4	28.6	28.4	28.3	28.4	28.4	28.4
24	17.2	17.4	17.4	17.4	16.7	17.4	17.3	17.5
25	15.6	15.7	15.7	15.8	15.5	15.8	15.7	15.6
26	17.4	17.5	17.7	17.6	17.5	17.6	17.5	16.6
27	26.3	26.3	26.3	26.2	26.1	26.2	26.3	26.2
28	180.3	180.3	180.5	176.6	176.5	176.6	180.3	180.4
29	33.3	33.4	33.3	33.3	33.1	33.2	33.3	33.3
30	23.8	23.9	23.9	23.8	23.4	23.5	23.5	23.9

at δ6.55 (1H, *br s*) and 4.82 (1H, *d*, J=7.2 Hz), and its ^{13}C NMR spectral data indicated the presence of a terminal α-L-rhamnopyranosyl unit and an inner β-D-xylopyranosyl unit (anomeric carbons: δ102.0, 106.2). In the ^{13}C NMR spectrum of **1**, the signal of C-2 due to xylose was shifted downfield to δ79.6, while the signals of C-1 and C-3 were displaced upfield to 106.2 and 78.0, respectively (Table 2). It was confirmed that the terminal rhamnose was attached to the C-2 position of the inner xylose. Thus, the structure of **1** was deduced as oleanolic acid 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranoside, which has already been obtained from *Cephalaria gigantea* and named giganteaside D [6].

Prosapogenin **2** afforded the same kinds of sugars as **1** following acid hydrolysis. The attachment sequence of sugars was determined by means of the negative ion FAB mass spectrum, in which a molecular ion peak was observed at *m/z* 866 $[M]^-$ ($C_{46}H_{74}O_{15}$) and the fragment ions at *m/z* 734 $[M-Xyl]^-$, 588 $[M-Xyl-Rha]^-$ and 456 $[M-2Xyl-Rha]^-$. By comparing the ^{13}C NMR data of **2** with those of **1**, the former showed a set of additional signals of a terminal xylose unit, and the C-3 of rhamnose of **1** was displaced downfield to δ83.1. This was in accordance with the presence of a 3-*O*-glycosylated-α-L-rhamnopyranoside [8, 9]. It was confirmed that the additional xylose was attached to the C-3 position of rhamnose. Accordingly, the structure of **2** was identified as oleanolic acid 3-*O*-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranoside.

On comparison of ^{13}C NMR spectra with those of **2**, compound **7** only showed a set of additional signals of a terminal glycopyranosyl unit which was proved to be attached at C-3 of the terminal xylose of **2**, for there was an obvious chemical shift (δ83.5) due to the C-3 position of the terminal xylose in **2**. In addition, the ^{13}C NMR spectral data (δ102.8, 61.8) of the terminal glucose in prosapogenin **7** was almost identical with those of α-D-glucopyranoside [10]. The ^{1}H NMR spectrum (δ4.96, *d*, J=5.6 Hz, Glc H-1) of **7** further supported this conclusion. From these results, the structure of **7** was elucidated to be oleanolic acid 3-*O*-α-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranoside.

Similarly, based on the comparison of the ^{13}C NMR spectrum of bretschnoside **B(6)** with that of **3**, saponin **6** showed a set of additional signals of β-D-gentiobiose which was proved to be attached to the 28-carboxyl group. Thus, the structure of bretschnoside **B(6)** is 3-*O*-α-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

Table 2. ^{13}C NMR spectral data of sugar moieties (in pyridine-d_5)

C	1	2	4	5	C	3	C	7	6
3-*O*-Xyl-1	106.2	106.2	106.2	106.2	3-*O*-GlcUA-1	105.4	3-*O*-Xyl-1	106.2	106.2
2	79.6	79.9	79.7	79.8	2	79.2	2	79.8	79.9
3	78.0	77.2	77.4	76.9	3	86.1	3	77.3	76.9
4	71.6	71.6	71.6	71.6	4	72.9	4	71.6	71.6
5	67.0	67.1	67.1	67.1	5	77.3	5	67.1	67.1
					6	171.9			
Rha-1	102.0	101.6	101.6	101.5	Glc-1	103.8	Rha-1	101.6	101.5
2	72.5	72.0	71.9	71.9	2	76.5	2	71.9	71.9
3	72.6	83.1	82.5	82.4	3	78.4	3	82.6	82.4
4	74.2	73.1	73.0	73.0	4	72.5	4	73.0	73.1
5	69.8	69.8	69.6	69.7	5	77.8	5	69.6	69.7
6	18.6	18.6	18.7	18.7	6	63.3	6	18.6	18.6
Xyl-1		107.6	107.0	107.1	Ara-1	105.2	Xyl-1	107.1	107.2
2		75.7	75.8	75.9	2	71.5	2	75.8	75.9
3		78.6	83.3	83.3	3	74.7	3	83.4	83.4
4		71.2	70.0	70.0	4	69.6	4	69.8	71.0
5		67.5	67.4	67.5	5	67.8	5	67.4	67.5
Rha-1			102.4	102.8			Glc-1	102.8	102.8
2			72.5	72.5			2	72.5	72.6
3			72.7	72.7			3	74.1	74.2
4			74.2	74.2			4	70.0	70.0
5			69.8	69.8			5	72.7	72.7
6			18.7	18.7			6	61.8	61.8
28-*O*-Glc-1				95.8		95.7			95.8
2				75.2		75.2			75.2
3				78.4		78.4			78.5
4				71.6		71.5			71.6
5				78.8		78.7			78.8
6				69.4		69.4			69.5
Glc-1				105.3		105.3			105.3
2				74.0		73.9			74.0
3				78.0		78.0			78.1
4				70.9		70.9			70.9
5				78.5		78.6			78.5
6				62.7		62.6			62.7

EXPERIMENTAL

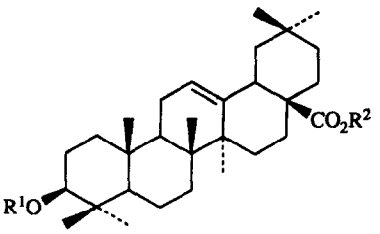
Mps.: uncorr; optical rotations: MeOH; ^{1}H and ^{13}C NMR: pyridine-d_5 with TMS as int. stand; TLC: silica gel G using solvent systems: $CHCl_3$–MeOH–H_2O (13:7:2, lower phase) and *n*-BuOH–HOAc–H_2O (4:1:5, upper phase). The spots were visualized by spraying $C_6H_5NH_2$–*o*-$C_6H_4(CO_2H)_2$–*n*-BuOH (2:3:200) in PC. CC: Kieselgel 60 (70–230 mesh, Merck) and Diaion HP-20 (Mitsubishi).

Plant material. Pterocephalus bretschneidri (Bat.) Pritz was collected at Xiangcheng (Sichuan), China, in August 1990 and identified by Prof. S. C. Xiao (Chengdu Institute of Biology, Academia Sinica). The voucher specimen is kept in CDBI.

Extraction and separation. The dried and powdered roots (4.2 kg) were extracted (× 3) with 95% EtOH. After the removal of solvent, the residue (334 g) was successively fractionated with petrol (bp 60–90°), EtOAc and *n*-BuOH. The *n*-BuOH extract (212 g) was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (from 50:10:1 to 10:10:1) to give frs A–G. Fr. E (30 g) was subjected to CC over silica gel developing with $CHCl_3$–MeOH–H_2O (16:6:1) to provide frs E1 and E2. Fr. E1 was again chromatographed on Diaion HP-20P (eluting with MeOH–H_2O, 7:3) to afford 5.1 g of **4**, whose yield was 0.12%. Fr. F (28.5 g) was subjected to CC over silica gel eluting with $CHCl_3$–MeOH–H_2O (10:5:1) to provide frs F1–F3. Fr. F2 was first chromatographed on Diaion HP-20P (eluting with MeOH–H_2O, 4:1) to give fractions Fa and Fb. Fr. Fb was then subjected to CC over reversed phase silica gel (RP-8) eluting with MeOH–H_2O (7:3) to afford 0.85 g of **6**, whose yield was 0.022%.

Bretschnoside A(**4**). Powder, mp 216–218°. $[\alpha]_D^{22}$ −39.64° (MeOH; *c* 0.312). IR ν_{max} cm^{-1}: 3300 (OH), 1720, 1630. (Found C, 55.86; H, 7.79 $C_{64}H_{104}O_{29}\cdot 2H_2O$ requires: C, 55.98; H, 7.87%). ^{1}H NMR: δ6.46 (1H, *s*, Rha H-1), 6.15 (1H, *d*, *J* = 7.8 Hz, Glc H-1), 6.12 (1H, *s*, Rha′ H-1); ^{13}C NMR: Tables 1 and 2.

Bretschnoside B(**6**). Powder, mp 209–212° (from MeOH–H_2O). $[\alpha]_D^{22}$ −26.67° (MeOH; *c* 0.305). IR ν_{max}



	R^1	R^2
1	-Xyl^{2}-Rha	-H
2	-Xyl^{2}-Rha—Xyl	-H
3	-GlcUA(^{2}Glc, ^{3}Ara)	-Glc^{6}-Glc
4	-Xyl^{2}-Rha^{3}-Xyl^{3}-Rha	-Glc^{6}-Glc
5	-Xyl^{2}-Rha^{3}-Xyl^{3}-Rha	-H
6	-Xyl^{2}-Rha^{3}-Xyl^{3}-Glc (α)	-Glc^{6}-Glc
7	-Xyl^{2}-Rha^{3}-Xyl^{3}-Glc (α)	-H
8	-H	-H

cm^{-1}: 3300 (OH), 1720, 1630. (Found C, 54.54; H, 7.76 $C_{64}H_{104}O_{30}\cdot 3H_2O$ requires: C, 54.62; H, 7.82%). ^{1}H NMR: δ6.58 (1H, *s*, Rha H-1), 6.26 (1H, *d*, $J=8.0$ Hz, Glc′ H-1), 5.36 (1H, *d*, $J=7.9$ Hz, Glc″ H-1), 5.03 (2H, *d*, $J=8.4$ Hz, Xyl H-1, Xyl′ H-1), 4.96 (1H, *d*, $J=5.6$ Hz, Glc H-1); ^{13}C NMR: Tables 1 and 2.

Acid hydrolysis of compounds **4** *and* **6**. Saponins **4** (80 mg) and **6** (50 mg) were hydrolysed with 2 M HCl in 5% MeOH under reflux for 2 hr, respectively. The reaction mixture was neutralized with a saturated soln of Na_2CO_3 and concd to dryness. The residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract was concd. to dryness and then recrystallized with MeOH to give **8**.

Aglycone **8**. Needles, mp 305–306°. ^{13}C NMR: Table 1.

Alkaline hydrolysis of compounds **4** *and* **6**. Saponins **4** (200 mg) and **6** (600 mg) in 1 M KOH (50% MeOH) were refluxed for 1 hr, respectively. The reaction mixture was neutralized with 36% HOAc and then concd to dryness. The residue was extracted with *n*-BuOH and then chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (30:10:1) to give **5** (76 mg) and **7** (480 mg).

Compound Ax(**5**). Powder from MeOH, mp 231–235° (dec.) {ref. [6] 224–230° (dec.)}. $[\alpha]_D^{22}-21.48°$ (MeOH, *c* 0.347). ^{1}H NMR: δ6.54 (1H, *s*, Rha H-1), 6.23 (1H, *s*, Rha′ H-1), 5.34 (2H, *d*, $J=7.8$ Hz, Xyl H-1, Xyl′ H-1); ^{13}C NMR: Tables 1 and 2.

Compound Bx(**7**). Powder, mp 228–231°. $[\alpha]_D^{22}-17.50$ (MeOH; *c* 0.413). ^{1}H NMR: δ6.55 (1H, *s*, Rha H-1), 5.33 (2H, *d*, $J=8.0$ Hz, Xyl H-1, Xyl′ H-1), 4.96 (1H, *d*, $J=5.6$ Hz, Glc H-1); ^{13}C NMR: Tables 1 and 2.

Partial acid hydrolysis of compound **7**. Compound **7** (450 mg) was hydrolysed with 0.25 M HCl in 50% EtOH under reflux for 40 min. The reaction mixture was neutralized with a saturated soln of $NaHCO_3$ and concd to dryness. The residue was partitioned between H_2O and *n*-BuOH. The *n*-BuOH extract was subjected to CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (50:10:1) to afford prosapogenins Bx-1 (18 mg) and 2 (25 mg), as well as aglycone (**8**).

Compound Bx-1(**1**). Powder, mp 254–258° (dec.) {ref. [6] 255–260° (dec.)}. ^{1}H NMR: δ6.55 (1H, *s*, Rha H-1), 4.82 (1H, *d*, $J=7.2$ Hz, Xyl H-1); ^{13}C NMR: Tables 1 and 2.

Compound Bx-2(**2**). Powder, mp 248–252° (dec.) {ref. [6] 245–250° (dec.)}. ^{1}H NMR: δ6.60 (1H, *s*, Rha H-1), 5.39 (1H, *d*, $J=7.6$ Hz, Xyl H-1), 4.82 (1H, *d*, $J=7.2$ Hz, Xyl′ H-1); ^{13}C NMR: Tables 1 and 2.

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