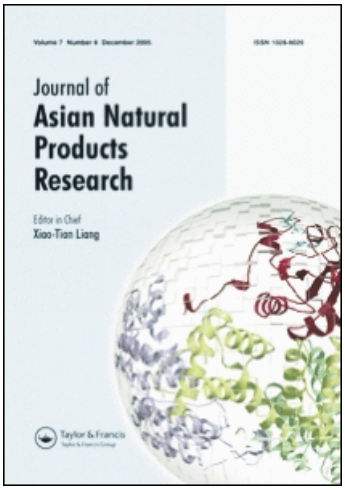

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Triterpenoid prosapogenols and prosapogenins from the husks of *Xanthoceras sorbifolia*

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Two new prosapogenins, 16-*O*-acetyl-21-*O*-(4-angeloyl)-α-L-rhamnopyranosyl barringtogenol C (**1**), 28-*O*-β-D-glucopyranosyl 16-deoxybarringtogenol C (**2**), were isolated from the acid hydrolyzate of the crude saponin obtained from the husks of *Xanthoceras sorbifolia* Bunge, along with six known triterpenoids. These structures were established on the basis of chemical and detailed spectral evidences. Compounds **1** and **2** showed cytotoxic activity against human cell lines (A375-S2, HeLa).

Keywords: *Xanthoceras sorbifolia*; Sapindaceae; Prosapogenol; Prosapogenin; Cytotoxic activity

1. Introduction

The wood and seeds of *X. sorbifolia* (Sapindaceae) are used as a folk remedy in China to cure rheumatism and enuresis in children [1]. Prior phytochemical studies yielded triterpenoids from the extract of the seeds of the titled plant [2–5]. In our previous work, a saponin and several sapogenols were identified [6,7]. This paper deals with the structural elucidation of two new prosapogenins **1** and **2** obtained in the continued research, and their cytotoxic activities.

2. Results and discussion

Compound **1** was obtained as an amorphous powder. The molecular formula of $C_{43}H_{68}O_{11}$ was determined by HRESI-MS (*m*/*z* 783.4643 $[M + Na]^+$) and NMR data. The positive reactions to Liebermann-Burchard and Molish tests characterised it as a triterpene glycoside. The ^{1}H NMR spectrum displayed the characteristic methyls and olefinic proton signals of olean-12-ene structural moiety, and the signals of H-21, H-22 at δ 4.11, 4.58

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(each 1H, d, $J = 9.5$ Hz) which were assigned by their correlations with the adjacent carbons in the HMBC spectrum. Additionally, the signals of an acetyl group at δ 2.20 (3H, s) and those of an angeloyl functionality at δ 5.84 (1H, m), 1.88 (3H, br. s) and 1.95 (3H, d, $J = 7.3$ Hz), along with an anomeric proton appearing at δ 5.46 (1H, br. s) and a methyl doublet at δ 1.35 (3H, d, $J = 6.3$ Hz) ascribable to a 6-deoxy hexose were observed. The ^{13}C NMR spectrum showed the signals according to the acetyl and angeloyl moieties above, together with six carbons of the sugar moiety at δ 104.3, 72.6, 72.0, 75.1, 68.2 and 18.1 assigned to rhamnose which was also detected on acid hydrolysis of **1**. The α-anomeric configuration of the sugar was ascertained based on its diagnostic C-5 signal at δ 68.2 [8]. Furthermore, 30 typical carbon signals in agreement with those of barringtogenol C (**3**), except for the remarkable downfield shifts of C-16 and C-21 were discerned.

The NMR data were assigned appropriately by the HSQC and HMBC experiments. In the HMBC spectrum, crosspeaks observed between H-1 of Rha at δ 5.46 and C-21 at δ 89.9, together with the long-range correlation between H-16 at δ 5.92 and the carbon at δ 169.7 (—O*C*OMe) suggested the rhamnose and acetyl moiety were located at C-21 and C-16, respectively. In addition, the correlation between H-4 of Rha at δ 5.84 and carbon at δ 168.0 (angeloyl C-1) defined the position of angeloyl group at C-4 (δ 75.1) of the rhamnose moiety. Consequently, compound **1** was identified as 16-*O*-acetyl-21-*O*-(4-*O*-angeloyl)-α-L-rhamnopyranosyl barringtogenol C (figure 1).

Compound **2**, positive to the Molish test, was obtained as white needles and assigned a molecular formula of $C_{36}H_{60}O_9$ established by HRESI-MS (*m*/*z* 659.4127 $[M + Na]^+$) and NMR data. ^{1}H NMR spectrum showed the signals of the aglycone similar to those of compound **4** (16-deoxybarringtogenol C) [9], except for the broad singlet at δ 4.16 (2H, br. s) instead of the AB coupling system in H-28 of **4** at δ 4.16, 3.81 (each 1H, d, $J = 10.3$ Hz). Moreover, an anomeric proton of the sugar moiety at δ 4.99 (1H, d, $J = 7.8$ Hz) was also observed. Among the 36 carbon signals in the ^{13}C NMR spectrum, 30 signals were assigned to the aglycone. The other six carbon signals at δ 105.5, 75.2, 78.7, 71.7, 78.5, and 62.8, combined with the result of the acid hydrolysis of **2**, were indicative of the presence of a glucose, whose β-anomeric configuration was determined by its large J_{H1-H2} value of 7.8 Hz.

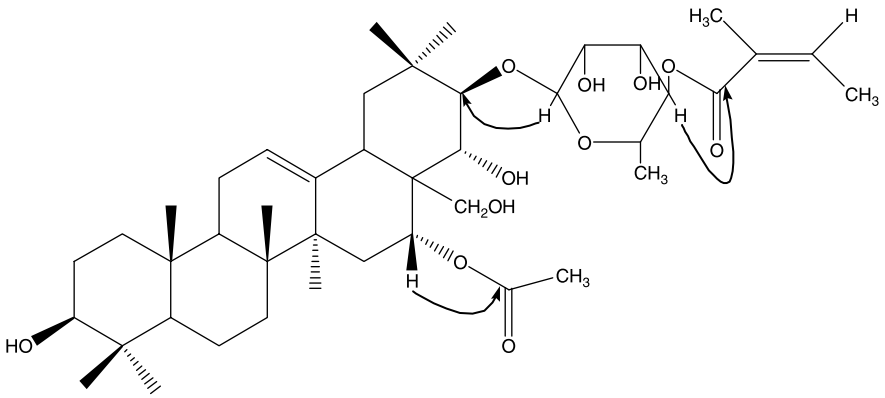

Figure 1. Structure and key HMBC correlations of **1**.

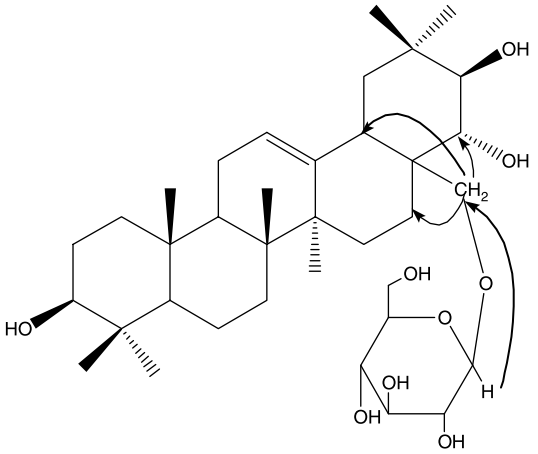

Figure 2. Structure and key HMBC correlations of **2**.

In the HMBC experiment, the proton signal at δ 4.16 was designated to the methylene at C-28 on the basis of its correlations with carbons at δ 18.5 (C-16), 42.2 (C-18), and 74.8 (C-22). Accordingly, the carbon signal at δ 74.4 was designated to C-28 by the HSQC experiment. Further analysis of the HMBC spectrum showed the correlation between the anomeric proton (δ 4.99) and C-28, which confirmed the attachment of the sugar moiety. Based on the observations above, compound **2** was characterised as 28-*O*-β-D-glucopyranosyl 16-deoxybarringtogenol C (figure 2).

Compounds **1**–**8** were tested for the cytotoxic activities *in vitro* against human tumour cell lines (A375-S2, HeLa), among which **1**–**6** showed activity with IC_{50} values from 15.1 to 74.5 μmol/L, compounds **7** and **8** were nearly inactive (see table 2). Therefore, the structure–activity relationship could be established as that the glycosidation at C-21 played positive role in the cytotoxic activity, while the hydroxy groups at C-15 or -16, and the sugar moieties attached to C-28 were negative to the activity.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco MP-S_3 apparatus and are uncorrected. ESI-MS were performed on an Agilent 1100 ion trap spectrometer. HRESI-MS were taken on QSTAR LCQ mass spectrometer. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. NMR spectra were taken on a Bruker-ARX-300 or Bruker-AV-600 spectrometer, using TMS as an internal standard. Macroporus resin (HPD-100) was produced by Cangzhou Bon Chemical Co. Ltd, P.R. China.

3.2 Plant material

The plant material was collected from Shenyang, Liaoning Province, China, in October 1997 and identified by Associate Professor Bai-Zhen Yang. A voucher specimen (No. 0154620) is

deposited in the Herbarium Department of the Institute of Applied Ecology, Chinese Academy of Sciences.

3.3 Extraction and isolation

The crushed husks of *X. sorbifolia* Bunge (15.0 kg) were extracted with 70% aqueous EtOH (120 L × 3). After evaporation of the solvent *in vacuo*, the extract was suspended in H_2O and partitioned with $CHCl_3$ (5 L × 3) and n-BuOH (5 L × 5), successively. A part of the n-BuOH extract (40.0 g), was dissolved in water (200 ml) and subjected to column chromatography on macroporus resin (HPD-100, 400 g), eluted with H_2O (1500 ml), 70% aqueous EtOH (1500 ml) and EtOH (1500 ml) successively. Evaporation of the 70% EtOH eluate *in vacuo* gave the crude saponin as pale powder (20.0 g).

A solution of crude saponin (3.0 g) in 1 M HCl/EtOH (1:1, 150 ml) was refluxed for 2 h. The reaction mixture was diluted with 50 ml of H_2O and concentrated to 100 ml to afford a brown precipitate, which was washed with H_2O to yield a mixture of sapogenols and prosapogenins (600 mg).

The mixture (500 mg) was chromatographed on silica gel (10.0 g) eluted in a stepwise manner with petroleum ether (PE, 60–90°C)/EtOAc/acetone (from 15:1:1 to 1:1:1, 100 ml each) to give **5** (11:1:1, 10 mg) and **8** (9:1:1, 150 mg). The subfraction (5:1:1, 180 mg) was further subjected to column chromatography on silica gel (5.0 g) eluted with $CHCl_3$/MeOH to afford **6** (100:1, 25 ml, 15 mg), **1** (100:2, 25 ml, 20 mg) and **4** (100:4, 50 ml, 30 mg). Rechromatography of the subfraction (1:1:1, 70 mg) on silica gel (5.0 g) with $CHCl_3$/MeOH (100:10) as the elution led to the isolation of **3** (25 ml, 15 mg), **7** (50 ml, 9 mg) and **2** (200 ml, 25 mg).

3.3.1 Compound 1. White amorphous powder, mp 230–232°C. $[\alpha]_D^{20}$ − 15 (*c* 0.10, MeOH). HRESI-MS *m/z* 783.4643 $[M + Na]^+$ (calcd for $C_{43}H_{68}O_{11}Na$, 783.4659). Positive ESI-MS: *m/z* = 783.5 $[M + Na]^+$, 723.4 $[M + Na\text{-}AcOH]^+$, 495.1 $[M + Na\text{-}AcOH\text{-}angeloxyl\text{-}Rha]^+$; negative ESI-MS: *m/z* = 795.9 $[M + Cl]^-$; DEPT, ^1H NMR and ^{13}C NMR data: see table 1.

3.3.2 Compound 2. White needles (MeOH), mp 272–274°C. $[\alpha]_D^{20}$ − 22 (*c* 0.10, MeOH/H_2O 1:1). HRESI-MS *m/z* 659.4127 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_9Na$, 659.4135). Positive ESI-MS: *m/z* = 659.4 $[M + Na]^+$, 258.2; negative ESI-MS: *m/z* = 671.2 $[M + Cl]^-$; DEPT, ^1H NMR and ^{13}C NMR data: see table 1.

The known compounds barringtogenol C (**3**), 16-deoxybarringtogenol C (**4**), 21,22-di-*O*-angeloyl barringtogenol C (**5**), 21-*O*-(3,4-di-*O*-angeloyl)-β-D-fucopyranosyl barringtogenol C (**6**), R_1-barrigenol (**7**), 21,22-di-*O*-angeloyl R_1-barrigenol (**8**), were identified by comparison of the physical and NMR data with those reported in the literature [2,4,9–11].

3.4 Cytotoxic activity assay

The MTT method was used as described [12] with six concentration levels (10, 20, 50, 60, 80, 100 μmol/L). Taxol was added paralleling with the tested compounds as the reference. Cell growth was measured by MTT with a plate reader. The percentage of cell inhibition was calculated as follows: Cell death (%) = $[A_{490}(\text{control}) - A_{490}(\text{test})]/A_{490}(\text{control}) \times 100\%$. IC_{50} values were calculated with the LOGIT method.

Table 1. ^{1}H NMR, ^{13}C NMR and DEPT (135°) spectral data of **1** and **2** (δ value, in C_5D_5N).

1			***2***		
Position	*^{1}H (J Hz)*	*^{13}C*	*Position*	*^{1}H (J Hz)*	*^{13}C*
1	1.00, 1.54	39.1t	1	1.00, 1.58	39.1t
2	1.50, 1.82	28.1t	2	1.82, 1.87	28.1t
3	3.40 (dd, 11.0, 5.0)	78.0d	3	3.43 (dd, 11.1, 4.7)	78.5d
4		39.4s	4		39.4s
5	0.80	55.8d	5	0.85 (d, 11.5)	55.8d
6	1.37, 1.57	18.7t	6	1.38, 1.54	18.8t
7	1.15, 1.40	33.2t	7	1.25, 1.51	32.9t
8		39.7s	8		40.2s
9	1.68	47.1d	9	1.65 (t, 8.8)	48.0d
10		37.2s	10		37.2s
11	1.80, 1.87	23.9t	11	1.86, 1.90	23.9t
12	5.47 br s	124.5d	12	5.34 br s	123.4d
13		141.9s	13		143.5s
14		41.4s	14		41.9s
15	1.66, 1.90	31.1t	15	1.05, 1.86	25.8t
16	5.92 br s	71.8d	16	1.96	18.5t
				2.27 (d, 13.5)	
17		47.8s	17		43.7s
18	2.89 (d, 13.6)	40.1d	18	2.66 (d, 13.6)	42.2d
19	1.34	47.3t	19	1.31 (dd, 13.6, 4.6)	46.7t
	2.55 (t, 13.6)			2.13 (t, 13.6)	
20		37.1s	20		36.3s
21	4.11 (d, 9.5)	89.9d	21	3.79 (d, 9.9)	76.9d
22	4.58 (d, 9.5)	70.4d	22	4.44 (d, 9.9)	74.8d
23	1.23	28.7q	23	1.24	28.8q
24	1.03	16.6q	24	1.05	16.6q
25	0.90	16.0q	25	0.98	15.8q
26	0.82	16.9q	26	0.92	17.1q
27	1.45	27.1q	27	1.26	26.3q
28	3.62, 3.92 (d, 10.2)	65.1t	28	4.16 br s	74.4t
29	1.11	30.6q	29	1.25	30.4q
30	1.19	19.9q	30	1.22	19.3q
16-acyl			28-sugar		
1′		169.7s	1′	4.99 (d, 7.8)	105.5d
2′	2.20	22.1q	2′	4.05 (t, 7.8)	75.2d
21-sugar			3′	4.19 (dd, 9.0, 7.8)	78.7d
1″	5.46 br s	104.3d	4′	4.24 (dd, 9.0, 8.0)	71.7d
2″	4.68 br s	72.6d	5′	3.95 m	78.5d
3″	4.58 (d, 9.5)	72.0d	6′	4.42 (dd, 11.4, 4.8)	62.8t
				4.53 (d, 11.4)	
4″	5.84	75.1d			
5″	4.74 (dd, 9.5, 6.3)	68.2d			
6″	1.35 (d, 6.3)	18.1q			
4″-acyl					
1‴		168.0s			
2‴		128.7s			
3‴	5.84	137.3d			
4‴	1.95 (d, 7.3)	15.8q			
5‴	1.88	20.9q			

Table 2. Inhibition on tumour cell lines of compounds **1**–**8** (IC_{50}, μmol/L).

	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	*Taxol*
A375-S2	25.0	70.3	74.4	52.4	70.3	34.1	>100	>100	39.5
HeLa	15.1	74.5	>100	49.4	>100	63.7	>100	>100	15.4

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