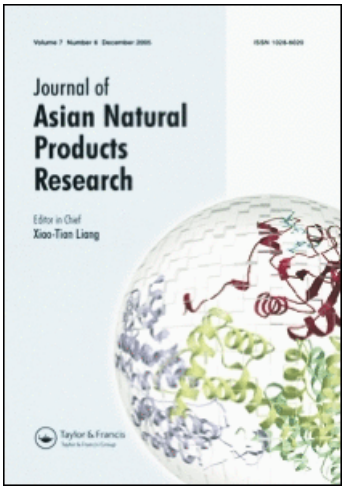

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Li, Wei , Li, Xian , Yang, Jia , Meng, Da-Li and Li, Ning(2008) 'Two new triterpenoid saponins from the carpophore of *Xanthoceras sorbifolia* Bunge', Journal of Asian Natural Products Research, 10: 3, 260 — 264
To link to this Article: DOI: 10.1080/10286020701605224
URL: http://dx.doi.org/10.1080/10286020701605224

Journal of Asian Natural Products Research
Vol. 10, No. 3, March 2008, 260–264

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Two new triterpenoid saponins from the carpophore of *Xanthoceras sorbifolia* Bunge

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(*Received 25 December 2006; final version received 18 April 2007*)

Two new triterpenoid saponins (**1**, **2**) and a known sapogenin (**3**) were isolated from the dried carpophore of *Xanthoceras sorbifolia* Bunge. (Sapindaceae), and their structures were established as 3-*O*-β-D-glucopyranosyl, 28-*O*-[α-L-rhamnosyl (1 → 2)]-β-D-glucopyranosyl 16-deoxybarrin-gtogenol C (**1**), 3-*O*-[β-D-glucopyranosyl(1 → 6)] 3′-angeloyl)-β-D-glucopyranosyl, 28-*O*-[α-L-rhamnosyl(1 → 2)]-β-D-glucopyranosyl 16-deoxybarringtogenol C (**2**), 16-deoxybarringtogenol C (**3**), by spectral and chemical methods. Compounds **1** and **2** were found to have activity of inhibiting the proliferation of two human tumour cell lines while compound **3** was inactive.

Keywords: *Xanthoceras sorbifolia*; Sapindaceae; triterpenoid saponins; cytotoxic

1. Introduction

Xanthoceras sorbifolia Bunge. (Sapindaceae) is a shrub distributed in Inner Mongolia, China and has been used as a folk medicine to treat rheumatism and enuresis of children. Previous phytochemical studies on this plant revealed the presence of saponins [1–6], flavonoids [7], coumarins [8] from the fruit, bark and husks of *X. sorbifolia*. In our preceding paper [9], we reported some chemical constituents of the carpophore of *X. sorbifolia*. Here we report the isolation and identification of two new triterpenoids together with a known sapogenin, as well as their inhibiting the proliferation activities against human tumour cell lines.

2. Results and discussion

Compound **1** was obtained as white powder from MeOH. Its molecular formula was determined as $C_{48}H_{80}O_{18}$ on the basis of its HREI-MS (*m/z* 944.5342 $[M]^+$). It was considered to be a triterpenoid glycoside due to a positive Liebermann–Burchard and Molish reactions, as well as its NMR spectral data. On hydrolysis, two kinds of monosaccharide units were obtained and identified as glucose and rhamnose by co-TLC with authentic sample. The ^{1}H NMR spectrum revealed seven tertiary methyl signals at δ 0.89, 1.01, 1.10, 1.18, 1.18, 1.30, 1.32, one olefinic proton at δ 5.44 and three anomeric protons at δ 4.94 (1H, d, $J = 7.7$ Hz), 4.73 (1H, d, $J = 7.7$ Hz), 6.60 (1H, s) which indicated the anomeric configurations of two glucoses were β orientation. The ^{13}C NMR spectrum showed three anomeric carbons at δ 107.0, 103.7, 100.6 as well as a pair of olefinic carbons at δ 123.9, 143.2. By comparison of the ^{13}C NMR spectral data of **1** with those of compound **3**, the distinguished downfield shift of C-3 and C-28 indicated that the two sugar moieties of **1** were linked at C-3 and C-28 positions. In the HMBC experiment, the long-range correlations occurred between H-1′ (δ 4.94) and C-3 (δ 88.8), H-1″ (δ 4.73) and C-28 (δ 74.9) while the linkage of the rhamnose was not observed. In the ROESY spectrum of **1**, the NOE interaction was found between the anomeric proton of the rhamnose (δ 6.60) and H-2″ (δ 4.30) of glucose. Besides, the deshielding of C-2″ (δ 80.4) of glucose also suggested the point of linkage of the rhamnose. From the above evidence and combined with the ^{1}H–^{1}H COSY, HMQC experiments, the structure of **1** was established as 3-*O*-β-D-glucopyranosyl, 28-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranosyl 16-deoxybarringtogenol C (Figure 1).

Compound **2** was obtained as white powder from MeOH and responded positively to Liebermann–Burchard and Molish reactions. The molecular formula of **2** was deduced as $C_{59}H_{96}O_{24}$ from HREI-MS (*m/z* 1188.6291 $[M]^+$) and NMR spectral data. The sugar moieties were identified as glucose and rhamnose by acid hydrolysis, and co-TLC with the authentic samples. In the ^{1}H NMR spectrum of **2**, the signals at δ 1.82 (3H, brs), 1.94 (3H, d, $J = 7.2$ Hz), 5.83 (1H, q, $J = 7.2$ Hz) could be assigned to an angeloyl function. Additionally, four anomeric protons at δ4.86 (1H, d, $J = 6.8$ Hz), 5.08 (1H, d, $J = 7.5$ Hz), 4.73 (1H, d, $J = 7.7$ Hz), 6.60 (1H, s) were observed which indicated the configurations of the three glucoses were β orientation. Besides, the ^{1}H NMR spectrum also gave seven singlets of methyls at δ 0.89, 0.97, 1.09, 1.19,

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020701605224
http://www.informaworld.com

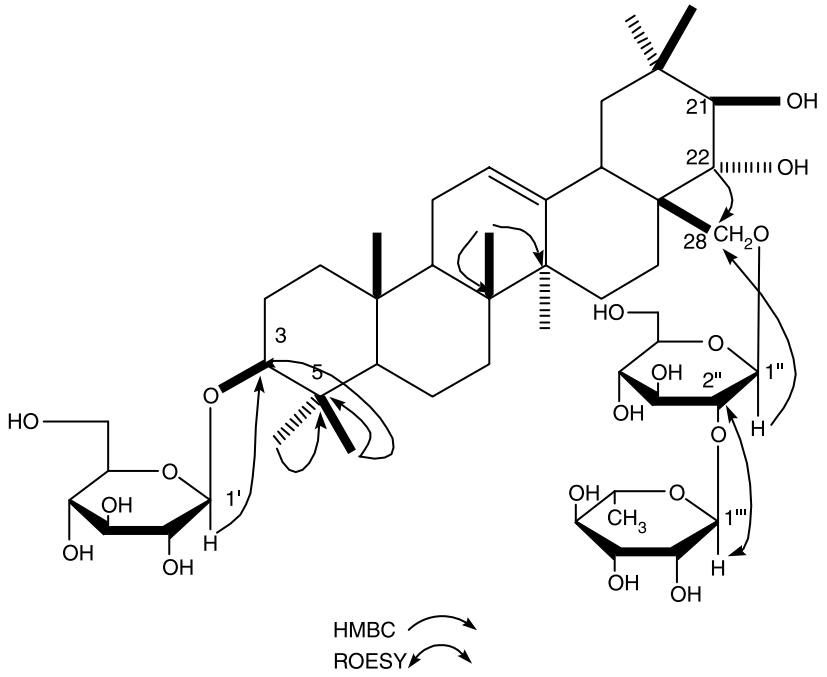

Figure 1. The important HMBC and ROESY correlations for compound **1**.

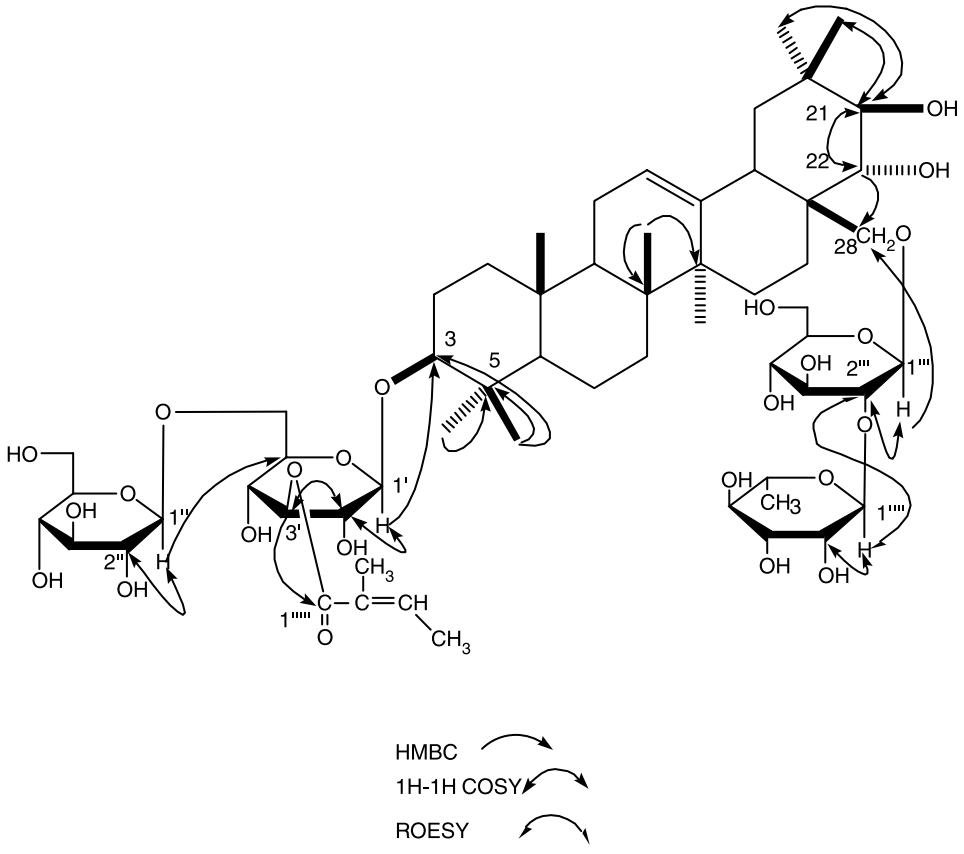

Figure 2. The important HMBC, ROESY and $^{1}H-^{1}H$ COSY correlations for compound **2**.

1.22, 1.23, 1.26. The ^{13}C NMR spectrum showed four anomeric carbons of sugars at δ 106.8, 105.6, 103.7, 100.6, two pairs of olefinic carbons at δ 123.9, 143.2, 128.9, 137.0 and a carboxyl carbon at δ 168.1. Comparison of the NMR spectral data of **2** with those of compound **1** indicated that compound **2** differed from compound **1** by the presence of an additional glucose and angeloyl group. Sequencing of the diglycosidic chains and the position of angeloyl group were confirmed by the HMBC spectrum, in which the long-range correlations were observed between H-1′ (δ 4.86) and C-3 (δ 89.3), H-1″ (δ 5.08) and C-6′ (δ 70.0), H-1‴ (δ 4.73) and C-28 (δ 74.9), H-3′ (δ 5.90) and C-1⁗ (δ 168.1). The linkage of the rhamnose was confirmed by the NOE interactions between the anomeric proton of the rhamnose (δ 6.60) and H-2‴ (δ 4.31) of glucose in the ROESY spectrum of **2** and the deshielding of C-2‴ (δ 80.4) of glucose, respectively. With the above data and combined with $^1H-^1H$ COSY, HMQC, and TOCSY experiments, the structure of **2** was established as 3-*O*-[β-D-glucopyranosyl(1 → 6)](3′-angeloyl)-β-D-glucopyranosyl, 28-*O*-[α-L-rhamnopyranosyl (1 → 2)]-β-D-glucopyranosyl 16-deoxybarringtogenol C (Figure 2).

The known compound **3** was identified as 16-deoxybarringtogenol C by comparison of its physical and NMR spectral data with those reported [10].

Using the MTT method, compounds **1**, **2**, and **3** were tested for their anti-tumour activity *in vitro* against two cell lines (A375-S2 and HeLa). **1** and **2** showed activity against A375-S2 cell line with IC_{50} values of 1191.09 μmol/L and 274.19 μmol/L, respectively, while inactive against HeLa. **3** was found inactive against both cell lines. The screening results indicated that the cytotoxic activities have some relationship with the glycoside of the aglycone and the substitute of the angeloyl group.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yanaco micro-hot-stage apparatus and are uncorrected. The UV spectra were recorded on a Shimadzu-2201 spectrometer. IR spectra were measured on a Perkin–Elmer 2000 FT-IR spectrometer as KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker-ARX-300 or an AV-600 spectrometer, using TMS as an internal standard. HREI-MS was measured with a Bruker AREX II mass spectrometer. The optical rotation was measured on Perkin–Elmer 241 polarimeter. The chromatographic silica gel (200–300 mesh) was produced by Qingdao Ocean Chemical Factory. Macroporous resin HPD100 was produced by Hebei Cangzhou BaoEn Chemical Factory.

3.2 Plant material

The carpophores of *Xanthoceras sorbifolia* Bunge. were collected in October 2003 at Shenhe district, Shenyang, China. A voucher specimen (No. 0187512) is deposited in the Herbarium Department of the Institute of Applied Ecology, Chinese Academy of Sciences.

3.3 Extraction and isolation

Dried carpophores of *Xanthoceras sorbifolia* (10 kg) were extracted with 70% ethanol (100 × 3). The extract (800 g) was concentrated *in vacuo*, and then half of the extract was eluted with H_2O, 70% ethanol and 95% ethanol on macroporous resin. The 70% ethanol fraction (110 g) was first subjected to column chromatography on silica gel, eluted with $CHCl_3$ and MeOH yielding fractions 1–26 (100:0–100:50). Fractions 3 and 4 were then rechromatographed on silica gel eluted with petroleum ether (PE, 60–90°C)/acetone (2:1) to give compound **3** (7 mg). Fractions 15–19 were then rechromatographed on ODS eluted with H_2O: MeOH (20:80, 40:60, 60:40, 80:20) to give fractions A-1–A-5. Then, fraction A-2 was further subjected to HPLC chromatography, eluted with H_2O: MeOH (40:60) to yield compounds **1** (10.0 mg) and **2** (15.8 mg).

3.3.1 Compound 1

White powder; mp 243–245°C; $[\alpha]_D^{24}$ −4.7 (*c* 0.0015, MeOH), UV (MeOH) λ_{max}: 204 nm. IR (KBr) ν_{max} (cm^{-1}): 3425, 2944, 1653, 1387, 1077, 912, 812. ^1H NMR and ^{13}C NMR spectral data are listed in Table 1. HREI-MS: *m/z* 944.5342 $[M]^+$ (calcd for $C_{48}H_{80}O_{18}$, 944.5347).

3.3.2 Compound 2

White powder; mp 254–255°C; $[\alpha]_D^{24}$ −24.0 (*c* 0.003, MeOH), UV (MeOH) λ_{max}: 223 nm. IR (KBr) ν_{max} (cm^{-1}): 3427, 2928, 1726, 1636, 1385, 1239, 1048. ^1H NMR and ^{13}C NMR spectral data are listed in (Table 1. HREI-MS: *m/z* 1188.6291 $[M]^+$ (calcd for $C_{59}H_{96}O_{24}$, 1188.6298).

3.4 Acid hydrolysis of compounds 1 and 2

Compounds **1** (3 mg) and **2** (5 mg) were refluxed in a mixture of 3 M HCl/H_2O/EtOH (2:1:2) (10 ml) in a water bath for 2 h. The hydrolysate was partitioned

Table 1. ^{1}h nmr (300 mhz) and ^{13}c nmr (75 mhz) spectral data of **1** and **2** (δ values, in c_5d_5n).

	1			**2**	
No.	δ_C	δ_H	No.	δ_C	δ_H
1	39.6		1	39.5	
2	25.8		2	25.9	
3	88.8		3	89.3	
4	38.8		4	38.8	
5	55.7		5	55.6	
6	18.5		6	18.7	
7	32.8		7	32.8	
8	40.3		8	40.3	
9	47.9		9	47.9	
10	36.9		10	36.8	
11	24.0		11	24.1	
12	123.9	5.44 (1H, brs)	12	123.9	5.41 (1H, brs)
13	143.2		13	143.2	
14	41.3		14	41.9	
15	26.6		15	26.6	
16	19.0		16	18.5	
17	43.3		17	43.2	
18	42.0		18	41.3	
19	46.4		19	46.5	
20	36.4		20	36.5	
21	76.8	3.74 (1H, d, $J = 9.8$ Hz)	21	76.8	
22	74.9	4.34 (1H, d, $J = 9.8$ Hz)	22	74.9	
23	28.2	1.32 (3H, s)	23	28.2	1.23 (3H, s)
24	17.1	1.01 (3H, s)	24	17.0	0.97 (3H, s)
25	15.8	0.89 (3H, s)	25	15.8	0.89 (3H, s)
26	16.8	1.10 (3H, s)	26	16.8	1.09 (3H, s)
27	26.3	1.30 (3H, s)	27	26.3	1.26 (3H, s)
28	74.9	4.07 (3H, s)	28	74.9	4.04 (3H, s)
29	30.4	1.21 (3H, s)	29	30.5	1.22 (3H, s)
30	19.7	1.18 (3H, s)	30	19.7	1.19 (3H, s)
3-o-glc-1			3-o-glc-1		
1′	107.0	4.94 (1H, d, $J = 7.7$ Hz)	1′	106.8	4.86 (1H, d, $J = 6.8$ Hz)
2′	75.9	4.04	2′	73.4	3.98
3′	78.4	4.01	3′	79.3	5.90
4′	71.8		4′	69.6	
5′	78.8		5′	76.8	
6′	62.7		6′	70.0	
28-o-glc			3-o-glc-2		
1″	103.7	4.73 (1H, d, $J = 7.7$ Hz)	1″	105.6	5.08 (1H, d, $J = 7.5$ Hz)
2″	80.4	4.30	2″	75.2	4.05
3″	77.9	3.80	3″	78.6	
4″	71.8		4″	71.6	
5″	78.8		5″	78.4	
6″	63.0		6″	62.6	
Rha			28-o-glc		
1‴	100.6	6.60 (1H, s)	1‴	103.7	4.73 (1H, d, $J = 7.7$ Hz)
2‴	72.6	4.71	2‴	80.4	4.31
3‴	72.4	4.67	3‴	77.9	3.79
4‴	74.4	4.33	4‴	71.7	
5‴	69.1	4.88	5‴	78.4	
6‴	19.0	1.84	6‴	62.6	
			Rha		
			1⁗	100.6	6.60 (1H, s)
			2⁗	72.6	4.73
			3⁗	72.4	4.67
			4⁗	74.4	4.33
			5⁗	69.1	4.86
			6⁗	19.0	1.84 (3H, d, $J = 5.9$ Hz)
			Ang.		
			1⁗′	168.1	

Table 1 – *continued*

	1			**2**	
No.	δ_C	δ_H	No.	δ_C	δ_H
			$2'''''$	128.9	
			$3'''''$	137.0	5.83 (1H, q, $J = 7.2$ Hz)
			$4'''''$	20.8	1.82 (3H, brs)
			$5'''''$	16.0	1.94 (3H, d, $J = 7.2$ Hz)

between EtOAc and H_2O, the aqueous layer was comprised with authentic samples on TLC with silica gel [n-BuOH/HOAc/H_2O (4:1:5), upper phase], which showed the sugars were D-glucose and rhamnose.

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