A New Triterpene Saponin from *Gynostemma pentaphyllum*

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Abstract: **Objective** To study the triterpene saponins from *Gynostemma pentaphyllum* with antitumor activities. **Methods** The 75% EtOH extract of *G. pentaphyllum* was used for isolation by silica gel column chromatography and preparative HPLC. The structures of pure compounds isolated were identified by the spectral analysis and chemical evidence. **Results** Two compounds were isolated and identified as 23(*S*)-3β,20ξ,21ξ-trihydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-α-*L*-rhamnopyranosyl (1→2)-[β-*D*-xylopyranosyl (1→3)]-β-*D*-arabinopyranoside (**1**) and 23(*S*)-21(*R*)-*O*-*n*-butyl-3β,20ξ-dihydroxy-21,23-epoxydammar-24-ene 3-*O*-α-*L*-rhamnopyranosyl (1→2)-[β-*D*-xylopyranosyl (1→3)]-β-*D*-arabinopyranoside (**2**). **Conclusion** Compound **2** is a new triterpene saponin with moderate antitumor activities against the HL-60, Colon205, and Du145 cell lines.

Key words: antitumor; *Gynostemma pentaphyllum*; gypenoside; triterpene saponin
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Introduction

Gynostemma pentaphyllum (Thunb.) Makino, belonging to the Cucurbitaceae family, is a famous herbal drug in traditional Chinese medicine. It is wildly distributed in China, Korea, and Japan. This plant has been reported to contain a number of dammarane-type glycosides called gypenosides (Nagai *et al*, 1981; Takemoto *et al*, 1984; Kuwahara *et al*, 1989; Piacente and Pizza, 1995; Hu, Chen, and Xie, 1997; Liu *et al*, 2004) and exhibit a variety of biological effects, such as antitumor activity (Hsiao *et al*, 2008; Lu *et al*, 2008), immunity effect (Duan and Chen, 2007), therapeutic effect on chronic liver injury (Chen, Tsai, and Chen, 2000), inhibition of ATPase (Han, Wei, and Zhang, 2006) prevention of high blood lipid, anti-arteriosclerosis, strengthening the body, and preventing diseases of the old people. As a continuation of our work for discovering more effective components (Zhao *et al*, 2007), we have now investigated the chemical constituents aimed at triterpene saponin of *G. pentaphyllum*.

From 75% EtOH extract of this plant, one new dammarane saponin, 23(*S*)-21(*R*)-*O*-*n*-butyl-3β,20ξ,21ξ-trihydroxy-21,23-epoxydammar-24-ene 3-*O*-α-*L*-rhamnopyranosyl (1→2)-[β-*D*-xylopyranosyl (1→3)]-β-*D*-arabinopyranoside (**2**), was isolated together with one known compound 23(*S*)-3β,20ξ,21ξ-trihydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-α-*L*-rhamnopyranosyl (1→2)-[β-*D*-xylopyranosyl(1→3)]-β-*D*-arabinopyranoside (**1**). The isolation and structural elucidation of the new antitumor gypenoside were described in this paper.

Materials and methods

Materials and instruments

Column chromatography: silica gel (200－300 mesh, Qingdao Hailang Chemical Co.); macroporous resin HPD_{100} (Hebei, Co.). IR Spectra: Bruker IFS—55 spectrophotometer; KBr pellets, υ in cm^{-1}. Optical rotations: Perkin—Elmer polarimeter. Prep. HPLC (Beijing CXTH3000 system): P3000 pump, UV3000

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spectrophotometric detector at 203 nm, Daisogel C_{18} reversed-phase column (250 mm × 30 mm, 10 μm; flow rate 14.0 mL/min). ^{1}H-NMR and ^{13}C-NMR spectra: Bruker AV—600 and ARX—300 spectrometer. HR-TOF-MS: BIC micro TOF-Q mass spectrometer; in *m/z*. GC: Agilent technologies 6890N apparatus. DMSO and MTT were purchased from Sigma Chemical Co., Ltd., USA.

Plant materials

The aerial parts of *Gynostemma pentaphyllum* (Thunb.) Makino were collected from Shaanxi Province, China by Xi'an Tianyi Co., Ltd. in September 2006, and were identified by Prof. SUN Qi-shi of Shenyang Pharmaceutical Univercity. A voucher specimen of the plant (No. 2007016) was deposited at our laboratory.

Extraction and isolation

Air-dried *G. pentaphyllum* (8.0 kg) was extracted with 75% EtOH for three times and the water soluble fraction of 75% EtOH extract was separated by macroporous resin column to get 70% EtOH eluate, from which upon drying the total saponins (80 g) were afforded. The total saponins were chromatographied repeatedly over silica gel to provide five fractions A−E. Fraction C was separated into five sub-fractions, frs. $C_{a\text{-}e}$, by prepparative HPLC (ODS, 80% MeOH). From frs. C_c, compounds **1** (35 mg) and **2** (20 mg) were obtained as white amorphous powder.

Antitumor bioassay

Antitumor activities were evaluated by MTT assay (Lammer, Wagerer, and Saffrich, 1998). The caricinoma HL-60, Colon205, and Du145 cell lines were used as the target cells in the cytotoxicity assay. For drug exposure experiments, after exposuring the drug with cells for 72 h, 10 μL of MTT solution (0.25 mg/mL) was added to each cell, and the tumor cells were incubated at 37 ℃ in a humidified atmosphere of 5% CO_2 air for 4 h. At the end of incubation, the growth medium was removed and replaced with 100 μL of DMSO (at room temperature). After agitating on a vortex for 10 min, the absorbance was determined at 490 nm as reference on a Bio-Rad (model 550) microplate reader to calculate IC_{50}.

Results and discussion

Compound **1**: white amorphous powder, mp (203−205) ℃, Liebermann-Burchard and Molish reactions were positive, indicating that compound **1** is a triterpene saponin. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 1.61 (3H, s, 26-Me), 1.54 (3H, s, 27-Me), 1.24 (3H, s, 28-Me), 1.10 (3H, s, 18-Me), 1.02 (3H, s, 29-Me), 0.92 (3H, s, 30-Me), 3.33 (1H, br d, J = 10.0 Hz), 5.98 (1H, d, J = 8.3 Hz, H-24), 10.24 (1H, m, H-19). ^{13}C-NMR (150 MHz, C_5D_5N) δ: 33.7 (C-1), 27.6 (C-2), 87.2 (C-3), 40.5 (C-4), 54.9 (C-5), 18.0 (C-6), 34.9 (C-7), 40.1 (C-8), 52.9 (C-9), 52.9 (C-10), 22.2 (C-11), 25.1 (C-12), 41.3 (C-13), 50.1 (C-14), 32.1 (C-15), 27.6 (C-16), 45.1 (C-17), 16.0 (C-18), 205.6 (C-19), 84.7 (C-20), 102.8 (C-21), 45.1 (C-22), 73.4 (C-23), 130.0 (C-24), 132.3 (C-25), 25.7 (C-26), 18.0 (C-27), 26.4 (C-28), 16.6 (C-29), 17.5 (C-30), 104.8 (C-1′), 74.6 (C-2′), 81.7 (C-3′), 68.6 (C-4′), 65.1 (C-5′), 102.1 (C-1″), 72.6 (C-2″), 72.5 (C-3″), 73.9 (C-4″), 70.1 (C-5″), 18.6 (C-6″), 105.3 (C-1‴), 74.5 (C-2‴), 77.8 (C-3‴), 70.9 (C-4‴), 67.1 (C-5‴). According to the literature (Yin *et al*, 2006), compound **1** was elucidated as 23(*S*)-3β,20ξ,21ξ-trihydroxy-19-oxo-21,23-epoxydammar-24-ene 3-*O*-α-*L*-rhamnopyranosyl (1→2)-[β-*D*-xylopyranosyl (1→3)]-β-*D*-arabino-pyranoside (Fig. 1).

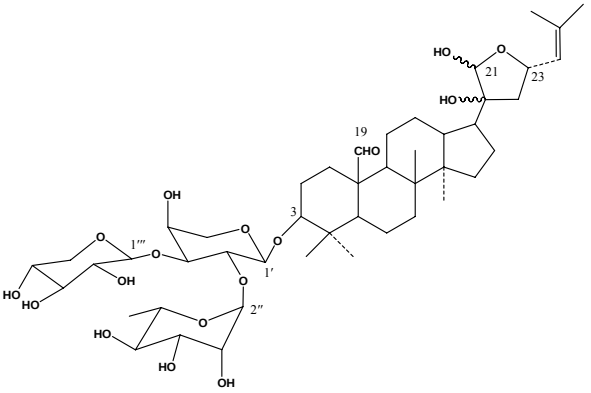

Fig. 1 Structure of compound 1

Compound **2**: white amorphous powder, mp (206−208) ℃, showed a peak at *m/z* 963.5669 [M + Na]$^{+}$ in HR-TOF-MS, indicating the molecular formula $C_{50}H_{84}O_{16}$. Liebermann-Burchard and Molish reactions were positive, showing the same property of triterpene saponin with compound **1**. $[\alpha]_D^{20}$ −7.6° (*c* 0.34, MeOH). The IR spectrum (KBr) showed peak at 3427 cm^{-1} (OH). The ^{1}H-NMR spectrum (Table 1) showed seven methyl groups δ 1.66 (3H, s, 27-Me), 1.63 (3H, s, 26-Me), 1.20 (3H, s, 28-Me), 1.16 (3H, s, 29-Me), 0.96 (3H, s, 18-Me), 0.95 (3H, s, 30-Me), 0.77 (3H, s, 19-Me), and a trisubstituted olefin part δ 5.62 (d, J = 9.0 Hz,

Table 1 ^{1}H-NMR (600 MHz in C_5D_5N) and ^{13}C-NMR (150 MHz in C_5D_5N) data for compound 2

No.	δ_C	δ_H	HMBC (H→C)
1	39.8	1.53 (m), 0.84 (m)	C-10
2	26.9	2.04 (m), 1.85 (m)	
3	88.4	3.38 (dd, *J* = 12.0, 4.2 Hz)	C-1′, C-4, C-28, C-29
4	39.6		
5	56.9	0.74 (m)	C-4, C-28, C-29
6	18.5	1.46 (m), 1.35 (m)	
7	35.7	1.52 (m), 1.23 (m)	
8	40.8		
9	51.0	1.29 (m)	
10	37.1		
11	21.7	1.47 (m), 1.38 (m)	
12	25.6	2.07 (m), 1.96 (m)	
13	41.9	2.25 (m)	C-14
14	50.9		
15	31.3	1.69 (m), 1.11 (m)	
16	27.7	2.02 (m), 1.33 (m)	
17	46.2	2.05 (m)	C-13, C-20
18	16.8	0.96 (s)	C-8, C-9, C-14
19	16.6	0.77 (s)	C-1, C-5, C-9, C-10
20	83.1		
21	104.5	5.07 (s)	C-17, C-22, C-1′′′′
22	43.6	2.43 (dd, *J* = 6.9, 12.3 Hz), 2.13(dd, *J* = 7.8, 12.6 Hz)	C-20, C-21, C-23, C-24
23	73.8	5.02 (m)	C-17, C-20, C-25
24	129.4	5.62 (d, *J* = 9.0 Hz)	C-23, C-26
25	133.9		
26	25.8	1.63 (s)	C-24, C-25, C-27
27	18.0	1.66 (s)	C-24, C-25, C-26
28	28.0	1.20 (s)	C-3, C-4, C-5, C-29
29	16.9	1.16 (s)	C-3, C-4, C-5, C-28
30	15.9	0.95 (s)	C-15
3-ara-1′	104.8	4.93 (d, *J* = 5.4 Hz)	C-3
2′	74.6	4.67 (t, *J* = 6.6 Hz)	C-1′′
3′	81.6	4.29 (m)	C-1′′′
4′	68.4	4.41 (m)	
5′	65.0	4.32 (m), 3.84 (d, *J* = 9.6 Hz)	
rha-1′′	102.1	6.14 (s)	C-2′
2′′	72.6	4.58 (m)	
3′′	72.5	4.73 (m)	
4′′	74.0	4.27 (m)	
5′′	70.1	4.58 (m)	
6′′	18.7	1.63 (d, *J* = 6.0 Hz)	
xyl-1′′′	105.2	5.01 (m)	C-3′
2′′′	74.7	3.93 (t, *J* = 7.8 Hz)	
3′′′	77.8	4.10 (m)	
4′′′	71.0	4.11 (m)	
5′′′	67.1	4.28 (m), 3.65 (t, *J* = 10.8 Hz)	
21-*O*-*n*-BuO-1′′′′	66.7	3.99 (m), 3.59 (m)	C-21, C-2′′′′, C-3′′′′
2′′′′	32.3	1.58-1.61 (m)	C-1′′′′, C-3′′′′, C-4′′′′
3′′′′	19.7	1.34-1.36 (m)	C-1′′′′, C-2′′′′, C-4′′′′
4′′′′	14.1	0.82 (t, *J* = 7.5 Hz)	C-2′′′′, C-3′′′′

H-24). The carbon signals assignable to the sugar moiety and the sapogenin part in the ^{13}C-NMR spectrum (Table 1) were closely similar to those of compound **1**, except that the C-19 (δ 205.6) signal due to the aldehyde group of compound **1** was replaced by a signal (δ 16.6) of a methyl group, which could be identified by the HMBC experiment. Namely, long-range correlations were observed between the H-19 and C-1, C-5, C-9, C-10 (Fig. 2). In addition, comparable to compound **1**, the *n*-butyl group as a substituent group is the main difference. Following long-range correlations in HMBC could testify the presentation of *n*-butoxy group located at C-21: between the H-1′′′′ and C-21, C-2′′′′, C-3′′′′. The absolute configuration at C-23 of compound **2** was deduced to be *S* on the basis of the literature (Yin *et al*, 2006). In the ROESY spectrum, ROESY cross-peaks were observed between the olefenic proton signal at δ 5.07 (H-21) and the signal at δ 5.02 (H-23) (Fig. 3). Up to this evidence, the aglycon part of compound **2** was determined as 23(*S*)-21(*R*)-*O*-*n*-butyl-3β,20ξ-dihydroxy-21,23-epoxy-dammar-24-ene 3-*O*-α-*L*-rhamnopyranosyl (1→2)-[β-*D*-xylopyranosyl (1→3)]- β-*D*-arabinopyranoside.

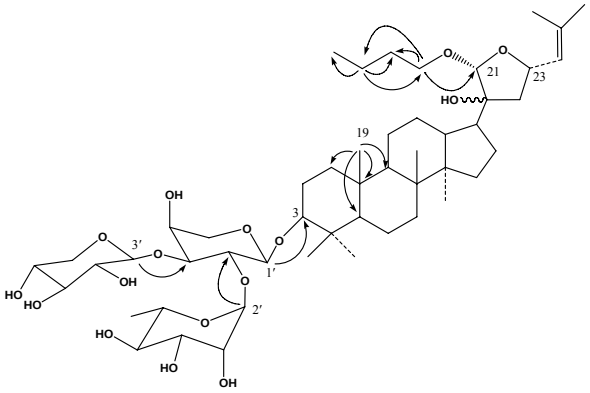

Fig. 2 Structure and key HMBC correlations of compound 2

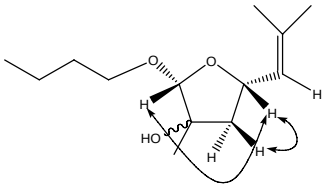

Fig. 3 Key ROE correlations in compound 2

By GC analysis of the trimethylsilyl ethers derivatives of the component monosaccharides (t_R: 6.20, 8.84, and 9.76 min), the standard monosaccharides were subjected to the same reaction and GC analysis under the same condition. Combining the analysis of the coupling NMR, it was clear that compound **2** contains a β-*D*-arabinose, a β-*D*-xylose, and an α-*L*-

rhamnose. The linkage sites and sequences of the three saccharides on the aglycon were confirmed by the 2D NMR experiments. In the HMBC (Fig. 2), the cross peaks between H-1′ of arabinose and C-3 of aglycon, H-1″ of rhamnose and C-2′, H-1‴ of xylose and C-3′, were displayed.

Thus, compound **2** was elucidated as 23(*S*)-21(*R*)-*O*-*n*-butyl-3β,20ξ-dihydroxy-21,23-epoxydammar-24-ene 3-*O*-α-*L*-rhamnopyranosyl(1→2)-[β-*D*-xylo-pyranosyl (1→3)]-β-*D*-arabinopyranoside.

Compound **2** showed moderate antitumor activities against HL-60, Colon205, and Du145 cell lines, and the IC_{50} were 17.44, 27.80, and 24.12 μg/mL, respectively.

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