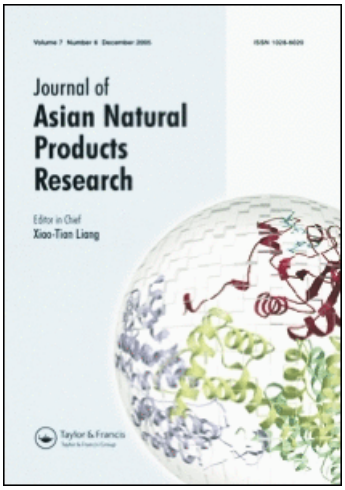

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New cycloartane glycosides from *Camptosorus sibiricus* Rupr.

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Two new cycloartane glycosides were isolated from the whole herbs of *Camptosorus sibiricus* Rupr. By means of chemical and spectroscopic methods (IR, 1D and 2D NMR, HRMS, ESI MS), the structures were established as (24*R*)-3β, 7β, 24, 25-tetrahydroxycycloartane 3-*O*-β-D-glucopyranosyl-24-*O*-β-D-glucopyranoside (**1**) and (24*R*)-3β, 7β, 24, 25-tetrahydroxycycloartane 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-24-*O*-β-D-glucopyranoside (**2**). At the same time, two new compounds were tested for their cytotoxicities *in vitro* against human tumor cell lines (A375-S2, Hela).

Keywords: *Camptosorus sibiricus*; Cycloartane glycosides; Cytotoxicity; Chemical and spectroscopic methods

1. Introduction

Camptosorus sibiricus is a herbal medicine widely distributed in the North China and Canada, which has good therapy effect on vascular inflammation, liver cancer and traumatism as a famous folk medicine [1]. Some flavonoids with activities of dilatation of blood vessels from the herb were reported in the literature [2]. In this paper, we report the isolation and structural elucidation of two new cycloartane glycosides (see figure 1), as well as their cytotoxicity.

2. Results and discussion

Compound **1** was isolated as white powder, mp 270–272°C. It showed a positive reaction with the Molish reagent. The sugar was identified as glucose by acid hydrolysis and HPLC analysis with authentic sample. The HRESIMS spectrum showed a quasi-molecular ion peak at *m/z* 823.4826 $[M + Na]^+$, compatible with the molecular formula $C_{42}H_{72}O_{14}$. In the ESIMS spectrum, the quasi-molecular ion peak $[M - H]^-$ at *m/z* 799.3 and the fragment

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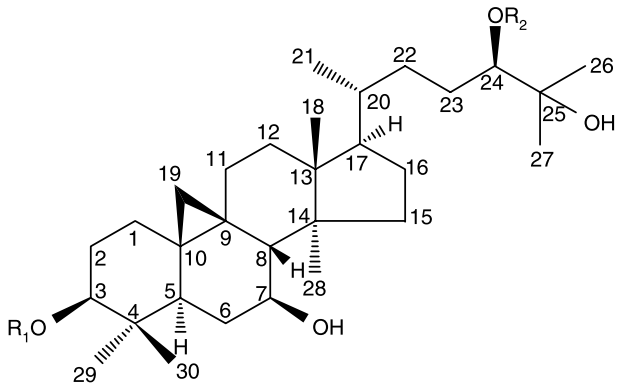

1 R_1 = glc R_2 = glc

2 R_1 = glc- (1→2) -glc R_2 = glc

Figure 1. The structures of compounds **1** and **2**.

peaks $[M - H - 162]^-$ at *m/z* 637.3, $[M - H - 2 \times 162]^-$ at *m/z* 475.2, representing the loss of 2 moles of hexoses from the parent molecular ion, were observed. ^{1}H NMR spectrum of **1** showed characteristic signals [3] of cyclopropane methylene protons at δ 0.20 (1H, d, $J = 4.0$ Hz, H-19a) and 0.47 (1H, d, $J = 4.0$ Hz, H-19b), six tertiary methyl and one secondary methyl groups at δ 0.95 (3H, s, 28-CH_3), 1.00 (3H, s, 18-CH_3), 1.08 (3H, s, 30-CH_3), 1.31 (3H, s, 29-CH_3), 1.60 (3H, s, 27-CH_3), 1.77 (3H, s, 26-CH_3), and 1.13 (3H, d, $J = 6.4$ Hz, 21-CH_3). Additionally, the signals of the anomeric protons at δ4.99 (1H, d, $J = 8.0$ Hz, H-1′), 5.21 (1H, d, $J = 7.9$ Hz, H-1″) were observed and the anomeric protons of the glucoses were both confirmed as β confirmation based on the coupling constants. The signals at δ 3.54 (1H, dd, $J = 5.5$, 11.9 Hz, H-3), 3.80 (1H, m, H-24), and 4.27 (1H, m, H-7) indicated the presence of the oxygenated carbon. In the ^{13}C NMR of **1**, 42 carbon signals were given, of which 4 oxygen-bearing carbons in aglycon moiety can be observed at δ 67.7 (C-7), 73.4 (C-25), 88.8 (C-3), and 94.1 (C-24), as well as the anomeric carbon signals at δ 106.9 (C-1′), 107.1 (C-1″).

The HMBC correlations (see figure 2) between H-29 (δ 1.31), H-30 (δ 1.08) and C-3 (δ 88.8) indicated that C-3 was substituted by the hydroxyl group. In addition, H-5 (δ 1.47) and H-8 (δ 1.30) presented long-range correlations with C-7 (δ 67.7), respectively, and in the NOESY spectrum of **1**, the correlations between H-5 and H-3, H-7 and H-28 were observed, so the presence of 7-OH and the β configurations of 3, 7-OH were determined. And the HMBC correlations between H-27 (δ 1.60) and C-25 (δ 73.4), C-24 (δ 94.1), and between H-26 (δ 1.77) and C-25 (δ 73.4), C-24 (δ 94.1) indicated the presence of 24-OH and 25-OH respectively. The ^{1}H and ^{13}C-NMR data for H-24 and C-24 of **1** were identical to those of analogs having a 24*R* configuration in the literature [4–6]. Combined with HMQC, NOESY, and ^{1}H–^{1}H COSY spectra, the aglycon of **1** was determined as (24*R*) -3β, 7β, 24, 25 -tetrahydroxycycloartane. The anomeric protons of β-D-glucoses at δ 4.99 (1H, d, $J = 8.0$ Hz, H-1′) showing long-range correlation with C-3 (δ 88.8), δ 5.21 (1H, d, $J = 7.9$ Hz, H-1″) with C-24 (94.0) suggested the sugars were connected to C-3 and C-24, respectively. With the data above, the structure of **1** was established as (24*R*) -3β, 7β, 24, 25-tetrahydroxy-cycloartane 3-*O*-β-D-glucopyranosyl -24-*O*-β-D-glucopyranoside.

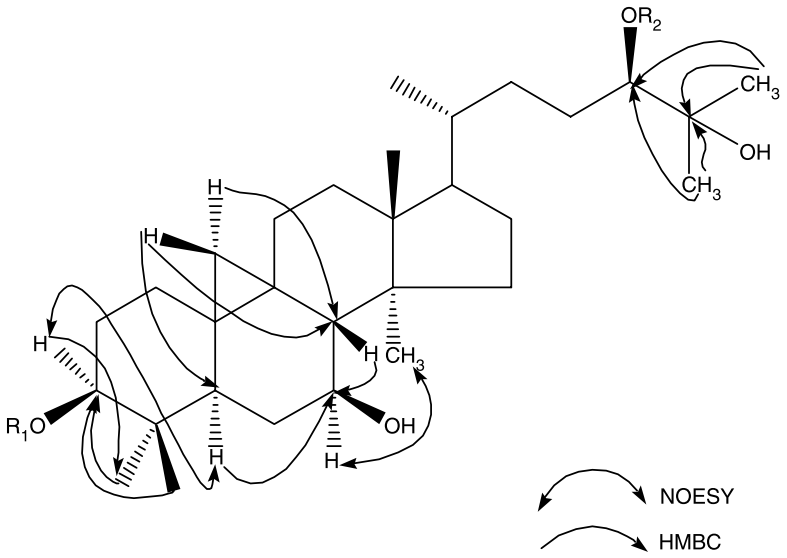

Figure 2. Important HMBC and NOSEY correlations for compounds **1** and **2**.

Compound **2** was isolated as white powder, mp 278–280°C. It showed a positive reaction with the Molish reagent. The sugar was identified as glucose by acid hydrolysis and HPLC analysis with authentic sample. The HRESIMS spectrum gave the quasi-molecular ion peak at *m/z* 985.5358 $[M + Na]^+$, compatible with the molecular formula $C_{48}H_{82}O_{19}$. In the ESIMS spectrum, the quasi-molecular ion peak $[M - H]^-$ at *m/z* 961.4 together with the fragment peaks $[M - H - 162]^-$ at *m/z* 799.4, $[M - H - 2 \times 162]^-$ at *m/z* 637.3, and $[M - H - 3 \times 162]^-$ at *m/z* 475.2, indicated the loss of 3 moles of hexoses from the parent molecular ion. Comparison of the NMR data (see table 1) of **2** with those of **1** showed that the structure of **2** was almost identical to that of **1**, except that one more set signals of glucose were observed in **2**. In the $^1H-^1H$ COSY spectrum, the correlation between H-1′ (δ 5.40) and H-2′ (δ 4.14) was observed. In addition, H-1′ (δ 5.40) and C-3 (δ 88.8), H-1″ (δ 4.97) and C-2′ (δ 83.6), H-1‴ (δ 5.22) and C-24 (δ 94.1), presenting long-range correlations respectively, indicated the sequencing and the linkage position of the sugar moieties. Combined the HMQC, HMBC, NOESY, and $^1H-^1H$ COSY spectra, the structure of **2** was established as (24*R*)-3β, 7β, 24, 25-tetrahydroxycycloartane 3-*O*-β-D-glucopyranosyl - (1 → 2) -β-D-glucopyranosyl-24-*O*-β-D-glucopyranoside.

Using MTT method, compounds **1** and **2** were tested their cytotoxicities *in vitro* against human tumor cell lines (A375-S2, Hela), and no compound showed activity.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yamaco-hot-stage uncorrected. NMR spectra were recorded on a JEOL JNM-LA 500 spectrometer, using TMS as an internal standard. ESIMS data were performed on a Finnigan LCQ mass spectrometer. HRESIMS data were performed on a QSTAR LCQ mass spectrometer. The optical rotations were measured on a

Table 1. The NMR data of compounds **1** and **2**.

	1		***2***			***1***		***2***	
No.	δ_H	δ_C	δ_H	δ_C	*No.*	δ_H	δ_C	δ_H	δ_C
1	1.12 (m), 1.48 (m)	32.1	1.10 (m), 1.43 (m)	29.9	25		73.4		73.4
2	1.90 (m), 2.10 (m)	30.0	1.88 (m), 2.12 (m)	29.9	26	1.77 (s)	26.4	1.77 (s)	26.4
3	3.54 (dd, 5.5, 11.9)	88.8	3.46 (dd, 3.7, 11.3)	88.8	27	1.60 (s)	27.0	1.60 (s)	27.0
4	–	41.3	–	41.3	28	0.95 (s)	19.5	0.94 (s)	19.5
5	1.47 (m)	47.9	1.33 (m)	47.5	29	1.31 (s)	25.8	1.35 (s)	25.8
6	1.10 (m), 1.50 (m)	32.1	1.11 (m), 1.44 (m)	32.1	30	1.08 (s)	15.5	1.18 (s)	15.4
7	4.27 (m)	67.7	4.27 (m)	67.7	1′	4.99 (d, 8.0)	106.9	5.40 (d, 7.7)	105.0
8	1.30 (m)	47.5	1.25 (m)	47.8	2′	–	76.0	4.14 (m)	83.6
9	–	20.0	–	20.0	3′	–	78.8	–	77.2
10	–	26.7	–	26.7	4′	–	71.8	–	71.4
11	1.63 (m)	28.0	1.66 (m)	28.5	5′	–	78.3	–	78.0
12	1.66 (m)	33.4	1.67 (m)	33.4	6′	–	63.0	–	62.8
13	–	45.7	–	45.7	1″	5.21 (d, 7.9)	107.1	4.97 (d, 7.6)	106.2
14	–	49.1	–	49.1	2″	–	75.8	–	76.0
15	1.24 (m), 1.30 (m)	35.8	1.30 (m)	35.7	3″	–	78.6	–	78.6
16	1.05 (m), 1.65 (m)	26.3	1.01 (m), 1.60 (m)	26.2	4″	–	71.6	–	71.6
17	1.74 (m)	53.7	1.73 (m)	53.7	5″	–	78.3	–	78.3
18		18.4	1.00 (s)	18.3	6″	–	62.8	–	62.8
19	0.20, 0.47 (d, 4.0)	29.6	0.19, 0.48 (d, 4.6)	29.6	1‴	–	–	5.22 (d, 7.9)	107.1
20	2.47 (m)	43.0	2.47 (m)	43.0	2‴	–	–	–	76.0
21	1.13 (d, 6.4)	18.3	1.13 (d, 6.1)	18.3	3‴	–	–	–	78.6
22	1.67 (m)	32.8	1.63 (m)	32.8	4‴	–	–	–	71.7
23	1.63 (m)	28.5	1.67 (m)	28.5	5‴	–	–	–	78.3
24	3.80 (m)	94.1	3.80 (m)	94.1	6‴	–	–	–	62.8

Measured in pyridine-d_5; coupling constants (J in Hz) are given in parentheses.

Perkin–Elmer 241 polarimeter. Silica gel for chromatography was produced by Qingdao Ocean Chemical Group Co., China. HPLC separations were performed on a Shim-pack PREP-ODS column (250 × 20 mm) equipped with a Shimadzu RID-6A refractive index detector and a Shimadzu LC-6AD series pumping system. The sugar part were analyzed on a Kaseisorb LC-NH2-60-5 column (250 × 4.6 mm) equipped with a Shodex OR-2 detector.

3.2 Plant material

The plant material was collected in Beining city, Liaoning Province, China, in July 2002, and identified by Prof. Qishi Sun, Shenyang Pharmaceutical University. A voucher specimen (No. 20020701) is deposited in Research Department of Natural Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

Dried whole herbs (4.2 kg) of *Camptosorus sibiricus* were extracted with 70% ethanol and concentrated *in vacuo*. Then the extract (596 g) was partitioned with petroleum ether, EtOAc and *n*-BuOH, successively. The *n*-BuOH extract (138 g) was subjected to column chromatography on silica gel gradiently eluted with $CHCl_3$: MeOH to give fraction 6 [$CHCl_3$: MeOH (100:14–20)]. Fraction 6 was chromatographied on ODS column eluted with MeOH: H_2O (0:100–100:0) to give two subfractions. The first subfraction [MeOH: H_2O (40:60)] was purfied on RP-HPLC with a ODS column (250 × 20 mm, flow rate 9 ml/min) with CH_3CN: H_2O (36:64) to yield **2** (24.0 mg), and the second subfraction [MeOH: H_2O (60:40)] was isolated on RP-HPLC column with MeOH: H_2O (70:30) to afford **1** (23.5 mg).

3.3.1 Compound **1**. white powder (MeOH), mp 270–272°C. $[\alpha]_D^{20} = +3.6$ (*c* 0.2, MeOH). IR (KBr pellet) ν_{max} 3407 (—OH), 2938 (CH), 1040 (C—O) cm^{-1}. The ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data see table 1. HRESIMS: *m/z* 823.4826 (calcd for $C_{42}H_{72}O_{14}Na$, 823.4820). ESI-MS: *m/z* 799.3 $[M - H]^-$.

3.3.2 Compound **2**. white powder (MeOH), mp 278–280°C. $[\alpha]_D^{20} = +2.2$ (*c* 0.1, MeOH). IR (KBr pellet) ν_{max} 3395 (—OH), 2930 (CH), 1045 (C—O) cm^{-1}. The ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data see table 1. HRESIMS: *m/z* 985.5358 (calcd for $C_{48}H_{82}O_{19}Na$, 985.5348). ESI-MS: *m/z* 961.4 $[M - H]^-$.

3.4 Acid hydrolysis of compounds 1 and 2

1 (2 mg) and **2** (2 mg) was respectively hydrolysed by refluxing with 2% (V/V) H_2SO_4 solution in a water bath for 2 h. The hydrolysate was neutralised and then chromatographied on ODS column eluted with H_2O and MeOH, respectively. The composition of the H_2O elute was identified as D-glucose by HPLC analysis with authentic sample.

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