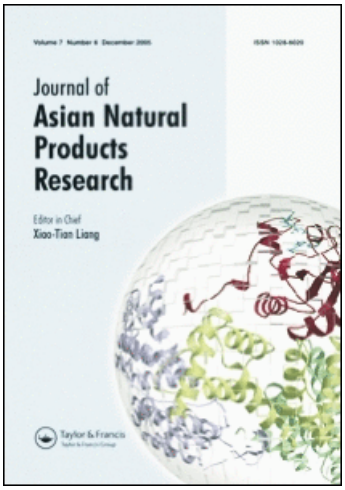

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New cycloartane glycosides from *Camptosorus sibiricus* Rupr

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Three 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, HR-MS, ESI-MS), the structures were established as (24*R*)-3β,7β,24,25, 30-pentahydroxycycloartane-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 2)-β-D-glucopyranosyl]-24-*O*-β-D-glucopyranoside (**1**), (24*R*)-3β,7β,24,25,30-pentahydroxycycloartane-3-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl]-24-*O*-β-D-glucopyranoside (**2**), (24*R*)-3β,7β,24,25,30-pentahydroxycycloartane-30-*O*-coumaroyl-3-*O*-β-D-glucopyranosyl-24-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**3**). At the same time, the new compounds were tested for their cytotoxicities *in vitro* against human tumor cell lines (A375-S2, Hela) using MTT method.

Keywords: *Camptosorus sibiricus* Rupr; triterpenoids; cycloartane glycosides; cytotoxicity

1. Introduction

Camptosorus sibiricus is a herbal medicine widely distributed in the North of China and Canada, which has good therapy effect on vascular inflammation, liver cancer, and traumatism as a famous folk medicine. It was used as tea in northeast China. Flavonoids and triterpenoids were isolated from the plant [1–4]. During the course of our studies on the bioactive constituents from *C. sibiricus*, we found three new cycloartane glycosides, which have the same aglycone structure as isolated triterpenoids [2–4] from the ethanolic extract of the plant. In this paper, we described the isolation and structural elucidation of three new cycloartane glycosides (Figure 1), as well as their results of a cytotoxicity test.

2. Results and discussion

Compound **1** was isolated as white powder, mp 280–282°C, showing a positive reaction with the Molish reagent. The sugars were identified as glucose and arabinose by acid hydrolysis and GLC methods. The HR-ESI-MS spectrum gave the quasi-molecular ion at *m/z* 1133.5713 $[M + Na]^+$, compatible with the molecular formula $C_{53}H_{90}O_{24}$. In the ESI-MS spectrum, the quasi-molecular ion $[M - H]^-$ at *m/z* 1109.3 together with the fragment peaks $[M - H - 132]^-$ at *m/z* 977.2, $[M - H - 132\text{-}162]^-$ at *m/z* 815.3 and $[M - H - 132\text{-}2 \times 162]^-$ at *m/z* 653.5, $[M - H - 132\text{-}3 \times 162]^-$ at *m/z* 491.4, corresponded to the loss of 3 mol of hexoses and 1 mol of pentose from the parent molecular ion. ^{1}H NMR spectrum of **1** showed characteristic signals [5] of cyclopropane methylene protons at δ 0.12 (1H, br s, H-19a) and 0.21 (1H, br s, H-19b), five tertiary methyl and one secondary methyl groups at δ 0.97 (3H, s, 28-CH_3), 0.98 (3H, s, 18-CH_3), 1.35 (3H, s, 29-CH_3), 1.60 (3H, s, 27-CH_3), 1.81 (3H, s, 26-CH_3) and δ 1.16 (3H, d, $J = 6.4$ Hz, 21-CH_3). Additionally,

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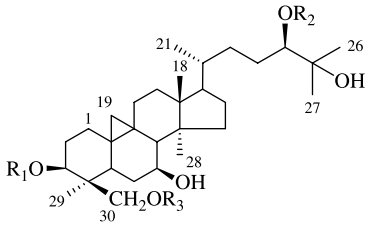

1 R_1 = –Glc (1→4) [Ara(1→2)] Glc, R_2 = Glc, R_3 = H
2 R_1 = –Glc (1→4) [Gal(1→2)] Glc, R_2 = Glc, R_3 = H
3 R_1 = –Glc, R_2 = Glc (1→6) Glc, R_3 = coumaroyl

Figure 1. Structures of compounds **1**–**3**.

the signals of the anomeric protons at δ 4.79 (1H, d, $J = 7.7$ Hz, H-1′), 5.20 (1H, d, $J = 7.9$ Hz, H-1″), 5.20 (1H, d, $J = 7.9$ Hz, H-1‴), and 5.58 (1H, d, $J = 8.0$ Hz, H-1⁗) were observed, and suggested anomeric centers of the glucoses were all β configuration. The signals at δ 3.52 (1H, dd, $J = 4.3$, 11.3 Hz, H-3), 3.76 (1H, br s, H-24), and 4.27 (1H, m, H-7) indicated the protons connected to the oxygenated carbons. In the ^{13}C NMR spectrum of **1**, 53 carbon signals were given, of which five oxygen-bearing carbons of the aglycone moiety can be observed at δ 63.7 (C-30), 67.6 (C-7), 73.8 (C-25), 91.1 (C-3), and 93.4 (C-24), and carbon signals at δ 104.4 (C-1′), 104.9 (C-1″), 106.9 (C-1‴), and 104.4 (C-1⁗).

In the HMBC experiment (Figure 2), the long-range correlations between H-29, H-30a, H-30b and C-3, as well as H-29 and C-30 indicated that C-3 and C-30 were substituted by hydroxyl groups. In addition, HMBC

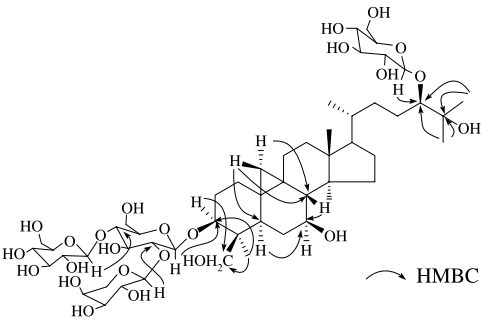

Figure 2. Important HMBC (H → C) correlations of compound **1**.

correlations of H-5, H-8 with C-7 and the NOESY correlations between H-5 and H-3, H-7 and H-28 suggested the presence of 7-OH and the β configurations of 3, 7-OH. The HMBC correlations between H-26, H-27 and C-24, C-25, respectively, indicated the presence of 24-OH and 25-OH. The ^{1}H and ^{13}C NMR spectral data for H-24 and C-24 of **1** were comparable with those reported analogous compounds having a 24*R* configuration [6–8]. Combined with HMQC, NOESY, and ^{1}H–^{1}H COSY spectra, the aglycone of **1** was determined as (24*R*)-3β,7β,24,25,30-pentahydroxycycloartane. The anomeric protons H-1′, H-1⁗, H-1″, and H-1‴ showed HMBC correlation to C-3, C-2′, C-4′, and C-24, respectively, which suggested the connection of sugars to C-3 and C-24. On the basis of HMQC, HMBC, NOESY, and ^{1}H–^{1}H COSY spectra, the structure of **1** was established as (24*R*)-3β,7β,24,25,30-pentahydroxycycloartane-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 2)-β-D-glucopyranosyl]-24-*O*-β-D-glucopyranoside (**1**).

Compound **2** was afforded as white powder, mp 283–285°C. It showed a positive reaction with the Molish reagent. The sugars were identified as glucose and galactose by acid hydrolysis and GLC methods. The HR-ESI-MS spectrum gave the quasi-molecular ion at *m/z* 1163.5815, corresponding to the molecular formula $C_{53}H_{90}O_{24}$. In the ESI-MS spectrum, the quasi-molecular ion $[M - H]^-$ at *m/z* 1139.5 together with the fragment ions $[M - H\text{-}162]^-$ at *m/z* 977.4, $[M - H\text{-}2 \times 162]^-$ at *m/z* 815.4, $[M–H\text{-}3 \times 162]^-$ at *m/z* 653.3, and $[M - H\text{-}4 \times 162]^-$ at *m/z* 491.2 showed the loss of 4 mol of hexoses from the parent molecular ion. Comparison of the NMR spectral data (Tables 1 and 2) of **2** with those of **1** indicated that the structure of **2** was almost identical to that of **1**, except for a set of signals of galactose at δ_H 5.56 (1H, d, $J = 7.7$ Hz) and δ_C 104.8 (C-1⁗), 74.7 (C-2⁗), 75.7 (C-3⁗), 69.7 (C-4⁗), 76.8 (C-5⁗), and 61.5 (C-6⁗) in compound **2** instead of the signals of arabinose in compound **1**. Combined with the HMQC, HMBC, NOESY, and ^{1}H–^{1}H COSY spectra, the structure of **2**

Table 1. ^{13}C NMR spectral data of **1**–**3**. (125 MHz in pyridine-d_5).

No.	**1**	**2**	**3**	No.	**1**	**2**	**3**
1	31.9	31.8	32.8	Glc			
2	31.9	31.8	32.8	1″	104.9	104.3	107.1
3	91.1	91.0	90.3	2″	75.8	76.3	76.0
4	44.4	44.3	45.2	3″	78.4	78.3	78.6
5	47.7	47.6	48.4	4″	71.4	71.3	71.7
6	30.1	29.9	30.0	5″	78.2	78.1	78.3
7	67.6	67.6	67.8	6″	62.2	62.1	71.1
8	48.4	48.3	48.4	Glc			
9	21.6	21.2	23.0	1‴	106.9	107.0	105.6
10	25.4	25.3	25.7	2‴	75.8	75.9	75.5
11	28.4	28.4	28.6	3‴	78.5	78.5	78.1
12	35.9	35.8	35.9	4‴	71.2	71.5	72.3
13	45.6	45.5	45.7	5‴	78.2	78.2	78.3
14	49.0	48.9	48.7	6‴	61.6	61.7	62.9
15	33.3	33.2	33.4	Gal			
16	26.9	26.4	27.0	1⁗		104.8	
17	53.8	53.7	53.8	2⁗		74.7	
18	18.5	18.5	18.3	3⁗		75.7	
19	30.0	29.6	29.7	4⁗		69.7	
20	43.2	42.9	43.0	5⁗		76.8	
21	18.5	18.2	18.6	6⁗		61.5	
22	32.7	32.7	32.8	Ara			
23	26.4	26.4	26.8	1⁗	104.4		
24	93.4	93.9	93.9	2⁗	74.8		
25	73.8	73.4	73.5	3⁗	75.1		
26	26.7	26.4	26.5	4⁗	70.0		
27	27.0	26.9	27.0	5⁗	65.0		
28	19.6	19.5	19.7	Cou			
29	20.8	20.7	20.5	1⁗			167.5
30	63.7	63.6	64.7	2⁗			115.3
Glc				3⁗			145.4
1′	104.4	104.3	107.4	4⁗			126.2
2′	80.2	80.0	75.6	5⁗			130.8
3′	76.5	78.1	78.6	6⁗			116.8
4′	80.4	80.4	71.9	7⁗			161.5
5′	78.5	78.2	78.6	8⁗			116.8
6′	61.8	62.7	62.9	9⁗			130.8

Glc, β-D-glucose; Ara, α-L-arabinose; Gal, β-D-galactose; Cou, coumaroyl.

was established as (24*R*)-3β, 7β, 24, 25, 30-pentahydroxycycloartane-3-*O*-β-D-glucopyranosyl-(1 → 4)-[β-D-galactopyranosyl-(1 →2)-β-D-glucopyranosyl]-24-*O*-β-D-glucopyranoside (**2**).

Compound **3** was obtained as white powder, mp 274–276°C, presenting a positive reaction with the Molish reagent. The sugars were identified as glucose by acid hydrolysis and GLC methods. The HR-ESI-MS spectrum showed the quasi-molecular ion at *m/z* 1147.5657 $[M + Na]^+$, compatible with the molecular formula $C_{57}H_{88}O_{22}$. In the ESI-MS spectrum, the quasi-molecular ion at *m/z* 1147.6 $[M + Na]^+$, together with the fragment ions at *m/z* 985.6 $[M + Na\text{-}162]^+$, 821.6 $[M + Na - 162\text{-}164]^+$, 659.6 $[M + Na - 164\text{-}2 \times 162]^+$, and 497.3 $[M + Na - 164\text{-}3 \times 162]^+$, indicated the loss of 3 mol of hexoses and 1 mol of coumaroyl from the parent molecular ion. Comparison of the NMR spectral data (Tables 1 and 2) of **3** with those of **1** showed that compound **3** possessed the identical aglycone part to that of **1**. But the

Table 2. The ^{1}H-NMR spectral data of compounds **1–3** (500 MHz in pyridine-d_5).

No.	**1**	**2**	**3**
3	3.52 (dd, 4.3, 11.3 Hz)	3.52 (m)	3.63 (t-like)
5	1.44 (m)	1.28 (m)	1.47 (m)
6	1.93, 2.35 (each m)	1.91, 2.35 (each m)	1.92, 2.42 (each m)
7	4.27 (m)	4.25 (m)	4.30 (m)
8	1.35 (m)	1.33 (m)	1.37 (m)
17	1.73 (m)	1.70 (m)	1.65 (m)
18	0.98 (s)	0.96 (s)	0.92 (s)
19	0.12, 0.21 (each br s)	0.11, 0.19 (d, 3.6 Hz)	0.18, 0.75 (each br s)
20	2.57 (m)	2.45 (m)	2.41 (t-like)
21	1.16 (d, 6.4 Hz)	1.12 (d, 6.1 Hz)	1.07 (d, 6.2 Hz)
24	3.76 (br s)	3.75 (m)	3.78 (m)
26	1.81 (s)	1.76 (s)	1.74 (s)
27	1.60 (s)	1.59 (s)	1.57 (s)
28	0.97 (s)	0.89 (s)	0.90 (s)
29	1.35 (s)	1.35 (s)	1.68 (s)
30	3.48, 4.45 (d, 11.0 Hz)	3.47, 4.43 (d, 10.7 Hz)	4.28, 4.66 (d, 10.2 Hz)
Glc-1′	4.79 (d, 7.7 Hz)	4.79 (d, 7.7 Hz)	4.93 (d, 7.9 Hz)
2′	4.15 (m)	4.14 (m)	
Glc-1″	5.20 (d, 7.9 Hz)	5.18 (d, 6.1 Hz)	5.19 (d, 7.7 Hz)
Glc-1″	5.20 (d, 7.9 Hz)	5.19 (d, 7.7 Hz)	4.77 (d, 7.7 Hz)
Gal-1″		5.56 (d, 7.7 Hz)	
Ara-1″	5.58 (d, 8.0 Hz)		
Cou″			α-H 6.72 (d, 16.0 Hz) β-H 8.00 (d, 16.0 Hz) 2, 6, 7.60 (d, 7.6 Hz) 3, 5, 7.15 (d, 7.6 Hz)

Glc, β-D-glucose; Ara, α-L-arabinose; Gal, β-D-galactose; Cou, coumaroyl.

signals of arabinose were absent. Furthermore, in the ^{1}H NMR spectrum of **1**, one set signals of coumaroyl were observed at δ 6.72 (1H, d, $J = 16.0$ Hz, H-α), 8.00 (1H, d $J = 16.0$ Hz, H-β), 7.60 (2H, d, $J = 7.6$ Hz, H-2″, 6″), 7.15 (2H, d, $J = 7.6$ Hz, H-3″, 5″). In the ^{13}C NMR spectrum, the signals of coumaroyl were displayed at δ 167.5 (C═O), 115.3 (C-α), 145.4 (C-β), 126.2 (C-1″), 130.8 (C-2″, 6″), 116.8 (C-3″, 5″), and 161.5 (C-4″). In the HMBC experiment, the long-range correlations between H-1′ and C-3, H-1″ and C-24, and H-1″ and C-6″ indicated the sequencing and the linkage position of the sugar moieties. The chemical shift of C-30 shifted downfield from 63.7 (in compound **1**) to 64.7 (in compound **3**), and the correlation between H-30 and C-1″ was observed in the HMBC spectrum, indicating that the coumaroyl moiety was connected with C-30. Combined with HMQC, HMBC, NOESY, and ^{1}H–^{1}H COSY spectra, the structure of **3** was established as (24*R*)-3β,7β,24,25,30-pentahydroxycycloartane-30-*O*-coumaroyl-3-*O*-β-D-glucopyranosyl-24-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**3**).

Using MTT method, the plant extract and compounds **1–3** were tested for their cytotoxicity *in vitro* against human tumor cell lines (A375-S2, Hela) and neither of them was found active ($IC_{50} > 100$ μg/ml).

3. Experimental

3.1 General experimental procedures

Melting point was measured on a Yamaco-hot-stage and is uncorrected. The optical rotation was measured on Perkin–Elmer 241 polarimeter. NMR spectra were recorded on JEOL JNM-LA 500 spectrometer, using TMS as an internal standard. ESI-MS was performed on Finnigan LCQ mass spectrometer. HR-MS

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

3.2 Plant material

Whole herbs of *C. sibiricus* were collected in Beining city, Liaoning province, China, in July 2006, and identified by Prof. Qishi Sun (Shenyang Pharmaceutical University). A voucher specimen (No. 20060701) is deposited in the Institute of Pharmaceutical Informatics, Zhejiang University.

3.3 Extraction and isolation

Dried whole herbs (4.2 kg) of *C. sibiricus* were extracted with 70% ethanol. The extract was concentrated *in vacuo*, and 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 chromatographed on ODS column eluted with MeOH/H_2O to give subfraction 1 (MeOH/H_2O 40:60), which was purified on RP-HPLC with an ODS column (250 × 20 mm, flow rate 9 ml/min) using CH_3CN/H_2O (36:64) as eluent to afford **3** (14.0 mg) ($t_R = 35$ min) and fractions a and b, which then were isolated by HPLC again with CH_3CN/MeOH/H_2O (28:16:64) to yield **1** (44.0 mg, $t_R = 24$ min) and **2** (32.0 mg, $t_R = 17$ min), respectively.

3.3.1 Compound *1*

White powder (MeOH), mp 280–282°C. $[\alpha]_D^{20} = +2.4$ (*c* 0.1, MeOH). IR (KBr pellet) ν_{max} 3407, 2938, 1040 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectral data see Tables 1 and 2. HR-ESI-MS *m/z* 1133.5713 $[M + Na]^+$ (calcd for $C_{53}H_{90}O_{24}Na$, 1133.5720). ESI-MS *m/z*: 1133.0 $[M + Na]^+$, 1109.3 $[M - H]^-$, 977.2 $[M - H - 132]^-$, 815.3 $[M - H - 132\text{-}162]^-$, 653.5 $[M - H - 132\text{-}2 \times 162]^-$, 491.4 $[M - H - 132\text{-}3 \times 162]^-$.

3.3.2 Compound *2*

White powder (MeOH), mp 283–285°C. $[\alpha]_D^{20} = +2.0$ (*c* 0.1, MeOH). IR (KBr pellet) ν_{max} 3404, 2941, 1040 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectral data see Tables 1 and 2. HR-ESI-MS *m/z* 1163.5815 $[M + Na]^+$ (calcd for $C_{54}H_{92}O_{25}Na$, 1163.5825). ESI-MS *m/z*: 1163.6 $[M + Na]^+$, 1139.5 $[M - H]^-$, 977.4 $[M - H\text{-}162]^-$, 815.4 $[M - H\text{-}2 \times 162]^-$, 653.3 $[M - H\text{-}3 \times 162]^-$, 491.2 $[M - H\text{-}4 \times 162]^-$.

3.3.3 Compound *3*

White powder (MeOH), mp 274–276°C. $[\alpha]_D^{20} = +6.4$ (*c* 0.05, MeOH). IR (KBr pellet) ν_{max} 3394, 2930, 1045 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectral data see Tables 1 and 2. HR-ESI-MS *m/z* 1147.5657 $[M + Na]^+$ (calcd for $C_{57}H_{88}O_{22}Na$, 1147.5665) ESI-MS *m/z*: 1147.6 $[M + Na]^+$, 1159.4 $[M + Cl]^-$, 1123.5 $[M - H]^-$, 985.6 $[M + Na - 162]^+$, 821.6 $[M + Na - 162\text{-}164]^+$, 659.6 $[M + Na - 164\text{-}2 \times 162]^+$.

3.4 Acid hydrolysis of 1–3

A solution of compounds **1**–**3** (2 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1:1, v/v, 1 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. After removal of the solvent

under reduced pressure from the filtrate, the residue was passed through a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (0.01 ml) in pyridine (0.02 ml) at 60°C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.01 ml) at 60°C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose (i), D-galactose (ii), L-arabinose (iii) from compounds **1**–**3**. GLC conditions: column, SupelcoTM-1, 0.25 mm (i.d.) 330 m; column temperature, 230°C; t_R: (i) 26.5 min; (ii) 25.6 min; (iii) 15.1 min.

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