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A New Cycloartane Glycoside from *Camptosorus sibiricus* Rupr.

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A new cycloartane glycoside was isolated from the dried whole herbs of *Camptosorus sibiricus* Rupr By means of chemical (hydrolysis) and spectroscopic methods (IR, 1D, and 2D NMR, ESI-MS), the structure was established as 3β, 7β, 24β, 25, 30-pentahydroxycycloartane-3-*O*-β-D-glucopyranoside-24-*O*-β-D-glucopyranoside (**1**).

Key words: *Camptosorus sibiricus*, Cycloartane glycoside

INTRODUCTION

Camptosorus sibiricus is a herbal medicine widely distributed in the North of China, which has activity in the dilatation of blood vessels. Some flavonoids with activities of dilatation of blood vessels from the herb were reported in the literature (Zhang *et al.*, 1979). In our intended research, **1** was obtained from dried whole herbs of the plant. This is the first time that cycloartane glycoside has been isolated from the plant. This paper describes the isolation and structural elucidation of a new cycloartane glycoside.

MATERIALS AND METHODS

General experimental procedures

Melting point was measured on a Yanaco-hot-stage and is uncorrected. NMR spectra were recorded on Bruker-ARX-300 spectrometer, using TMS as an internal standard. IR spectra were measured on a Perkin-Elmer 2000 FT-IR spectrometer as KBr pellets. ESI-MS was performed on Finnigan LCQ mass spectrometer. HRMS was performed on QSTAR LCQ mass spectrometer. The optical rotation was measured on Perkin-Elmer 241 polarimeter. Silica gel for chromatography was produced by Qingdao Ocean Chemical Group Co. of China.

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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.

Extraction and isolation

Dried whole herbs (4.2 kg) of *Camptosorus sibiricus* were extracted with 70% ethanol. The extract was concentrated *in vacuo*, then the extract (596.0 g) was partitioned with petroleum ether (3000 mL), EtOAc (3000 mL) and BuOH (5000 mL) successively. The BuOH fraction (138.0 g) was subjected to column chromatography on silica gel gradiently eluted with $CHCl_3$: MeOH, subfraction 16 [$CHCl_3$: MeOH (100 : 22), 600 mg] was rechromatographed on a silica gel column eluted with EtOAc : MeOH (100 : 14) to give compound **1** (18.0 mg).

Compound **1**: white needle (MeOH), mp 255-257°C. [α]= +23.0 (*c* 0.002, MeOH/H_2O). The ^{1}H-NMR (300 MHz, pyridine-d_5) and ^{13}C-NMR (75 MHz, pyridine-d_5) data: see Table I. ESI-MS: $[M+Na]^+$ at *m/z* 839.5, $[M+Cl]^-$ at *m/z* 851.6, $[M-H]^-$ at *m/z* 815.5, $[M-H-162]^-$ at *m/z* 653.4, $[M-H-2\times162]^-$ at *m/z* 491.3.

RESULTS AND DISCUSSION

An 70% ethanol extract of the air-dried whole herbs of *C. sibiricus* was separated using liquid-liquid extraction.

Table I. The ^1H and ^{13}C-NMR data of compound **1** (in Pyridine-d_5, ^1H 300 MHz, ^{13}C 75 MHz)

No.	δ_H	δ_C	No	δ_H	δ_C
1		32.8	22		31.5
2	2.07 (1H, m), 1.62 (1H, m)	32.2	23		29.5
3	3.79 (1H, m)	88.8	24	4.10 (1H, m)	93.5
4		45.1	25		72.8
5	2.41 (1H, br.s)	42.4	26	1.76 (3H, s)	25.8
6		32.8	27	1.60 (3H, s)	26.4
7	4.27 (1H, m)	67.1	28	0.91 (3H, s)	19.0
8	1.43 (1H, m)	47.3	29	1.58 (3H, s)	20.6
9		20.7	30	3.85 (1H, d, J = 10.7 Hz) 4.56 (1H, d, J = 10.1 Hz)	62.8
10		25.0	1'	5.07 (1H, d, J = 7.8 Hz)	105.7
11		27.9	2'		75.1
12		35.3	3'		78.0
13		44.5	4'		71.1
14		47.9	5'		77.7
15		31.5	6'		62.2
16		27.9	1"	5.22 (1H, d, J = 7.8 Hz)	106.5
17	1.67 (1H, m)	53.2	2"	4.12 (1H)	75.4
18	0.95 (3H, s)	18.0	3"		78.2
19	0.17, 0.39 (each 1 H, d, J = 3.6 Hz)	29.1	4"		71.1
20		35.3	5"		78.0
21	1.10 (3H, d, J = 6.2 Hz)	17.7	6"		62.2

Further purification of the resulting BuOH fraction by repeated silica gel column chromatography with $CHCl_3$: MeOH and EtOAc : MeOH eluting led to the isolation of compound **1**.

Compound **1** was isolated as white needle, mp 255-257°C. It showed a positive reaction with the Molish reagent. The sugar was identified as glucose by acid hydrolysis (Shanghai Institute of Materia medica 1972) and co-TLC with authentic sample ($CHCl_3$-MeOH = 4 : 1, Rf ≈ 0.4).

The ESI-MS spectrum gave quasi-molecular ion peak $[M-H]^-$ at *m/z* 815.4 and $[M+Na]^+$ at m/z 839.5, $[M+Cl]^-$ at *m/z* 851.6 compatible with the molecular formula $C_{42}H_{72}O_{15}$, along with the fragment peaks $[M-H-162]^-$ at *m/z* 653.4, $[M-H-2\times162]^-$ at *m/z* 491.3 representing that its sugar moiety was composed of two glucoses. ^1H-NMR spectrum of **1** displayed characteristic signals (Hua, 2001) of cyclopropane methylene protons at δ 0.17 (1H, d, J = 3.6 Hz, H-19a) and 0.39 (1H, d, J = 3.6 Hz, H-19b), five tertiary methyl and one secondary methyl groups at δ 0.91 (3H, s, 28-CH_3), 0.95 (3H, s, 18-CH_3), 1.58 (3H, s, 29-CH_3), 1.60 (3H, s, 27-CH_3), 1.76 (3H, s, 26-CH_3), and 1.10 (3H, d, J = 6.2 Hz, 21-CH_3). Additionally, the signals of the

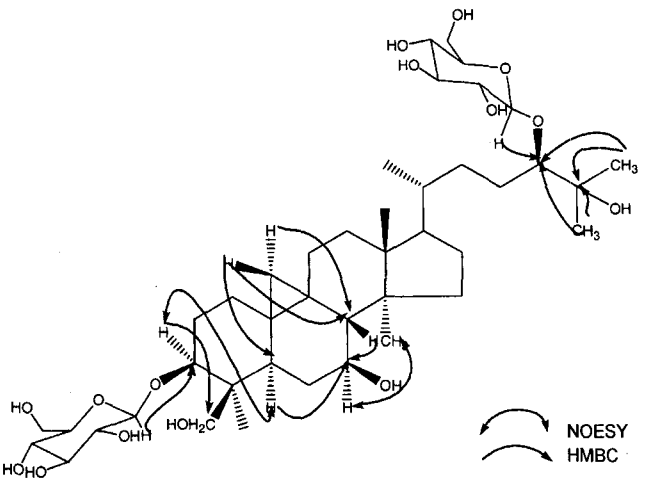

Fig. 1. Important HMBC and NOSEY correlations of **1**

anomeric protons at δ 5.07 (1H, d, J = 7.8 Hz, H-1'), 5.22 (1H, d, J = 7.8 Hz, H-1") were observed. These spectra data indicated that **1** was a cycloartane diglycoside derivative and the centers of glucoses were all β orientation. In the ^{13}C-NMR of **1**, 42 carbon signals were given, of which 5 oxygen bearing carbons of aglycone can be observed at δ 88.8 (C-3), 67.1 (C-7), 93.5 (C-24), 72.8 (C-25), and 62.8 (C-30). And it also presented the carbon signals ascribed to the sugar unit at δ 105.7 (C-1'), 106.5 (C-1"), 62.2~78.2 (C-2'- C-6").

In the HMBC experiment (see Fig. 1), the long-range correlations between δ 1.58 (H-29), 3.85 (H-30a), 4.56 (H-30b) and δ 88.8 (C-3), as well as δ 1.58 (H-29) and δ 62.8 (C-30) indicated that C-3 and C-30 were substituted by hydroxyl groups. In addition, δ 2.41 (H-5), 1.43 (H-8) presented long-range correlations with δ 67.1 (C-7), and in the NOESY spectrum of **1**, the correlations between proton H-5 and H-3, H-7, and H-28 were observed, so the presence of 7-OH was determined. Furthermore, the configurations of 3, 7-OH were determined as β face. At the same time from correlation, the partial fragments deduced from HMBC (see Fig. 1), combined with HMQC, NOESY and ^1H-^1H COSY spectra, the aglycone of **1** was determined as 3β, 7β, 24, 25, 30-pentahydroxy-9, 19-cycloartane. The anomeric protons of β-D-glucoses at δ 5.22 (1H, d, J = 7.8 Hz, H-1") and δ 5.07 (1H, d, J = 7.8 Hz, H-1') showed long-range correlation to δ 93.5 (C-24) and δ 88.8 (C-3) respetively, which suggested the connections of sugars to C-24 and C-3. The ^1H and ^{13}C-NMR data for H-24 and C-24 of **1** were comparable to those reported for analogous compounds having a 24*R* configuration (Isaev *et al.*, 1992; Calis *et al.*, 1996). With the data above, the structure of **1** was established as 3β, 7β, 24β, 25, 30-pentahydroxycycloartane-3-*O*-β-D-glucopyranoside-24-*O*-β-D-glucopyranoside (**1**).

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