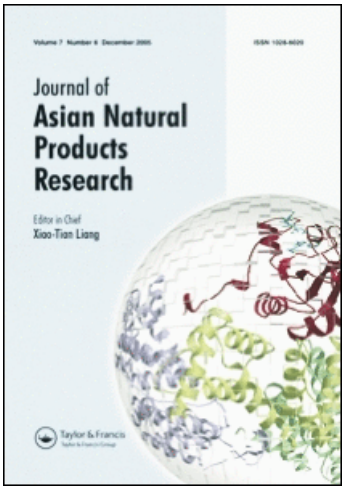

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A NEW TRITERPENOID FROM *STELMATOCRYPTON KHASIANUM*

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A new triterpenoid, 2α-,3β-,19α-trihydroxy-urs-12-ene-24,28-dioic acid (**1**), along with two known compounds, 3β-acetoxy-urs-12-ene-11-one (**2**) and vomifoliol (**3**), was isolated from stems of *Stelmatocrypton khasianum* for the first time. Their structures were elucidated on the basis of chemical and spectroscopic methods.

Keywords: Stelmatocrypton khasianum; Asclepiadaceae; 2α-,3β-,19α-Trihydroxy-urs-12-ene-24,28-dioic acid

INTRODUCTION

Stelmatocrypton khasianum (Benth.) Bail.(Asclepiadaceae) is distributed in India, and Yunnan, Guizhou and Sichuan Provinces of China. It is used in Chinese folk medicine for the treatment of cold, tracheitis, stomachache and rheumatic ache. However, only one compound, 4-methoxy salyclic aldehyde, was obtained from its roots by steam distillation [1]. We have taken this plant for systematic phytochemical investigations and some compounds have been isolated. This paper reports the isolation and structural elucidation of a new triterpenoid, 2α-,3β-,19α-trihydroxy-urs-12-ene-24,28-dioic acid (**1**), together with two known compounds, 3β-acetoxy-urs-12-ene-11-one (**2**) and vomifoliol (**3**).

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RESULTS AND DISCUSSION

Ninety-five percent ethanol extracts of stems of *S. khasianum* were suspended in H_2O and then extracted with petroleum ether, ethyl ether and *n*-BuOH, respectively. The ethyl ether soluble part was separated by usual chromatographic methods to afford compound **1**.

Compound **1** was obtained as a white powder. A positive Libermann–Buchard reaction and its NMR spectra suggested it to be a triterpenoid. The ^{1}H-NMR spectrum showed signals for six methyl groups at δ 1.05, 1.11, 1.39, 1.65, 1.68 (each 3H, s) and 1.06 (3H, d, $J=8.5$ Hz), two carbinol protons at δ 3.35 (1H, d, $J=8.0$ Hz) and 4.69 (1H, m), and one trisubstituted olefinic proton at δ 5.51 (1H, brs). The ^{13}C-NMR spectrum revealed the presence of three oxygenated carbons at δ 68.2, 72.3 and 83.7, a pair of olefinic carbons at δ 127.6 and 139.5, and two carbonyl carbons at δ 180.3 and 180.5. The DEPT spectrum showed the presence of nine quaternary carbons (C), seven tertiary carbons (CH), eight secondary carbons (CH_2) and six primary carbons (CH_3). The FAB-MS exhibited quasimolecular ion peaks at m/z 519 ($M^+ + 1$) and 541 ($M^+ + Na$), corresponding to a molecular formula of $C_{30}H_{46}O_7$. The above evidences suggested that compound **1** was a trihydroxy-substituted urs-12-ene type triterpenoid with two carboxylic groups, and one of the three hydroxyl groups was linked at 19 or 20 position because there was only one doublet methyl group. The presence of a singlet at δ 2.96 attributed to H-18 in the ^{1}H-NMR spectrum indicated that the hydroxyl group was located at 19 position which was further confirmed by the absence of cross peaks of H-18 in the ^{1}H–^{1}H COSY spectrum. In the ^{1}H-NMR spectrum, two one proton signals for carbinol protons at δ 3.35 and 4.69 were ascribed to H-3 and H-2, respectively, on the basis of biogenetic analogy and analysis of NMR spectra, and the large coupling constant of $J_{2,3}$ (8.0 Hz) indicated that 2-OH and 3-OH were α- and β-orientated, respectively. The HMBC spectrum showed strong cross peaks between δ 180.3 (C<u>O</u>OH) and δ 3.34 (H-3), 1.18 (H-5) and δ 1.68 (C<u>H</u>$_3$), and δ 180.5 (C<u>O</u>OH) and 2.96 (H-18), suggesting that the carboxyl carbon at δ 180.3 be linked at 4 position and the carboxyl carbon at δ 180.5 be linked at 17 position (Fig. 1). Therefore, the structure of compound **1** must be either 2α-,3β-,19α-trihydroxy-urs-12-ene-23,28-dioic acid or 2α-,3β-,19α-trihydroxy-urs-12-ene-24,28-dioic acid. However, the ^{13}C-NMR data of compound **1** were not in agreement with the published data [2] of 2α-,3β-,19α-trihydroxy-urs-12-ene-23,28-dioic acid (Table I), which suggested that the structure of compound **1** should be 2α-,3β-,19α-trihydroxy-urs-12-ene-24,28-dioic acid. The conclusive evidence for the structure of compound **1**

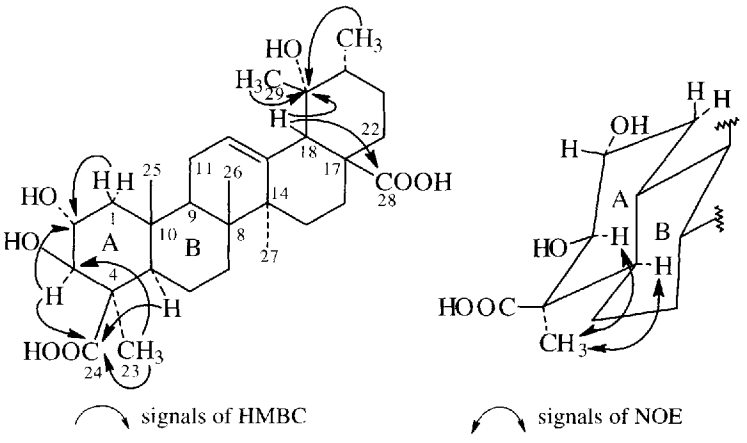

FIGURE 1 The key HMBC and NOE correlations of compound **1**.

TABLE 1 ^{13}C-NMR data of 2α-,3β-,19α-trihydroxy-urs-12-ene-23,28-dioic acid (C_5D_5N) and NMR data of compound **1** (C_5D_5N, 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR)

No.	**8***	*Compound* **1**			
	δ C	*δ C*	*DEPT*	*HMBC*	*δ H*
1	48.1	47.9	CH_2	25-H	2.33 dd (3.5, 12.5), 1β-H
2	68.6	68.2	CH	1-H, 3-H	4.69 m
3	80.9	83.7	CH	1-H, 23-H	3.34 d (9.0)
4	54.7	49.7	C	23-H, 3-H, 5-H	
5	52.2	56.5	CH	1-H, 23-H, 25-H	1.18 m
6	21.4	20.6	CH_2	5-H	
7	33.3	33.4	CH_2	26-H	
8	40.5	39.9	C	15-H, 27-H, 26-H	
9	48.1	46.8	CH	12-H, 25-H, 26-H	1.87 m
10	38.5	38.6	C	1-H, 9-H, 5-H	
11	24.2	23.9	CH_2	12-H	
12	127.7	127.6	CH	18-H	5.51 brs
13	139.9	139.5	C	18-H, 27-H	
14	42.0	41.9	C	12-H, 18-H	
15	29.1	28.9	CH_2	27-H, 16-H	2.23 m, 15β-H
16	26.3	26.0	CH_2	18-H, 15-H	3.02 m, 16α-H
17	48.6	47.9	C	16-H, 18-H	
18	54.7	54.3	CH	12-H, 29-H	2.96 s
19	72.7	72.3	C	18-H, 29-H, 30-H	
20	42.0	42.0	CH	29-H	1.42 m
21	27.1	26.5	CH_2	30-H	
22	38.5	38.1	CH_2		
23	180.6	24.7	CH_3	3-H, 5-H	1.68 s
24	13.4	180.3	C	3-H, 5-H, 23-H	
25	17.3	14.9	CH_3	1-H, 9-H	1.11 s
26	17.1	16.8	CH_3	9-H	1.05 s
27	24.7	24.2	CH_3	15-H	1.65 s
28	180.0	180.5	C	18-H	
29	27.1	26.7	CH_3		1.39 s
30	16.8	16.4	CH_3		1.06 d (6.0)

*2α-,3β-,19α-trihydroxy-urs-12-ene-23,28-dioic acid was numbered as compound **8** in Ref. [2].

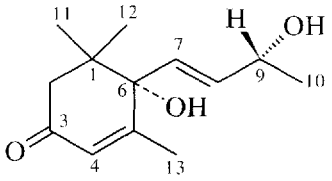

FIGURE 2 The structure of compound **3**.

was derived from the results of NOESY spectrum which showed cross peaks between 23-H and 3-H, and 23-H and 5-H (Fig. 1). Thus the structure of compound **1** was established as 2α-,3β-,19α-trihydroxy-urs-12-ene-24,28-dioic acid unambiguously.

The structures of compounds **2** and **3** were determined by comparison of the physical and spectral data with those of the Refs. [3–5] and further confirmed by 2D NMR spectra (Fig. 2). Moreover, the ^{13}C-NMR signals of compounds **2** and **3** were assigned totally for the first time on the basis of 2D NMR spectra because of the absence of ^{13}C-NMR data of compound **2** and the incomplete assignment of compound **3** [5].

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on an X_4 micro-melting point apparatus and were uncorrected. NMR spectra were recorded on a Bruker-AM 500 spectrometer or a INOVA-500 spectrometer. EI-MS were recorded on an AEI MS-50 mass spectrometer and FAB-MS were taken on a ZABspec mass spectrometer. Optical rotation was determined on an AA-IOR Automatic Polarimeter. TLC was performed on silica gel GF_{254} (10–40 μm, Qingdao). Separation and purification were performed by column chromatography on silica gel (200–300 mesh, Qindao) and Sephadex LH-20 (Pharmacia).

Plant Materials

Dried stems of *S. khasianum* were purchased from Xishuangbanna of Yunnan Province in October 1997. A voucher specimen was identified by Prof. H.B. Chen and deposited in the Herbarium of Division of Natural Medicinal Chemistry of Beijing Medical University.

Extraction and Isolation

Air-dried powered stems of *S. khasianum* (9.5 kg) were percolated with 95% EtOH. After evaporation of the solvent, the residues were suspended in H_2O and then extracted with petroleum ether, ethyl ether and *n*-BuOH, respectively.

The ethyl ether extracts (104 g) were subjected to silica gel column chromatography using $CHCl_3$–CH_3OH (100 : 0 to 65 : 35) as gradient eluting system to afford 91 fractions (500 ml/fraction). Fractions 55–56 were further subjected to silica gel column chromatography eluted with $CHCl_3$–$(CH_3)_2CO$ (100 : 0 to 60 : 40) as gradient solvent system to yield 428 fractions (400 ml/fraction). Fractions 131–140 (600 mg) and fractions 208–250 (1.2 g) were further purified by repeated Sephadex LH-20 and silica gel column chromatography to furnish compound **3** (45 mg) and compound **1** (20 mg) respectively.

The petroleum ether extracts (144 g) were subjected to silica gel column chromatography eluted with petroleum ether–acetone (100 : 0 to 60 : 40) as gradient eluting system to yield 321 fractions (500 ml/fraction). Fractions 110–130 (4.5 g) were further chromatographed on silica gel column with petroleum ether–acetyl acetate (95 : 5) as eluent to afford compound **2** (58 mg).

2α-,3β-,19α-Trihydroxy-urs-12-ene-24,28-dioic acid (**1**) white powder, m.p. 280–283°C. $[\alpha]_D$ −45.45 (in pyridine). FAB-MS *m/z* 541 ($M^+ + Na$), 519 ($M^+ + 1$). For NMR data see Table I.

3β-Acetoxy-urs-12-ene-11-one (**2**) white needles, m.p. 286–288°C. EI-MS *m/z* 482 (M^+), 422, 287, 273, 232. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.80 (3H, d, $J = 6.0$ Hz, H-29), 0.81, 0.87, 0.88, 1.16, 1.18, 1.29 (each 3H, s, H-28, 23, 24, 26, 25, 27), 0.94 (3H, d, $J = 6.0$ Hz, H-30), 2.04 (3H, s, $COC\underline{H}_3$), 2.34 (1H, s, H-9), 2.75 (1H, ddd, $J = 3.5, 3.5, 13.5$ Hz, H-1β), 4.51 (1H, dd, $J = 4.6, 11.7$ Hz, H-3), 5.54 (1H, s, H-12). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 38.9 (C-1), 23.6 (C-2), 80.7 (C-3), 38.1 (C-4), 55.1 (C-5), 17.5 (C-6), 32.8 (C-7), 45.2 (C-8), 61.5 (C-9), 36.7 (C-10), 199.5 (C-11), 130.5 (C-12), 164.8 (C-13), 43.7 (C-14), 27.2 (C-15), 27.3 (C-16), 33.9 (C-17), 59.1 (C-18), 39.2 (C-19), 39.3 (C-20), 30.9 (C-21), 40.9 (C-22), 28.1 (C-23), 16.7 (C-24), 16.5 (C-25), 18.6 (C-26), 20.5 (C-27), 28.9 (C-28), 17.5 (C-29), 21.2 (C-30), 21.1 ($COC\underline{H}_3$), 170.9 ($\underline{C}OCH_3$).

Vomifoliol (**3**) white needles, m.p. 108–110°C. EI-MS *m/z* 206 ($M^+ - H_2O$), 168, 150, 135, 124, 122, 111, 107, 79, 77, 69, 55, 43. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.02, 1.08 (each 3H, s, H-11, 12 or H-12, 11), 1.30 (3H, d, $J = 6.5$ Hz, H-10), 1.90 (3H, d, $J = 1.0$ Hz, H-13), 2.19 (1H, brs, O$\underline{H}$), 2.24,

2.45 (each 1H, d, $J = 17.0$ Hz, H-2), 4.41 (1H, m, H-9), 5.79 (1H, d, $J = 15.0$ Hz, H-7), 5.85 (1H, dd, $J = 5.0, 15.0$ Hz, H-8), 5.90 (1H, d, $J = 1.0$ Hz, H-4). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 41.1 (C-1), 49.6 (C-2), 198.4 (C-3), 128.9 (C-4), 163.4 (C-5), 79.0 (C-6), 126.7 (C-7), 135.7 (C-8), 67.9 (C-9), 23.7 (C-10), 24.0, 22.9 (C-11, 12 or C-12, 11), 19.0 (C-13).

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