New Triterpenoid Saponins from *Stelmatocrypton khasianum*

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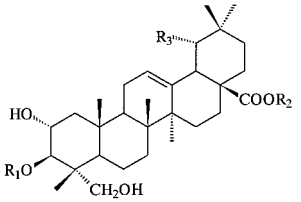
Four new triterpenoid saponins, designated as stelmatotriterpenosides E—H (1—4), together with three known compounds, asterbatanoside B (5), 2α,3β,19α,23-tetrahydroxy-olean-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl ester (6) and 2α,3β,19α,23-tetrahydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl ester (7), were isolated from the stems of *Stelmatocrypton khasianum.* On the basis of chemical and spectral evidence, the structures of 1—4 were established as 2α,3β,23-trihydroxy-olean-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (1), 2α,3β,23-trihydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (2), 2α,3β,19α-trihydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl ester (3), and 2β,3β,19α-trihydroxy-urs-12-en-24,28-dioic acid-24-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl diester (4).

Key words *Stelmatocrypton khasianum*; Asclepiadaceae; stelmatotriterpenoside; triterpenoid saponin

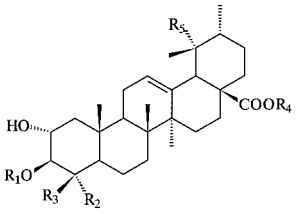
Stelmatocrypton khasianum (BENTH.) BAIL. (Family: Asclepiadaceae) is distributed in Yunnan, Guizhou, and Sichuan Provinces of China and has been used in Chinese folk medicine for the treatment of colds, tracheitis, stomachaches, and rheumatic aches. The preliminary screening test for anti-cancer activity *in vitro* showed that the crude extracts of *S. khasianum* had significant cytotoxic activity against some human cancer cell lines. As part of our search for anti-cancer agents from plants, the chemical constituents of *S. khasianum* were investigated and four new pregnane glycosides, stelmatocryptonosides A—D, were reported.[1] This paper deals with the isolation and structural elucidation of four new triterpenoid saponins, stelmatotriterpenosides E—H (**1**—**4**), together with three known compounds (**5**—**7**), from this plant.

The stems of *S. khasianum* (9.5 kg) were extracted with 95% EtOH. After evaporation of the solvent, the residues were suspended in H_2O and extracted with petroleum ether, EtOAc, and *n*-BuOH, successively. The *n*-BuOH extracts were separated by a combination of column chromatography on silica gel, D101 macroporous resin, Rp C_{18}, Sephadex LH-20, and finally semipreparative HPLC to afford four new compounds (**1**—**4**) and three known compounds (**5**—**7**). The three known compounds were identified as asterbatanoside B [2α,3β,23-trihydroxy-olean-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester] (**5**),[2] 2α,3β,19α,23-tetrahydroxy-olean-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl ester (**6**),[3] and 2α,3β,19α,23-tetrahydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl ester (**7**),[4] respectively, by comparing the spectra and chemical data with the literature.

Stelmatotriterpenoside E (**1**) was isolated as a white powder and showed positive Liebermann–Burchard and Molish reactions. The molecular formula $C_{48}H_{78}O_{20}$ was determined by a combination of NMR spectra and HRSI-MS (High Resolution Second Ion Mass Spectrum) (neg.) which exhibited a quasi-molecular ion peak at *m*/*z* 973.5006 $[M-H]^-$ (Calcd for $C_{48}H_{77}O_{20}$: 973.5014). On acid hydrolysis, **1** afforded only glucose as the sugar moiety that was identical to an authentic sample. The ^{13}C-NMR spectrum revealed 48 signals, of which 30 were assigned to the aglycone and 18 to the sugars. The ^{1}H-NMR spectrum showed signals for six methyl protons [δ 0.82, 0.83, 0.99, 1.02, 1.10, 1.12 (each 3H, s)], one olefinic methine proton [δ 5.36 (1H, t-like)], and three anomeric protons [δ 5.02 (1H, d, J=8.0 Hz), 5.16 (1H, d, J=7.0 Hz), 6.25 (1H, d, J=8.0 Hz)]. In the ^{13}C-NMR spectrum, the signals for a pair of characteristic olefinic carbons of olean-12-ene type triterpenoid (δ 122.74, 144.07) and three anomeric carbons (δ 95.59, 105.26, 105.73) were observed. The forgoing evidence suggested that **1** may be an olean-12-ene type triterpenoid glycoside with three glucose residues. The ^{13}C signals at δ 63.84, 66.96, 88.48, and 176.47, assigned to the aglycone moiety by careful examination of the NMR spectra, indicated the presence of three carbinyl carbons and one carboxyl carbon in the aglycone. Comparison of the ^{13}C-NMR data of the aglycone moiety of



	R_1	R_2	R_3
1	β-D-glc	β-D-glc-(1→6)-β-D-glc	H
5	H	β-D-glc-(1→6)-β-D-glc	H
6	β-D-glc	β-D-glc	OH



	R_1	R_2	R_3	R_4	R_5
2	β-D-glc	CH_2OH	CH_3	β-D-glc-(1→6)-β-D-glc	H
3	β-D-glc	CH_3	CH_3	β-D-glc-(1→2)-β-D-glc	OH
4	H	CH_3	COO-β-D-glc	β-D-glc	OH
7	β-D-glc	CH_2OH	CH_3	β-D-glc	OH

Fig. 1. Structures of Compounds **1**—**7**

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Table 1. Selected ^{1}H-NMR Data for Compounds **1**—**4** (500 MHz, C_5D_5N)

H	1	2	3	4
2	4.23	4.21	4.01	4.89
3	4.25	4.25	3.23 (d, 9.2)	3.44 (d, 9.5)
12	5.36 (t-like)	5.39 (t-like)	5.52 (t-like)	5.50 (t-like)
18	3.14 (dd, 12.5, 3.0)	2.46 (d, 12.0)	2.91 (s)	2.90 (s)
23	3.67 (d, 10.0), 4.54	3.67 (d, 10.0), 4.54	1.32 (s)	1.81 (s)
24	0.99 (s)	0.99 (s)	0.98 (s)	
25	1.02 (s)	1.03 (s)	0.91 (s)	1.34 (s)
26	1.10 (s)	1.14 (s)	1.08 (s)	1.23 (s)
27	1.12 (s)	1.06 (s)	1.68 (s)	1.65 (s)
29	0.82 (s)	0.86 (d, 6.0)	1.40 (s)	1.37 (s)
30	0.83 (s)	0.80 (d, 6.0)	1.07 (d, 7.0)	1.05 (d, 6.0)
3-*O*-glc				
1	5.16 (d, 7.0)	5.16 (d, 8.0)	4.92 (d, 7.8)	
2	4.10	4.10	4.08	
3	4.24	4.12	4.25	
4	4.21	4.20	4.21	
5	3.90	3.90	4.09	
6	4.32, 4.50	4.31, 4.54	4.33, 4.59	
24-*O*-glc				
1				6.33 (d, 7.8)
2				4.19
3				4.24
4				4.29
5				4.03
6				4.28, 4.39
28-*O*-sugars				
inner-glc′				
1′	6.25 (d, 8.0)	6.19 (d, 8.5)	6.19 (d, 8.2)	6.24 (d, 8.0)
2′	4.10	4.08	4.46 (t, 8.5)	4.21
3′	4.21	4.21	4.30 (t, 8.5)	4.27
4′	4.34	4.34	4.25	4.24
5′	4.10	4.08	3.95	4.01
6′	4.36, 4.71	4.32, 4.70	4.36, 4.43	4.39, 4.47
outer-glc″				
1″	5.02 (d, 8.0)	5.03 (d, 8.0)	5.67 (d, 7.7)	
2″	4.00 (t, 8.0)	4.00 (t, 8.0)	4.09	
3″	4.21	4.20	4.22	
4″	4.10	4.14	4.12	
5″	3.87	3.89	4.00	
6″	4.36, 4.48	4.34, 4.49	4.40, 4.62	

Overlapped proton signals are reported without designated multiplicity.

1 with those of 2α,3β,23-trihydroxy-olean-12-en-28-oic acid[5] showed that the signal for C-3 of **1** was significantly shifted downfield by +9.8 ppm to 88.48, and the C-28 signal was shifted upfield by −2.1 ppm to 176.47, while the others were very similar. Furthermore, the ^{13}C-NMR data of **1** were in good agreement with those of arijunolitin (2α,3β,23-trihydroxy-olean-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl ester)[6] except for the presence of a set of signals for one additional glucose in the spectra of **1**. From these observations, it was deduced that **1** was a 3,28-bis-desmoside of 2α,3β,23-trihydroxy-olean-12-en-28-oic acid with three units of glucose, which was further confirmed by the two-dimensional (2D)-NMR spectra. 1D- and 2D-NMR techniques (correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), total correlation spectroscopy (TOCSY), and heteronuclear multiple bond correlation (HMBC)) permitted assignment of all the ^{1}H- and ^{13}C-NMR signals for the aglycone and sugar moieties of **1** (Tables 1, 2). The exact sugar arrangement was determined to be 3-*O*-glucose and 28-*O*-glucosyl-(1→6)-glucose on the basis of the HMBC correlations between signals at δ 5.16 (glc H-1) and δ 88.48 (aglycone C-3), δ 6.25 (glc′ H-1′) and δ 176.47 (aglycone C-28), and δ 5.02 (glc″ H-1″) and δ 69.29 (glc′ C-6′). The HRSI-MS (neg.) spectrum that revealed the prominent fragment ion peak at *m*/*z* 649.3939 $[M-2glc]^-$ provided additional evidence for the proposed sequence of the sugar units because the ester glycosidic linkage was more easily broken than the *O*-glycosidic linkage. The D-configuration has been assumed for the glucoses in keeping with Massiot and Lavaud's assertion regarding the D-sugars commonly found in the plant kingdom: "The enantiomers of these sugars (glucose, galactose, *etc.*) are not found in plants, a fact used as a clue in the determination of these sugars".[7] Evaluation of the spin-spin couplings and chemical shifts allowed the identification of β-D-glucopyranoses. Thus, the structure of stelmatotriterpenoside E (**1**) was unambiguously established as 2α,3β,23-trihydroxy-olean-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Stelmatotriterpenoside F (**2**), a white powder, showed positive Liebermann–Burchard and Molish reactions and yielded

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**4** (125 MHz, C_5D_5N)

C	1	2	3	4	C	1	2	3	4
1	46.93	47.98	47.82	48.70	3-*O*-glc				
2	66.96	66.98	66.75	68.17	1	105.73	105.72	106.51	
3	88.48	88.46	95.65	84.22	2	75.10	75.14	75.65	
4	44.62	44.62	40.79	51.04	3	78.68	78.34	78.78	
5	48.05	47.17	55.84	57.51	4	70.82	70.94	71.76	
6	18.19	18.15	18.72	20.91	5	78.34	78.34	78.37	
7	32.61	33.00	33.59	33.81	6	62.52	62.57	62.70	
8	39.87	40.08	40.66	40.54	24-*O*-glc				
9	47.23	47.54	47.64	47.49	1				95.96
10	37.78	37.67	37.48	39.05	2				74.17
11	23.77	23.68	24.18	24.42	3				78.88
12	122.74	125.79	128.28	128.49	4				71.48
13	144.07	138.37	139.47	139.14	5				79.18
14	42.07	42.44	42.18	42.30	6				62.63
15	28.19	28.64	29.86	29.15	28-*O*-sugars				
16	23.86	24.50	26.00	26.33	inner-glc′				
17	47.23	48.32	48.67	48.80	1′	95.59	95.56	93.77	95.84
18	41.55	53.09	54.60	54.48	2′	73.80	73.74	79.44	74.12
19	46.05	39.21	72.94	72.72	3′	78.51	78.66	79.09	78.91
20	30.65	38.95	42.23	42.12	4′	71.41	71.44	70.99	71.39
21	33.87	30.68	26.82	26.74	5′	77.92	77.87	79.10	79.18
22	32.50	36.72	37.87	37.62	6′	69.29	69.46	62.37	62.50
23	63.84	63.80	28.61	24.82	outer-glc″				
24	14.70	14.74	18.17	175.87	1″	105.26	105.27	104.90	
25	17.51	17.58	16.82	15.56	2″	75.39	75.40	76.00	
26	17.40	17.67	17.39	17.25	3″	78.34	78.45	78.52	
27	25.97	23.65	24.68	24.38	4″	71.29	71.30	72.94	
28	176.47	176.23	176.96	176.86	5″	78.43	78.51	78.14	
29	32.99	17.26	27.07	27.06	6″	62.25	62.26	63.94	
30	23.57	21.17	16.69	16.69					

glucose as the only sugar moiety on acid hydrolysis. Its TOF-MS (Time of Fly Mass Spectrum) (posit.) showed $[M+K]^+$ and $[M+Na]^+$ ion peaks at *m/z* 1012.6 and 996.6, respectively. The ^{13}C-NMR spectrum revealed 48 signals, of which 30 were assigned to the aglycone moiety and 18 to the sugar part. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **1** showed that the signals due to the sugar moiety were in such good agreement that it can be proposed that compound **2** had the same sugar arrangement as that of **1** and they differentiated only in the aglycone part. In the ^{1}H-NMR spectrum, **2** exhibited six methyl signals of which two were doublet [δ 0.80 (3H, d, J=6.0 Hz), 0.86 (3H, d, J=6.0 Hz)], four were singlet [δ 0.99, 1.03, 1.06 and 1.14 (each 3H, s)], and one olefinic methine signal [δ 5.39 (1H, t-like)]. The ^{13}C-NMR spectrum of **2** showed the signals for a pair of characteristic olefinic carbons of urs-12-ene type triterpenoid at δ 125.79 and 138.37. These data suggested that **2** was a urs-12-en type tritrpenoid. The ^{13}C signals of 63.80, 66.98, 88.46, and 176.23 corresponding to the aglycone of **2** indicated the presence of three carbinyl carbons and one carboxyl carbon in the aglycone moiety. Detailed comparison of the ^{13}C-NMR data of the aglycone of **2** with those of asiatic acid (2α,3β,23-trihydroxy-urs-12-en-28-oic acid)[8] showed glycosylation shifts at C-3 (+9.6 ppm) and C-28 (−3.6 ppm) while other data were very similar, from which **2** was deduced to be a 3,28-bisdesmoside of asiatic acid. The ^{1}H- and ^{13}C-NMR signals for the aglycone and sugar moieties of **2** were assigned based on the 1D- and 2D-NMR spectra (COSY, DEPT, HMQC, TOCSY, and HMBC) and the sugar sequence was unambiguously confirmed to be 3-*O*-β-D-glucopyranose and 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranose by the HMBC results which showed the correlations between signals at δ 5.16 (glc H-1) and δ 88.46 (aglycone C-3), δ 6.19 (glc′ H-1′) and δ 176.23 (aglycone C-28), and δ 5.03 (glc″ H-1″) and δ 69.46 (glc′ C-6′). The HRSI-MS (neg.) spectrum failed to give the molecular ion peak but revealed a prominent fragment ion peak at 649.3935 [M−2glc]$^-$, which further proved the sequence of the sugars. Therefore, the structure of stelmatotriterpenoside F (**2**) was elucidated as 2α,3β,23-trihydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Stelmatotriterpenoside G (**3**) was obtained as a white powder and showed positive Liebermann–Burchard and Molish reactions. Its TOF-MS (posit.) spectrum exhibited $[M+K]^+$ and $[M+Na]^+$ as quasi-molecular ion peaks at *m/z* 1013.8 and 997.8, respectively. On acid hydrolysis, **3** yielded glucose as the only sugar moiety. The ^{13}C-NMR spectrum exhibited 48 signals, of which 30 were assigned to the aglycone moiety and 18 to the sugars. In the ^{1}H-NMR spectrum, **3** showed signals for seven methyl protons [δ 0.91, 0.98, 1.08, 1.32, 1.40, 1.68 (each 3H, s), and 1.07 (3H, d, J=7.0 Hz)], one olefinic methine proton [δ 5.52 (1H, t-like)], and three anomeric protons [δ 4.92 (1H, d, J=7.8 Hz), 5.67 (1H, d, J=7.7 Hz), 6.19 (1H, d, J=8.2 Hz)]. The ^{13}C-NMR spectrum revealed the signals for a pair of characteristic olefinic carbons of urs-12-ene type triterpenoid (δ 128.28, 139.47) and three anomeric carbons (δ 93.77, 104.90, 106.51). Based on the above data, it was deduced that **3** was a urs-12-ene type triterpenoid glycoside with three units of glucose. On de-

tailed examination of the NMR spectra, the ^{13}C signals at δ 66.75, 72.94, 95.65 and 176.96 were assigned to the aglycone moiety, indicating the presence of three carbinyl carbons and one carboxyl carbon in the aglycone of **3**. Comparing the ^{13}C-NMR data of the aglycone moiety of **3** with those of 2α,3β,19α-trihydroxy-urs-12-en-28-oic acid[9] revealed that the data were almost the same except for the glycosylation shifts at C-3 (+11.7 ppm) and C-28 (−3.8 ppm), implying that **3** was a 3,28-bisdesmoside of 2α,3β,19α-trihydroxy-urs-12-en-28-oic acid. In the HMBC spectrum, significant correlations between signals at δ 4.92 (glc H-1) and δ 95.65 (aglycone C-3), δ 6.19 (glc′ H-1′) and δ 176.96 (aglycone C-28), and δ 5.67 (glc″ H-1″) and δ 79.44 (glc′ C-2′) were observed and therefore the structure of stelmatotriterpenoside G (**3**) was concluded to be 2α,3β,19α-trihydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl ester.

Stelmatotriterpenoside H (**4**), a white powder, showed positive Liebermann–Burchard and Molish reactions. The TOF-MS (posit.) spectrum gave quasi-molecular ion peaks at *m*/*z* 881.8 $[M+K]^+$ and 865.8 $[M+Na]^+$. Acid hydrolysis of **4** afforded glucose as the only sugar. The ^{13}C-NMR spectrum revealed 42 signals, of which 30 were assigned to the aglycone and 12 to the sugars. The ^1H-NMR spectrum showed signals for six methyl protons [δ 1.23, 1.34, 1.37, 1.65, 1.81 (each 3H, s), 1.05 (3H, d, J=6.0 Hz)], one olefinic methine proton [δ 5.50 (1H, t-like)], and two anomeric protons [δ 6.24 (1H, d, J=8.0 Hz), 6.33 (1H, d, J=7.8 Hz)]. In the ^{13}C-NMR spectrum the signals for a pair of characteristic olefinic carbons of urs-12-ene type triterpenoid (δ 128.49, 139.14) and two anomeric carbons (δ 95.96, 95.84) were observed. The forgoing data indicated that **4** was a urs-12-ene type triterpenoid glycoside with two glucoses. The ^{13}C signals at δ 68.17, 72.72, 84.22, 175.87, and 176.86, assigned to the aglycone moiety by detailed analysis of the NMR spectra, implied the presence of three carbinyl carbons and two carboxyl carbons in the aglycone of **4**. The ^{13}C-NMR data of the alycone of **4** were similar to those of stelmatocryptonoic acid,[10] which is a new triterpenoid genin also obtained from this plant, except for the glycosylation shifts at C-24 (−4.4 ppm) and C-28 (−3.6 ppm), suggesting that **4** was a 24,28-bisdesmoside of stelmatocryptonoic acid with two glucose residues. This hypothesis was also in agreement with the relatively downfield shifts of anomeric protons (δ 6.33, 6.24) and the corresponding upfield shifts of anomeric carbons (δ 95.96, 95.84). All the ^1H- and ^{13}C-NMR signals for the aglycone and sugar moieties of **4** were assigned based on the 2D-NMR spectra and the exact glycosidic linkages at C-24 and C-28 were unambiguously confirmed by the following HMBC cross-peaks between δ 6.33 (glc H-1) and 175.87 (C-24), and δ 6.24 (glc′ H-1′) and 176.86 (C-28). Therefore, the structure of stelmatotriterpenoside H (**4**) was identified as stelmatocryptonoic acid-24-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl diester.

Experimental

General Procedures Melting points were determined on an X_4-A micro-melting point apparatus and uncorrected. Optical rotations were recorded on an AA-IOR Automatic Polarimeter. NMR spectra were taken on a Bruker DRX-500 spectrometer. HRSI-MS spectra were obtained on an APEX II mass spectrometer and TOF-MS spectra were recorded on a BIFLEX II MADLI-TOF mass spectrometer. TLC was performed on silica gel GF_{254} (10—40 μm, QingdaoHaiyang). Separation and purification were performed by column chromatography on macroporous resin D101 (Nanda), silica gel (200—300 mesh, QingdaoHaiyang), Sephadex LH-20 (Pharmacia), and Rp C_{18} silica gel (100—200 mesh, Pharmacia). HPLC was carried out using a Gilson automatic HPLC system with an Unicom C_{18} column (5 μm, 100 Å, 7.8×300 mm) for semi-preparative or a Waters 600 analytical HPLC system with a Phenomenex C_{18} column (5 μm, 100 Å, 4.6×250 mm) for analysis.

Plant Material Dried stems of *S. khasianum* were purchased from Xishuangbanna Autonomous Prefecture of Yunnan Province, China in October 1997. A voucher specimen was identified by Professor Hubiao Chen of the Department of Natural Medicines, Peking University and deposited at the Herbarium of the Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University.

Isolation and Identification Air-dried, powdered 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 extracted with petroleum ether, EtOAc, and *n*-BuOH, successively. The *n*-BuOH extracts (370 g) were subjected to column chromatography on silica gel (3 kg) and eluted with $CHCl_3$–MeOH in order of increasing MeOH concentration (100 : 0→60 : 40) to give 520 fractions (each fraction, 500 ml). Fractions 257—265 were subjected to Rp C_{18} column chromatography to yield **5** (15 mg); Fractions 266—520 were separated on a D101 macroporous resin column and eluted sequentially with 100% H_2O, 30% EtOH, 50% EtOH, 70% EtOH, and 95% EtOH to give the corresponding fractions. The fraction from the 30% EtOH elution (57 g) was subjected to column chromatography on silica gel using $CHCl_3$–MeOH (4 : 1→1 : 1) as a gradient eluent to afford 145 fractions (each fraction 400 ml). Fractions 20—30 were chromatographyed on Rp C_{18} (50% MeOH→70% MeOH) and Sephadex LH-20 (MeOH) and finally, semipreparative HPLC (20% CH_3CN, 2.5 ml/min, detection at 210 nm) to obtain **6** (12 mg) and **7** (40 mg). Fractions 41—62 were isolated using Rp C_{18} (50% MeOH→70% MeOH), Sephadex LH-20 (MeOH) column chromatography and semipreparative HPLC (50% MeOH, 2.5 ml/min, detection at 210 nm) to give **3** (10 mg) and **4** (4 mg). Fractions 63—80 were subjected to Rp C_{18} (50% MeOH→70% MeOH), Sephadex LH-20 (MeOH) column chromatography and semipreparative HPLC (23% CH_3CN, 3.0 ml/min, detection at 210 nm) to yield **1** (24 mg) and **2** (10 mg).

Stelmatotriterpenoside E (**1**): White powder, mp 226—229 °C (dec.). $[\alpha]_D^{25}$ −43.5° (*c*=0.18, MeOH). HRSI-MS (neg.): *m*/*z* 973.5006 $[M-H]^-$ (Calcd for $C_{48}H_{77}O_{20}$: 973.5014), 649.3939 $[M-2glc]^-$. The ^1H- and ^{13}C-NMR data are shown in Tables 1 and 2.

Stelmatotriterpenoside F (**2**): White powder, mp 217—220 °C (dec.). $[\alpha]_D^{25}$ −14.9° (*c*=0.27, MeOH). TOF-MS (posit.): *m*/*z* 1012.6 $[M+K]^+$, 996.6 $[M+Na]^+$. HRSI-MS (neg.): *m*/*z* 649.3935 $[M-2glc]^-$ (Calcd for $C_{36}H_{57}O_{10}$: 649.3957). The ^1H- and ^{13}C-NMR data are shown in Tables 1 and 2.

Stelmatotriterpenoside G (**3**): White powder, mp 185—187 °C. $[\alpha]_D^{25}$ −138.0° (*c*=0.06, MeOH). TOF-MS (posit.): *m*/*z* 881.8 $[M+K]^+$, 865.8 $[M+Na]^+$. The ^1H- and ^{13}C-NMR data are shown in Tables 1 and 2.

Stelmatotriterpenoside H (**4**): White powder, mp 219—221 °C. $[\alpha]_D^{25}$ +200.0° (*c*=0.02, MeOH). TOF-MS (posit.): *m*/*z* 1013.8 $[M+K]^+$, 997.8 $[M+Na]^+$. The ^1H- and ^{13}C-NMR data are shown in Tables 1 and 2.

Acidic Hydrolysis on TLC Plate A sample (1 mg) was dissolved in 1 ml of MeOH and loaded on a TLC plate. The plate was suspended over a solution of 10 ml of 6 N HCl at a temperature of 60 °C for 30 min. After hydrolysis, HCl absorbed by the silica gel on the plate was evaporated. Then the plate was chromatographyed using $CHCl_3$–CH_3OH–H_2O–HOAc (30 : 12 : 4 : 6) as the development system and visualized by spraying with phenylamine-*ortho*-benzenedicarboxylic acid reagent followed by heating. This was used for identifying the sugars by comparison with authentic samples.

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