Three New Pentacyclic Triterpenoids from *Centella asiatica*

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Three new pentacyclic triterpenoids, named centellasaponin I (**1**), centellasaponin J (**2**), and centellasaponin E (**3**), together with three known compounds, were isolated from the whole plants of *Centella asiatica*. Their structures were elucidated on the basis of chemical and spectral analysis, including 1D- and 2D-NMR and HR-MS experiments, and by comparison with literature data.

Introduction. – *Centella asiatica* (L.) URBAN is a traditional herbal medicine used in China, India, Sri Lanka, Southeast Asia, and Africa. It has been widely used for wound healing, and treatment of mental disorders, inflammation, and leprosy. In addition, numerous clinical reports verified the antibacterial, ulcer-preventive, and antidepressive sedative effects of *C. asiatica* preparations, as well as their potential to improve venous insufficiency and microangiopathy [1–4]. Previous chemical studies revealed that pentacyclic triterpenes were the main chemical constituents of this plant [5]. As a part of our continuing search for bioactive components from *Centella asiatica*, our investigations led to the isolation of three new pentacyclic triterpenoids, named centellasaponin I (**1**), centellasaponin J (**2**) and centellasaponin E (**3**), along with three known triterpenes **4**–**6** (*Fig. 1*). Compounds **1** and **2** are the first two pentacyclic triterpenes with C(5)=C(6) bonds. Compound **1** represents the second pentacyclic skeleton containing two C=C bonds found in this plant. Centellasaponin E (**3**) is a new pentacyclic triterpene with a C(3)=O group.

Results and Discussion. – The crude extract of *C. asiatica* was repeatedly subjected to column chromatography (silica gel) and preparative HPLC to afford six compounds. Compounds **1**–**3** were new, and their structures were elucidated on the basis of 1D- and 2D-NMR data in combination with MS studies. The other three compounds were identified as centelloside D (**4**) [6], centellasaponin B (**5**) [7], and isoasiaticoside (**6**) [8], by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was isolated as white amorphous powder. Its HR-ESI mass spectrum exhibited a *quasi*-molecular-ion peak at *m*/*z* 979.4872 ($[M+Na]^+$; calc. 979.4879) consistent with the molecular formula $C_{48}H_{76}O_{19}$. The IR spectrum displayed characteristic absorptions for OH (3441 cm^{-1}), C=O (1731 cm^{-1}), and olefin moieties (1637 cm^{-1}). The ^{1}H-NMR data (*Table*) revealed the presence of seven Me groups. The

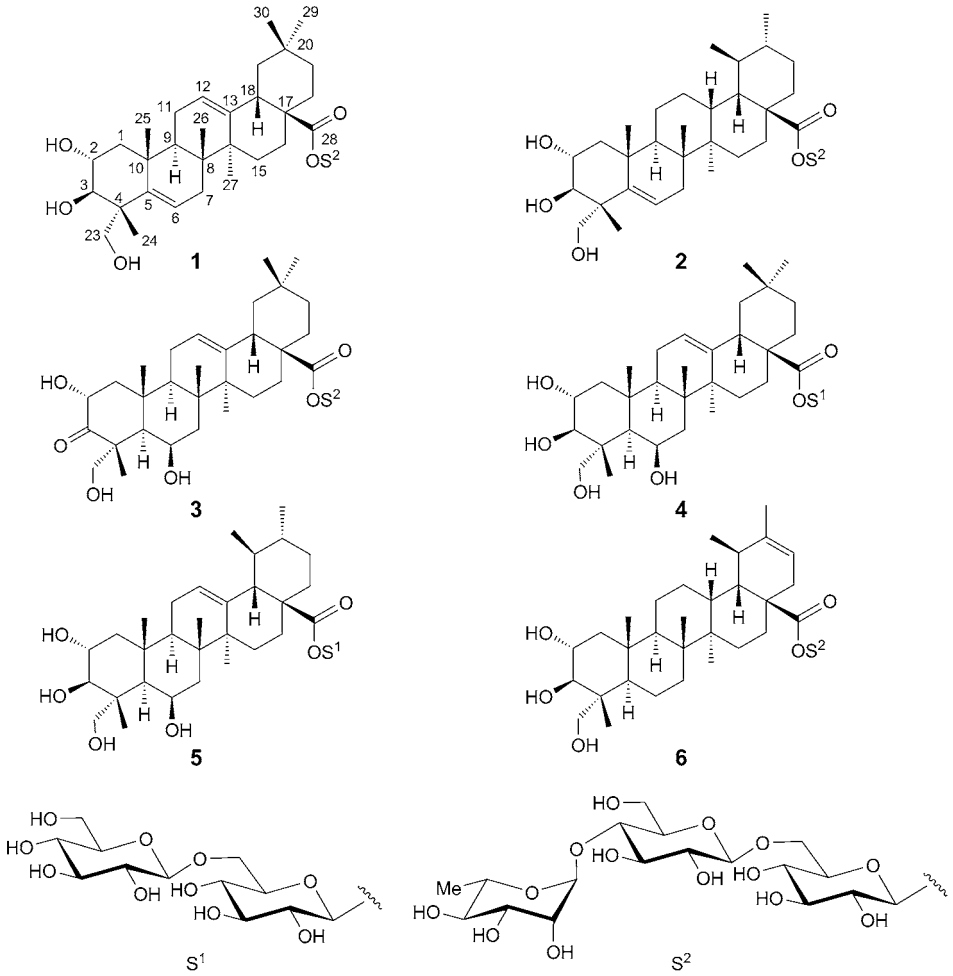

Fig. 1. *Compounds* **1**–**6**, *isolated from* Centella asiatica

chemical shifts of Me(29) and Me(30) displayed a single peak at 0.86 (*s*, 6 H), while they both showed the long-range correlations to CH_2(19) (δ(C) 46.0), CH_2(21) (δ(C) 32.4), and CH_2(22) (δ(C) 33.9) (*Fig. 2*). The structure of compound **1** was deduced to contain an oleanane-type skeleton as its aglycon part. The ^{13}C-NMR spectrum showed two groups of olefinic signals at δ(C) 121.0, 123.8, 144.3, and 146.9. The correlation δ(H) 1.07 (Me(27)/δ(C)144.3 indicated the location of the C=C bond between C(12) (δ(C) 123.8) and C(13) (δ(C) 144.3). Further analysis of HMBCs of both Me(24) (δ(H) 1.44) and Me(25) (δ(H) 1.31) to δ(C) 146.9 revealed that the other C=C bond (δ(C) 121.0 and 146.9) was between C(5) and C(6). The long-range correlations H–C(1′) (δ(H) 6.28)/C(28) (δ(C) 176.5), H–C(1″) (δ(H) 5.01–5.03)/C(6′) (δ(C) 69.2); and H–C(1‴) (δ(H) 5.92)/C(4″) (δ(C) 78.1) revealed the linkage sequence of the

Table. *^{1}H- and ^{13}C-NMR Data of* **1** (in (D_5)pyridine, at 500 and 125 MHz, resp.), **2** (in (D_5)pyridine, at 400 and 100 MHz, resp.), *and* **3** (in CD_3OD, at 400 and 100 MHz, resp.)). δ in ppm, J in Hz.

Position	Centellasaponin I (**1**)		Centellasaponin J (**2**)		Centellasaponin E (**3**)	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	2.29–2.41 (*m*)	47.0 (*t*)	1.15–1.20 (*m*), 2.20–2.24 (*m*)	48.1 (*t*)	1.81 (*d*, *J* = 12.8), 2.67 (*d*, *J* = 12.8)	51.2 (*t*)
2	4.37–4.43 (*m*)	67.9 (*d*)	4.07–4.12 (*m*)	69.1 (*d*)	3.46–3.48 (*m*)	76.9 (*d*)
3	4.20–4.24 (*m*)	78.1 (*d*)	4.01–4.07 (*m*)	78.3 (*d*)		214.8 (*s*)
4		47.1 (*s*)		49.3 (*s*)		43.2 (*s*)
5		146.9 (*s*)		143.1 (*s*)	2.70 (*s*)	58.6 (*d*)
6	5.92 (br. *s*)	121.0 (*d*)	5.33 (*d*, *J* = 6.4)	117.4 (*d*)	3.67 (*d*, *J* = 4.4)	69.0 (*d*)
7		46.8 (*t*)	1.74–1.80 (*m*), 2.39–2.43 (*m*)	37.7 (*t*)	2.00–2.07 (*m*)	48.2 (*t*)
8		38.2 (*s*)		41.4 (*s*)		47.6 (*s*)
9	1.09–1.14 (*m*)	45.6 (*d*)	2.51–2.55 (*m*)	39.3 (*d*)	2.23–2.27 (*m*)	49.2(*d*)
10		38.3 (*s*)		38.6 (*s*)		44.8 (*s*)
11	1.20–1.21 (*m*), 1.37–1.40 (*m*)	29.2 (*t*)	1.57–1.61 (*m*)	27.8 (*t*)	2.00–2.07 (*m*)	25.0 (*t*)
12	5.48 (*s*)	123.8 (*d*)	1.68–1.76 (*m*)	26.0 (*t*)	5.33 (*s*)	123.4 (*t*)
13		144.3 (*s*)	2.51–2.55 (*m*)	39.5 (*d*)		144.6 (*s*)
14		42.6 (*s*)		42.8 (*s*)		43.4 (*s*)
15		27.7 (*t*)	1.02–1.07 (*m*), 1.82–1.94 (*m*)	30.1 (*t*)	1.69–1.75 (*m*)	28.8 (*t*)
16		30.0 (*t*)	0.82–1.05 (*m*), 1.25–1.31 (*m*)	32.2 (*t*)	1.69–1.75 (*m*)	23.9 (*t*)
17		47.1 (*s*)		41.2 (*s*)		48.1 (*s*)
18		41.8 (*d*)	1.38–1.44 (*m*)	51.0 (*d*)	2.90 (*dd*, *J* = 13.6, 4)	42.5 (*d*)
19		46.0 (*t*)	2.51–2.55 (*m*)	38.4 (*d*)	1.69–1.75 (*m*)	47.0 (*t*)
20		30.7 (*s*)	2.25–2.29 (*m*)	37.6 (*d*)		31.6 (*s*)
21		32.4 (*t*)	1.20–1.24 (*m*), 1.37–1.42 (*m*)	34.3 (*t*)	1.22–1.26 (*m*), 1.39 (*d*, *J* = 7.6)	34.9 (*t*)
22		33.9 (*t*)	1.57–1.61 (*m*)	35.7 (*t*)	1.58–1.62 (br. *d*)	33.2 (*t*)
23	4.37–4.43 (*m*)	68.2 (*t*)	3.52–3.58 (*m*), 4.01–4.07 (*m*)	66.7 (*t*)	3.32–3.34 (*m*), 3.48–3.50 (*m*)	65.9 (*t*)
24	1.44 (*s*)	21.7 (*q*)	0.93 (*s*)	23.4 (*q*)	1.08 (*s*)	13.9 (*q*)
25	1.31 (*s*)	22.9 (*q*)	1.15 (*s*)	23.1 (*q*)	1.04 (*s*)	18.9 (*q*)
26	1.26 (*s*)	21.0 (*q*)	0.74 (*s*)	20.4 (*q*)	0.83 (*s*)	18.5 (*q*)
27	1.07 (*s*)	26.0 (*q*)	0.83 (*s*)	14.8 (*q*)	1.30 (*s*)	26.7 (*q*)
28		176.5 (*s*)		174.9 (*s*)		178.0 (*s*)
29	0.86 (*s*)	23.7 (*q*)	1.05 (*s*)	16.4 (*q*)	0.92 (*s*)	33.4 (*q*)
30	0.86 (*s*)	33.1 (*q*)	0.90 (*s*)	18.1 (*q*)	0.95 (*s*)	24.1 (*q*)
Glc I						
1′	6.28 (*d*, *J* = 10)	95.6 (*d*)	6.16–6.19 (*m*)	95.2 (*d*)	5.34 (*d*, *J* = 2.8)	95.9 (*d*)
2′	4.37–4.43 (*m*)	74.1 (*d*)	3.89–4.01 (*m*)	74.0 (*d*)	3.37–3.39 (*m*)	73.8 (*d*)
3′	4.20–4.24 (*m*)	78.8 (*d*)	4.01–4.07 (*m*)	78.5 (*d*)	3.38–3.42 (*m*)	78.3 (*d*)
4′	4.37–4.43 (*m*)	70.3 (*d*)	4.07–4.12 (*m*)	71.0 (*d*)	3.38–3.42 (*m*)	71.1 (*d*)
5′	4.45–4.50 (*m*)	77.7 (*d*)	3.77–3.81 (*m*)	77.9 (*d*)	3.48–3.50 (*m*)	78.1 (*d*)
6′	4.28–4.31 (*m*), 4.69–4.72 (*m*)	69.2 (*t*)	4.07–4.12 (*m*), 4.52–4.55 (*m*)	69.5 (*t*)	3.75–3.80 (*m*), 4.06 (*dd*, *J* = 10.8, 1.2)	69.5 (*t*)

Table (cont.)

Position	Centellasaponin I (**1**)		Centellasaponin J (**2**)		Centellasaponin E (**3**)	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Glc II						
1″	5.01–5.03 (*m*)	104.9 (*d*)	4.80–4.84 (*m*)	105.0 (*d*)	4.37 (*d*, *J* = 8)	104.4 (*d*)
2″	3.96–3.99 (*m*)	75.4 (*d*)	3.77–3.81 (*m*)	75.2 (*d*)	3.20–3.24 (*m*)	75.3 (*d*)
3″	4.20–4.24 (*m*)	76.5 (*d*)	3.89–4.01 (*m*)	76.5 (*d*)	3.46–3.50 (*m*)	76.8 (*d*)
4″	4.11–4.15 (*m*)	78.1 (*d*)	4.24–4.26 (*m*)	78.7 (*d*)	3.50–3.54 (*m*)	79.9 (*d*)
5″	3.67–3.69 (*m*)	77.2 (*d*)	3.52–3.58 (*m*)	77.1 (*d*)	3.48–3.50 (*m*)	77.1 (*d*)
6″	4.11–4.15 (*m*), 4.20–4.24 (*m*)	61.2 (*t*)	3.89–4.01 (*m*), 4.07–4.12 (*m*)	61.4 (*t*)	3.79–3.80 (*m*), 3.64–3.65 (*m*)	62.1 (*t*)
Rha III						
1‴	5.92 (br. *s*)	102.8 (*d*)	5.67 (br. *s*)	102.6 (*d*)	4.84 (br. *d*)	103.0 (*d*)
2‴	4.69–4.72 (*m*)	72.7 (*d*)	4.37–4.39 (*m*)	72.7 (*d*)	3.82–3.84 (*m*)	72.5 (*d*)
3‴	4.58–4.62 (*m*)	72.8 (*d*)	4.52–4.55 (*m*)	72.5 (*d*)	3.61–3.62 (*m*)	72.3 (*d*)
4‴	4.11–4.15 (*m*)	73.9 (*d*)	4.12–4.16 (*m*)	73.9 (*d*)	3.37–3.39 (*m*)	73.9 (*d*)
5‴	4.37–4.44 (*m*)	70.8 (*d*)	4.74–4.76 (*m*)	70.3 (*d*)	3.93–3.97 (*m*)	70.7 (*d*)
6‴	1.73 (*d*, *J* = 10)	18.6 (*q*)	1.57 (*s*)	18.3 (*q*)	1.26 (*s*)	17.9 (*q*)

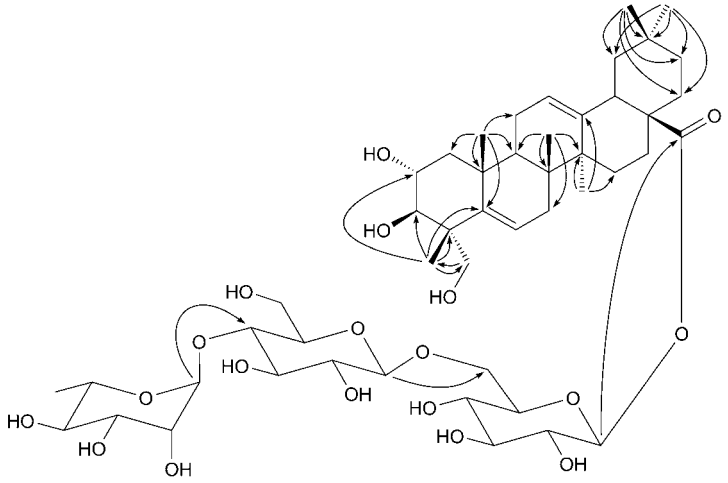

Fig. 2. *Key HMBCs* (H → C) *for* **1**

sugar units. From the above evidence, the structure of compound **1**, named centellasaponin I, was elucidated as depicted in *Fig. 1*.

Compound **2** was isolated as white amorphous powder. Its HR-ESI mass spectrum exhibited a *quasi*-molecular-ion peak at *m*/*z* 981.5042 ($[M+Na]^+$; calc. 981.5035) consistent with the molecular formula $C_{48}H_{78}O_{19}$. The IR spectrum displayed characteristic absorptions for OH (3441 cm^{-1}), C=O (1733 cm^{-1}), and olefin moieties (1637 cm^{-1}). The ^{13}C-NMR spectrum of **2** only showed one group of olefinic signals at

δ(C) 117.4 and 143.1. The correlations δ(H) 0.93 (Me(24))/δ(C) 143.1; δ(H) 1.15(Me(25))/δ(C) 143.1; δ(H) 2.39–2.43 (CH_2(7))/δ(C) 117.4 and 143.1 indicated the location of the C=C bond between C(5) (δ(C) 143.1) and C(6) (δ(C) 17.4) (*Fig. 3*). The long-range correlations Me(29) (δ(H) 1.05)/C(17) (δ(C) 41.2); Me(29) (δ(H) 1.05)/C(18) (δ(C) 51.0); Me(30) (δ(H) 0.90)/C(19) (δ(C) 38.4); Me(30) (δ(H) 0.90)/C(20) (δ(C) 37.6); and Me(30)/CH_2(21) (δ(C) 34.3) revealed that compound **2** possessed an ursane-type skeleton. The long-range correlations H–C(1′) (δ(H) 6.16–6.19)/C(28) (δ(C) 174.9); H–C(1″) (δ(H) 4.80–4.84)/C(6′) (δ(C) 69.5), and H–C(1‴) (δ(H) 5.67)/C(4″) (δ(C) 78.7) established the linkage sequence of the sugar units. From the above evidence, the structure of compound **2**, named centellasaponin J, was elucidated as depicted in *Fig. 1*.

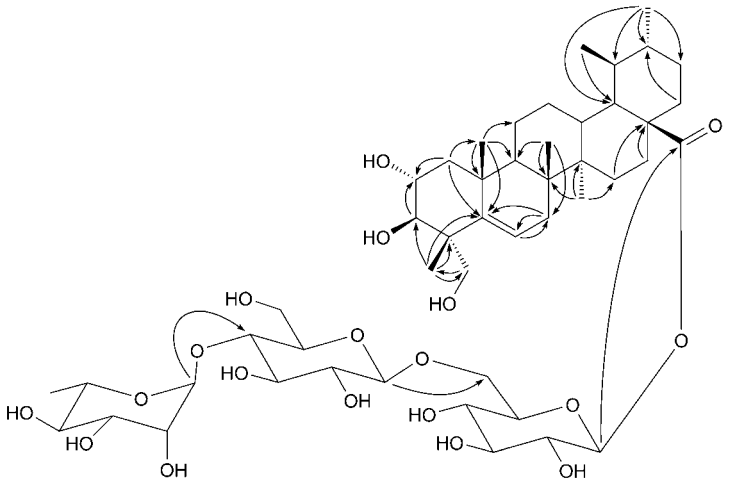

Fig. 3. *Key HMBCs* (H → C) *for* **2**

Compound **3** was isolated as white amorphous powder. Its HR-ESI mass spectrum exhibited a *quasi*-molecular-ion peak at *m*/*z* 995.4832 ($[M + Na]^+$; calc. 995.4828), consistent with the molecular formula $C_{48}H_{76}O_{20}$. The IR spectrum displayed characteristic absorptions for OH (3421 cm^{-1}), C=O (1686 cm^{-1} and 1749 cm^{-1}), and olefin moieties (1637 cm^{-1}). The long-range HMBCs δ(H) 1.81, 2.67 (CH_2(1)/δ(C) 214.8; and δ(H) 2.70 (CH_2(5))/δ(C) 214.8 were observed (*Fig. 4*), suggesting the presence of the C(3)=O group (δ(C) 214.8). The chemical shifts of Me(29) and Me(30) displayed a single peak, while they both showed the long-range correlations to CH_2(19) (δ(C) 47.0), C(20) (δ(C) 31.6), and CH_2(21) (δ(C) 34.9). The structure of compound **3** was deduced to possess an oleanane-type skeleton. The long-range correlations H–C(1′) (δ(H) 5.34)/C(28) (δ(C) 178.0); H–C(1″) (δ(H) 4.37)/C(6′) (δ(C) 69.5); and H–C(1‴) (δ(H) 4.84)/C(4″) (δ(C) 79.9) established the linkage sequence of the sugar units. From the above evidence, the structure of compound **3**, as named centellasaponin E, was elucidated as depicted in *Fig. 1*.

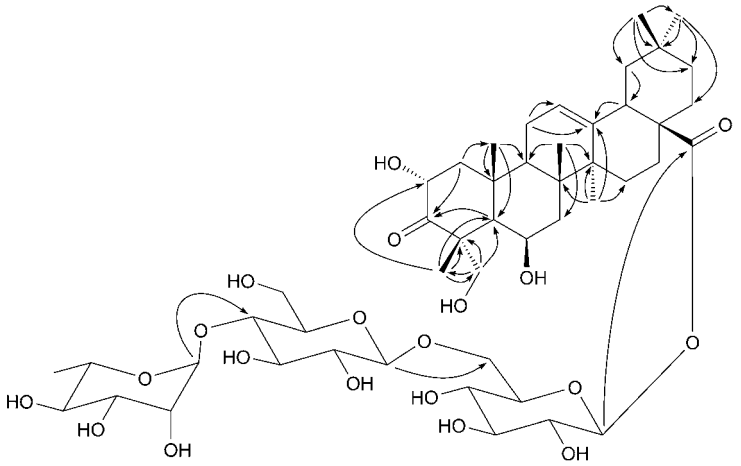

Fig. 4. *Key HMBCs* (H → C) *for* **3**

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Shanghai Sanpont Co., Ltd.*, P. R. China). TLC: Silica gel *HSGF*$_{254}$ (*Yantai Jiangyou Guijiao Kaifa Co., Ltd.*, P. R. China); detection by spraying with 10% H_2SO_4 in EtOH, followed by heating. Prep. HPLC: *CXTH LC3000* HPLC system (*P3000* pump, *UV 3000* scanning spectrophotometer, P. R. China); column, *YMC-Pack ODS-AQ*, 5 μm, i.d. 20 × 250 mm; *Inertsil ODS-3*, 5 μm, i.d. 7.6 × 250 mm. Optical rotations: *PerkinElmer-341* polarimeter. IR Spectra: *Nicolet-NEXUS-670-FTIR* spectrophotometer, KBr pellets; in cm^{-1}. NMR Spectra: *Varian INOVA-400/500* instrument at 400/500 MHz (^1H) and 100/125 MHz (^{13}C) in (D_5)pyridine or CD_3OD; δ in ppm rel. to Me_4Si; J in Hz. MS: *Waters Q-Tof micro YA019* mass spectrometer; in m/z.

Plant Material. The whole-plant material of *Centella asiatica* (L.) URBAN was collected in the Guangxi Zhuang Autonomous Region, P. R. China, in July 2008, and identified by Dr. *Tong Wu* at the Shanghai Institute of Pharmaceutical Industry. A voucher specimen (SIPITCM-080711) has been deposited with the institute.

Extraction and Isolation. The air-dried whole plants of *C. asiatica* (10 kg) were extracted two times with H_2O (100 l for 1.5 h; 80 l for 1 h). The extracts were combined and concentrated to 3 l, and then 95% EtOH (15 l) was added, and the mixture was kept for 24 h. The precipitate was removed by filtration. After solvent removal from the filtrate to reach a volume of 3 l, the crude extract was suspended in H_2O and extracted two times with BuOH (6 and 4.8 l). The BuOH extract (165 g) was subjected to CC (SiO_2; $CHCl_2$/MeOH/H_2O 10 : 2 : 0.2, 10 : 4 : 0.4, 10 : 5 : 0.6, and 10 : 6 : 1, finally EtOH, each 6 l): *Frs. 1–12*. *Fr. 6* (3.5 g) was purified by prep. HPLC (MeCN/H_2O 22 : 53, 6 ml/min, 204 nm) to afford *Frs. A–F*. *Fr. D* (273 mg) was further separated by prep. HPLC (MeCN/H_2O 24 : 76; 2 mmol/l β-cyclodextrin, 6 ml/min, 204 nm): **1** (t_R 52 min, 3 mg), **2** (t_R 70 min, 10 mg), **4** (t_R 40 min, 20 mg), **5** (t_R 60 min, 49 mg), and **6** (t_R 74 min, 68 mg). Similarly, **3** (t_R 16 min, 11 mg) was isolated from *Fr. C* (140 mg) by prep. HPLC (MeCN/H_2O 22 : 78, 2 mmol/l β-cyclodextrin, 6 ml/min, 204 nm).

Centellasaponin I (= *α-L-Rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-1-O-[(2α,3β)-2,3,23-trihydroxy-28-oxooleana-5,12-dien-28-yl]-β-D-glucopyranose*; **1**). White amorphous powder. $[\alpha]_D^{20} = -18.313$ ($c = 0.081$, MeOH). IR (KBr): 3441, 2928, 1731, 1637, 1459, 1384, 1273, 1121, 1071, 576. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 979.50 ($[M + Na]^+$). ESI-MS (neg.): 991.54 ($[M + Cl]^-$). HR-ESI-MS: 979.4872 ($[M + Na]^+$, $C_{48}H_{76}NaO_{19}^+$; calc. 979.4879).

Centellasaponin J (= *α-L-Rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-1-O-[(2α,3β)-2,3,23-trihydroxy-28-oxours-5-en-28-yl]-β-D-glucopyranose*; **2**). White amorphous powder. $[\alpha]_D^{20} =$

-48.012 ($c = 0.327$, MeOH). IR (KBr): 3441, 2931, 1733, 1637, 1458, 1384, 1062, 880, 811, 561. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 981.32 ($[M+Na]^+$). ESI-MS (neg.): 957.42 ($[M-H]^-$). HR-ESI-MS: 981.5042 ($[M+Na]^+$, $C_{48}H_{78}NaO_{19}^+$; calc. 981.5035).

Centellasaponin E (=*α*-L-*Rhamnopyranosyl-(1 → 4)-β*-D-*glucopyranosyl-(1 → 6)-1-O-[(2α,6β)-2,6,23-trihydroxy-3,28-dioxoolean-12-en-28-yl]-β*-D-*glucopyranose*; **3**). White amorphous powder. $[\alpha]_D^{20} = -8.130$ ($c = 0.287$, MeOH). IR (KBr): 3421, 2925, 1749,1686, 1637, 1458, 1383, 1261, 1063, 1037, 811, 577. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 995.35 ($[M+Na]^+$). ESI-MS (neg.): 971.31 ($[M-H]^-$). HR-ESI-MS: 995.4832 ($[M+Na]^+$, $C_{48}H_{76}NaO_{20}^+$; calc. 995.4828).

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