Two New Pentacyclic Triterpenoids from *Centella asiatica*

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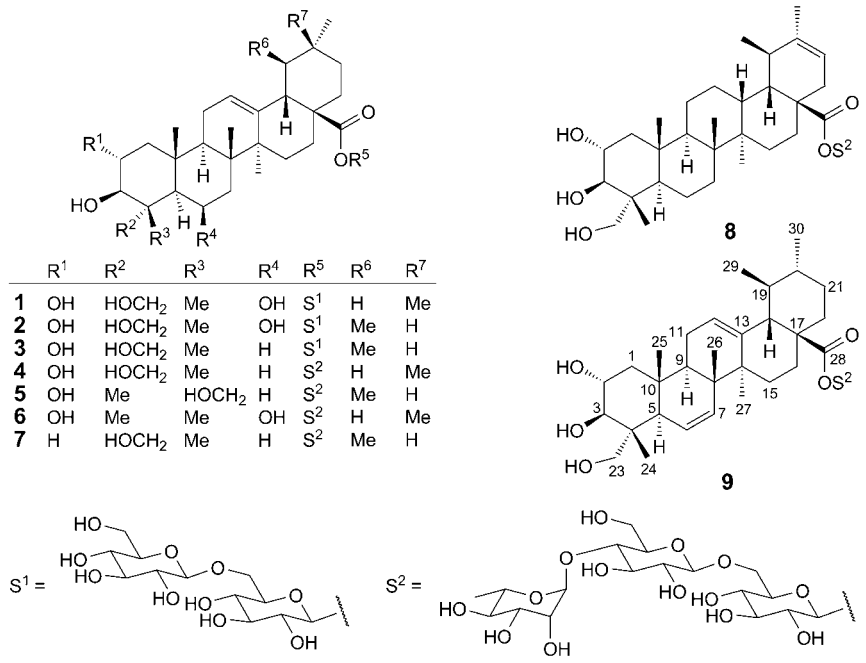
Two new pentacyclic triterpenoids, named centelloside D (**1**) and centelloside E (**9**), together with the seven known compounds **2**–**8**, were isolated from the whole plants of *Centella asiatica*. Compound **5** was reported for the first time from this genus. Their structures were elucidated on the basis of chemical and spectral analysis, including 1D- and 2D-NMR, and HR-MS experiments, and by comparison with literature data. Compounds **1**–**4**, **6**, and **8** did not show any cytotoxicity against L929 (mouse embryonic fibroblast).

Introduction. – *Centella asiatica* (L.) URBAN, a plant of the family Umbelliferae, is a traditional herbal medicine used in China, Southeast Asia, India, Sri Lanka, and Africa. Experimental and clinical investigations showed that it had a number of medicinal properties, *e.g.*, for the treatment of venous insufficiency, striae gravidarum, and wound healing disturbances [1]. Previous chemical studies showed that pentacyclic triterpenes which contained many pairs of corresponding ursane- and oleanane-type triterpenes were the main components of this plant [2–4]. Our phytochemical investigation of this plant now revealed the presence of two new pentacyclic triterpenoids, named centelloside D (**1**) and centelloside E (**9**), besides that of the seven known triterpenes **2**–**8** (*Fig. 1*). Compound **5** was reported for the first time from this genus. Centelloside E (**9**) is the first pentacyclic skeleton with two C=C bonds discovered in this plant as well as with a C=C bond between C(6) and C(7). Compounds **1**–**4**, **6**, and **8** were evaluated *in vitro* for cytotoxicity.

Results and Discussion. – The crude extract of *C. asiatica* was repeatedly subjected to column chromatography (silica gel) and prep. HPLC to afford compounds **1**–**9**. Compounds **1** and **9** were found to be new, and their structures were elucidated by 1D- and 2D-NMR data in combination with MS studies. The other seven compounds were identified as centellasaponin B (**2**) [4], asiaticoside E (**3**) [5], scheffoleoside A (**4**) [6], scheffursoside F (**5**) [6], (2α,3β,6β)-2,3,6-trihydroxyolean-12-en-28-oic acid 28-[*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester (**6**) [3], asiaticoside F (**7**) [5], and isoasiaticoside (**8**) [7], by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was obtained as a white amorphous powder. Its HR-ESI-MS showed a quasi-molecular-ion peak at *m*/*z* 851.4409 ([*M* + Na]$^+$), in accord with the molecular formula $C_{42}H_{68}O_{16}$. The IR spectrum showed the presence of OH (3424 cm^{-1}), C=O



	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	OH	$HOCH_2$	Me	OH	S^1	H	Me
2	OH	$HOCH_2$	Me	OH	S^1	Me	H
3	OH	$HOCH_2$	Me	H	S^1	Me	H
4	OH	$HOCH_2$	Me	H	S^2	H	Me
5	OH	Me	$HOCH_2$	H	S^2	Me	H
6	OH	Me	Me	OH	S^2	H	Me
7	H	$HOCH_2$	Me	H	S^2	Me	H

Fig. 1. *Compounds* **1**–**9**, *isolated from* Centella asiatica

(1733 cm^{-1}), and olefin moieties (1660 cm^{-1}). The resonances for two anomeric CH groups at δ(H) 4.90 (*d*, $J = 7.6$ Hz)/δ(C) 105.2 and δ(H) 6.08 (*d*, $J = 8.0$ Hz)/δ(C) 95.7 showed signals assignable to two β-configured sugar moieties. The NMR data (*Table*) were similar to those of **2** for rings *A* – *D* and the sugar moiety. The ^{1}H-NMR data revealed six Me groups, and an olefinic H-atom at δ(H) 5.38 (br. *s*, H–C(12)) and a signal at δ(H) 3.09 (*dd*, $J = 4.0$, 13.6 Hz, H–C(18)) were characteristic for an olean-12-en-28-oic derivative. The difference of the NMR data of an ursane- and oleanane-type glycoside mainly concerns the δ(C) of C(12), C(13), C(27), C(29), C(30), and the *E*-ring C-atoms, and the δ(H) of Me(29) and Me(30) show splitting for an ursane derivative, while they display a single peak for an oleanane derivative [6][8]; comparison of the NMR data of **1** and **2** showed that they exactly matched these typical spectral features. The structure was further assigned by HMQC, HMBC, ^{1}H,^{1}H-COSY, and NOESY experiments. The HMBC spectrum (*Fig. 2*) established the location and sequence of the sugar moieties, with the key correlations H–C(1′) (δ(H) 6.08)/C(28) (δ(C) 176.5) and H–C(1″) (δ(H) 4.90)/C(6′) (δ(C) 69.4). The structure of compound **1** was, thus, determined as terminolic acid 28-[*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester, and named centelloside D.

Compound **9** was isolated as a white amorphous powder. The HR-ESI-MS exhibited a quasi-molecular-ion peak at *m*/*z* 979.4875 ([M + Na]$^+$) consistent with the molecular formula $C_{48}H_{76}O_{19}$. The IR spectrum displayed characteristic absorptions for OH (3413 cm^{-1}), C=O (1733 cm^{-1}), and olefin moieties (1645 cm^{-1}). Its ^{13}C-NMR

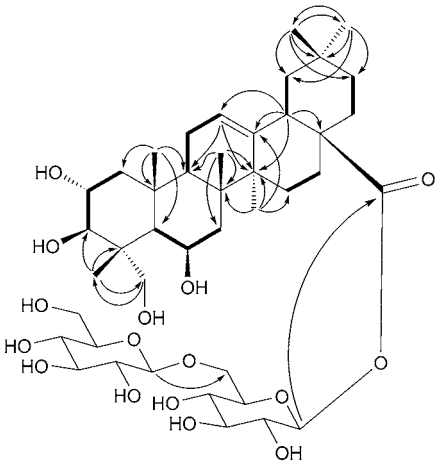


Fig. 2. *Selected HMBC* (H → C) *and* ^{1}*H,*^{1}*H-COSY* (—) *features of* **1**

spectrum showed two groups of olefinic signals at δ(C) 127.3, 127.5, 134.5, and 139.6. The ^{1}H,^{1}H-COSY plot showed the correlations H–C(6) δ(H) 5.98/δ(C) 127.3) and H–C(7) (δ(H) 5.5–5.60/δ(C) 134.5 (*cf. Fig. 3*) and, therefore, δ(C) 127.5 and 139.6 arose from another C=C bond than C(12)=C(13). The ^{1}H- and ^{13}C-NMR data (*Table*) were very similar to those of asiaticoside [6], except for the appearance of a set of olefinic signals (δ(C) 127.3 and 134.5) in **9**. The HMBC spectrum (*Fig. 3*) showed the long-range correlations δ(H) 5.98/δ(C) 44.1 and δ(H) 1.29/δ(C) 134.5. Further analysis of the ^{1}H,^{1}H-COSY cross-peaks δ(H) 5.58 and 5.98/δ(H) 2.70 established that a C=C bond (δ(C) 127.3 and 134.5) existed between C(6) and C(7). The key correlations H–C(18) (δ(H) 2.53)/δ(C) 127.5 and 139.6, and Me(27) (δ(H) 1.23)/δ(C) 139.6 demonstrated the location of the C=C bond between C(12) and C(13). In the HMBC

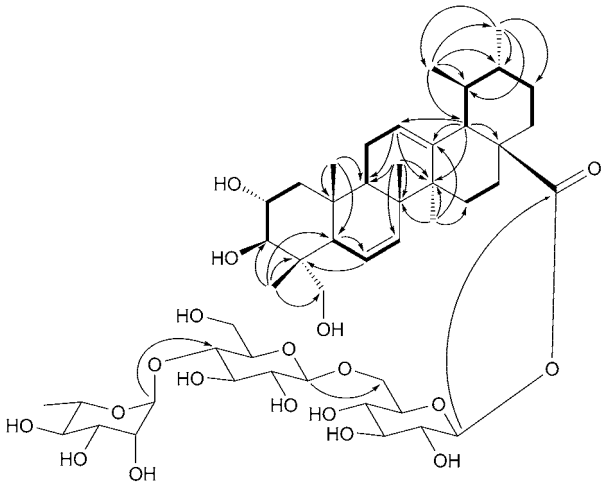


Fig. 3. *Key HMBC* (H → C) *and* ^{1}*H,*^{1}*H-COSY* (—) *features of* **9**

Table. *^{1}H- and ^{13}C-NMR Data* ((D_5)pyridine) *of* **1** (400 and 100 MHz, resp.) *and* **9** (500 and 125 MHz, resp.). δ in ppm, J in Hz.

	Centelloside D (**1**)		Centelloside E (**9**)	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.31–1.38 (*m*), 2.23–2.29 (*m*)	50.1 (*t*)	1.40–1.43 (*m*), 2.26 (*d*, *J* = 11.0)	45.8 (*t*)
H–C(2)	4.22–4.30 (*m*)	69.0 (*d*)	4.66–4.70 (*m*)	69.5 (*d*)
H–C(3)	4.03–4.12 (*m*)	78.5 (*d*)	4.07–4.13 (*m*)	78.4 (*d*)
C(4)		44.5 (*s*)		44.1 (*s*)
H–C(5)	1.90–1.95 (*m*)	48.7 (*d*)	2.70 (br. *s*)	47.6 (*d*)
H–C(6)	4.95 (br. *s*)	67.6 (*d*)	5.98 (*d*, *J* = 10.0)	127.3 (*d*)
CH_2(7)	1.73–1.81 (*m*), 1.83–1.90 (*m*)	41.0 (*t*)	5.55–5.60 (*m*)	134.5 (*d*)
or H–C(7)				
C(8)		39.4 (*s*)		44.9 (*s*)
H–C(9)	1.83–1.89 (*m*)	48.8 (*d*)	2.10–2.15 (*m*)	48.3 (*d*)
C(10)		38.1 (*s*)		37.4 (*s*)
CH_2(11)	1.77–1.84 (*m*), 1.89–1.98 (*m*)	23.5 (*t*)	1.18–1.22 (*m*), 1.97–2.02 (*m*)	24.8 (*t*)
H–C(12)	5.38 (br. *s*)	123.1 (*d*)	5.59–5.62 (*m*)	127.5 (*d*)
C(13)		143.5 (*s*)		139.6 (*s*)
C(14)		42.8 (*s*)		43.1 (*t*)
CH_2(15)	1.05–1.11 (*m*), 2.27–2.33 (*m*)	28.2 (*t*)	1.21–1.24 (*m*), 1.24–1.27 (*m*)	29.0 (*t*)
CH_2(16)	2.01–2.06 (*m*), 2.16–2.23 (*m*)	24.0 (*t*)	1.14–1.16 (*m*), 1.98–2.02 (*m*)	23.6 (*t*)
C(17)		47.0 (*s*)		49.3 (*s*)
H–C(18)	3.09 (*dd*, *J* = 4.0, 13.6)	41.7 (*d*)	2.51–2.55 (*m*)	54.7 (*d*)
CH_2(19)	1.11–1.18 (*m*), 1.60–1.66 (*m*)	46.3 (*t*)	0.83–0.85 (*m*)	39.4 (*d*)
or H–C(19)				
C(20)		30.7 (*s*)	1.31–1.35 (*m*)	38.8 (*d*)
or H–C(20)				
CH_2(21)	0.96–1.04 (*m*), 1.18–1.24 (*m*)	34.0 (*t*)	1.21–1.24 (*m*), 1.30–1.35 (*m*)	30.3 (*t*)
CH_2(22)	1.63–1.68 (*m*), 1.78–1.84 (*m*)	32.5 (*t*)	1.75–1.77 (*m*), 1.88–1.90 (*m*)	37.0 (*t*)
CH_2(23)	3.93–4.00 (*m*), 4.26–4.35 (*m*)	66.2 (*t*)	3.78–3.82 (*m*), 4.19–4.24 (*m*)	66.3 (*t*)
Me(24)	1.63 (*s*)	15.9 (*q*)	1.12–1.14 (*m*)	14.9 (*q*)
Me(25)	1.60 (*s*)	18.9 (*q*)	1.09–1.11 (*m*)	18.5 (*q*)
Me(26)	1.69 (*s*)	19.0 (*q*)	1.29 (*s*)	18.6 (*q*)
Me(27)	1.06 (*s*)	26.0 (*q*)	1.23 (*s*)	24.5 (*q*)
C(28)		176.5 (*s*)		176.7 (*s*)
Me(29)	0.73 (*s*)	33.0 (*q*)	0.90–0.94 (*m*)	17.9 (*q*)
Me(30)	0.75 (*s*)	23.6 (*q*)	0.85–0.90 (*m*)	21.5 (*q*)
Glc I				
H–C(1′)	6.08 (*d*, *J* = 8.0)	95.7 (*d*)	6.21 (*d*, *J* = 8.0)	95.9 (*d*)
H–C(2′)	4.01–4.10 (*m*)	73.8 (*d*)	4.33–4.39 (*m*)	74.4 (*d*)
H–C(3′)	4.04–4.12 (*m*)	78.3 (*d*)	4.43–4.47 (*m*)	78.3 (*d*)
H–C(4′)	4.13–4.23 (*m*)	71.0 (*d*)	4.30–4.36 (*m*)	71.3 (*d*)
H–C(5′)	3.94–4.01 (*m*)	77.9 (*d*)	3.66–3.70 (*m*)	77.5 (*d*)
CH_2(6′)	4.18–4.25 (*m*), 4.58 (*dd*, *J* = 1.6, 9.6)	69.4 (*t*)	4.28–4.31 (*m*), 4.66–4.70 (*m*)	69.8 (*t*)
Glc II				
H–C(1″)	4.90 (*d*, *J* = 7.6)	105.2 (*d*)	4.97–5.01 (*m*)	105.4 (*d*)
H–C(2″)	3.83–3.91 (*m*)	75.1 (*d*)	3.97 (*t*, *J* = 8.5)	75.7 (*d*)
H–C(3″)	4.04–4.13 (*m*)	78.3 (*d*)	4.15–4.19 (*m*)	76.8 (*d*)
H–C(4″)	4.05–4.13 (*m*)	71.5 (*d*)	4.21–4.25 (*m*)	79.1 (*d*)
H–C(5″)	3.74–3.81 (*m*)	78.3 (*d*)	4.07–4.12 (*m*)	78.5 (*d*)
CH_2(6″)	4.20–4.28 (*m*), 4.32–4.40 (*m*)	62.6 (*t*)	4.07–4.13 (*m*), 4.20–4.24 (*m*)	61.6 (*t*)

Table (cont.)

	Centelloside D (**1**)		Centelloside E (**9**)	
	δ(H)	δ(C)	δ(H)	δ(C)
Rha III				
H–C(1‴)			5.88 (br. *s*)	103.0 (*d*)
H–C(2‴)			4.69 – 4.72 (*m*)	73.0 (*d*)
H–C(3‴)			4.56 – 4.60 (*m*)	73.1 (*d*)
H–C(4‴)			4.11 – 4.17 (*m*)	74.2 (*d*)
H–C(5‴)			4.99 – 5.04 (*m*)	70.6 (*d*)
Me(6‴)			1.72 (*d*, $J = 5.0$)	18.9 (*q*)

spectrum, the long-range correlations H–C(1′) (δ(H) 6.21)/C(28) (δ(C) 176.7), H–C(1″) (δ(H) 4.97 – 5.01)/C(6′) (δ(C) 69.8), and H–C(1‴) (δ(H) 5.88)/C(4″) (δ(C) 79.1), established the linkage sequence of the sugar units. From the above evidence, the structure of compound **9** was elucidated as (2α,3β,23α)-2,3,23-trihydroxyursa-6,12-diene 28-[*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester, and was named centelloside E.

This work was sponsored by the *Program of State 'Created a Significant New Drug' Science and Technology Funding*, P. R. China (Grant No. 2009ZX09301-007).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh; *Shanghai Sanpont Co., Ltd.*, P. R. China). TLC: SiO_2 *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: *Shimadzu* HPLC system (*LC-8A* pump, *SPD-M10A* detector, Japan); *Shimadzu-PRC-ODS* column (15 μm, i.d. 20 × 250 mm); t_R in min. Optical rotations: *Perkin–Elmer-341* polarimeter. IR Spectra: *Nicolet-Nexus-670* FT-IR spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *Varian Inova-400 or -500* instrument; at 400 or 500 MHz (^1H) and 100 or 125 MHz (^{13}C); in (D_5)pyridine; 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 2 times with H_2O (100 l for 1.5 h; 80 l for1 h). The extracts were combined and concentrated to 3 l, and then 95% EtOH (15 l) was added and the mixture 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 2 × 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, and finally EtOH, each 6 l): *Frs. 1 – 12*. *Fr. 6* (2.0 g) was purified by prep. HPLC (MeOH/H_2O 70 : 30, 6 ml/min, 204 nm): *Frs.* $A_6 - E_6$. *Fr.* B_6 (1.5 g) was further subjected to prep. HPLC (MeCN/H_2O 27 : 73, 6 ml/min, 204 nm): *Frs.* $B_{6\text{-}1} - B_{6\text{-}4}$. *Fr.* $B_{6\text{-}1}$ (138 mg) was separated by prep. HPLC (MeCN/H_2O 24 : 76, 6 ml/min, 204 nm): **1** (17 mg; t_R 27), **2** (43 mg; t_R 44), and **8** (57 mg; t_R 56). Similarly, **4** (19 mg; t_R 33) and **9** (8 mg; t_R 50) were isolated from *Fr.* $B_{6\text{-}2}$ (42 mg). *Fr.* $B_{6\text{-}4}$ (44 mg) was further purified by prep. HPLC (MeCN/H_2O 28 : 72, 6 ml/min, 204 nm): **3** (35 mg, t_R 42). *Fr.* C_6 (70 mg) was submitted to prep. HPLC (MeCN/H_2O 29 : 71, 6 ml/min,

204 nm): **6** (37 mg, t_R 29). Compounds **5** (10 mg, t_R 57) and **7** (26 mg, t_R 96) were isolated from *Fr. E*$_6$ (80 mg) by prep. HPLC (MeCN/H_2O[1]) 3 : 7, 6 ml/min, 204 nm).

Centelloside D (*= (2α,3β,4α,6β)-2,3,6,23-Tetrahydroxyolean-12-en-28-oic Acid 6-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranoyl Ester*; **1**): White amorphous powder. $[\alpha]_D^{24} = -1.3$ ($c = 0.64$, MeOH). IR (KBr): 3424, 2925, 1733, 1660, 1462, 1384, 1263, 1162, 1062, 534. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 851 ($[M + Na]^+$). ESI-MS (neg.): 863 ($[M + Cl]^-$). HR-ESI-MS: 851.4409 ($[M + Na]^+$, $C_{42}H_{68}NaO_{16}^+$; calc. 851.4405).

Centelloside E (*= (2α,3β,4α)-2,3,23-Trihydroxyursa-6,12-dien-28-oic Acid* O*-6-Deoxy-α-*L*-mannopyranosyl-(1 → 4)-*O*-β-*D*-glucopyranosyl-(1 → 6)-β-*D*-glucopyranosyl Ester*; **9**): White amorphous powder. $[\alpha]_D^{24} = -15$ ($c = 0.26$, MeOH). IR (KBr): 3413, 2925, 2856, 1733, 1645, 1456, 1384, 1067, 1036, 813, 535. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 979 ($[M + Na]^+$). ESI-MS (neg.): 991 ($[M + Cl]^-$). HR-ESI-MS: 979.4875 ($[M + Na]^+$, $C_{48}H_{76}NaO_{19}^+$; calc. 979.4879).

MTT Cytotoxicity Assay. Compounds **1**–**4**, **6**, and **8** were evaluated *in vitro* for cytotoxicity against the L929 cell lines by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method. All of them showed no inhibitory activity against L929 cells with IC_{50} values of 100 μg/ml. Cisplatin was used as a positive control which exhibited inhibitory activity with an IC_{50} value of 9.12 μg/ml.

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Received July 12, 2011

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[1]) β-Cyclodextrin was added to H_2O (4 g/l) for separating compounds **5** and **7**, and can be removed by means of extraction.