New Triterpenoid Glycosides from *Centella asiatica*

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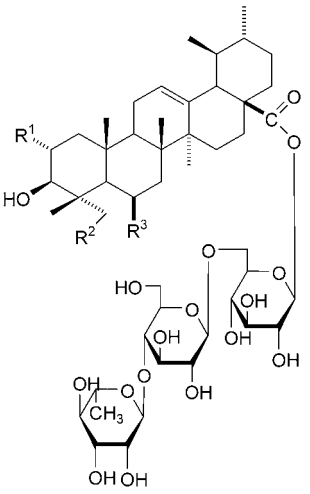
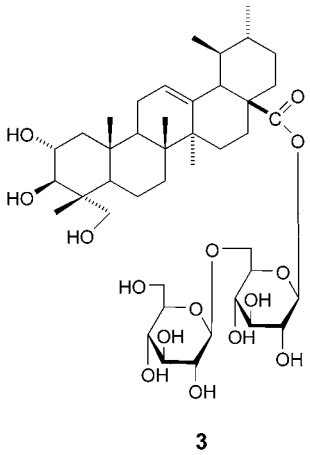
Four new triterpenoid glycosides named asiaticoside C (**1**), D (**2**), E (**3**), and F (**4**) were isolated from the BuOH fraction of the EtOH extract of whole plants of *Centella asiatica*, together with three known compounds, asiaticoside (**5**), madecassoside (**6**), and scheffuroside B (**7**). Based on FAB-MS, IR, ^{1}H- and ^{13}C-NMR, and 2D-NMR data (HMQC, HMBC, COSY), the structures of the new compounds were determined as (2α,3β,4α)-23-(acetyloxy)-2,3-dihydroxyurs-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**1**), (2α,3β)-2,3-dihydroxyurs-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**2**), asiatic acid 6-*O*-β-D-glycopyranosyl-β-D-glucopyranosyl ester (**3**), (3β,4α)-3,23-dihydroxyurs-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**4**).

Introduction. – The Umbelliferae *Centella asiatica* (L.) URBAN has been widely cultivated as a vegetable or spice in China, Southeast Asia, India, Sri Lanka, Africa, and Oceanic countries, and used for skin diseases, diarrhea, eye diseases, inflammation, asthma, leprosy, and hypertension in southeast Asia [1]. To characterize an active compound for traditional effects of *C. asiatica*, the whole plants were extracted with EtOH under reflux to give a residue, which was suspended in H_2O and partioned between $CHCl_3$ and BuOH. The BuOH fraction was separated by chromatographic techniques to give four new triterpenoid glycosides, named asiaticoside C (**1**), D (**2**), E (**3**), and F (**4**), together with three known compounds, asiaticoside (**5**) [2][3], madecassoside (**6**) [4], and scheffuroside B (**7**) [5] (*Fig.*). Herein, we report the isolation and the structure identification by FAB-MS, IR, ^{1}H- and ^{13}C-NMR, and 2D-NMR data (HMQC, HMBC, COSY) of asiaticoside C (**1**), D (**2**), E (**3**), and F (**4**).

Results and Dicussion. – Asiaticoside C (**1**) was obtained as colorless amorphous powder. The IR spectrum of **1** showed absorptions for OH groups at 3441 cm^{-1}, an ester carbonyl at 1738 cm^{-1}, and an olefin moiety at 1655 cm^{-1} besides a stronger absorption at 1063 cm^{-1} suggesting a glycosidic linkage in the molecule. The high-resolution (HR) FAB-MS exhibited an accurate ion peak at *m*/*z* 999.5164 ([M – H]$^-$) in accordance with an empirical molecular formula $C_{50}H_{80}O_{20}$. The negative-ion FAB-MS of **1** gave a quasi-molecular ion and fragment-ion peaks at *m*/*z* 999 ([M – H]$^-$), 853 ([M – $C_6H_{11}O_4$]$^-$), 529 ([M – $C_{18}H_{31}O_{14}$]$^-$), and 487 ([M – $C_{18}H_{31}O_{14}$ – C_2H_2O]$^-$), suggesting the presence of three sugar moieties in the molecule. After hydrolysis of **1** with 5% H_2SO_4 in MeOH, glucose and rhamnose were identified by comparison with authentic samples on PC (paper chromatography). The quasi-molecular-ion peak established





3

	R^1	R^2	R^3
1	OH	AcO	H
2	OH	H	H
4	H	OH	H
5	OH	OH	H
6	OH	OH	OH
7	OH	CHO	H

Figure. *Structures of compounds* **1**–**7**

that compound **1** had 42 mass units more than asiaticoside (**5**) [2][3]. Moreover, the signals of an acetyl group were observed in the ^{1}H- and ^{13}C-NMR spectra of **1** (*Tables 1* and *2*, resp.), which were absent in the spectra of asiaticoside (**5**). The structure of **1** was deduced as (2α,3β,4α)-23-(acetyloxy)-2,3-dihydroxyurs-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.

In the ^{1}H-NMR spectrum of **1** (*Table 1*), 6 Me signals (2*d* and 4*s*) assignable to an aglycone, an olefinic proton (H–C(12) at δ(H) 5.41 (br. *s*)), and 1*d* ascribable to H–C(18) at δ(H) 2.49 (J = 11.2 Hz) were observed, which suggested that **1** is an urs-12-en-28-oic acid derivative. The three anomeric-proton signals of H–C (1″), H–C (1′), and H–C(1‴) at δ(H) 4.96 (*d*, J = 8.0 Hz), 6.17 (*d*, J = 8.0 Hz) and 5.82 (br. *s*), respectively, suggested that there were two β-D-linkages and one α-L-linkage in the sugar moiety. The ^{13}C-NMR spectrum showed two olefinic C-signals at δ 126.0 (C(12)) and 138.5 (C(13)) and three signals of anomeric C-atoms at δ 95.7 (C(1′)), 102.7 (C(1‴)), and 105.0 (C(1″)), supporting three sugar moieties in the molecule. In the HMBC spectrum of **1**, a long-range correlation between H–C(1′) (δ(H) 6.17) and C(28) (δ(C) 176.4) was observed, together with the correlations H–C(1″) (δ(H) 4.96)/C(6′) (δ(C) 69.5) and H–C(1‴) (δ(H) 5.82)/C(4″) (δ(C) 78.8), which revealed that the inner glucose unit was linked at C(28), the middle glucose unit at C(6′) of the inner glucose unit, and the terminal rhamnose unit at C(4″) of the middle glucose unit. Furthermore, the long-range correlation between H–C(23) and the C=O of the Ac group (δ(C) 170.8) was also observed, which showed that the AcO group was attached at C(23).

Table 1. *Selected ^{1}H-NMR Data for Compounds* **1**–**4** *in C_5D_5N*[a]). δ in ppm, J in Hz.

	1[b])	**2**[c])	**3**[c])	**4**[c])
H–C(2)	4.27 (*m*)	4.12 (*m*)	4.34 (*m*)	overlapped
H–C(3)	3.91 (*d*, *J* = 9.2)	3.66 (*d*, *J* = 9.3)	4.18 (*d*, *J* = 9.5)	4.10 (*m*)
H–C(12)	5.41 (br. *s*)	5.12 (br. *s*)	5.42 (br. *s*)	5.41 (br. *s*)
H–C(18)	2.49 (*d*, *J* = 11.2)	2.48 (*d*, *J* = 11.3)	2.48 (*d*, *J* = 11.3)	2.48 (*d*, *J* = 11.0)
H–C(23)	4.20 (*d*, *J* = 11.2)	1.07 (*s*)	4.50 (*d*, *J* = 10.6)	4.20 (*d*, *J* = 9.8)
	4.25 (*d*, *J* = 11.2)	–	4.18 (*d*, *J* = 10.6)	4.28 (*d*, *J* = 9.8)
Me(24)	0.98 (*s*)	1.03 (*s*)	1.05 (*s*)	1.01 (*s*)
Me(25)	1.05 (*s*)	1.14 (*s*)	1.07 (*s*)	1.11 (*s*)
Me(26)	1.13 (*s*)	1.14 (*s*)	1.14 (*s*)	1.03 (*s*)
Me(27)	1.14 (*s*)	1.25 (*s*)	1.18 (*s*)	1.16 (*s*)
Me(29)	0.91 (*d*, *J* = 6.4)	0.91 (*d*, *J* = 6.4)	0.88 (*d*, *J* = 6.3)	0.91 (*d*, *J* = 6.0)
Me(30)	0.87 (*d*, *J* = 6.0)	0.89 (*d*, *J* = 6.2)	0.82 (*d*, *J* = 6.2)	0.87 (*d*, *J* = 6.1)
H–C(1′)	6.17 (*d*, *J* = 8.0)	6.17 (*d*, *J* = 8.1)	6.20 (*d*, *J* = 8.1)	6.17 (*d*, *J* = 8.0)
H–C(1″)	4.96 (*d*, *J* = 8.0)	4.96 (*d*, *J* = 8.0)	5.04 (*d*, *J* = 7.7)	4.96 (*d*, *J* = 7.8)
H–C(1‴)	5.82 (br. *s*)	5.77 (br. *s*)		5.81 (br. *s*)
Me(6‴)	1.68 (*d*, *J* = 6.0)	1.68 (*d*, *J* = 6.4)		1.67 (*d*, *J* = 6.1)
MeCO	1.99 (*s*)			

[a]) Assignments based on HMQC and HMBC correlations. [b]) Recorded at 500 MHz. [c]) Recorded at 400 MHz.

Asiaticoside D (**2**) was obtained as colorless amorphous powder. The negative-ion FAB-MS of **2** gave quasi-molecular- and fragment-ion peaks at *m*/*z* 941 ($[M-H]^-$), 795 ($[M-C_6H_{11}O_4]^-$), and 471 ($[M-C_{18}H_{31}O_{14}]^-$). The HR-FAB-MS analysis revealed the molecular formula of **2** to be $C_{48}H_{78}O_{18}$ (*m*/*z* 941.5091 $[M-H]^-$). The IR spectrum showed absorptions for OH (3438 cm^{-1}), ester-carbonyl (1733 cm^{-1}), and olefinic groups (1655 cm^{-1}). Acidic hydrolysis of **2** with 5% H_2SO_4 in MeOH liberated glucose and rhamnose, identified by comparison with authentic samples on PC. The structure of **2** was determined to be (2α,3β)-2,3-dihydroxyurs-12-en-28-oic acid *O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester.

The ^{1}H-NMR spectrum of **2** (*Table 1*) showed 2*d* and 5*s* Me signals assignable to an aglycone moiety, one proton signal ascribable to H–C(18), and one olefinic proton H–C(12), which are the characteristics of urs-12-en-28-oic acid derivatives. Additionally, two signals of protons attached to an O-bearing C-atom, H–C(2) (δ(H) 4.12 (*m*)) and H–C(3) (δ(H) 3.66 (*d*, *J* = 9.3 Hz)), were observed, with coupling constants establishing the (2α)- and (3β)-configurations of the OH groups. The anomeric-proton signals of H–C(1″) at δ(H) 4.96 (*d*, *J* = 8.0 Hz), H–C(1′) at δ(H) 6.17 (*d*, *J* = 8.1 Hz), and H–C(1‴) at δ(H) 5.77 (br. *s*) showed two β-D-linkages and one α-L-linkage for the sugar moiety of **2**. Compared with asiaticoside (**5**) [2][3], there was one more Me group in **2** as the DEPT showed, and the δ(H) and δ(C) of ring A were essentially the same as those of the known compound centellasaponin C [4], suggesting the presence of OH_α-C(2) and OH_β-C(3) but the absence of an OH at C(23). The HMBC experiment of **2** gave the following long-range correlations: H–C(1′) (δ(H) 6.17)/C(28) (δ(C) 176.3), H–C(1″) (δ(H) 4.96)/C(6′) (δ(C) 69.5), and H–C(1‴) (δ(H) 5.77) and C(4″) (δ(C) 78.8), which showed that the inner glucose unit was linked at C(28), the middle glucose unit at C(6′) of the inner glucose unit, and the terminal rhamnose unit at C(4″) of the middle glucose unit.

Asiaticoside E (**3**) was obtained as colorless amorphous powder. The HR-FAB-MS revealed the molecular formula of **3** to be $C_{48}H_{78}O_{18}$ (*m*/*z* 811.4465 ($[M-H]^-$). The negative-ion FAB-MS spectrum showed quasi-molecular and fragment-ion peaks at *m*/*z* 811 ($[M-H]^-$) and 487 ($[M-C_{12}H_{21}O_{10}]^-$). In the IR spectrum, absorptions for OH (3424 cm^{-1}), ester-carbonyl (1733 cm^{-1}), and olefinic functions (1637 cm^{-1}) were

Table 2. *^{13}C-NMR Data for Compounds* **1**–**4** *in C_5D_5N*[a]). δ in ppm.

	1[b])	**2**[c])	**3**[c])	**4**[c])		**1**[b])	**2**[c])	**3**[c])	**4**[c])
C(1)	48.0	48.2	48.0	39.1	C(1′)	95.7	95.7	95.6	95.7
C(2)	68.5	68.7	68.9	27.8	C(2′)	73.8	73.8	73.8	73.9
C(3)	78.0	83.9	78.0	73.6	C(3′)	78.3	78.3	78.5	78.3
C(4)	42.9	40.2	43.6	43.0	C(4′)	71.0	71.0	71.1	71.0
C(5)	48.4	56.0	47.9	48.6	C(5′)	77.2	77.2	77.9	78.0
C(6)	18.7	18.9	18.5	18.7	C(6′)	69.5	69.5	69.6	69.5
C(7)	33.3	33.5	33.2	33.4					
C(8)	40.3	18.9	40.2	40.2	C(1″)	105.0	105.0	105.3	105.0
C(9)	48.3	48.5	48.2	48.5	C(2″)	75.4	75.4	75.2	75.4
C(10)	38.2	38.5	38.3	37.2	C(3″)	76.6	76.6	78.5	76.6
C(11)	23.8	23.8	23.8	23.8	C(4″)	78.8	78.8	71.5	78.8
C(12)	126.0	126.0	126.0	126.2	C(5″)	77.2	77.1	78.2	77.2
C(13)	138.5	138.5	138.5	138.6	C(6″)	61.3	61.3	62.7	61.3
C(14)	42.6	42.6	42.6	43.0					
C(15)	28.7	28.7	28.7	28.9	C(1‴)	102.7	102.7		102.8
C(16)	24.7	24.7	24.6	24.7	C(2‴)	72.6	72.6		72.6
C(17)	48.5	48.5	48.4	48.6	C(3‴)	72.8	72.8		72.8
C(18)	53.3	53.2	53.2	53.4	C(4‴)	74.0	74.0		74.0
C(19)	39.4	39.4	39.3	39.4	C(5‴)	70.4	70.3		70.4
C(20)	39.2	39.1	39.0	39.2	C(6‴)	18.6	18.6		18.6
C(21)	30.9	30.9	30.8	30.9					
C(22)	36.9	36.9	36.8	36.9					
C(23)	66.7	29.7	66.5	67.8					
C(24)	14.0	18.6	14.4	13.2					
C(25)	17.7	17.2	17.7	16.4					
C(26)	17.9	17.8	17.8	17.9					
C(27)	23.6	23.8	23.7	23.8					
C(28)	176.4	176.3	176.3	176.4					
C(29)	17.4	17.4	17.3	17.5					
C(30)	21.3	21.4	21.2	21.4					
Me*C*=O	170.8								
*Me*CO	20.8								

[a]) Assignments based on HMQC and HMBC correlations. [b]) Recorded at 125 MHz. [c]) Recorded at 100 MHz.

present. Acid hydrolysis of **3** furnished glucose and asiatic acid, identified by comparison with authentic samples on PC and TLC. The structure of **3** was formulated as asiatic acid 6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl ester.

The ^{1}H-NMR spectrum of **3** (*Table 1*) gave 6 Me signals, of which 2*d* and 4*s*, together with signals for H–C(18) (δ(H) 2.48 (*d*, J = 11.3 Hz)) and H–C(12) (δ(H) 5.42 (br. *s*)), as expected for an urs-12-en-28-oic acid skeleton. The anomeric protons with signals at (δ(H) 5.04 (*d*, J = 7.7 Hz, H–C(1′ and 6.20 (*d*, J = 8.1 Hz, H–C(1″)) were assignable to two-β-D-linked glucopyranosyl moieties. In the HMBC experiment, the long-range correlations H–C(1′) (δ(H) 6.20)/C(28) (δ(C) 176.3) and H–C(1″) (δ(H) 5.04)/C(6′) (δ(C) 69.6) were observed, revealing that the inner glucose unit was linked at C(28) of the aglycone and terminal glucose unit at C(6′) of the inner glucose unit. The δ(H) and δ(C) signals assignable to the oligoglucoside moiety were almost the same as those of centellasaponin B [4].

Asiaticoside F (**4**) was obtained as colorless powder. The negative-ion FAB-MS of **4** gave quasi-molecular-ion and fragment ions at *m*/*z* 941 ($[M-H]^-$), 795 ($[M-C_6H_{11}O_4]^-$), and 471 ($[M-C_{18}H_{31}O_{14}]^-$). The HR-FAB-MS was consistent with the

molecular formula $C_{48}H_{78}O_{18}$ (*m/z* 941.5092 ($[M-H]^-$). The IR spectrum showed OH, ester-carbonyl, and olefin functions at 3438, 1733 and 1652 cm^{-1}. On acid hydrolysis of **4**, glucose and rhamnose were identified by comparison with authentic samples on PC. The structure of **4** was elucidated to be (3*β*,4*α*)-3,23-dihydroxyurs-12-en-28-oic acid *O*-*α*-L-rhamnopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester.

In the ^{1}H-NMR spectrum of **4** (*Table 1*), 2*d* and 4*s* Me signals were observed, together with the olefinic H–C(12) signal at *δ*(H) 5.41 (br. *s*), and the H–C(18) signal at *δ*(H) 2.48 (*d*, *J* = 11.0 Hz), typical of an urs-12-en-28-oic acid derivative. The three anomeric-proton signals at *δ*(H) 4.96 (*d*, *J* = 7.8 Hz, H–C(1″)), 6.17 (*d*, *J* = 8.0 Hz, H–C(1′)), and 5.81 (br. *s*, H–C(1‴)) suggested two *β*-D-linked and one *α*-L-linked sugar moieties. The ^{13}C-NMR signals of **4** and asiaticoside (**5**) were almost the same, except that C(2), C(1), and C(3) were shifted upfield in **4** to *δ*(C) 39.1 (C(2)), 27.8 (C(1)), and 73.6 (C(3)), as compared to the corresponding *δ*(C) 69.7 (C(2)), 48.3 (C(1)), and 78.2 (C(3)) of **5** [2][3]. This established that **4** had no OH group at C(2) and contained the same sugar sequence as **5**.

Comparison of the spectral data (see *Table 3* for ^{13}C-NMR) with the values reported in [2–6] allowed us to identify compounds **5**–**7** to be asiaticoside (**5**),

Table 3. *^{13}C-NMR Data for Compounds* **5**–**7** *in C_5D_5N.*[a]). *δ* in ppm.

	5	**6**	**7**		**5**	**6**	**7**
C(1)	48.2	50.5	48.0	C(1′)	95.8	95.8	95.7
C(2)	69.0	69.1	68.1	C(2′)	73.8	73.8	73.8
C(3)	78.0	78.2	78.0	C(3′)	78.4	78.4	78.2
C(4)	43.6	44.6	56.6	C(4′)	70.9	71.1	71.0
C(5)	48.0	48.7	48.1	C(5′)	78.0	77.9	77.2
C(6)	18.6	67.6	20.6	C(6′)	69.6	69.5	69.5
C(7)	33.2	41.4	32.8				
C(8)	40.2	39.7	40.2	C(1″)	105.1	104.9	105.0
C(9)	48.2	48.5	48.1	C(2″)	75.4	75.4	75.4
C(10)	38.4	38.2	38.3	C(3″)	76.5	76.5	76.6
C(11)	23.9	23.8	23.8	C(4″)	78.8	78.6	78.8
C(12)	126.0	126.4	125.8	C(5″)	77.2	77.2	77.1
C(13)	138.6	137.9	138.6	C(6″)	61.3	61.3	61.4
C(14)	42.6	43.2	42.6				
C(15)	28.8	28.8	28.6	C(1‴)	102.6	102.6	102.7
C(16)	24.6	24.7	24.6	C(2‴)	72.7	72.6	72.6
C(17)	48.4	48.5	48.4	C(3‴)	72.8	72.8	72.8
C(18)	53.2	53.4	53.2	C(4‴)	74.0	74.0	74.0
C(19)	39.3	39.4	39.3	C(5‴)	70.3	70.3	70.3
C(20)	39.1	39.2	39.1	C(6‴)	18.6	18.6	18.5
C(21)	30.8	30.9	30.8				
C(22)	36.9	36.8	36.8				
C(23)	66.7	66.3	206.4				
C(24)	14.4	16.0	10.8				
C(25)	17.8	19.3	17.4				
C(26)	17.7	19.3	17.4				
C(27)	23.8	23.8	23.8				
C(28)	176.4	176.3	176.3				
C(29)	17.4	17.4	17.7				
C(30)	21.3	21.3	21.3				

[a]) Assignments based on HMQC and HMBC correlations and recorded at 100 MHz.

madecassoside (**6**), and scheffuroside B (**7**). Compounds **5** and **6** were the characteristic compounds from *C. asiatica*, and both of them were found to contribute to wound healing [7][8]. Compounds **1**–**4** were new triterpenoid glycosides, and compound **7** was obtained for the first time from *C. asiatica*, which provided new natural sources and probable candidates for the study of the traditional function of *C. asiatica*.

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Experimental Part

General. Column chromatography (CC): *Qingdao* silica gel (200–300 mesh); *MCI* gel *CHP-20P* (70-150 μ; *Mitsubishi Chemical Corporation*, Tokyo, Japan), *Lichrospher Rp-18* or *Rp-8* gel (40–63 μ; *Merck*, Germany), and *Sephadex LH-20*. TLC: silica gel; detection by 10% H_2SO_4 in EtOH, followed by heating. Optical rotations: *Horiba Sepa-300* high sensitive polarimeter. M.p.: *XRC-1* apparatus; uncorrected. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; ν in cm^{-1}. 1D- and 2D-NMR Experiments: *Bruker AM-400* (^1H and ^{13}C, at 400 and 100 MHz, resp.) or *DRX-500* (^1H and ^{13}C, 500 and 125 MHz, resp.) spectrometer; δ in ppm rel. to $SiMe_4$ as internal reference, coupling constants J in Hz. FAB-MS and HR-FAB-MS: *VG Auto-Spec-3000* instrument.

Plant Material. The plant used in this experiment was collected in Xishuanbanna, Yunnan Province, P.R. China, in May 2002, and was identified as *Centella asiatica* (L.) URBAN by senior engineer Mr. Jing-Yun Cui, Xishuangbanna Tropic Botanical Garden, Chinese Academy of Sciences, where a voucher specimen is deposited.

Extraction and Isolation. The whole plant of *C. asiatica* (9.5 kg) was extracted 3 × with 90% EtOH for 2 h under reflux. After evaporation, the residue was suspended in H_2O and partioned between $CHCl_3$ and BuOH to give a $CHCl_3$ fraction (200 g) and a BuOH fraction (296 g). The BuOH fraction was subjected to CC (silica gel (1.5 kg; 200–300 mesh), gradients $CHCl_3$/MeOH 95:5 → 80:20 and $CHCl_3$/MeOH/H_2O 7:3:0.3 → 6:4:0.5): *Fractions 1–40* (500 ml each). *Fr. 15–28* (with $CHCl_3$/MeOH 8:2 and $CHCl_3$/MeOH/H_2O 7:3:0.3) gave 59 g of a residue, which was repeatedly submitted to CC (silica gel (500 g), $CHCl_3$/MeOH 9:1 and 8:2), $CHCl_3$/MeOH/H_2O (7:3:0.3): *Fr. A* (8 g), *B* (5 g), *C* (10 g), and *D* (15 g). *Fr. A* was submitted to CC (*MCI* gel *CHP-20P* (100 g), (MeOH/H_2O 6:4 → 8:2, 250-ml fractions): *Fr. A1–A9*. *Fr. A2* was subjected to CC (*RP-8* gel): **4** (50 mg). *Fr. A3* was subjected to CC (*Rp-18* gel, MeOH/H_2O 7.5:2.5): **3** (20 mg). *Frs. B* and *C* were respectively submitted to CC (*MCI* gel *CHP-20P* (100 g), MeOH/H_2O 7:3 → 9:1): *Frs. B2–B4* and *Fr. C1* with MeOH/H_2O 7:3 → 8:2. *Fr. B2* was subjected to CC (*RP-8 gel*, MeOH/H_2O 7:3): **1** (35 mg) and **7** (15 mg). *Fr. C1* was subjected to CC (*RP-8 gel*, MeOH/H_2O 7:3): *Fr. C1.1–C1.3*. Compound **5** (2.5 g) was obtained from *Fr. C1.2* by CC (silica gel, $CHCl_3$/MeOH/H_2O 7:3:0.3). Compound **2** (10 mg) was obtained from *Fr. C1.3* by further purification by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1). *Fr. D* was submitted to CC (silica gel, $CHCl_3$/MeOH/H_2O, 8:2:0.2 → 7:3:0.3): *Fr. D1–D4*. *Fr. D3* (9 g) was subjected to CC (*MCI* gel *CHP-20P*, MeOH/H_2O, 6.5:3.5): *Fr. D3.1–Fr. D3.3*. *Fr. D3.3* was subjected repeatedly to CC (silica gel, $CHCl_3$/MeOH/H_2O, 7:3:0.3): **6** (2.0 g).

Asiaticoside C (= *(2α,3β,4α)-23-(Acetyloxy)-2,3-dihydroxyurs-12-en-28-oic Acid* O-α-L-*Rhamnopyranosyl-(1 → 4)*-O-β-D-*glucopyranosyl-(1 → 6)*-β-D-*glucopyranosyl ester*; **1**): Colorless amorphous powder. M.p. 202–206°; $[\alpha]_D^{26.2} = -14.6$ ($c = 0.4$, MeOH). IR (KBr): 3441, 2928, 1738, 1655, 1266, 1063. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 999 (91, $[M-H]^-$), 957 (4), 853 (7), 529 (100), 487 (30). HR-FAB-MS (neg.): 999.5164 ($[M-H]^-$, $C_{50}H_{79}O_{20}^-$; calc. 999.5174).

Asiaticoside D (= *(2α,3β)-2,3-Dihydroxyurs-12-en-28-oic Acid* O-α-L-*Rhamnopyranosyl-(1 → 4)*-O-β-D-*glucopyranosyl-(1 → 6)*-β-D-*glucopyranosyl Ester*; **2**): Colorless amorphous powder. M.p. 206–209°; $[\alpha]_D^{20.8} = -22.7$ ($c = 0.2$, MeOH). IR (KBr): 3438, 2928, 1733, 1655, 1064. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 941 (100, $[M-H]^-$), 795 (8), 471 (92). HR-FAB-MS (neg.): 941.5091 ($[M-H]^-$, $C_{48}H_{77}O_{18}^-$; calc. 941.5109).

Asiaticoside E (= *(2α,3β,4α)-2,3,23-Trihydroxyurs-12-en-28-oic Acid 6*-O-β-D-*Glucopyranosyl*-β-D-*glucopyranosyl Ester*; **3**): Colorless amorphous powder. M.p. 198–201°; $[\alpha]_D^{20.9} = -2.0$ ($c = 0.2$, MeOH). IR (KBr): 3424, 2926, 1733, 1637, 1064. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 811 (100, $[M-H]^-$, 487 (35). HR-FAB-MS (neg.): 811.4465 ($[M-H]^-$, $C_{42}H_{67}O_{15}^-$; calc. 811.4479).

Asiaticoside F (=*(3β,4α)-3,23-Dihydroxyurs-12-en-28-oic Acid* O-α-L-*Rhamnopyranosyl-(1→4)*-O-β-D-*glucopyranosyl-(1→6)*-β-D-*glucopyranosyl Ester*; **4**): Colorless amorphous powder. M.p. 219–222°; $[\alpha]_D^{26.0} = -13.3$ ($c = 0.5$, MeOH). IR (KBr): 3438, 2929, 1733, 1652, 1064. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 941 (100, $[M-H]^-$), 795 (7), 471 (93). HR-FAB-MS (neg.): 941.5092 ($[M-H]^-$, $C_{48}H_{77}O_{18}^-$; calc. 941.5109).

Acidic Hydrolysis: Each of compounds **1**–**4** (3 mg) was dissolved in MeOH (2.0 ml) and 5% H_2SO_4 soln. (2.0 ml) and hydrolyzed under reflux for 2 h. The hydrolyzate was allowed to cool, diluted 2-fold with H_2O, and partitioned between $CHCl_3$ and H_2O. The aq. layer was neutralized with aq. $Ba(OH)_2$ soln. and evaporated: glucose/rhamnose (from **1**, **2**, and **4**) or glucose (from **3**). Identification by PC comparison with authentic samples (BuOH/AcOEt/H_2O 4:1:5, upper layer). Asiatic acid (from **3**) was isolated from the $CHCl_3$ layer by TLC comparison with an authentic sample (silica gel, $CHCl_3$/MeOH 90:10).

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