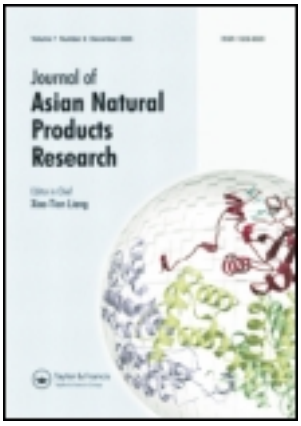

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Two new dammarane monodesmosides from *Centella asiatica*

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Two new dammarane monodesmosides from *Centella asiatica*

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Two new dammarane monodesmosides centellosides A (**1**) and B (**2**), and two new natural products ginsenosides Mc (**10**) and Y (**11**), together with 11 known compounds (**3**–**9** and **12**–**15**) reported for the first time from this genus, were isolated from the whole plants of *Centella asiatica*. All structures were elucidated by spectroscopic techniques and chemical methods, and compared with literature values. All the isolated compounds were evaluated *in vitro* for cytotoxicity.

Keywords: *Centella asiatica*; centelloside; ginsenoside; cytotoxicity

1. Introduction

Centella asiatica (L.) Urban (Apiaceae) is a traditional herbal medicine used in Asiatic countries. Experimental and clinical investigations showed that it had a wide spectrum of medicinal effects, e.g. the use in the treatment of venous insufficiency, striae gravidarum, and wound-healing disturbances [1]. Previous studies of this plant have resulted in the isolation of more than 70 constituents, which mainly contain pentacyclic triterpenes [2,3]. Although no tetracyclic triterpenoid saponin has been reported so far, a dammarenediol synthase was isolated and characterized from this plant [4]. This observation prompted us to discover new tetracyclic triterpenes with potential bioactivity. In the present research, two new dammarane saponins named as centelloside A (**1**) and centelloside B (**2**), two new natural products named as ginsenoside Mc (**10**) and ginsenoside Y (**11**), and 11 further related known derivatives (tetracyclic) were isolated from the whole plant of *C. asiatica* (Figure 1). These 13 known compounds were isolated for the first time from this genus. Some of the compounds exhibited mild inhibitory activity against HepG2 (human hepatocellular carcinoma), K562 (human chronic myeloid leukemia), and BGC-823 (human gastric carcinoma) cells.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its HR–ESI–MS showed a quasi-molecular ion peak $[M + Na]^+$ at *m/z* 921.5194, in accord with the molecular formula $C_{47}H_{78}O_{16}$. The IR spectrum showed absorption bands at 3407 (OH) and 1638 (C=C) cm^{-1}. The negative ESI–MS–MS of **1** exhibited fragment ion peaks at *m/z* 765 ($[M - H\text{-}132(pentosyl)]^-$ and 603 ($[M - H\text{-}132\text{-}162(hexosyl)]^-$) suggesting the presence of pentosyl and hexosyl units in **1** and a pentosyl unit as the terminal sugar moiety. The resonances for three anomeric methines at δ_H 4.81 (d, $J = 6.8$ Hz)/δ_C 104.7, δ_H 5.37 (d, $J = 7.6$ Hz)/δ_C 103.2, and δ_H 5.24 (d, $J = 6.4$ Hz)/δ_C 106.4 revealed the

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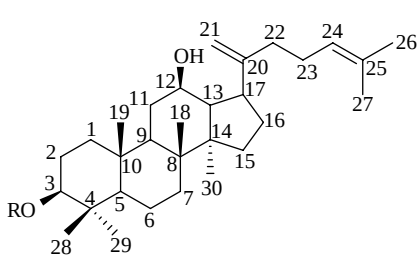

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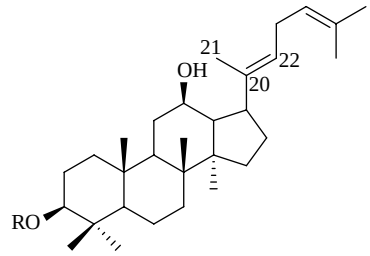
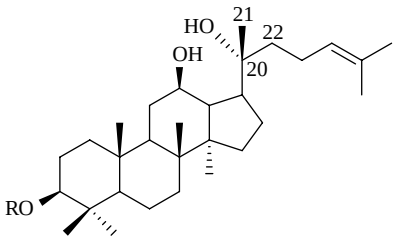
3 R = Glc (1→2) Glc



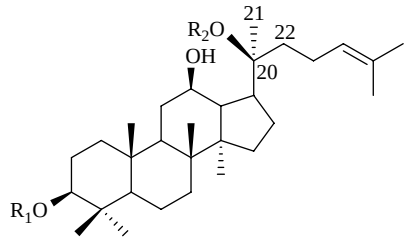
2 R = Xyl (1→2) Glc (1→2) Glc

4 R = Glc (1→2) Glc



5 R = Xyl (1→2) Glc (1→2) Glc

6 R = Glc (1→2) Glc



7—15

Compounds	R_1	R_2
7	Glc	Glc
8	Glc(1→2)Glc	H
9	Xyl(1→2)Glc(1→2)Glc	H
10	H	Araf(1→6)Glc
11	H	Arap(1→6)Glc
12	H	Xyl(1→6)Glc
13	Glc	Araf(1→6)Glc
14	Glc	Arap(1→6)Glc
15	Glc	Xyl(1→6)Glc

Glc = β-D-glucopyranosyl, Xyl = β-D-xylopyranosyl, Araf = α-L-arabinofuranosyl, Arap = α-L-arabinopyranosyl.

Figure 1. Chemical structures of compounds **1**–**15**.

presence of three sugar moieties with β-orientation. Comparative study of the sugar resonances [5] indicated the existence of two β-glucopyranose units and one β-xylopyranose unit. Further comparison of the NMR spectral data of **1** with those of **3** indicated that **1** had one more xylopyranosyl unit than **3** [6]. The structure was further assigned by HMQC, HMBC, TOCSY, and $^1H-^1H$ COSY experiments. In the HMBC spectrum of **1**, the location and the sequence of sugar moieties were demonstrated by the key correlations from H-1′ (δ 4.81) to C-3 (δ 89.0), H-1″ (δ 5.37) to C-2′ (δ 82.9), and H-1‴ (δ 5.24) to C-2″ (δ 84.6). The HMBC correlations of H-11 and H-13 with C-12 (δ 72.5) confirmed that the hydroxy was linked at C-12. Other

key HMBC and $^1H-^1H$ COSY correlations are shown in Figure 2. From these data, the structure of compound **1** was, thus, determined as 3β,12β-dihydroxydammar-20(21),24-diene 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside, and named centelloside A.

Compound **2** was obtained as a white amorphous powder. The HR–ESI–MS of **2** displayed a quasi-molecular ion peak at *m/z* 921.5191 $[M + Na]^+$, in agreement with the molecular formula $C_{47}H_{78}O_{16}$. Its IR spectrum showed absorption bands at 3422 (OH) and 1640 (C=C) cm^{-1}. Pentosyl and hexosyl units in **2** and a pentosyl unit as the terminal sugar moiety were deduced from the characteristic fragment ion peaks (negative ESI–MS–MS) at *m/z* 765 ($[M - H\text{-}132(pentosyl)]^-$ and 603 ($[M - H\text{-}132\text{-}162(hexosyl)]^-$). The ^1H and ^{13}C NMR spectral data (Table 1) of **2** were very similar to those of **4**, except for the appearance of a set of additional signals in **2** ascribed to one xylopyranosyl unit [δ_H 5.23 (d, $J = 6.4$ Hz)/δ_C 106.4]. The ^{13}C NMR spectral data (Table 1) of the sugar moieties of **2** were in good agreement with those of **1**. The chemical shift of C-21 at δ 13.1 indicated the presence of an *E*-type double bond at C-20, as present in the ginsenosides Rg_5, Rh_3, and Rh_4 [6], while the methyl carbon of *Z* type was usually observed at lower field around 30 ppm [7]. In the HMBC spectrum of **2**, the long-range correlations from H-1′ (δ 4.80) to C-3 (δ 89.0), H-1″ (δ 5.36) to C-2′ (δ 82.9), and H-1‴ (δ 5.23) to C-2″ (δ 84.6), proved the linkage sequence of sugar units. The key HMBC and $^1H-^1H$ COSY correlations for side chain at C-17 are shown in Figure 2. From the above evidence, the structure of compound **2** was elucidated as (20*E*)-3β,12β-dihydroxydammar-20(22),24-diene 3-*O*-β-d-xylopyranosyl-(1 → 2)-*O*-β-d-glucopyranosyl-(1 → 2)-*O*-β-d-glucopyranoside, and named centelloside B.

The other 13 known compounds were identified as ginsenoside Rk_1 (**3**) [6], ginsenoside Rg_5 (**4**) [8], (20*R*)-ginsenoside Rg_3 (**5**) [9], notoginsenoside Ft_1 (**6**) [5], ginsenoside F_2 (**7**) [10,11], (20*S*)-ginsenoside Rg_3 (**8**) [9], notoginsenoside ST-4 (**9**) [12], ginsenoside Mc (**10**) [13], ginsenoside Y (**11**) [14], gypenoside ιβ (**12**) [15], notoginsenoside Fe (**13**) [16], ginsenoside Rd_2 (**14**) [17,18], and gypenoside η (**15**) [19] by comparison of their spectroscopic data with those reported in the literature. Ginsenosides Mc and Y are two new natural products among them.

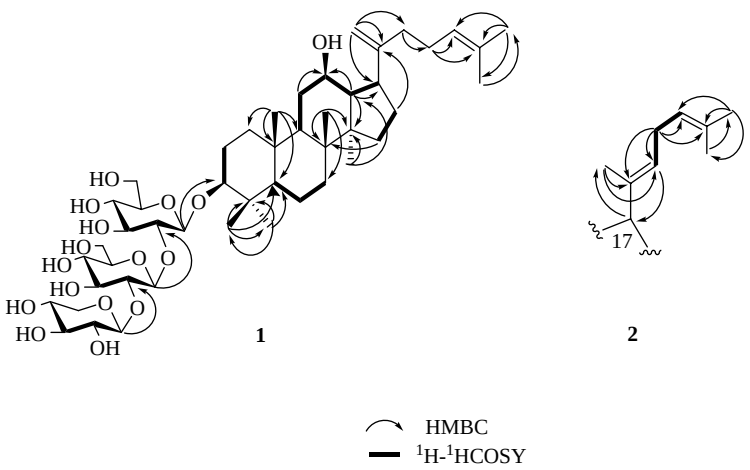


Figure 2. Selected HMBC and $^1H-^1H$ COSY correlations for **1** and **2**.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** and **2** (C_5D_5N).

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
1	39.3 t	0.68 (m), 1.38 (m)	39.3 t	0.72 (m), 1.41 (m)
2	26.7 t	1.72 (br.d, 11.6), 2.08 (dd, 10.0, 3.6)	28.8 t	1.40 (m), 1.88 (m)
3	89.0 d	3.20 (dd, 11.6, 3.6)	89.0 d	3.20 (dd, 11.6, 4.4)
4	39.8 s		40.3 s	
5	56.5 d	0.63 (d, 11.6)	56.5 d	0.62 (d, 11.2)
6	18.5 t	1.32 (m), 1.45(m)	18.5 t	1.37 (m), 1.47 (m)
7	35.4 t	1.43 (m), 1.19 (m)	35.4 t	1.17 (m), 1.40 (m)
8	40.2 s		39.8 s	
9	50.8 d	1.32 (m)	50.8 d	1.31 (m)
10	37.1 s		37.1 s	
11	32.6 t	1.32 (m), 1.81 (m)	32.2 t	1.35 (m), 1.84 (m)
12	72.5 d	3.80 (m)	72.6 d	3.81 (m)
13	52.5 d	1.97 (t, 10.8)	50.4 d	2.68 (t, 7.6)
14	51.2 s		50.9 s	
15	32.6 t	0.98 (m), 1.60 (m)	32.6 t	1.01 (m), 1.60 (m)
16	30.7 t	1.46 (m), 1.95 (m)	26.7 t	1.74 (m), 2.10 (m)
17	48.2 d	2.70 (m)	51.1 d	1.89 (m)
18	16.4 q	0.92 (s)	16.4 q	0.72 (s)
19	15.8 q	0.72 (s)	16.6 q	1.00 (s)
20	155.6 s		140.2 s	
21	108.2 t	4.80 (m), 5.04 (br.s)	13.1 q	1.71 (s)
22	33.9 t	2.20 (m), 2.28 (m)	123.6 d	5.40 (t, 7.2)
23	27.1 t	2.22 (m), 2.86 (t, 6.8)	27.4 t	2.68 (t, 7.6)
24	125.3 d	5.20 (m)	123.8 d	5.13 (t, 7.2)
25	131.2 s		131.3 s	
26	25.7 q	1.57 (s)	25.6 q	1.53 (s)
27	17.7 q	1.51 (s)	17.7 q	1.49 (s)
28	28.1 q	1.19 (s)	28.1 q	1.18 (s)
29	16.6 q	1.01 (s)	15.9 q	0.92 (s)
30	17.0 q	0.86 (s)	17.1 q	0.86 (s)
1′	104.7 d	4.81 (d, 6.8)	104.7 d	4.80 (d, 7.6)
2′	82.9 d	3.95 (m)	82.9 d	3.95 (m)
3′	77.9 d	4.15 (m)	77.9 d	3.99 (m)
4′	71.9 d	4.03 (m)	71.9 d	4.03 (m)
5′	77.6 d	3.98 (m)	77.6 d	4.15 (m)
6′	63.0 t	4.13 (m), 4.34 (br.d, 10.4)	63.0 t	4.13 (m), 4.33 (br.d, 8.8)
1″	103.2 d	5.37 (d, 7.6)	103.2 d	5.36 (d, 7.6)
2″	84.6 d	4.05 (m)	84.6 d	4.05 (m)
3″	78.1 d	3.80 (m)	78.1 d	3.78 (m)
4″	71.1 d	3.95 (m)	71.2 d	4.00 (m)
5″	77.6 d	3.72 (m)	77.6 d	3.73 (m)
6″	63.0 t	4.21 (m), 4.41 (br.d, 11.6)	63.0 t	4.20 (m), 4.41 (br.d, 11.2)
1‴	106.4 d	5.24 (d, 6.4)	106.4 d	5.23 (d, 6.4)
2‴	75.8 d	3.96 (m)	75.8 d	3.96 (m)
3‴	78.6 d	4.19 (m)	78.6 d	4.18 (m)
4‴	70.7 d	3.96 (m)	70.7 d	3.98 (m)
5‴	67.3 t	3.57 (t, 10.0), 4.23 (m)	67.3 t	3.56 (t, 9.6), 4.20 (m)

Compounds **1**, **3**, **11**, and **12** showed mild *in vitro* cytotoxicity against HepG2 and K562 cells, with IC_{50} values in the range of 19.93–54.59 μM.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter (San Jose, CA, USA). IR spectra were recorded in KBr pellets on a Nicolet-NEXUS-670-FTIR spectrophotometer (Madison, WI, USA). NMR spectra were determined on a Varian INOVA-400 instrument (Palo Alto, CA, USA). HR–ESI–MS and ESI–MS/MS spectra were recorded on a Waters Q-TOF micro YA019 mass spectrometer (Leederville, WA, USA). Preparative HPLC was performed on a Shimadzu HPLC system (LC-8A pump, SPD-M10A detector; Kyoto, Japan) with a Shimadzu PRC-ODS column (15 μ, i.d. 20 × 250 mm; Kyoto, Japan). Analytical GC was carried out on an Agilent 6890N system (H_2 flame ionization detector; Minneapolis, MN, USA) and a capillary column (30 m × 0.32 mm × 0.25 μm; Abel AB-5; Appleton, WI, USA). Materials for column chromatography were silica gel (200–300 mesh; Shanghai Sanpont Co., Shanghai, China) and Sephadex LH-20 (40–150 μm; Amersham Pharmacia Biotech, Uppsala, Sweden).

3.2 Plant material

The whole plant materials of *C. asiatica* were collected at Guangxi Zhuang Autonomous Region, China, in July 2008 and identified by Dr Tong Wu of Shanghai Institute of Pharmaceutical Industry. A voucher specimen (SIPITCM-080711) has been deposited at the institute.

3.3 Extraction and isolation

The air-dried whole plants of *C. asiatica* (10 kg) were extracted with water two times (100 L × 1.5 h and 80 L × 1 h). The extracts were combined and concentrated to a certain volume (3 L), and then 95% EtOH (15 L) was added and allowed to stand for 24 h. The precipitate was removed by filtration. After solvent removal of the filtrate to 3 L, the crude extract was suspended in H_2O and extracted with BuOH for two times (6 and 4.8 L). The BuOH extracts (165 g) were subjected to silica gel column chromatography (200–300 mesh, 1.1 kg, 8.5 × 43 cm) using gradient elution with a mixture of $CHCl_2$–MeOH–H_2O (10:2:0.2, 10:4:0.4, 10:5:0.6, 10:6:1, and 100%EtOH, each 6 L) to give fractions 1–12. Fraction 3 (2.0 g) was further purified by preparative HPLC [MeOH–H_2O (90:10, 6 ml/min, 204 nm)] to yield fractions A_3–E_3. Fraction C_3 (86 mg) was further applied to preparative HPLC [CH_3CN–H_2O (48:52, 6 ml/min, 204 nm)] to afford **10** (55 mg, t_R: 42 min). Fraction D_3 (188 mg) was subjected to preparative HPLC [CH_3CN–H_2O (44:56, 6 ml/min, 204 nm)] to obtain **11** (47 mg, t_R: 87 min) and **12** (120 mg, t_R: 96 min). From fraction E_3 (180 mg), **3** (56 mg, t_R: 78 min) and **4** (50 mg, t_R: 86 min) were obtained by preparative HPLC [MeOH–H_2O (82:18, 6 ml/min, 204 nm)]. Fraction 4 (3.2 g) was separated by Sephadex LH-20 (35 g, 2.5 × 44 cm, MeOH) and then further purified by preparative HPLC [MeOH–H_2O (83:17, 6 ml/min, 204 nm)] to give fractions A_4–E_4. Fraction C_4 (312 mg) was subjected to preparative HPLC [CH_3CN–H_2O (50:50, 6 ml/min, 204 nm)] to provide **6** (55 mg, t_R: 33 min), **7** (77 mg, t_R: 20 min), and **8** (107 mg, t_R: 30 min). Fraction D_4 (143 mg) was separated over preparative HPLC [CH_3CN–H_2O (53:47, 6 ml/min, 204 nm)] to yield **1** (15 mg, t_R: 37 min) and **2** (35 mg, t_R: 44 min). Similarly, fraction 5 (1.5 g) was submitted to preparative HPLC [MeOH–H_2O (83:17, 6 ml/min, 204 nm)] to give fractions A_5–F_5. Then, **13** (104 mg, t_R: 45 min) was obtained from fraction D_5 (138 mg) by preparative HPLC [CH_3CN–H_2O

(40:60, 6 ml/min, 204 nm)]. Fraction E_5 (132 mg) was purified by preparative HPLC [CH_3CN-H_2O (37:63, 6 ml/min, 204 nm)] to provide **14** (24 mg, t_R: 83 min) and **15** (56 mg, t_R: 94 min). From fraction F_5 (134 mg), **5** (33 mg, t_R: 55 min) and **9** (80 mg, t_R: 52 min) were isolated using preparative HPLC [CH_3CN-H_2O (42:58, 6 ml/min, 204 nm)].

3.3.1 *Centelloside A (**1**)*

White amorphous powder. $[\alpha]_D^{24}$ + 1.5 (*c* 0.66, MeOH); IR (KBr) ν_{max}: 3407, 2930, 1638, 1454, 1389, 1174, 1077, 1043, 894, 618 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI–MS (positive): *m/z* 921.30 $[M + Na]^+$; ESI–MS (negative): *m/z* 897.46 $[M - H]^-$; HR–ESI–MS (positive): *m/z* 921.5194 $[M + Na]^+$ (calculated for $C_{47}H_{78}O_{16}Na$, 921.5188).

3.3.2 *Centelloside B (**2**)*

White amorphous powder. $[\alpha]_D^{24}$ + 1.8 (*c* 1.10, MeOH); IR (KBr) ν_{max}: 3422, 2945, 1640, 1454, 1389, 1077, 1043, 895, 617 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI–MS (positive): *m/z* 921.35 $[M + Na]^+$; ESI–MS (negative): *m/z* 897.35 $[M - H]^-$; HR–ESI–MS (positive): *m/z* 921.5191 $[M + Na]^+$ (calculated for $C_{47}H_{78}O_{16}Na$, 921.5188).

3.4 *Acid hydrolysis of 1 and 2*

Compounds **1** and **2** (5 mg each) were refluxed in 2M HCl (2 ml) for 3 h. The mixture was neutralized with NH_4OH and then partitioned with $CHCl_3$. To the dried aqueous layer, pyridine and acetic anhydride (1 ml each) were added, and the mixture was kept overnight. The acetylated derivatives were subjected to GC analysis to identify the sugars. Conditions for GC were: cap. column: AB-5 (30 m × 0.32 mm × 0.25 μm); column temp.: 100°C/250°C; programmed increase: 10°C/min; injector and detector temp.: 250°C; injection volume: 2.0 μl; split ratio: 1:20; carrier gas: N_2 at 1 ml/min. D-glucose (t_R: 14.442 min)/D-xylose (t_R: 11.728 min) were detected from **1** and **2** (identical to authentic materials).

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