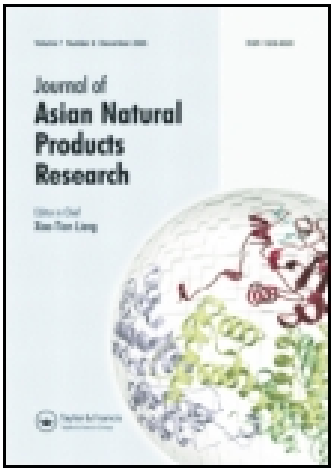

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Two new terpene glucosides and antitumor agents from Centipeda minima

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Two new terpene glucosides and antitumor agents from *Centipeda minima*

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One new monoterpene glucoside minimaoside A (**1**) and one new sesquiterpene glucoside minimaoside B (**2**), together with four known terpenoids, were isolated from the whole plants of *Centipeda minima* (L.) A. Braun et Ashers. Their structures were determined by spectroscopic methods. Compounds **5** and **6** showed weak or moderate cytotoxic activity toward several tumor cell lines.

Keywords: *Centipeda minima*; Compositae; terpene glucosides; cytotoxicity

1. Introduction

Centipeda minima L. (Compositae), distributed in South Asia and Oceania, is a traditional Chinese medicine used for cold, nasal allergy, diarrhea, malaria, asthma, and several forms of tumors such as rhinopharyngocele and encephaloma in China [1]. Flavonoids and mono-, sesqui-, and triterpenoids in this plant have been shown to have various biological activities [2,3]. During our search for antitumor constituents from medicinal plants, we conducted an investigation and isolated two new terpene glucosides and four known terpenoids (Figure 1). Herein, we describe the isolation, structural elucidation of two new compounds, and cytotoxic activities of the isolates from *C. minima* (L.) A. Braun et Ashers.

2. Results and discussion

Minimaoside A (**1**) was purified as a white powder. The molecular formula of **1** was determined to be $C_{16}H_{24}O_7$ from its HR-APPI-MS at m/z 327.1449 $[M-H]^-$ (calcd 327.1443). The ^{13}C NMR spectrum indicated the presence of a glucose moiety, and 10 additional carbon signals were reminiscent of a thymol analog. The ^1H NMR spectrum showed three methyl singlets, a typical ABX system in a benzene ring, and an anomeric proton at δ 4.64 (1H, d, $J = 7.8$ Hz, H-1′), suggesting **1** to be a glucoside of thymol derivative [3]. HMBC correlations of H-2 (δ 6.89) with C-3 (δ 153.2) and C-4 (δ 136.5), H-7 (δ 2.28) with C-2 (δ 124.5) and C-6 (δ 126.4), and H-9 (δ 1.60) with C-8 (δ 79.9) and C-10 (δ 27.4), H-10 (δ 1.62) with C-4 and C-9 (δ 30.6),

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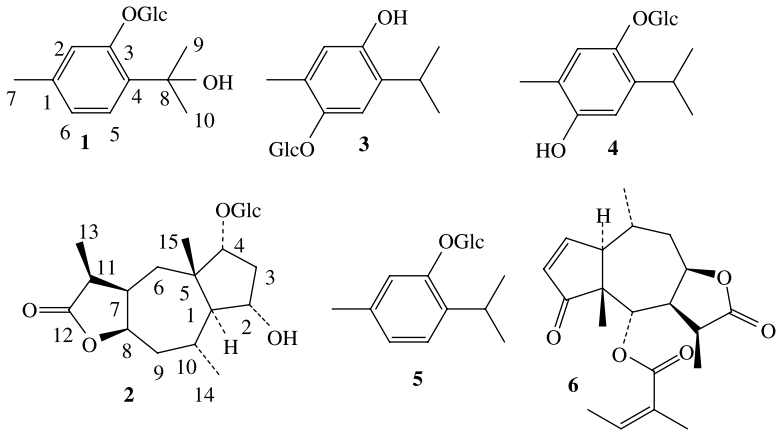

Figure 1. Structures of compounds **1**–**6**.

indicated that the aglycone of **1** was a 3,8-dihydroxythymol. Further HMBC correlation of H-1′ with C-3 (δ 153.2) implied that the glucosyl group was connected to C-3. The D-form of the glucosyl group was established by the positive sign of the optical rotation of sugar after acid hydrolysis. Accordingly, the structure of **1** was established as 8-hydroxythymol 3-*O*-β-D-glucopyranoside.

Minimaoside B (**2**) was isolated as a white oil. The molecular formula of **2** was assigned as $C_{21}H_{34}O_9$ by HR-ESI-MS at *m*/*z* 465.1901 $[M+Cl]^-$. The ^{13}C NMR and DEPT spectra displayed a typical glucose moiety and 15 additional carbon signals including three methyls, three methylenes, seven methines, and two quaternary carbons, which suggested that **2** was a sesquiterpene glucoside. $^1H-^1H$ COSY spectrum revealed the following fragments: H-1/H-2/H-3/H-4; H-6/H-7/H-8/H-9/H-10/H-1; H-10/H-14; and H-7/H-11/H-13. HMBC interactions of H-1 (δ 1.73), H-4 (δ 3.75), H-6a (δ 2.32), and H-15 (δ 0.85) all with C-5 (δ 48.5), H-7 (δ 2.67), H-11 (δ 2.26), and H-13 (δ 1.29), all with a carbonyl group (δ 182.7), in combination with Me-15 correlating with C-1 (δ 55.0), C-4 (δ 88.3), C-5 and C-6 (δ 39.5), Me-14 (δ 1.22) with C-1, C-9 (δ 36.1), and C-10 (δ 30.7) assembled the structure of aglycone of **2**. Additional HMBC correlation of H-1′ (δ 4.22) with C-4 implied that the glucosyl group was positioned at C-4. The stereochemistry of **2** was established by ROESY correlations and NOE irradiation experiments. ROESY correlations of both H-2 (δ 3.98) and H-4 with Me-15, both H-2 and Me-15 with H-10 (δ 1.91) suggested α-orientations of OH-2 and Me-14, and β-position of H-4; ROESY cross-peaks of H-1 with H-7, H-8 (δ 4.85) and H-9a (δ 2.11), and H-6b (δ 1.35) with H-9a and H-11 suggested that H-1, H-7, H-8, and H-11 were cofacial. Irradiation of H-1 and the enhancement of Me-14, H-9a, and H-7 were observed, which indicated that H-1, H-7, H-8, and H-11 were all α-oriented. The relative configuration of aglycone of **2** was thus identified. Actually, the aglycone of **2** has been previously identified as dihydroneopulchellin [4]. Acid hydrolysis of **2** yielded glucose, which was assigned as D-form by the positive sign of optical rotation recorded in water.

Four known compounds were identified as 6-*O*-β-D-glucopyranoside (**3**) [5], 6-hydroxythymol 3-*O*-β-D-glucopyranoside (**4**) [5], thymol-β-glucopyranoside (**5**) [6], and 6-*O*-angeloylplenolin (**6**) [7], respectively, by comparison of their spectroscopic data with those reported in

the literature. Compounds **3**–**5** were isolated from this plant for the first time.

Compounds **2**–**6** were tested for their *in vitro* cytotoxic activity. With respect to sesquiterpene lactones, compound **6** exhibited moderate activity toward A549, K562, and Kasumi-1 (K-1) cell lines with IC_{50} values of 7.1 (adriamycin: IC_{50} value = 0.43 μM), 7.2 (imatinib mesylate: IC_{50} value = 0.10 μM), and 7.9 μM (bortezomib: IC_{50} value = 5 nM), respectively. For the thymol derivatives, only compound **5** showed negligible activity against A549 cells with an IC_{50} value of 46.20 μM.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter. The UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer, λ_{max} in nm. The IR spectra were obtained on a Bio-Rad FTS-135 spectrometer. The ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, and HMBC spectra were recorded on a DRX-500 spectrometer, δ in ppm relative to TMS as an internal reference, and *J* in Hz. FAB-MS were recorded on a VG Auto Spec-3000 spectrometer, and HR-ESI-MS were determined on an API QSTAR Pulsar 1 spectrometer, and HR-APPI-MS was recorded on a QSTAR LCQ-TOF spectrometer. Silica gel (200–300 mesh and 10–40 μm; Qingdao Marine Chemical Factory, Qingdao, China), RP-18 silica gel (40–63 μm; Daiso Co., Osaka, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden), and MCI gel CHP 20P (75–150 μm; Mitsubishikasei, Tokyo, Japan) were used for column chromatography. Silica gel GF_{254} (10–40 μm; Qingdao Marine Chemical Factory) was used for TLC.

3.2 Plant material

The whole plants of *C. minima* were purchased from Yunnan Corporation of Materia Medica, Yunnan Province, China, and identified by Mr Hong-Yan Sun at Yunnan Corporation of Materia Medica. A voucher specimen (CHYX0159) is deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

3.3 Extraction and isolation

The dried and powdered whole plants of *C. minima* (10 kg) were extracted with 95% EtOH under reflux, the combined extracts were suspended in water, and partitioned by petroleum ether, EtOAc, and *n*-BuOH successively. The EtOAc extract (170 g) was submitted to column chromatography over silica gel ($CHCl_3$/MeOH, 1:0–0:1) to afford fractions 1–4. Fraction 3 (6 g) was decolorized over MCI gel CHP 20P (gradient aqueous MeOH) to give fractions 3.1–3.4. Repeated chromatography of fraction 3.2 (1.2 g) over silica gel with gradient $CHCl_3$/MeOH (15:1–7:1), RP-18 (gradient aqueous MeOH), and Sephadex LH-20 (MeOH) yielded compounds **1** (8 mg), **5** (380 mg), and **6** (64 mg). The *n*-BuOH-soluble material (45 g) was fractionated over silica gel ($CHCl_3$/MeOH, 5:1) to afford fractions A–C. Fraction B (3.1 g) was passed through an MCI gel CHP 20P column (MeOH/H_2O, 20:80–50:50), and then a Sephadex LH-20 column (MeOH) to yield **4** (386 mg). Fraction C (2.8 g) was chromatographed over Sephadex LH-20 (MeOH) and RP-18 (MeOH/H_2O, 70:30) to give compounds **2** (170 mg) and **3** (41 mg).

3.3.1 8-Hydroxythymol 3-O-β-D-glucopyranoside (*1*)

A white powder; $[\alpha]_D^{18}$ −80.4 (c = 0.06, MeOH). UV (MeOH) λ_{max} (log ε): 274 (2.85), 213 (4.65), 202 (4.16) nm. IR (KBr) ν_{max}: 3444, 2922, 2851, 1636, 1068, 669 cm^{-1}. ^{1}H and ^{13}C NMR spectral data, see Table 1. FAB-MS (negative) *m/z*: 309 $[M-H_2O-H]^-$. HR-APPI-MS

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz; in CD_3OD).

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
1		140.2	1.73 (dd, 8.0, 4.0)	55.0
2	6.89 (br s)	124.5	3.98 (m)	77.3
3a		153.2	2.46 (ddd, 15.5, 9.0, 5.0)	41.8
3b			1.61 (dd, 15.5, 2.5)	
4		136.5	3.75 (br d, 5.0)	88.3
5	7.16 (d, 10.0)	127.5		48.5
6a	6.93 (br d, 10.0)	126.4	2.32 (dd, 14.0, 4.8)	39.5
6b			1.35 (br d, 14.0)	
7	2.28 (s)	20.7	2.67 (m)	45.1
8		79.9	4.85 (m)	79.8
9a	1.60 (s)	30.6	2.11 (m)	36.1
9b			1.70 (m)	
10	1.62 (s)	27.4	1.91 (m)	30.7
11			2.26 (br q, 7.5)	44.0
12				182.7
13			1.29 (d, 7.5)	17.4
14			1.22 (d, 6.5)	21.1
15			0.85 (s)	22.3
1′	4.64 (d, 7.8)	103.2	4.22 (d, 7.5)	101.9
2′	3.42 (m)	76.1	3.14 (dd, 9.0, 7.5)	75.4
3′	3.47 (m)	77.0	3.44 (m)	77.9
4′	3.26 (m)	71.6	3.21 (m)	72.1
5′	3.35 (m)	79.4	3.23 (m)	77.8
6′a	3.88 (dd, 14.6, 3.5)	62.7	3.88 (dd, 11.5, 1.5)	63.1
6′b	3.67 (dd, 14.5, 7.0)		3.63 (dd, 11.5, 5.5)	

m/z: 327.1449 $[M-H]^-$ (calcd for $C_{16}H_{23}O_7$, 327.1443).

3.3.2 2α-Hydroxy-4α-O-glucopyranosyl-10βH-pseudoguai-12,8β-olide (2)

A white oil; $[\alpha]_D^{18}$ −5.6 ($c = 0.09$, MeOH). UV (MeOH) λ_{max} (log ε): 202 (3.88) nm. IR (film) ν_{max}: 3423, 2960, 2929, 2877, 1747, 1632, 1458, 1381, 1230, 1214, 1163, 1075, 1043, 609 cm^{-1}. ^{1}H and ^{13}C NMR spectral data, see Table 1. FAB-MS (negative) *m/z*: 429 $[M-H]^-$. HR-ESI-MS *m/z*: 465.1901 $[M+Cl]^-$ (calcd for $C_{21}H_{34}O_9Cl$, 465.1891).

3.4 Acid hydrolysis of compounds 1 and 2

A solution of **1** (5 mg) or **2** (10 mg) was dissolved in a mixture of MeOH (2 ml) and 2 M HCl (2 ml), and heated in a water bath at 70°C for 6 h. After cooling, the mixture was neutralized with $NaHCO_3$ and extracted with EtOAc. TLC comparison (silica gel, *n*-BuOH/AcOH/H_2O, 4:1:1) with an authentic sample revealed the presence of glucose in the water layer ($R_f = 0.22$). Furthermore, the D-form of glucose was established by optical rotation measured in water.

3.5 Antiproliferative assay

The human leukemia cell lines K562 and K-1 were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in RPMI medium 1640 containing 10–15% fetal bovine serum (FBS), 2 mM L-glutamine, penicillin G (100 units/ml), and streptomycin (100 μg/ml). The lung cancer cell lines

A549 and SPCA1 were cultured in DMEM medium supplemented with 10% FBS, 2 mM L-glutamine, penicillin G (100 units/ml), and streptomycin (100 μg/ml). All cell lines were incubated in a humidified CO_2 incubator (5% CO_2, 37°C). In the cell proliferation assay, cells were incubated with or without the indicated drugs for 48 h. The K562 and K-1 cell proliferation was determined using Cell Counting Kit-8 (Dojin Laboratories, Kumamoto, Japan); A549 and SPCA1 cell proliferation was determined using MTT colorimetric assay (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer's instruction. The IC_{50} values were calculated.

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Note

1. These authors contributed equally to this paper.

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