One New Iridoid Glycoside from *Hedyotis tenelliflora*

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Abstract: **Objective** To study the chemical constituents of *Hedyotis tenelliflora*. **Methods** The compounds were isolated by chromatographic separation technology. The structures were identified on the basis of chemical and spectral data. **Results** Four iridoid glycosides were isolated from the whole plant of *H. tenelliflora*. On the basis of the chemical and spectral methods, their structures were elucidated as teneoside C (**1**), harpagoside (**2**), harpagide (**3**), and asperulosidic acid (**4**). **Conclusion** Compound **1** is a new compound, and compounds **2** and **3** are isolated from *H. tenelliflora* for the first time.

Key words: harpagoside; *Hedyotis tenelliflora*; iridoid glycoside; Rubiaceae; teneoside C

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Introduction

The whole plant of *Hedyotis tenelliflora* Blume is a traditional Chinese herbal medicine called *Xiazicao* (Chinese Academy of Sciences China Flora Editing Group, 1999). It is used for the treatment of snake wounds, nephritis, hepatitis, rheumatic arthritis, and inflammations (Li, 1990). Iridoid glycosides, triterpenoids (Yuan, Zhao, and Li, 2001), lignan glycosides, flavonoids, and anthraquinones (Permana *et al*, 1999) had been reported from several species of *Hedyotis* L. (Yuan, Zhao, and Li, 2001).

In two previous papers, we reported the isolation and structure elucidation of two new iridoid glycosides from the roots of *H. tenelliflora* (Zhao *et al*, 2005) and eight compounds from the whole plant of *H. tenelliflora* (Yuan *et al*, 2004). In this paper, one new iridoid glycoside from the whole plant of *H. tenelliflora*, teneoside C (**1**), together with three known iridoid glycosides, harpagoside (**2**), harpagide (**3**) and asperulosidic acid (**4**), was isolated. Compounds **2** and **3** were isolated from *H. tenelliflora* for the first time. Their structures were elucidated by spectroscopic analyses.

Materials and methods

Column chromatography (CC): silica gel (100－200 or 200－300 mesh; Qingdao) or Sephadex LH-20 gel (Amersham Pharmacia). TLC: silica gel GF_{254} plates (Qingdao). D-101 macroporous adsorption resin (Tianjin). All solvents were of industrial grade and redistilled before use. Melting point is measured on Kofler Apparatus. UV Spectra: Shimadzu UV—210A Apparatus; ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), and 2D-NMR (COSY, HMBC, HMQC, and NOESY) spectra were recorded on a DRX—500 Spectrometer. HR-FAB-MS (positive mode): VG Auto Spec—3000 Spectrometer; in *m/z*.

The plants were collected from Wenshan, Yunnan, China, and identified by Prof. HU Zhi-hao, Department of Biology, Yunnan University, China. A voucher specimen was deposited at the Key Laboratory of Medicinal Chemistry for Natural Resource, Yunnan University, China.

Extraction and isolation

The air-dried and powdered plant material (4.0 kg) was repeatedly extracted with 95% EtOH at room temperature. The extracts were combined and concentrated *in vacuo*. The resulted residue was suspended in H_2O, and then successively extracted with petroleum ether (PE), EtOAc, and *n*-BuOH. The EtOAc-

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soluble extract (40 g) was subjected to CC ($CHCl_3$-MeOH) to afford three fractions (Fr.1－3). Fr. 3 (8 g) was separated by vacuum CC ($CHCl_3$-MeOH). The fraction eluted with $CHCl_3$-MeOH (9:1) was re-chromatographed to afford compounds **1** (80 mg), **2** (34 mg), and **3** (50 mg). The *n*-BuOH-soluble extract (80 g) was subjected to macroporous resin (D-101) absorption chromatography. The fraction eluted with 95% EtOH was re-chromatographed with $CHCl_3$-MeOH (6:1) to yield compound **4** (20 mg).

Results

Compound **1**, amorphous powder, mp 131－135 ℃, $[\alpha]_D^{25} = -58.34°$ (*c* 0.95, MeOH), UV λ_{max}^{MeOH} (nm): 205 (4.48), 217 (4.44), 223 (4.37), 280 (4.60), molecular formula $C_{25}H_{32}O_{11}$, as established by HR-FAB-MS (*m*/*z*: 508.4895, calcd. 508.4903). The IR spectrum indicated the present of OH (3400), C=O (1685), C=C group (1630), and benzene ring (1490, 1445). The ^{1}H-NMR and ^{13}C-NMR spectra of compound **1** (Table 1) displayed the typical signals of an iridoid glycoside. ^{1}H-NMR and ^{13}C-NMR assignments were made with the help of ^{1}H-NMR, ^{1}H-COSY, and HMQC experiments, starting with the easily distinguishable acetal C-1 at δ 94.91 and H-1 at δ 6.81, C-9 at δ 55.55 and H-1 at δ 3.64, C-3 at δ_C 142.40 and H-3 at δ 6.53, C-4 at δ_C 108.11 and H-4 at δ 5.17. A simple AB system (J = 16.3 Hz) characteristic of *trans* olefinic protons and an A_2B_2 system characteristic of phenyl were easily recognized from the signals at δ_H 7.72 (δ_C 144.58) and δ_H 6.55 (δ_C 120.39), and signals at δ_H 7.45 (δ_C 129.28) and δ_H 7.30 (δ_C 130.52). These features indicated the presence of cinnamoyl group in compound **1**. As observed in the HMBC spectrum, the correlations of δ 87.28 to H-1, H-3, H-7β and H-9 and δ 77.03 to H-4 and H-7β confirmed that the δ 87.28 could be assigned as C-5 and the δ 77.03 as C-6. And the correlations of δ 68.77 to H-7β and H-9 confirmed that the δ 68.77 could be assigned as C-8.

Table 1 ^{1}H-NMR and ^{13}C-NMR data of teneoside C and harpagoside in C_5D_5N

No.	teneoside C		harpagoside	
	δ_H	δ_C	δ_H	δ_C
1	6.81 (s)	94.91	6.19 (s)	94.71
3	6.53 (d, J = 7.3 Hz)	142.40	6.42 (d, J = 6.4 Hz)	143.90
4	5.17 (dd, J = 6.2, 1.16 Hz)	108.11	4.95 (dd, J = 6.3, 1.5 Hz)	106.91
5	—	87.28		73.45
6	4.12 (d, J = 3.6 Hz)	77.03	3.93	77.72
7	2.15 (dd, J = 14.8, 4.5 Hz)	46.12	2.02 (d, J = 2.3 Hz)	46.24
8		68.77		88.78
9	3.64 (s)	55.55	2.94 (s)	55.65
10	1.69 (s)	22.85	1.55 (s)	22.66
11	3.54 (s)	51.45		
1′	5.03 (d, J = 7.9 Hz)	99.45	4.63 (d, J = 7.9 Hz)	99.54
2′	3.27 (dd, J = 7.9, 9.3 Hz)	74.92		74.90
3′	3.40 (t, J = 9.1 Hz)	78.72		78.79
4′	3.25 (t, J = 9.1 Hz)	71.93		71.81
5′	3.44 (m)	78.57		77.92
6′	3.67 (dd, J = 11.9, 6.7 Hz)	63.05		63.02
	3.94 (dd, J = 11.9, 2.1 Hz)			
1″		135.10		135.82
2″	7.45 (m)	129.28	7.50	130.20
3″	7.30 (m)	128.57	7.40 (m)	129.02
4″	7.30 (m)	130.52	7.40 (m)	131.50
5″	7.30 (m)	128.57	7.40 (m)	129.23
6″	7.45 (m)	129.28	7.50	130.20
C=O	—	166.95		168.75
C=C-a	7.72 (d, J = 16.3 Hz)	144.58	7.70 (d, J = 16.1 Hz)	145.58
C=C-β	6.55 (d, J = 16.3 Hz)	120.39	6.52 (d, J = 16.1 Hz)	120.29

Compounds **1** and **2** (harpagoside) have similar data of ^{1}H-NMR and ^{13}C-NMR (Table 1), IR, UV, and MS (Zhang *et al*, 1994). However, compound **1** has a methoxyl group at δ_H 3.54 (δ_C 51.45). In the HMBC spectrum, the correlations of C-8 to δ_H 3.54 suggested linkage between C-8 and methoxyl group. The ^{1}H-NMR and ^{13}C-NMR spectra of compound **1** (Table 1) displayed C-5 at δ 87.28, C-8 at δ 68.77, H-1 at δ 6.81 (s), and H-9 at δ 3.64 (s), whereas those of compound **2** are C-5 at δ 73.45, C-8 at δ 88.80, H-1 at δ 6.18 (s), and H-9 at δ 2.93 (s) (Zhang *et al*, 1994). In the NOESY spectrum, the correlations of H-1 to H-9, H-10, and H-7α to H-6, H-10 implied the same space distribution of H-1, H-6, H-9, CH_3-10, and H-7α. From these data, the structure of compound **1** was identified as Fig. 1, and trivially named teneoside C.

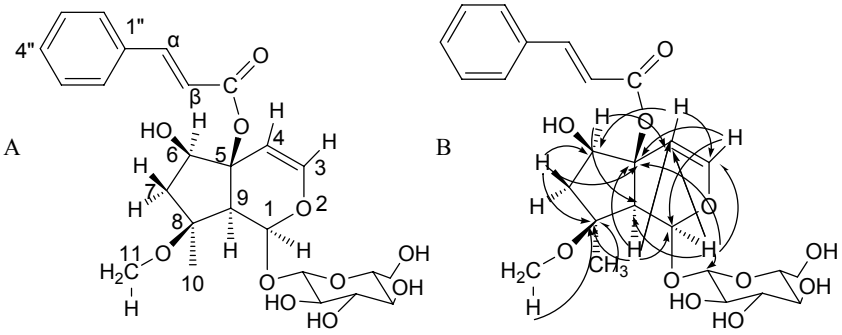

Fig. 1 Structure (A) and the key HMBC (B) of teneoside C

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