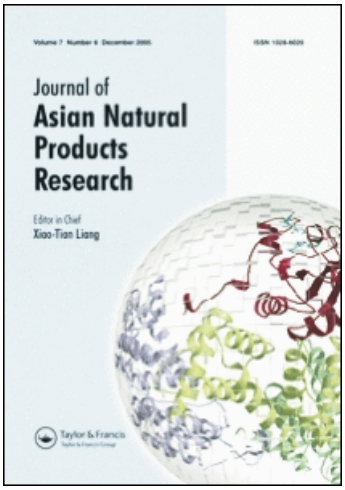

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Two new *ent*-labdane diterpenoid glycosides from the aerial parts of *Andrographis paniculata*

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Two new *ent*-labdane diterpenoid glycosides were isolated from the aerial parts of *Andrographis paniculata*. Their structures were elucidated as 3-*O*-β-D-glucosyl-14-deoxyandrographiside (**1**) and 3-*O*-β-D-glucosyl-14-deoxy-11,12-didehydroandrographiside (**2**) by means of 1D and 2D NMR spectral and chemical methods.

Keywords: *Andrographis paniculata*; *ent*-labdane diterpenoids; 3-*O*-β-D-glucosyl-14-deoxy andrographiside; 3-*O*-β-D-glucosyl-14-deoxy-11,12-didehydroandrographiside

1. Introduction

Andrographis paniculata (Burm.f.) Nees (Acanthaceae), a well-known herbal medicine, is found throughout Southeast Asia and India [1]. It is widely used in the clinic for the treatment of infectious diseases such as fever, cold, laryngitis, diarrhea, and inflammation [2]. Extensive chemical and pharmaceutical investigations on this species have demonstrated that *ent*-labdane diterpenoids are the main bioactive compounds [3–9]. In our previous study, 18 *ent*-labdane diterpenoids have been isolated from the aerial parts of *A. paniculata* [10]. This paper describes the isolation and structural elucidation of two new *ent*-labdane diterpenoids from the alcohol extract of *A. paniculata*.

2. Results and discussion

Compound **1** was obtained as colorless needles (MeOH). The positive HRESIMS analysis (*m/z* 519.2571 $[M + Na]^+$) determined the molecular formula to be $C_{26}H_{40}O_9$ and supported by the NMR spectral data (Table 1). The IR spectrum of **1** showed the presence of hydroxyl groups (3358 cm^{-1}), α,β-unsaturated γ-lactone (1737 and 1645 cm^{-1}), and *exo*-methylidene (895 cm^{-1}) groups. Positive Legal and Kedde color reactions further confirmed the presence of an α,β-unsaturated γ-lactone. The characteristic NMR spectral data indicated that compound **1** was a labdane-type diterpene with an α,β-unsaturated γ-lactone [6]. The ^1H and ^{13}C NMR spectra of **1** showed the signals due to a β-glucopyranosyl group [δ_H 5.01 (1H, d, $J = 7.8$ Hz, H-1′) and δ_C 101.6, 79.2, 79.2, 75.7, 72.4, and 63.6]. The presence of β-glucopyranosyl moiety was further confirmed by enzymatic hydrolysis. Compared with the ^{13}C NMR spectral data of the known compound 14-deoxyandrographolide isolated from this species [6], the chemical shifts of the aglycone moiety of **1** were similar to those literature values except for the signal of C-3 at δ 86.0, which was shifted downfield by 6 ppm. In the HMBC spectrum, the anomeric proton signals at δ 5.01 correlated with the carbon signal at δ 86.0 (C-3; Figure 1),

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Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data for the compounds **1** and **2** in pyridine-d_5.

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1	1.79 (1H, o) 0.99 (1H, m)	37.4	2.41 (1H, o) 2.00 (1H, m)	37.5
2	1.74 (2H, o)	25.5	1.79 (2H, o)	24.4
3	3.82 (1H, dd, 3.9, 11.9 Hz)	86.0	3.86 (1H, dd, 4.0, 11.6 Hz)	86.1
4		43.9		44.0
5	1.13 (1H, d, 12.0 Hz)	56.1	1.16 (1H, d, 11.6 Hz)	55.6
6	2.20 (1H, o) 1.86 (1H, o)	24.2	2.11 (1H, o) 1.80 (1H, o)	24.0
7	2.37 (1H, m) 1.90 (1H, o)	39.1	1.55 (1H, o) 1.02 (1H, m)	38.9
8		148.4		149.7
9	1.59 (1H, o)	56.7	2.33 (1H, m)	62.1
10		39.8		39.3
11	1.66 (1H, o) 1.70 (1H, o)	22.8	7.14 (1H, dd, 10.2, 15.8 Hz)	136.3
12	2.25 (1H, o) 2.55 (1H, brt, 12.0 Hz)	25.4	6.30 (1H, d, 15.8 Hz)	122.5
13		134.5		129.3
14	7.22 (1H, brs)	146.1	7.35 (1H, brs)	145.6
15	4.78 (2H, brs)	71.1	4.80 (2H, brs)	70.8
16		175.1		173.3
17	4.93 (1H, brs) 4.75 (1H, brs)	107.7	4.87 (1H, brs) 4.77 (1H, brs)	109.3
18	1.49 (3H, s)	24.0	1.51 (3H, s)	24.0
19	3.63 (1H, d, 11.0 Hz) 4.28 (1H, o)	64.2	3.64 (1H, d, 11.0 Hz) 4.30 (1H, o)	64.2
20	0.67 (3H, s)	15.5	0.88 (3H, s)	16.1
1′	5.01 (1H, d, 7.8 Hz)	101.6	5.04 (1H, d, 7.5 Hz)	101.6
2′	4.03 (1H, d, 7.8 Hz)	75.7	3.97 (1H, d, 7.5 Hz)	75.7
3′	4.30 (1H, o)	79.2	4.31 (1H, o)	79.2
4′	4.26 (1H, o)	72.4	4.23 (1H, o)	72.4
5′	4.05 (1H, o)	79.2	4.03 (1H, o)	79.1
6′	4.45 (1H, dd, 11.1, 2.5 Hz) 4.65 (1H, dd, 11.1, 5.1 Hz)	63.6	4.43 (1H, dd, 11.2, 2.5 Hz) 4.65 (1H, dd, 11.2, 5.4 Hz)	63.6

"o" Indicated overlapped.

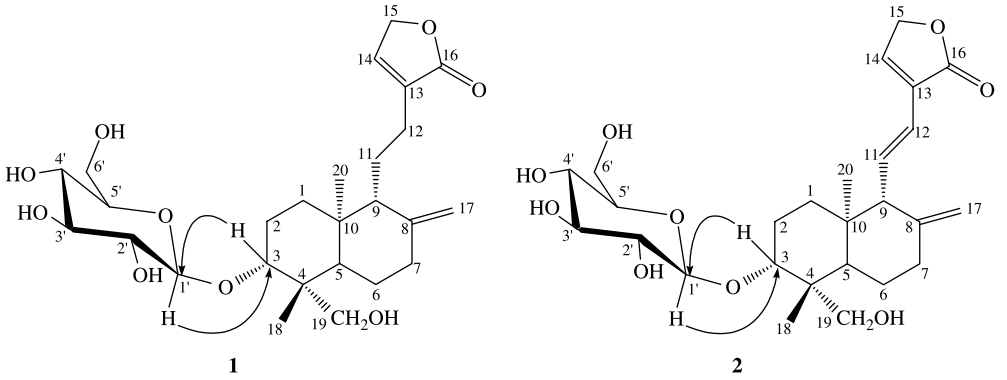

Figure 1. Chemical structures and selected HMBC correlations of compounds **1** and **2**.

indicating that the sugar unit was linked at C-3. Therefore, compound **1** was identified as 3-*O*-β-D-glucosyl-14-deoxyandrographiside. By the analyses of HSQC and HMBC spectra, the ^{1}H and ^{13}C NMR signals of compound **1** were assigned and are completely given in Table 1.

Compound **2** was obtained as a colorless amorphous powder (MeOH). The molecular formula $C_{26}H_{38}O_9$ was inferred from the positive HRESIMS (*m/z* 517.2411 $[M + Na]^+$) and supported by the NMR spectral data. It also showed positive results for the Legal and Kedde reactions, which suggested the presence of an α,β-unsaturated γ-lactone moiety. The IR absorptions revealed hydroxyl groups (3414 cm^{-1}), α,β-unsaturated γ-lactone group (1751 and 1643 cm^{-1}), and *exo*-methylidene groups (893 cm^{-1}). The characteristic NMR spectral data of **2** indicated that it was also a labdane-type diterpene with an α,β-unsaturated γ-lactone [6]. The ^{1}H and ^{13}C NMR spectra of **2** exhibited the presence of a β-glucopyranosyl group [δ_H 5.04 (1H, d, $J = 7.5$ Hz, H-1′) and δ_C 101.6, 79.2, 79.1, 75.7, 72.4, and 63.6], which was further confirmed by enzymatic hydrolysis. In the HMBC spectrum, correlations between H-11 and C-13, H-12 and C-14 and C-16 were observed, indicating that the lactone moiety was attached to the labdane skeleton *via* a C=C bond between C-11 (δ 136.3) and C-12 (δ 122.5). It was obvious that the aglycone moiety of compound **2** was similar to that of the compound 14-deoxy-11,12-didehydroandrographolide [6]. The significant difference between the two was the carbon signal of C-3 in **2** shifting downfield by 6 ppm. Additionally, the HMBC spectrum showed the correlation between H-1′ and C-3, suggesting that the glucose moiety was linked at C-3 (Figure 1). On the basis of the above evidence, compound **2** was established as 3-*O*-β-D-glucosyl-14-deoxy-11,12-didehydroandrographiside. With the aid of HSQC and HMBC spectra, all the ^{1}H and ^{13}C NMR signals of compound **2** were assigned and are given in Table 1.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco MP-S3 micro-melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The IR spectra were recorded with a Bruker IFS 55 spectrometer. The NMR spectrum was recorded on a Bruker ARX-600 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) in pyridine-d_5 with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm and coupling constants (*J*) were reported in Hertz. TLC was carried out on silica gel 60 and the spots were visualized by spraying with the Kedde reagent. Silica gel 60 (Qingdao Marine Chemical Co., Ltd, Qingdao, China), Sephadex LH-20 (Pharmacia Co., Uppsala, Sweden), and ODS (40–75 μm; Fuji Silysia Chemical Co.,

Ltd, Kasugai, Japan) were used for open column chromatography. Preparative HPLC was performed with an ODS column (C-18, 250 mm × 20 mm, 10 μm; GL Science Inc., Torrance, CA, USA) in a Waters 600 liquid chromatograph apparatus equipped with a Waters 490 UV detector with a flow rate of 8.0 ml/min. CH_3CN was an HPLC-grade solvent and water was double distilled in our laboratory. All the analytic reagents were of analytical grade and purchased from Tianjin Damao Chemical Company (Tianjin, China).

3.2 Plant material

The dried aerial parts of *A. paniculata* Nees were collected from Fujian province, China. A voucher specimen (AP-2003-824) was identified by Professor Qi-Shi Sun, and has been deposited at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, China.

3.3 Extraction and isolation

The dried aerial parts of *A. paniculata* (10 kg) were cut into small pieces and heated at reflux with 85% aq. EtOH for three times. The resulting EtOH extract was then concentrated, suspended in H_2O, and partitioned successively with cyclohexane, EtOAc, and *n*-BuOH. The *n*-BuOH extract (135 g) was subjected to column chromatography on silica gel and eluted with $CHCl_3$–CH_3OH (100:1, 50:1, 30:1, 20:1, 10:1, 5:1, 3:1, and 2:1) to give 15 fractions according to the R_f values of TLC. Fraction 8 (7.5 g) was followed by Sephadex LH-20 column chromatography with $CHCl_3$–CH_3OH (1:1) and ODS open column with the gradient CH_3OH–H_2O (10, 30, 50, and 70%) to afford four subfractions. Moreover, subfraction 3 (385 mg) was then separated by preparative HPLC with CH_3CN–H_2O (30:70) as the mobile phase to yield compounds **1** (27.0 mg) and **2** (17.5 mg).

3.3.1 Compound *1*

Colorless needles (MeOH); m.p. 245–250°C; $[\alpha]_D^{25}$ −58.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (nm): 202; IR (KBr) ν_{max} (cm^{-1}): 3358, 1737, 1645, 895; ^1H and ^{13}C NMR (pyridine-d_5) spectral data: see Table 1; ESIMS: *m/z* 519 $[M + Na]^+$, 495 $[M\text{-}H]^-$, HRESIMS: *m/z* 519.2571 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_9Na$, 519.2570).

3.3.2 Compound *2*

Colorless amorphous powder (MeOH); m.p. 152–158°C; $[\alpha]_D^{25}$ −68.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (nm): 202, 250; IR (KBr) ν_{max} (cm^{-1}): 3414, 1751, 1643, 893; ^1H and ^{13}C NMR (pyridine-d_5) spectral data: see Table 1; ESIMS: *m/z* 517$[M + Na]^+$, 493$[M\text{-}H]^-$, HRESIMS: *m/z* 517.2411 $[M + Na]^+$ (calcd for $C_{26}H_{38}O_9Na$, 517.2414).

3.4 Enzymatic hydrolysis of 1 and 2

The solutions of **1** and **2** (each 1 mg) in acetate buffer (pH 4.4, 2 ml) were treated respectively with 5 mg β-glucosidase [emulsion (EC 3.2.1.21; Sigma Co., St Louis, MO, USA)], and the solutions were incubated at 37°C for 48 h. The reaction solutions were extracted with the same volume of EtOAc. The aglycone was analyzed by the ESIMS spectroscopy, and identified by direct comparisons of UV spectra and retention times with the authentic samples in 3D HPLC.

Acknowledgements

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