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Two New Coumarin Glucosides from the Roots of *Angelica apaensis* and Their Anti-Platelet Aggregation Activity

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Two new coumarin glucosides, 11-*O*-β-D-glucopyranosyl thamnosmonin (**1**) and 12-*O*-β-D-glucopyranosyl gosferol (**2**), were isolated from the roots of *Angelica apaensis*. Their structures were elucidated spectroscopically. Both compounds showed weak inhibitory effects on rabbit platelet aggregation induced by PAF, AA and APD.

Key words: Anti-platelet aggregation, 11-*O*-β-D-glucopyranosyl thamnosmonin, 12-*O*-β-D-glucopyranosyl gosferol, Coumarin, *Angelica apaensis*, Umbelliferae

INTRODUCTION

Angelica apaensis Shan et Yuan, which belongs to the family Umbelliferae, is a perennial herb widely distributed in the northeast of Yunnan, China. The roots of this plant have been used extensively as folk medicines to treat tympanites, cough, asthma, wind colic and flatulency (Sun *et al.*, 1981; Sun *et al.*, 1978). Previously, we isolated a number of closely related furanocoumarins from the roots of this plant (Sun *et al.*, 1981; Sun *et al.*, 1978). In the continuing search for bioactive constituents, two new minor coumarin glucosides, 11-*O*-β-D-glucopyranosyl thamnosmonin (**1**) and 12-*O*-β-D-glucopyranosyl gosferol (**2**), were isolated from the acetone extract of this plant. The present paper discusses their structure elucidation and anti-platelet aggregation activities.

MATERIALS AND METHODS

General experimental procedure

1D- and 2D-NMR spectra were recorded on Brucker AM-400 and DRX-500 spectrometers. Unless otherwise specified, chemical shifts (d) were expressed in ppm with reference to the solvent signals. MS was performed on a VG Autospec-3000 spectrometer or on an API Qstar Pulsar I instrument. Uncorrected melting point was obtained on a XRC-1 micro melting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. A Tenor27 spectrophotometer was used for scanning IR spectroscopy of compounds with KBr pellets. HPLC separations were performed on a HP 1100 apparatus equipped with a UV detector and Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. Column chromatography was performed with silica gel (200-300 mesh, Qing-dao Marine Chemical Inc., Qing-dao, Peoples Republic of China), silica gel H (60 μm, Qing-dao Marine Chemical Inc.) and Diaion HP-20. Fractions were monitored by TLC and spots were visualized under UV light (256 nm) or by heating Si gel plates sprayed with 10% H_2SO_4 in EtOH.

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Plant materials

Angelica apaensis roots were purchased from the local market in Kunming, Yunnan, in 2002. The identity of the plant material was verified by Prof. Zhong-Wen Lin at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

Powdered *Angelica apaensis* roots (8.0 kg) were exhaustively extracted with 70% acetone (5×15 L) at room temperature and filtered. The filtrate was concentrated and the resulting residue was partitioned between H_2O and EtOAc to yield 745 g of EtOAc-soluble fraction. The

water layer was directly subjected to column chromatography over Diaion HP-20 and eluted with H_2O, aqueous MeOH (90%) and MeOH, stepwise. The aqueous MeOH eluate (127 g) was subjected to column chromatography over silica gel and eluted with a gradient system of $CHCl_3$/MeOH (6:1, 3:1, 1:1) and Me_2CO to yield four parts. Part 2 (10 g) was further chromatographed over silica gel and eluted with $CHCl_3$/Me_2CO (5:1 to 1:2 and 0:1) to give eight fractions. Fraction 4 afforded **1** (7 mg) and fraction 6 afforded **2** (8 mg) by RP-HPLC with 35% and 37% MeOH in H_2O as the mobile phase, respectively.

11-*O*-β-D-glucopyranosyl thamnosmonin (1)

Light-brown amorphous solid; $[\alpha]_D^{20.6}$ +70.25 (*c* 0.103, MeOH); UV_{max} (MeOH): 206, 292, 326 nm; IR bands (KBr): 3440, 2925, 2855, 2063, 1717, 1695, 1622, 1565, 1500, 1448, 1384, 1278, 1209, 1131, 1077, 1035, 904, 828, 575 cm^{-1}; negative ESIMS: *m/z* 437 $[M-H]^-$; negative-ion HR-ESIMS: *m/z* 438.1450 (calcd. for $C_{21}H_{26}O_{10}$ 438.1447); For ^1H- and ^{13}C-NMR data see Table I.

12-*O*-β-D-glucopyranosyl gosferol (2)

Light-yellow crystals; m.p. 123-125°C; $[\alpha]_D^{20.9}$ -12.82 (*c* 0.195, MeOH); UV_{max} (MeOH): 221, 249.8, 266.8, 307.6 nm; IR bands (KBr): 3432, 2923, 2044, 1723, 1625, 1579, 1547, 1458, 1351, 1285, 1257, 1208, 1157, 1133, 1100, 1077, 1039, 901, 827, 752, 720, 573 cm^{-1}; negative FABMS: *m/z* 447 $[M-H]^-$; negative-ion HR-FABMS: *m/z* 448.1320 (calcd. for $C_{22}H_{24}O_{10}$ 448.1369); For ^1H- and ^{13}C-NMR data see Table I.

Acid hydrolysis of compounds 1 and 2

A solution of **1** (3.5 mg) in 2 M HCl (3 mL) was heated in a water bath at 70°C for 6 h. After cooling, the reaction mixture was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and concentrated to give compound **1a** (1.8 mg) with $[\alpha]_D^{20.3}$ +170.65 (*c* 0.099, MeOH). A solution of **2** (4.1 mg) was treated by the same method to afford **2a** (1.9 mg) with $[\alpha]_D^{21.2}$ - 4.12 (*c* 0.096, EtOH). The water layers of **1** and **2**, together with authentic sugars, were individually spotted on a HPTLC plate and developed with $CHCl_3$-MeOH-H_2O-HOAC (7:3:0.8:1). The HPTLC plate was sprayed with 10% H_2SO_4 for detection. D-glucose was detected with R_f value of 0.49.

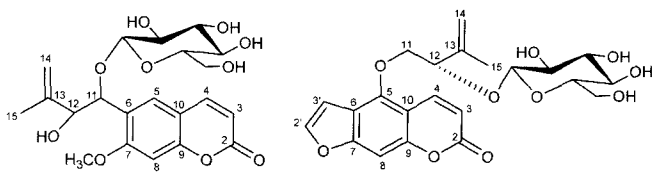
Anti-platelet aggregation assay

The anti-platelet aggregation activity was evaluated *in vitro* as the inhibition of rabbit platelet aggregation induced by PAF (platelet activating factor), AA (arachidonic acid), and ADP (adenosine diphosphate), using previously described bioassay methods (Shen *et al.*, 1999). Ginkgolide B (BN52021) and aspirin were used as positive controls, and 0.3% DMSO was used as a negative control.

RESULTS AND DISCUSSION

Two new coumarin glucosides were isolated by repeated chromatography of the water-soluble fraction of the 70% acetone extract of powdered *Angelica apaensis* roots.

Compound **1**, a light-brown amorphous solid, was assigned a molecular formula of $C_{21}H_{26}O_{10}$ by negative-ion HR-ESI-MS (*m/z* 438.1450, calcd. 438.1447). The ^1H- and ^{13}C-NMR spectra, coupled with UV absorptions at 206.0, 291.6, 326.0 nm and IR bands at 1717 and 1622 cm^{-1}, revealed that **1** has a coumarin skeleton. The presence of two typical aromatic proton singlets at *d* 6.73 (H-8) and 7.94 (H-5) in the ^1H-NMR spectrum of **1** indicated a 6, 7-disubstituted coumarin. A methoxyl group at δ 3.66 with ^1H-^{13}C long-range correlation to the carbon signal at δ 150.2 (C-7) in the HMBC spectrum suggested this methoxy was attached to C-7 of the coumarin nucleus. In addition, the NMR spectra also showed proton signals at δ 5.66 (1H, *d*, *J* = 4.4 Hz, H-11), 5.36 (1H, *d*, *J* = 4.4 Hz, H-12), 4.78 (1H, *s*, H-14), 4.83 (1H, *s*, H-14), and 2.01 (3H, *s*, H-15) and carbon signals at δ 80.9 (C-11), 80.0 (C-12), 145.2 (C-13), 112.8 (C-14) and 18.5 (C-15), indicating the existence of a 3, 4-dioxygenated-2-methyl-butylene chain; HMBC cross-peaks from a proton at δ 5.36 (H-12) to C-6 and from a proton at δ 5.66 (H-11) to C-5 and C-7 (Fig. 2) indicated this chain was attached to C-6. Furthermore, a β-glucose [δ 5.44 (1H, *d*, *J* 7.5 Hz, glc-H-1), other signals overlapped between δ 4.15-4.58, *d* 106.1, 76.1, 78.5, 71.8, 78.5, 62.6] was also obvious from the ^1H- and ^{13}C-NMR spectra. A comparison of the NMR data of **1** with those of thamnosmonin (Chang *et al.*, 1976) revealed that the two compounds were similar except for an additional glucose in **1**. Acid hydrolysis of compound **1** afforded thamnosmonin (**1a**) as its aglycone (Table I), as well as D-glucose, confirming this similarity. The glucose was unambiguously assigned to C-11 (δ 80.9) due to the obvious ^1H-^{13}C long-range correlations from the anomeric proton of glucose to C-11 and from H-11 to the anomeric carbon of glucose (Fig. 2). Compound **1** was deduced to have a *threo*-configuration in the side chain from the small coupling constant between H-11 and H-12 (*J* = 4.1 Hz) in its aglycone (**1a**). Thus, the structure of **1** was established



1 **2**

Fig. 1. The structures of 1 and 2

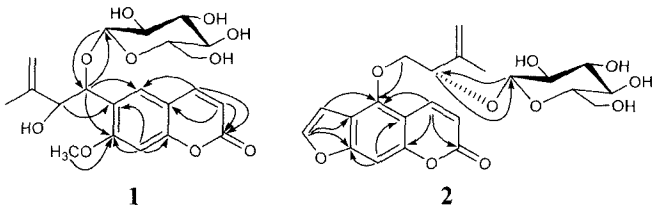


Fig. 2. The key HMBC correlations of **1** and **2**

as 11-*O*-β-D-glucopyranosyl thamnosmonin (Fig. 1).

Compound **2** was obtained as light-yellow crystals with m.p.123-125°C and had a molecular formula of $C_{22}H_{24}O_{10}$ as deduced by high-resolution FABMS spectrum (*m/z* 448.1320 $[M-H]^-$, calcd. 448.1369). Its strong yellowish-green fluorescence under UV light (256 nm) and the ^1H- and ^{13}C signals in the aromatic region of its NMR spectra were typical of a linear furocoumarin (Ahluwalis *et al.*, 1988). Comparison of the ^1H-NMR data of **2** with those of gosferol, a previously described linear furocoumarin (Kutney *et al.*, 1972; Adebajo *et al.*, 2000), revealed that they were similar except for the presence of additional signals due to glucose in **2.** Acid hydrolysis of compound **2** afforded gosferol (**2a**) as its aglycone (Table I) and D-glucose, confirming this result. Furthermore, the obvious HMBC interaction observed between H-11 and C-5 revealed a side chain attached to C-5 (δ149.3) (Fig. 2), which further supported gosferol as the aglycone of **2**. In addition, ^1H-^{13}C long-range correlations from the anomeric proton of glucose to C-12 and from H-12 to the anomeric carbon of glucose indicated that the glucose was located at C-12 (Fig. 2). Full assignments were established by 2D-NMR including ^1H-^1H COSY, HMQC and HMBC experiments. Thus, compound **2** was identified as 12-*O*-β-D-glucopyranosyl gosferol (Fig. 1).

Recently, our group has paid great attentation on anti-platelet aggregation bioactive constituents from natural products and we found that some coumarins showed remarkable bioactivity both *in vitro* and *ex vivo* (Li *et al.*, 2005). Compounds **1** and **2** were accordingly evaluated for their *in vitro* inhibitory activity against rabbit platelet

Table I. The NMR Data of **1** (C_5D_5N), **1a** ($CDCl_3$), **2** (C_5D_5N) and **2a** ($CDCl_3$)

Position.	**1**[a]		**1a**[a]	**2**[a]		**2a**[a]
	δ_H [mult., J(Hz)][b]	δ_C	δ_H [mult., J(Hz)][b]	δ_H [mult., J(Hz)][b]	δ_C	δ_H [mult., J(Hz)][b]
2	/	160.4 (s)	/	/	160.9 (s)	/
3	6.26 (d, 9.4)	113.2 (d)	6.31 (d, 9.1)	6.36 (d, 9.8)	113.6 (d)	6.31 (d, 9.7)
4	7.60 (d, 9.4)	144.3 (d)	7.74 (d, 9.1)	8.53 (d, 9.8)	140.3 (d)	8.26 (d, 9.7)
5	7.94 (s)	128.6 (d)	7.66 (s)	/	149.3 (s)	/
6	/	127.2 (s)	/	/	114.2 (s)	/
7	/	150.2 (s)	/	/	158.5 (s)	/
8	6.73 (s)	99.0 (d)	6.84 (s)	7.25 (s)	94.2 (d)	7.22 (s)
9	/	149.9 (s)	/	/	153.3 (s)	/
10	/	112.6 (s)	/	/	107.5 (s)	/
11	5.66 (d, 4.4)	80.9 (d)	5.20 (d, 4.1)	4.78 (m)	74.5 (t)	4.47 (m, 2H)
12	5.36 (d, 4.4)	80.0 (d)	4.33 (d, 4.1)	4.96 (t, 5.4)	81.6 (d)	4.56 (m)
13	/	145.2 (s)	/	/	143.7 (s)	/
14	4.78, 4.83 (s)	112.8 (t)	5.08 (br s, 2H)	5.39, 5.04 (s)	114.2 (t)	5.10, 5.23 (br s)
15	2.01 (s, 3H)	18.5 (q)	1.97 (s, 3H)	1.90 (s, 3 H)	19.2 (q)	1.84 (s, 3 H)
2'	/	/	/	7.83 (d, 2.4)	145.8 (d)	7.59 (d, 2.4)
3'	/	/	/	7.24 (d, 2.4)	105.7 (d)	7.01 (d, 2.4)
OMe	3.66 (s, 3H)	55.5 (q)	3.88 (s, 3H)	/	/	/
Glu1	5.44 (d, 7.5)	106.1 (d)	/	5.19 (d, 7.8)	104.4 (d)	/
Glu2	4.15 (m)	76.1 (d)	/	4.01 (m)	75.5 (d)	/
Glu3	4.37 (overlap)	78.5 (d)	/	4.35 (m)	78.7 (d)	/
Glu4	4.19 (m)	71.8 (d)	/	3.99 (m)	71.8 (d)	/
Glu5	4.24 (m)	78.5 (d)	/	4.26 (overlap)	78.7 (d)	/
Glu6	4.36 (overlap) 4.58 (m)	62.6 (t)	/	4.52 (m) 4.26 (overlap)	62.9 (t)	/

[a]^1H and ^{13}C NMR spectra were obtained at 500 and 125 MHz respectively.

[b]Coupling constants were presented in Hertz, δ in ppm. Unless otherwise indicated, all proton signals integrated to 1H.

Table II. Percentage inhibition of compounds **1-2** on the aggregation of rabbit platelets induced by PAF, AA, and ADP

Compound (10 mg/L)	Aggregation %		
	PAF (7.2 nmol/L)	AA (0.35 μmol/L)	ADP (3 μmol/L)
DMSO	60.3±2.9	72.6±3.3	69.5±3.2
1	56.3±3.8	66.5±1.0	69.2±3.4
2	60.1±2.8	71.6±5.2	65.3±2.8
BN52021	0.6±0.1[a]		
Aspirin		4.7±0.8[a]	65.9±5.3

[a]$P < 0.05$, as compared with control (*t*-test). The data were expressed as means ± S.D. of 4 rabbits.

aggregation induced by PAF (platelet activating factor), AA (arachidonic acid), and ADP (adenosine diphosphate). Both compounds showed weak inhibitory activities (Table II).

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