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A NEW COUMARIN GLYCOSIDE FROM *Physochlaina physaloides*

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A new coumarin glycoside, namely physochloside A, together with four known compounds, was isolated from the ethanol extract of Physochlaina physaloides. *The structure of physochloside A was identified by UV, IR, ESI-MS, 1D NMR, and 2D NMR.*

Keywords: *Physochlaina physaloides*, Solanaceae, physochloside A.

Physochlaina physaloides (Solanaceae) is found predominantly in the hill, gully, and grassland of Humeng and Ximeng, Inner Mongolia. The roots of *P. physaloides* are utilized to treat many diseases, such as swelling, analgesia, and spasmolysis [1]. Secondary metabolites, including alkaloids [2–4] and coumarins [5], have been reported from *P. physaloides*. Alkaloids and coumarins are important secondary metabolites in plant that exhibit significant pharmacological activities [6–8], including antitumor, anti-inflammatory, analgesic, hypotensive, and anticoagulant, which prompted us to further study this plant.

From the EtOH extract of *P. physaloides*, five compounds were obtained. On the basis of ^{1}H, ^{13}C NMR, COSY, HSQC, and HMBC spectra, as well as by comparison with previous reports, compound (**1**) was identified as a new compound while the remaining four compounds were found to be the known compounds 6-hydroxycoumarin-7-*O*-*β*-D-glucoside (**2**) [9], 6-methoxycoumarin-7-*O*-*β*-D-glucoside (**3**) [9], scopolamine (**4**) [10], and hyoscyamine (**5**) [10].

Compound **1** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{40}H_{58}O_{29}$ by HR-ESI-MS at *m/z* 1001.8622 $[M - H]^-$ (calcd for $C_{40}H_{58}O_{29}$, 1002.8632). In the ^{1}H NMR spectrum (Table 1), there are two characteristic resonances for H-3 and H-4 of coumarin at δ 6.33 (1H, d, J = 9.5 Hz, H-3) and 7.97 (1H, d, J = 9.5 Hz, H-4). The remaining aromatic signals at δ 7.32 (1H, s) and 7.20 (1H, s) were assigned to H-5 and H-8 based on the HMBC correlations from H-5 (δ 7.32) to C-4 (δ 144.7), C-6 (δ 146.4), C-7 (δ 150.2), and C-9 (δ 149.4), and H-8 (δ 7.20) to C-6 (δ 146.4), C-7 (δ 150.2), C-9 (δ 149.4), and C-10 (δ 112.8).

The ^{13}C NMR spectrum showed 40 carbon signals, of which 10 were assigned to the aglycon part and 30 to the sugar moiety. In the aglycon part, a methoxy carbon signal was present in addition to a coumarin skeleton carbon signals at δ 161.0 (C-2), 113.8 (C-3), 144.7 (C-4), 110.1 (C-5), 146.4 (C-6), 150.2 (C-7), 103.4 (C-8), 149.4 (C-9), and 112.8 (C-10). The HMBC correlation (Fig. 1) from CH_3O- (δ 3.83) to C-6 (δ 146.4) indicated that the methoxyl group was attached to C-6.

Acid hydrolysis [11, 12] of compound **1** afforded sugar components identified as D-glucose and D-galactose by GC analysis. The peaks of authentic samples of D-glucose and D-galactose after treatment in the same manner were detected at 19.6 and 20.7 min. The presence of five anomeric proton signals at δ 5.10 (1H, d, J = 7.5 Hz, H-1′), 4.26 (1H, d, J = 7.5 Hz, H-1″), 4.12 (1H, d, J = 7.5 Hz, H-1‴), 4.90 (1H, d, J = 3.5 Hz, H-1⁗), and 5.18 (1H, d, J = 3.5 Hz, H-1⁗′) and of five corresponding carbon signals at δ 99.9, 97.4, 104.5, 92.7, and 92.2 also indicated that compound **1** contained a pentasaccharide of three D-glucoses and two D-galactoses. The *α*-anomeric configuration of the two D-galactoses was revealed by the coupling constant (3.5 Hz). The large coupling constant (7.5 Hz) suggested that the anomeric configuration of the three D-glucoses was *β* [13].

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TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compound **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2	–	161.0	5″	3.40 (m)	77.2
3	6.33 (d, J = 9.5)	113.8	6″	3.68 (dd, J = 8.0, 5.0)	66.1
4	7.97 (d, J = 9.5)	144.7		3.01 (d, J = 8.0)	
5	7.32 (s)	110.1	1‴	4.12 (d, J = 7.5)	104.5
6	–	146.4	2‴	3.02 (m)	73.5
7	–	150.2	3‴	3.07 (m)	77.0
8	7.20 (s)	103.4	4‴	3.13 (m)	70.7
9	–	149.4	5‴	3.16 (m)	77.5
10	–	112.8	6‴	3.64 (d, J = 9.0); 3.42 (m)	61.0
OCH_3	3.83 (s)	56.5	1⁗	4.90 (d, J = 3.5)	92.7
1′	5.10 (d, J = 7.5)	99.9	2⁗	3.27 (m)	69.9
2′	3.53 (m)	83.0	3⁗	3.01 (m)	71.0
3′	3.57 (m)	75.8	4⁗	2.98 (m)	73.8
4′	3.14 (m)	72.4	5⁗	3.18 (m)	72.0
5′	3.39 (m)	77.1	6⁗	3.56(d, J = 8.0); 3.37 (m)	62.5
6′	3.88 (dd, J = 8.5, 5.0)	68.6	1⁗′	5.18 (d, J = 3.5)	92.2
	3.67 (d, J = 8.5)		2⁗′	3.30 (m)	69.6
1″	4.26 (d, J = 7.5)	97.4	3⁗′	3.12 (m)	70.3
2″	3.52 (m)	82.4	4⁗′	2.97 (m)	73.5
3″	3.59 (m)	75.6	5⁗′	3.20(dd, J = 7.0, 6.5)	71.8
4″	3.38 (m)	72.8	6⁗′	3.54 (d, J = 8.0); 3.38 (m)	62.5

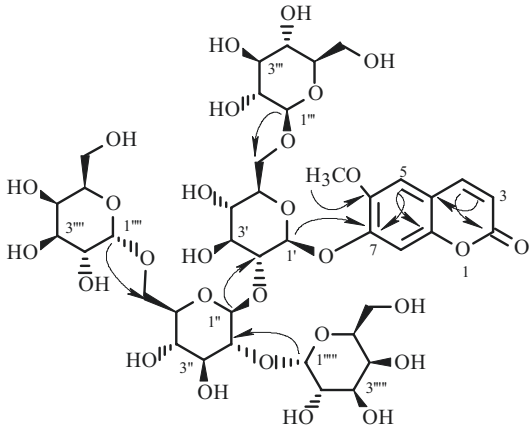

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Fig. 1. Selected HMBC correlations for **1**.

The sequence of the pentasaccharide moiety was determined by the long-range correlations of H-1′ (δ 5.10) of the *β*-glucose unit 1 and C-7 (δ 150.2) of the aglycon, H-1″ (δ 4.26) of the *β*-glucose unit 2 with C-2′ (δ 83.0) of the *β*-glucose unit 1, H-1‴ (δ 4.12) of the *β*-glucose unit 3 with C-6′ (δ 68.6) of the *β*-glucose unit 1, H-1⁗ (δ 4.90) of the D-galactose unit 1 with C-6″ (δ 66.1) of the *β*-glucose unit 2, and H-1⁗′ (δ 5.18) of the D-galactose unit 2 with C-2″ (δ 82.4) of the *β*-glucose unit 2 in the HMBC spectrum. Mutual coupling between H-1′ and H-2′, H-1″ and H-2″, H-1‴ and H-2‴, H-1⁗ and H-2⁗, and H-1⁗′ and H-2⁗′ observed in the ^{1}H–^{1}H COSY spectrum, and the coupling between H-1′ and C-1′ (δ 99.9), H-2′ and C-2′ (δ 83.0), H-1″ and C-1″ (δ 97.4), H-2″ and C-2″ (δ 82.4), H-1‴ and C-1‴ (δ 104.5), H-2‴ and C-2‴ (δ 73.5), H-1⁗ and C-1⁗ (δ 92.7), H-2⁗ and C-2⁗ (δ 69.9), H-1⁗′ and C-1⁗′ (δ 92.2), and H-2⁗′ and C-2⁗′ (δ 69.6) was observed in the HMQC spectrum. Thus, the structure of compound **1** was determined to be 6-methoxycoumarin-7-*O*-[6-*O*-*β*-D-glucopyranosyl-2-*O*-(2-*O*-*α*-D-galactopyranosyl-6-*O*-*α*-D-galactopyranosyl)-*β*-D-glucopyranosyl]-*β*-D-glucopyranoside and named physochloside A.

EXPERIMENTAL

General Experimental Procedures. IR spectra were recorded on a Thermo Nicolet 200 double beam spectrophotometer. HR-ESI-MS spectra were measured on a Waters Xevo G2-S QTOF spectrometer. The LC system consisted of a LC-20AT pump (Shimadzu, Kyoto, Japan), Shimadzu SPD-20A detector, and Shimadzu CBM-20A software for data processing. ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC spectra were recorded in CD_3OD using a 500 Hz Bruker NMR Avance spectrometer.

Plant Material. The roots of *P. physaloides* were collected in Humeng, Inner Mongolia of China, in July 2016. The plant material was identified by Prof. Wuxiangjie (Inner Mongolia University for Nationalities) and a voucher (No. 20160720) has been deposited in the School of Traditional Mongolian Medicine of Inner Mongolia University for Nationalities.

Extraction and Isolation of Five Compounds. The roots of *P. physaloides* (1000 g) were crushed and extracted twice with EtOH under reflux. The filtrates were combined and concentrated to dryness under reduced pressure. The EtOH extract (10.0 g) was divided by column chromatography on silica gel and gradiently eluted with $CHCl_3$–MeOH (50:1 to 5:1) to give five fractions (Frs. C_{1-5}). Fraction C_3 (206 mg, $CHCl_3$–MeOH, 20:1 eluate) was further chromatographed on a Sephadex LH-20 column, eluting with MeOH, and then separated by semipreparative HPLC (CH_3OH–H_2O, 45:55) yielding **5** (28 mg) and **4** (32 mg). Fraction C_4 (420 mg, $CHCl_3$–MeOH, 10:1 eluate) was further separated by semipreparative HPLC (CH_3OH–H_2O, 39:61) to yield **3** (53 mg) and **2** (27 mg). Fraction C_5 (510 mg, $CHCl_3$–MeOH, 10:1 eluate) was further separated by semipreparative HPLC (CH_3OH–H_2O, 29:71) to give **1** (61 mg). The purity of each compound was determined to be above 98% by normalization of the peak areas detected by HPLC.

Acid Hydrolysis of 1 and Sugar Analysis. A solution of compound **1** (2.0 mg) in 2 M HCl (2 mL) was stirred at 80°C in a stoppered vial for 2 h. The solution after cooling was evaporated under a stream of N_2. Anhydrous pyridine solutions (1.0 mL) of the residue and L-cysteine methyl ester hydrochloride (1.5 mg) were mixed and warmed at 60°C for 2 h. After drying the solution, trimethylsilyl imidazole (150 μL) was added to the mixture, which was warmed at 60°C for another 1 h and then partitioned between H_2O (500 μL) and cyclohexane (500 μL). The cyclohexane layer was concentrated and analyzed by GC using an HP-5 column. The temperatures of the injector and detector were 260 and 280°C, respectively. A temperature gradient system was used for the oven, starting at 100°C and increasing up to 140°C at a rate of 4°C/min, and then increasing up to 170°C for 8 min at a rate of 13°C/min, and finally, increasing up to 200°C at a rate of 5°C/min.

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