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Two novel iridoid derivatives isolated from *Phlomis likiangensis*

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ABSTRACT

The extract of the aerial and underground parts of *Phlomis likiangensis* afforded two new iridoid derivatives, namely as phloloside H (**1**) and phloline (**2**), along with four known compounds (**3**–**6**), and compound **2** was a novel normonoterpene. Their structures were elucidated on the basis of spectroscopic studies and chemical methods. Six compounds were assayed for cytotoxic, antibacterial and antioxidative activities, but were either inactive or very weakly active.

Introduction

Phlomis likiangensis C. Y. Wu belongs to the plant family Labiatae. It is a native perennial herb, 0.6–1.5 m tall, growing in wet meadows and woods at an altitude of 3500 m throughout the northwest of Yunnan, China.[1,2]

In our previous contributions,[3,4] we have reported the chemical structures and *in vitro* activities of seventeen iridoid glycosides from the aerial parts of *P. likiangensis*. This paper describes the continued study of the same plant, from which a rare 4′-substituted iridoid glycoside, and a novel normonoterpene, named phloloside H and phloline, respectively, and four known iridoid compounds have been isolated (Fig. 1). The aim of this paper was to introduce the isolation and structural elucidation of the new iridoid compounds and their cytotoxic, antibacterial and antioxidative activities.

Results and discussion

Compound **1** was purified to be a white amorphous powder. The HR-ESIMS showed the sodiated molecular ion peak at *m/z* 543.1687 (calcd 543.1690), corresponding to the molecular formula $C_{22}H_{32}O_{14}$. Its IR spectrum demonstrated absorption bands for hydroxyl (3424 cm^{-1}) and ester carbonyl (1730 cm^{-1}) groups and the UV spectrum revealed the presence of an α,β-unsaturated esters (233 nm). A glucose unit could be proposed from the ^1H, ^{13}C NMR and DEPT spectra (Table 1) on basis of three proton signals at δ_H 4.66 (1H, d, J = 8.0 Hz), 3.66 (1H, dd, J = 12.0, 2.0 Hz) and 3.57 (1H, m), an anomeric carbon (δ_C 100.2), a methylene (δ_C 62.4), and four methines signals between δ_C 74.7 and 76.1 ppm. Besides of the sugar unit, its ^1H NMR spectrum showed one oxygenated methine [δ_H 4.33 (1H, m)], one acetal methine [δ_H 5.90 (1H, d, J = 2.0 Hz)], one tertiary methyl [δ_H 1.50 (3H, s)], one acetyl methyl [δ_H 2.00 (3H, s)], one methoxyl [δ_H 3.70 (3H, s)], one ethoxyl [δ_H 4.17 (2H, q, J = 7.0 Hz), 1.28 (3H, t, J = 7.0 Hz)], and one oxygenated olefin methine [δ_H 7.42 (1H, s)]. The ^{13}C NMR and DEPT spectra showed three ester carbonyl carbons at δ_C 173.2, 168.9 and 156.3, three methine carbons at δ_C 153.6, 95.7 and 75.9, considered to be attached to oxygen functionalities, an oxygenated mythylene carbon at δ_C 65.4, four methyl carbon signals, two methine carbon signals, two quaternary carbon signals, and one mythylene carbon signal. The ^1H and ^{13}C NMR data of **1** displayed signals characteristic of an iridoid glycoside with a carbonate ester substituent, as in the case of phlolosides A–G.[3,4] Its ^{13}C NMR spectrum was very similar to those reported for phloloside B, with the assignments of the ^{13}C NMR spectral data being aided by examination of 2D NMR spectra. In this way, it was found that the difference between compound **1** and phloloside B was the position of the carbonate ester substituent group. The downfield carbon signal at C-4′

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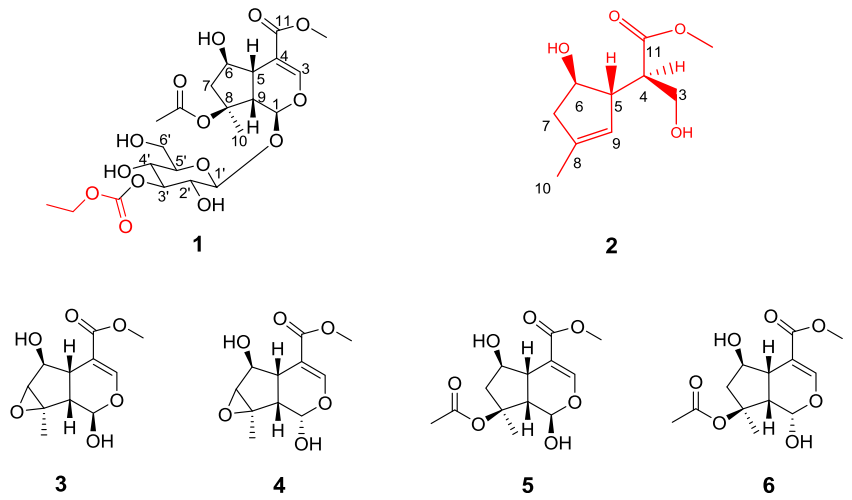

Fig. 1. Structures of **1**–**6**.

Table 1
^{1}H, ^{13}C and HMBC NMR Data of Phloloside H in Methanol-d_4.[α]

Position	δ_H	δ_C	HMBC (^{1}H)
1	5.90, d (2.0)	95.7 d	H-3, H-5, H-9, H-1′
3	7.42, s	153.6 d	H-1, H-5
4		109.8 s	H-3, H-5, H-9
5	3.05, dd (9.0, 1.5)	42.3 d	H-1, H-3, H-7β, H-9
6	4.33, m	75.9 d	H-5, H-7β
7α	2.00, dd (15.0, 6.0)	47.7 t	H-5, H-10
7β	2.18, dd (15.0, 3.0)		
8		89.7 s	H-1, H-5, H-6, H-7β, H-9, H-10
8-OCO–		173.2 s	H-CH$_3$ (Ac)
–CH$_3$	2.0, s	22.2 q	–
9	3.00, dd (9.0, 2.0)	49.9 d	H-5, H-6, H-7β, H-10
10	1.50, s	22.2 q	H-7α, H-9
11		168.9 s	H-3, H-5, H-CH$_3$(MeO)
11-OCH$_3$	3.70, s	51.8 q	–
1′	4.66, d (8.0)	100.2 d	H-1, H-2′, H-3′
2′	3.24, m	74.7 d	H-3′, H-4′
3′	3.55, m	75.6 d	H-1′, H-2′ H-4′
4′	4.54, m	76.1 d	H-3′, H-5′
5′	3.50, m	76.0 d	H-1′, H-3′, H-4′, H-6′a, H-6′b
6′a	3.66, dd (12.0, 2.0)	62.4 t	H-4′
6′b	3.57, m		
1″		156.3 s	H-4′, H-2″
2″	4.17, q (7.0)	65.4 t	H-3″
3″	1.28, t (7.0)	14.5 q	H-2″

[α] ^{1}H NMR data (δ) were measured at 500 MHz and ^{13}C NMR data (δ) were measured at 125 MHz, and the assignments were based on ^{1}H–^{1}H COSY, NOESY (ROESY), HSQC, and HMBC experiments. Chemical shifts and coupling constants (in parentheses) are given in ppm and Hz, respectively.

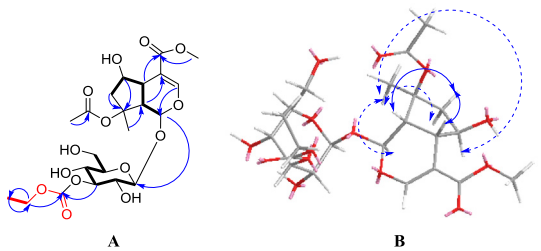

Fig. 2. ^{1}H–^{1}H COSY (A, bold –), selected HMBC (A, →), and ROESY (B, ↔) correlations of **1**.

(δ_C 76.1 from 71.2) and upfield carbon signal at C-6′ (δ_C 62.4 from 67.5) indicated the connection of the ethoxycarbonyl group to the C-4′ hydroxyl instead of the C-6′ hydroxyl of the glucose unit in compound **1**. In the HMBC spectrum (Fig. 2A), the proton signals at δ_H 4.54 (H-4′) and 4.17 (H-2″) were correlated with the carbon signal at δ_C 156.3 (C-1″). From these observations, it was concluded that the carbonate ester substituent group (ethoxycarbonyl group) was assigned at position C-4′.

The relative stereochemistry of compound **1** was resolved by analysis of the ROESY spectrum (Fig. 2B) and the *J*-coupling pattern. In the ROESY spectrum of **1**, the proton signal at δ_H 1.50 (H$_3$-10) correlated with the signals at δ_H 5.90 (H-1), 4.33 (H-6) and 2.00 (H-7α), while the proton signal at δ_H 2.18 (H-7β) correlated with the signals at δ_H 3.05 (H-5), and 3.00 (H-9). Thus, the relative configurations of the H were determined as 1α, 5β, 6α, 9β and 10α. The absolute configurations of sugars were elucidated as D-glucose through acid hydrolysis and HPLC analysis as we reported before.[3,5] The β-anomeric configuration for the glucosyl unit was established from its ^{3}J coupling constant (d, J = 8.0 Hz).[6] Based on the above observations, compound **1** was elucidated as shown and named phloloside H.

Compound **2** was initially isolated as a colorless oil. The molecular formula of $C_{10}H_{16}O_4$ was established by ^{13}C NMR data (Table 2) and HR-ESIMS, the molecular ion M$^+$ at *m*/*z* 200.1047 (calcd for $C_{10}H_{16}O_4$, 200.1049) and accounted for 3 indices of hydrogen deficiency. In the IR spectrum of **2**, absorption bands at 3417 and 1732 cm^{-1} indicated the presence of hydroxyl and ester carbonyl groups. Its ^{1}H NMR data suggested one olefin methine [δ_H 5.25 (1H, m)], one oxygenated methine [δ_H 4.21 (1H, dt, J = 7.0, 3.5 Hz)], one oxygenated methylene [δ_H 3.78 (1H, dd, J = 11.0, 9.0 Hz), 3.66 (1H, dd, J = 11.0, 5.0 Hz)], one methoxyl [δ_H 3.68 (3H, s)], and one tertiary methyl [δ_H 1.70 (3H, m)] attached to a double bond. The ^{13}C and DEPT spectrum made clear one ester carbonyl carbons at δ_C 176.2, one olefin methine carbon at δ_C 124.3, one olefinic quaternary carbon at δ_C 141.0, one oxygenated methine carbon at δ_C 76.4, an oxygenated mythylene carbon at δ_C 62.7, two methyl carbon signals, one mythylene carbon signal, one methine carbon signal, and one quaternary carbon signal. The absence of other *sp* or *sp*^{2} carbon signals indicated that compound **2** contained one ring to satisfy its indices of hydrogen deficiency.

The ^{1}H and ^{13}C NMR assignments were supported by 2D NMR analysis, including HSQC, ^{1}H–^{1}H COSY and HMBC analyses (Fig. 3). The ^{1}H–^{1}H COSY of **2** revealed one separated spin-spin system of H-3/H-4/H-5/H-6/H-7 and H-5/H-9 (Fig. 3A). In the HMBC spectra, the proton signals at δ_H 2.72 (H-5), 4.21 (H-6), 2.10 (H-7α), 2.57 (H-7β), 5.25 (H-9) and 1.70 (H-10) were all correlated with the carbon signal at δ_C 141.0 (C-8), suggesting the connection of C-7, C-9 and C-10 through the carbon of C-8 and the location of