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A NEW PRENYLATED FLAVONE FROM THE LEAVES OF *GLYCYRRHIZA URALENSIS* CULTIVATED IN CHINA

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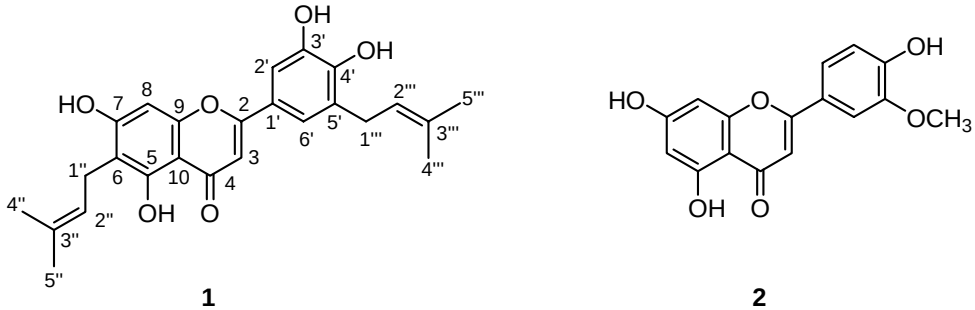
Abstract - A new prenylated flavone, 6,5′-diprenylluteolin (**1**), together with three known compounds chrysoeriol (**2**), kaempferol 3-*O*-glucoside (**3**), quercetin 3-*O*-glucoside (**4**) was isolated from the leaves of *Glycyrrhiza uralensis* cultivated in China. The structure of **1** was established on the basis of spectroscopic evidence.

The roots of *Glycyrrhiza* species are one of the most important crude drugs in China and Europe. Many biological activities were reported, such as antimutagenic activity,[1] antitumor promoting activity,[2] antimicrobial activity,[3] and antioxidant activity.[4] These activities are reported due to two kinds of main constituents, the saponins and flavonoids. The aerial parts of the plant are scarcely used and always considered as waste products. However, studies on the aerial parts belonging to different species of this genus have highlighted the antimicrobial[5] and anti-HIV[6] properties of their extracts.

Recently, in China, the cultivated licorice is serving as the main resource for the exhaustion of the wild licorice. As part of our current interest in the cultivated licorice, we have carried out a phytochemical investigation on the roots of *G. uralensis*, one of the most important species of licorice, which resulted in a new biflavonoid, licobichalcone, along with twelve known flavonoids.[7] In a further study on the leaves of this species, a new prenylated flavone, named 6,5′-diprenylluteolin (**1**), was isolated together with three known flavonoids, chrysoeriol (**2**),[8] kaempferol 3-*O*-glucoside (**3**),[9] and quercetin 3-*O*-glucoside (**4**).[10] All these four flavonoids isolated from the leaves could be classified as 5-hydroxyflavonoids, on the contrary to the fact that the flavonoids from the roots were 5-dehydroxyflavonoids. This paper deals with the structural characterization of the new compound by spectroscopic analysis.

The leaves of *G. uralensis* collected in September 2002 from Yanchi of Ningxia Province, China, were extracted with 95% ethanol. The ethanolic extract was partitioned between ethyl acetate and water. The EtOAc extract was then subjected to normal-phase silica gel column eluted using a stepwise gradient of $CHCl_3$ and MeOH. Further separation by repeated silica gel, Sephadex LH-20, and ODS column chromatography gave four compounds (**1** – **4**).



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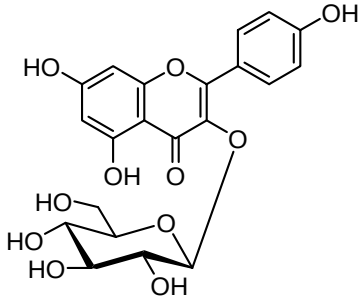

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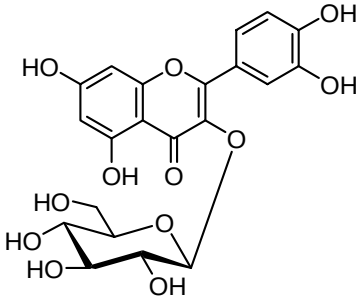

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Compound (**1**) was obtained as a yellow powder. The molecular formula, $C_{25}H_{26}O_6$, was established on the basis of *pseudo* molecular ion peak in HR-FAB-MS at *m/z* 423.1786 $[M+H]^+$. The IR spectrum showed absorptions at 3425 and 1647 cm^{-1} due to hydroxyl and carbonyl groups, respectively. The UV spectrum showed absorptions at 349, 273, 215 nm, suggesting the presence of the flavone moiety in the structure.[11] The ^{1}H-NMR spectrum of **1** (Table 1) showed a sharp singlet at δ 6.53 (1H, s) characteristic of C-3 proton of flavone. The spectrum also exhibited a set of *meta*-coupled aromatic signals at δ 7.38 (1H, d, *J* = 2.1 Hz) and 7.37 (1H, d, *J* = 2.1 Hz), an isolated signal at δ 6.56 (1H, s), as well as two prenyl groups signals at δ 5.28 (1H, m), 3.35 (2H, d, *J* = 7.1 Hz), 1.65 (3H, d, *J* = 0.9 Hz) and 1.78 (3H, br s), and δ 5.40 (1H, m), 3.42 (2H, d, *J* = 7.3 Hz), 1.75 (3H, d, *J* = 1.1 Hz) and 1.76 (3H, br s), which suggested the similar substituents and substitution pattern of **1** as those of papyriflavonol A.[12] In the ^{13}C-NMR spectrum, the methylene carbon signal of one prenyl group was observed at δ 22.0, which indicated that both *ortho*-positions to the prenyl group were occupied by the oxygenated substituents, while another methylene carbon signal of the prenyl group was observed at δ 29.0, which revealed that one of the *ortho*-positions to the prenyl group was replaced by an oxygenated substituent and the other by a hydrogen atom.[13] In addition, the proton signals at δ 3.35 (H-1″) and 6.56 (H-8),[14] as well as the 5-OH

signal at δ 13.31[15] suggested that **1** was a 6,5′-diprenylated flavone instead of 8,5′-diprenylated flavone. In order to confirm the substitution pattern and also for more accurate assignment of ^{1}H-NMR and ^{13}C-NMR spectral data, the HMBC spectrum was measured to give long range correlation as shown in Figure 1. The methylene proton at δ 3.35 (H-1″) was correlated to aromatic carbon δ 112.3 (C-6) through $^{2}J_{CH}$, and the hydroxyl proton at δ 13.31 (5-OH) was also correlated to the same carbon through $^{3}J_{CH}$, which supported that C-6 was substituted by a prenyl group. The location of another prenyl group at C-5′ was assigned by the correlation observed between methylene proton at δ 3.42 (H-1‴) and the aromatic carbon at δ 148.1 (C-4′). On the basis of the above evidence, the structure of **1** was determined as 6,5′-diprenylluteolin.

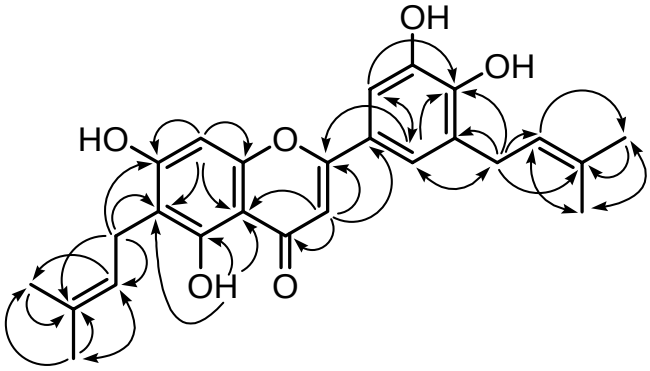

Figure 1 HMBC correlations of **1**.

Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data for **1** in acetone-d_6.

Position	δ_H, mult., *J* in Hz	δ_C	Position	δ_H, mult., *J* in Hz	δ_C
2		165.1	1"	3.35, d, 7.1	22.0
3	6.53, s	104.1	2"	5.28, m	123.2
4		183.1	3"		131.6
5		160.2	4"	1.78, br s	17.9
6		112.3	5"	1.65, d, 0.9	25.9
7		162.4	1‴	3.42, d, 7.3	29.0
8	6.56, s	94.0	2‴	5.40, m	123.1
9		156.6	3‴		133.1
10		105.3	4‴	1.76, br s	17.9
1'		123.0	5‴	1.75, d, 1.1	25.9
2'	7.37, d, 2.1	111.6	5-OH	13.31, s	
3'		145.7			
4'		148.1			
5'		129.9			
6'	7.38, d, 2.1	120.6			

EXPERIMENTAL

General Experiment Procedures. UV spectra were obtained with a SHIMADZU BIOSPEC-MINI spectrophotometer, whereas the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrophotometer. High-resolution FAB-MS was taken on a JEOL JMS-700 Mstation spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL ECP-500 spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm).

Extraction and Isolation. *G. uralensis* was collected in September 2002 from Yanchi of Ningxia Province, China. The air-dried leaves (5 kg) were extracted repeatedly with 95% ethanol (50 L × 3) at rt for 72 h. The extract fractions were combined and evaporated to give the residue (1 kg). This residue (1 kg) was partitioned between EtOAc and water. Removal of the solvent from EtOAc phase yielded the EtOAc soluble extract (289 g). The EtOAc soluble fraction (140 g) was chromatographed on a silica gel column, and eluted with $CHCl_3$, followed by a gradient of MeOH to 50% to give twelve fractions (fractions 1 – 12). Further separation of fraction 3 was achieved by repeated silica gel, Sephadex LH-20, and ODS column chromatography to give **2** (4 mg), of fraction 6 to give **1** (5 mg), of fraction 10 to give **3** (45 mg), and of fraction 11 to give **4** (19 mg).

6,5′-diprenylluteolin (**1**): Yellow powder. UV (MeOH) λ_{max} nm (log ε): 349 (4.27), 273 (4.14), 215 (4.56). IR (KBr) ν_{max}: 3425, 1647, 1552 cm^{-1}. ^{1}H-NMR (500 MHz, acetone-d_6) and ^{13}C-NMR (125 MHz, acetone-d_6): See Table 1. FAB-MS (positive) *m/z* 423 $[M+H]^+$. HR-FAB-MS (positive) *m/z* 423.1786 $[M+H]^+$ (calcd for $C_{25}H_{27}O_6$, 423.1807).

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