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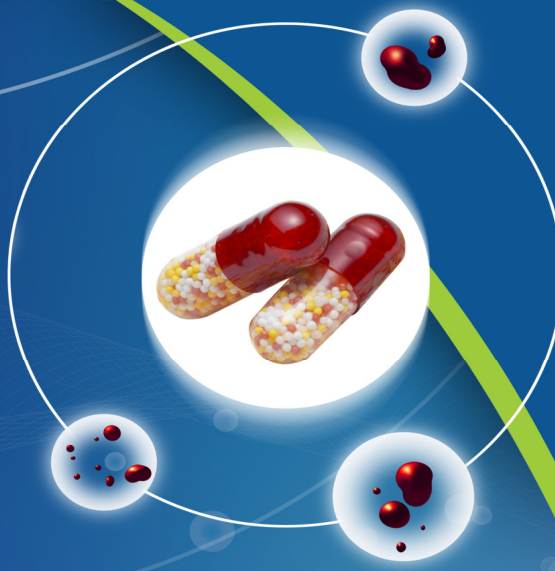

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Research Article

ISOLATION AND STRUCTURE ELUCIDATION OF DAIDZEIN AND GENISTEIN FROM *SIRAITIA GROSVENORII*

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ABSTRACT

Two isoflavones were isolated by the purification of the commercial extract of Luo Han Guo (Siraitia grosvenorii) on a C-18 column using a Biotage Flash Chromatography system. The structure of the isolated compounds were characterized as 4', 7-dihydroxyisoflavone (Daidzein) and 4',5,7,-trihydroxyisoflavone (Genistein) on the basis of extensive NMR and Mass spectral data. The complete ^{1}H and ^{13}C NMR spectral assignments are herewith assigned for Daidzein and Genistein on the basis of 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC) NMR and high resolution mass (HRMS) spectroscopic data.

Keywords: *Siraitia grosvenorii, Curcubitaceae, Luo Han Guo, Isofalvones, NMR, MS*

INTRODUCTION

The fruit of *Siraitia grosvenorii* (Swingle) Lu & Zhang (*Momordica grosvenorii*; Cucurbitaceae) also known as Luo Han Guo grows in Guangxi, People's Republic of China, and is has been used for centuries in traditional Chinese medicine for the treatment of pulmonary demulcent and emollient for the treatment of dry cough, sore throat, and constipation [1-4]. Luo Han Guo is well known now throughout the world due to its intense sweet taste and has been used as a non-caloric natural sweetener in some countries.

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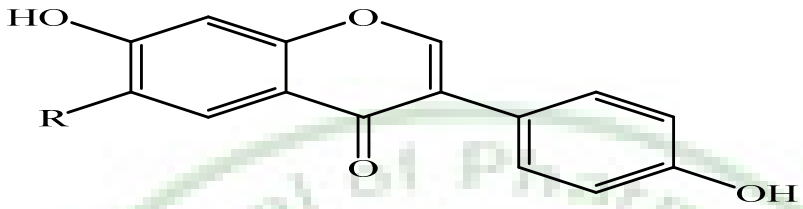
Previous chemical investigation of this species resulted in the isolation of several triterpenoid glycosides also known as mogrosides [1-4]. Some of the compounds among them are sweet such as mogroside V and mogroside IVa and their relative sweetness was about 250-300 times to that of sucrose.

In continuation of our study on the isolation of natural sweeteners from various plant extracts, we have recently reported several steviol glycosides from *Stevia rebaudiana*, [5-12] and several cucurbitane glycosides namely mogrosides III A2, 11-deoxymogroside III mogroside IVa, mogrosides V & VI, isomogroside V, 11-oxomogroside V, siamenoside I from the aqueous alcoholic extract of Luo Han Guo extract based apart from several kaempferol glycosides [13-15]. The structures of

all the isolated compounds were characterized on the extensive NMR and Mass spectroscopic studies. In this paper we are describing the isolation and purification of two isoflavones known as daidzein (**1**) and genistein (**2**) that were characterized on the basis of COSY, HSQC, and HMBC spectral data.



1: R = H

2; R = OH

Figure 1: Structure of Daidzein (1) and Genistein (2)

EXPERIMENTAL WORK

General Methods

NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. IR spectral data was acquired using a Perkin Elmer 400 Fourier Transform Infrared (FT-IR) Spectrometer with Universal attenuated total reflectance (UATR) polarization accessory. HRMS data was generated with a Thermo LTQ Orbitrap Discovery mass spectrometer in the positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramark 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples. Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 50 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the on-board syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 µm). TLC was performed on Baker Si-C_{18}F plates with mobile phase H_2O-MeOH (35:65). Identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80^o C.

Materials

The Luo Han Guo commercial extract was purchased from Chengdu Biopurify Phytochemicals, China. A voucher specimen is deposited at The Coca Cola Company, No. VSPC-3166-99.

Isolation and Purification

The Luo Han Guo extract (50 g) was purified on a C-18 column using a Biotage Flash chromatography system (Solvent system: gradient from 20-80% MeOH-water, 60 mL/min. Detection at UV 210 nm). Fractions 21-38 furnished the earlier describe compounds namely mogroside IVa, mogrosides V & VI, isomogroside V, 11-oxomogroside V and siamenoside I. Fractions 39-48 yielded mogroside III A2, 11-deoxymogroside III, and two glycosides Kaempferol-3-O-α-L-rhamnoside and kaempferol-3,7-O-α-L-dirhamnoside. Fractions 49-52 (1.2 g) were combined and further subjected by flash chromatography purification two times (Solvent system: 80% MeOH-water, 20 mL/min, Detection: UV 210 nm) yielded Daidzein (**1**, 68 mg) and Geneistein (**2**, 86 mg).

*Daidzein (**1**):* amorphous powder, IR ν_{max}: 3355, 2927, 1650, 1610, 843 cm^{-1}; UV (MeOH): 268, 314, and 342 nm; ^{1}H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 1; HRMS *m/z* [M+Na^+] calcd for $C_{15}H_{10}O_4Na$: *m/z* 277.0462; found 277.0477.

*Genistein (**2**):* amorphous powder, IR ν_{max}: 3352, 2935, 1654, 1613, 848 cm^{-1}; UV (MeOH): 261, 313, and 341 nm; ^{1}H NMR (600 MHz, C_5D_5N, δ ppm) and ^{13}C NMR (150 MHz, C_5D_5N, δ ppm) spectroscopic data see Table 1; HRMS *m/z* [M+Na^+] calcd for $C_{15}H_{10}O_5Na$: *m/z* 293.0421; found 293.0426.

RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder and its molecular formula was established as $C_{15}H_{10}O_5$ from its HRMS spectral data that showed [M+$Na]^+$ ion at *m/z* 277.0462; this was supported by the ^{13}C NMR spectral data. The UV spectrum of **1** showed absorption maxima at 268, 314, and 342 nm suggested a flavonoid structure [15-17]. The ^{1}H NMR spectrum of **1** showed the presence of a meta-coupled aromatic proton at δ 6.91 as a doublet having coupling constants 2.1 Hz, an ortho-coupled aromatic proton at δ 7.86 as a doublet having coupling constants 9.4 Hz, an ortho/meta-coupled aromatic proton at δ 6.95 as a doublet of doublets having coupling constants 10.2 and 2.1 Hz, and a singlet at δ 8.32. In addition, the ^{1}H NMR spectrum of **1** also showed the presence of ortho/meta-coupled aromatic protons as doublet of doublets at δ 6.84 and 7.46 with coupling constants 8.4/2.4 and 8.7/2.1 Hz respectively corresponds to the 4 aromatic protons of ring B; characteristic for a disubstituted isoflavone skeleton. The presence of isoflavone skeleton was supported by the ^{13}C NMR values of 1 which showed corresponding aromatic carbons and an unsaturated carbonyl group at δ 176.8. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations (Table 1).

Based on the above spectral and chemical studies it was suggested that compound **1** is having an isoflavonoid skeleton having two phenolic hydroxyl groups. The placement of the phenolic hydroxyl groups were identified at C-5 and C-4' positions on the basis of key COSY and HMBC correlations as shown in Figure 2. Thus, based on the above spectral data, structure of **1** was assigned as 4',7-dihydroxyisoflavone (Daidzein) consistent to the reported literature values [16].

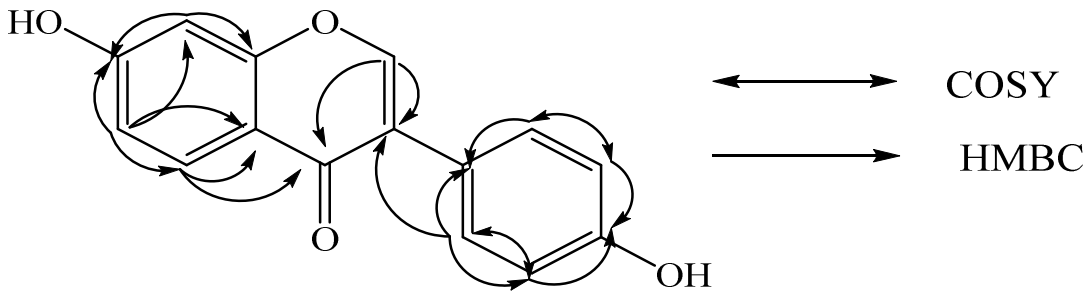


Figure 2: Key COSY and HMBC correlations of **1**

Compound **2** was also obtained as an amorphous powder and its molecular formula was established as $C_{15}H_{10}O_5$ from its HRMS spectral data which showed $[M+Na]^+$ ion at *m/z* 293.0421. The UV spectrum of **2** also showed absorption maxima at 261, 313, and 341 nm suggested an isoflavonoid structure similar to **1** and as reported in the literature [15-17]. The ^1H NMR spectrum of **2** showed the presence of two meta coupled aromatic protons at δ 6.25 and 6.46 corresponds to H-6 and H-8 protons, two doublet of doublets at δ 6.98 and 7.81 for H-3'/H-5' and H-2'/H-6' protons of ring B; characteristic for the 4', 5,7-trisubstituted isoflavone. The ^1H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations and are given in Table 1. A search in the literature suggested the assigned proton and carbon values were consistent with 4',5,7,-trihydroxyisoflavone, also known as genistein [17]. The structure was further supported by the COSY and HMBC correlations as shown in Figure 3.

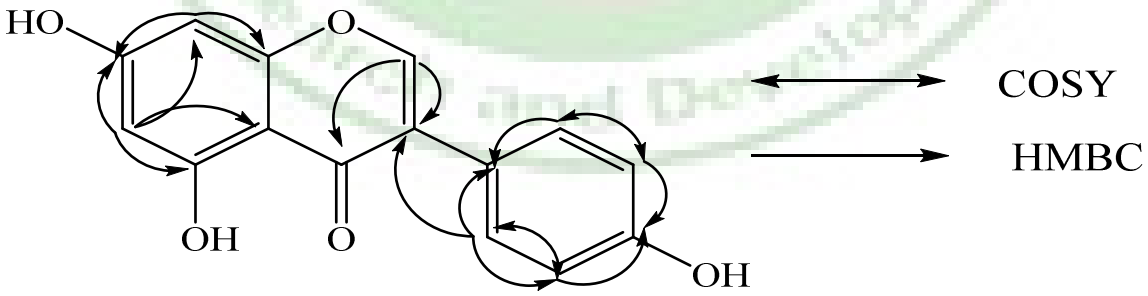


Figure 3: Key COSY and HMBC correlations of **2**

Table 1. ^{1}H and ^{13}C NMR chemical shift values for compounds **1** and **2** in C_5D_5N [a-c]

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2	8.32 s, 1H	153.2	8.42 s, 1H	153.6
3		124.3		123.6
4		176.8		179.8
5	7.86 d, 1H, 9.4	127.3		163.6
6	6.95 dd, 1H, 10.2, 2.1	114.8	6.25 d, 1H, 2.4	98.9
7		162.6		164.6
8	6.91 d, 1H, 2.1	102.6	6.46 d, 1H, 2.4	94.1
9		157.6		157.6
10		117.1		104.5
1'		122.4		121.6
2'	7.46 dd, 1H, 8.7, 2.1	130.6	7.48 dd, 1H, 8.4, 1.8	130.5
3'	6.84 dd, 1H, 8.4, 2.4	115.4	6.86 dd, 1H, 8.7, 2.1	115.4
4'		158.4		158.8
5'	6.84 dd, 1H, 8.4, 2.4	115.4	6.86 dd, 1H, 8.7, 2.1	115.4
6'	7.46 dd, 1H, 8.7, 2.1	130.6	7.48 dd, 1H, 8.4, 1.8	130.5

[a] assignments made on the basis of COSY, HSQC and HMBC correlations; [b] Chemical shift values are in δ (ppm); [c] Coupling constants are in Hz.

CONCLUSION

Two known isoflavones were isolated from the commercial extract o of *Siraitia grosvenorii* obtained from Chengdu Biopurify Phytochemicals Limited, China. The structures of the isolated compounds were identified as 7,4'-dihydroxy-isoflavone (Daidzein) (**1**) and 5,7,4'-trihydroxy-isoflavone (Genistein) (**2**) on the basis of spectroscopic and chemical studies as well as by comparing their physical and spectral properties reported in the literature. The complete ^{1}H and ^{13}C NMR spectral assignments of the two isolated compounds are reported herewith in CD_5N_5 based on 1D and 2D NMR, and HRMS data as well as chemical studies.

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