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STUDIES OF THE EGYPTIAN TRADITIONAL FOLK MEDICINES. IV. [1] NEW ISOFLAVONOID ISOLATED FROM EGYPTIAN LICORICE

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Abstract – A new isoflavonoid, 2′,4′-dihydroxy[6″,6″-dimethylpyrano (2″,3″:7,8)]isoflavone, named isoglabrone, was isolated from the ethyl acetate extract of Egyptian licorice, *Glycyrrhiza glabra*, together with 10 known phenolic compounds. The structure was elucidated on the basis of spectrometric evidence.

INTRODUCTION

The roots of *glycyrrhiza* genus plants (Leguminosae), licorice, have been used for a long time as not only one of the most important crude drugs, but raw material for the flavor, sweetening agent, and the drug glycyrrhizic acid. Chinese licorice is prized for its pure sweetness without bitter taste, however, in these days, the quantity of production has been decreasing for desertification. In Japan, almost all the licorice had depended on import from China, but for this reason, the quantity of importation from China has been growing down. On the contrary, that from Afghanistan, Australia, Turkey, is increasing.

During our study on Egyptian and Turkish traditional folk medicines[1] and from the point of view of keeping licorice resource, we examined the constituents of Egyptian licorice which is commonly used in Egypt. The material was identified as *G. glabra* by HPLC analysis under the same method as described report.[2] In previous study, we reported the structural elucidation of a new 3-arylcoumarin, named licocoumarin A, isolated from this material.[3] After that, in continuous study, we could isolate some other phenolics, including a new compound. Here, we report the isolation and the structural elucidation of a new isoflavone from Egyptian licorice.

RESULTS AND DISCUSSION

Thin crushed powder of licorice were extracted with ethyl acetate, dried *in vacuo* gave ethyl acetate extract. The ethyl acetate extract was subjected to silica gel column chromatography and followed by

normal phase- and reversed phase-HPLC purification to afford a new isoflavone, isoglabrone (**1**), and 10 known phenolic compounds. The known compounds were characterized by comparison with their spectral data with those in the literatures.[3-10]

Compound **1** was isolated as a pale yellow amorphous solid. The molecular fomula was estimated as $C_{20}H_{16}O_5$ by HR-EIMS spectrum (*m/z* 336.0995). A UV absorption maxima (λ max 263 nm) and a singlet signal at δ 8.21 on ^{1}H-NMR indicated that the skeleton should be isoflavone.

The ^{1}H-NMR spectrum of **1** also shows the signals due to dimethyl protons at δ 1.46 (6H, s), a pair of olefinic protons at δ 6.81 (1H, d, *J*=10.2) and 5.94 (1H, d, *J*=10.2), a pair of ortho-coupled aromatic protons at δ 7.86 (1H, d, two *J*=8.8) and 6.92 (1H, d, *J*=8.8), ABX-type protons, δ 6.98 (1H, d, *J*=8.3),

Table 1. ^{1}H-NMR Data of Compounds 1-3

	1	**2**	**3***
2	8.21 (1H, s)	8.26 (1H, s)	8.09 (1H, s)
5	7.86 (1H, d, *J*=8.8)	7.99 (1H, d, *J*=8.6)	
6	6.92 (1H, d, *J*=8.8)	6.98 (1H, dd, *J*=8.6, 2.3)	6.20 (1H, s)
8		6.92 (1H, d, *J*=2.3)	
3'	6.36 (1H, d, *J*=2.3)		6.40 (1H, m)
5'	6.27 (1H, dd, *J*=8.3, 2.3)	6.34 (1H, d, *J*=8.4)	6.37 (1H, dd, J=8.3, 2.0)
6'	6.98 (1H, d, *J*=8.3)	6.93 (1H, d, *J*=8.4)	7.04 (1H, d, *J*=8.3)
4"	6.81 (1H, d, *J*=10.2)	6.68 (1H, d, *J*=9.9)	6.72 (1H, d, J=9.75)
5"	5.94 (1H, d, *J*=10.2)	5.69 (1H, d, *J*=9.9)	5.69 (1H, d, J=9.75)
-CH_3	1.46 (6H, s)	1.38 (6H, s)	1.50 (6H, s)
-OH	9.33 (1H, brs)	9.12 (1H, brs)	
	9.23 (1H, brs)		

Data are expressed as ppm from TMS in DMSO-d_6

* Data are adopted from Maver *et al* (ref. 11), expressed as ppm from TMS in $CDCl_3$

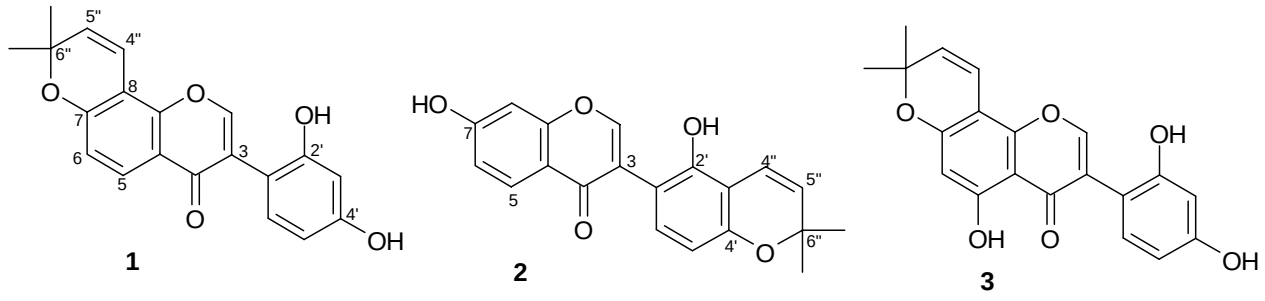

Figure 1. Structures of Compounds **1**~**3**

Table 2. ^{13}C-NMR Data of Compounds 1-3

	1	2	3*
C-2	154.0 d	155.1 d	155.2 d
3	121.6 s	122.0 s	121.6 s
4	174.7 s	176.0 s	181.7 s
5	125.7 d	127.2 d	159.7 s
6	114.5 d	115.3 d	99.6 d
7	156.0 s	162.7 s	161.9 s
8	108.7 s	102.0 d	101.2 s
9	151.3 s	157.5 s	152.3 s
10	117.5 s	116.1 s	105.7 s
1'	109.6 s	112.7 s	109.3 s
2'	156.1 s	153.4 s	156.1 s
3'	102.6 d	110.0 s	103.0 d
4'	158.1 s	153.5 s	159.1 s
5'	106.0 d	107.6 d	106.9 d
6'	131.8 d	130.9 d	132.0 d
4"	114.0 d	116.9 d	114.2 d
5"	131.0 d	128.8 d	127.6 d
6"	77.6 s	75.4 s	78.2 s
-CH_3	27.6 q	27.4 q	27.2 q

Data are expressed as ppm from TMS in DMSO-d_6

* Data are adopted from Maver *et al* (ref. 11), expressed as ppm from TMS in $CDCl_3$

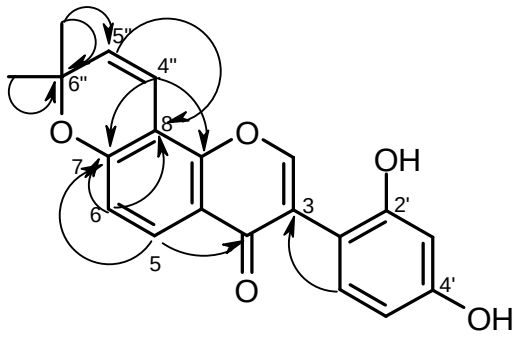

Figure 2. HMBC correlation of compound **1**

6.36 (1H, d, *J*=2.3), 6.27 (1H, dd, *J*=8.3, 2.3), hydroxyl protons at δ 9.33 and 9.23 (each 1H, brs) (Table 1).

The ^{13}C-NMR spectrum and their multiplicities based on the DEPT spectrum showed two methyl, eight methine, and ten quaternary carbons (Table 2). The assignment of ^{1}H- and ^{13}C-NMR signals was made by ^{1}H-^{1}H and ^{1}H-^{13}C COSY, and hetero nuclear multiple-bond correlation (HMBC) experiments.

Observations of the ^{1}H- and ^{13}C-NMR signals [δ_H 1.46 (6H, s), 6.81 (1H, d, *J*=10.2), 5.94 (1H, d, *J*=10.2), δ_C 27.6 (q), 77.6 (s), 114.0 (d), 131.0 (d)] indicated the presence of dimethylpyrane ring in the molecule, and these spectral data are similar to those of glabrone (**2**) isolated from the same material by Kinoshita *et al* [6] or parvisoflavone A (**3**) isolated from *Eriophorum scheuchzeri* by Maver *et al*.[11] (see Tables 1 and 2, Figure 1)

The HMBC spectrum showed the correlation of dimethyl protons (δ 1.46, 6H, s) with C5″ (δ 131.0, d) and C6″ (δ 77.6, s), olefinic protons H5″ (δ 5.94, 1H, d, *J*=10.2) with C8 (δ 108.7, s), H4″ (δ 6.81, 1H, d, *J*=10.2) with C7 (δ 156.0, s) and C9 (δ 151.3, s), aromatic protons H6 (δ 6.92, 1H, d, *J*=8.8) with C7 (δ 156.0, s) and C8 (δ 108.7, s), H5 (δ 7.86 ,1H, d, *J*=8.8) with C7 (δ 156.0, s) and carbonyl carbon (C4, 174.7, s), respectively (Figure 2). These results led to 2′,4′-dihydroxy[6″,6″-dimethylpyrano(2″,3″:7,8)]isoflavone, and was named isoglabrone. (Figure 1)

EXPERIMENTAL

General Procedure

^{1}H-, ^{13}C-NMR and 2D NMR spectra were taken with JNM-EX-270, JNM-BM-400 and JNM-LA-500 spectrometer (JEOL) in DMSO-d_6. Chemical shifts are given in δ-values (ppm) with TMS as an internal standard. MS were recorded using JMS-DX 302 Mass spectrometer (JEOL). The UV spectrum was measured in methanol using Shimadzu UV-1600 spectrophotometer. Preparative HPLC were performed by using Shimadzu HPLC system (Detector SPD-20A, Pump LC-6AD) with ODS column (Nacalai Tesque COSMOSIL 5C18MSII 10 x 250 mm) or silica gel column (Nacalai Tesque COSMOSIL 5SLII 10 x 250 mm).

Materials

The thin crashed powder of Egyptian licorice was purchased from Tachibana Japan (Tokyo). A voucher specimen was deposited at the Dept. of Natural Medicine and Phytochemistry, Meiji Pharmaceutical University, Tokyo, Japan (No.97G0001). The specimen was collected by Prof. Nasr El-Emary in Egypt and the materials were analyzed by HPLC under the same condition as described by Shibano *et al*.[2] The HPLC profiles of specimen, *G. glabra* and the materials used in this study were very close each other, moreover, the characteristic peaks were found to be glabridin and 3,4-dihydroglabridin used as index compounds of *G. glabra*. From these findings, the original plant of the Egyptian licorice was identified as *G. glabra*.

Extraction and Isolation

The powdered licorice (2 kg) was extracted with ethyl acetate (AcOEt 3 L) six times for 12 h at rt. The AcOEt solution was concentrated and then dried *in vacuo* to give the dark brown resin (AcOEt extract 50.2 g). AcOEt extract (50 g) was separated into 12 fractions by silica gel column (600 g) chromatography with gradient mixture of *n*-hexane and AcOEt. The Fr. 8 (5.9 g) was carried out using silica gel column chromatography with gradient mixture of *n*-hexane and AcOEt, followed by HPLC using ODS column with mixture of methanol and water (10:3) to give a new compound (**1**, 5.3 mg), together with 3 known compounds, glabridin (**5**, 7.3 mg),[4] licocoumarin A (**7**, 13.5 mg),[3] [6″,6″-dimethylpyrano(2″,3″:4,5)]-3′-γ,γ-dimethylallyl-2′,3,4′-trihydroxychalcone (**4**, 9.4 mg),[5] respectively. The Fr.7 (2.7 g) was also separated by silica gel column chromatography, followed by normal- and reverse-phase HPLC with mixture of n-hexane and AcOEt (10:3), MeOH and water (5:1), gave 5 known compounds, glabrone (**2**, 10.4 mg),[6] 3′-hydroxy-4′-*O*-methylglabridin (**6**, 21.6 mg),[4] plicadin (**9**, 4.2 mg),[7] medicarpin (**10**, 18.9 mg),[8] glabrocoumarone B (**11**, 3.6 mg),[5] respectively. The Fr. 9 (12.8 g) and Fr.10 (6.8 g) were also separated by silica gel column chromatography, followed by

reversed-phase HPLC with mixture of MeOH and water (5:2), gave kanzonol W (**12**, 72.1 mg)[9] and echinatin (**13**, 15.2 mg),[10] respectively.

Isoglabrone (2′,4′-dihydroxy[6″,6″-dimethylpyrano(2″,3″:7,8)]isoflavone) (1)

A pale yellow amorphous solid, mp 202~205 ºC (decomp), EI-MS *m/z* (rel.Int.%): 336$[M]^+$(65), 322(21), 321(100), 188 (10), 187(84), 161(11) ; HR-EI-MS: *m/z* 336.0995 (calcd for $C_{20}H_{16}O_5$ 336.0998) UVλmax(MeOH)nm(logε) : 263(3.60), 225(3.21), 202(3.64); ^{1}H-NMR and ^{13}C-NMR spectra are shown in Tables 1, 2.

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