STUDIES OF THE EGYPTIAN TRADITIONAL FOLK MEDICINES. III.[1] A NEW DIPRENYLATED 3-ARYLCOUMARIN FROM EGYPTIAN LICORICE

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Abstract - A new diprenylated 3-arylcoumarin, named licocoumarin A, was isolated from Egyptian licorice together with two known compounds (kanzonol W, isoliquiritigenin), and the structure was confirmed to be 3', 8-diprenyl- 7, 2', 4'-trihydroxy-3- arylcoumarin.

Licorice, the roots and long-stalks of various species of *Glycyrrhiza* genus, has been used as traditional folk medicine and foodstuff all over the world.[2] In Kampo prescription, licorice is one of the essential components.[3] In Japan, about 3000 to 6000 tons of licorice are imported every year, 20 % of which are used as clinical and others are used as raw material for the flavor, sweetening agent, and the drug glycyrrhizic acid.[4] Chinese licorice is prized for its pure sweetness without bitter taste, however, the quantity of import from China is declining, on the contrary, the import from other areas (*e.g.* Russian Asia, the Middle East, *etc*) is increasing. There are many reports for the isolation of various phenolic compounds and triterpenes from licorice.[5]

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 paper.[6] In this note, we report the isolation and the structural elucidation of a new compound as well as two reported compounds.

The ethyl acetate extract of Egyptian licorice was subjected to column chromatography on silica gel followed by HPLC purification to afford kanzonol W (**4**), isoliquiritigenin (**5**) and a new compound, licocoumarin A (**1**). The former two compounds were characterized by comparison of their spectral data with those in the literatures.[7,8]

Licocoumarin A (**1**) was isolated as yellow amorphous solid and negative to ferric chloride test. The molecular formula $C_{25}H_{26}O_5$ was established by the HR-MS (*m/z* 406.1785, M^+).

The ^{1}H-NMR spectrum of **1** exhibited the signals due to four methyls, two methylenes, two vinyl protons, two pairs of *ortho*-coupled aromatic protons, a double bond -conjugated proton, and three hydroxyl protons (Table I). The ^{13}C-NMR spectrum and their multiplicities based on the distortionless enhancement by polarization transfer (DEPT) spectrum showed four methyl, two methylene, seven methine, and twelve quaternary carbons (Table II). 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. These NMR data and the UV absorption maxima (λ_{max} 252 , 352 nm) are resemble to those of 3-arylcoumarin derivatives, espcially to gancaonin W (**2**) isolated from Xibei licorice (*G. aspera*)[9] (Tables I and II). Observations of the ^{1}H- and ^{13}C-NMR signals [δ_H 1.64 (3H, d, *J*=1.22), 1.67 (3H, d, *J*=0.92), 1.78 (3H, d, *J*=0.92), 1.86 (3H, d, *J*=0.92), δ_C 17.95 (q), 18.06 (q), 25.90 (q), 25.92 (q); δ_H 3.44 (2H, d, *J*=7.02), 3.57 (2H, d, *J*=7.32), δ_C 22.59 (t), 23.41 (t); δ_H 5.32 (1H, m) x 2, δ_C 122.34 (d), 124.26 (d); δ_C 130.88 (s), 132.70 (s)] (see Tables I and II) indicated the presence of two 3-methyl-2-butenyl (prenyl) groups in the molecule, and these spectral data are similar to those of kanzonol X (**3**) isolated from *G. glabra* by Fukai *et al.*[7] (Tables I, II). The chemical shifts of carbonyl carbon (δ 163.75, s) and 2- dimensional NMR experiments (^{1}H-^{1}H, ^{1}H-^{13}C COSY) indicated that **1** is diprenyl- trihydroxy- arylcoumarin.

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

H	1	2[a]	3[a]
2			3.95 (1H, t, *J*=10) 4.28 (1H, ddd, *J*=2, 3, 10)
3			3.45 (1H, m)
4	7.89 (1H, s)	8.00 (1H, d, *J*=0.6)	2.78 (1H, ddd, *J*=2, 5, 15.5) 2.88 (1H, ddd, *J*=1, 10, 15.5)
5	7.45 (1H, d, *J*=8.55)		6.69 (1H, br d, *J*=8)
6	6.97 (1H, d, *J*=8.54)	6.48 (1H, d, *J*=2)	6.38 (1H, d, *J*=8)
8		6.46 (1H, dd, *J*=0.6, 2)	
9	3.57 (2H, d, *J*=7.32)		3.30 (2H, br d, *J*=7)
10	5.32 (1H, m)[b]		5.24 (1H, m)[b]
12	1.67 (3H, d, *J*=0.92)		1.65 (3H, br s)
13	1.86 (3H, d, *J*=0.92)		1.76 (3H, br s)
5'	6.52 (1H, d, *J*=8.55)	6.52 (1H, d, *J*=8)	6.43 (1H, d, *J*=8)
6'	6.99 (1H, d, *J*=8.54)	6.97 (1H, d, *J*=8)	6.81 (1H, d, *J*=8)
7'	3.44 (2H, d, *J*=7.02)	3.43 (2H, br d, *J*=7)	3.43 (2H, br d, *J*=7)
8'	5.32 (1H, m)[b]	5.32 (1H, br t, *J*=7)	5.24 (1H, m)[b]
10'	1.64 (3H, d, *J*=1.22)	1.64 (3H, br s)	1.62 (3H, br s)
11'	1.78 (3H, d, *J*=0.92)	1.77 (3H, br s)	1.73 (3H, br s)
-OH	8.03 (1H, br s)	7.12 (1H, s)	7.95 (2H, br s)
	8.39 (1H, br s)	7.90 (1H, s)	8.39 (1H, br s)
	9.42 (1H, br s)	8.08 (1H, s)	

Data are expressed as ppm from TMS in acetone-*d6*

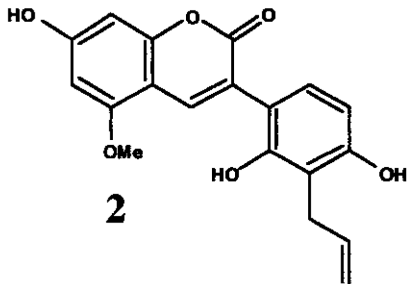
a; Data from T. Fukai *et al.* (ref. 7, 9)

b; $H_{8'}$ and H_{10} signals were overlapped, that resulted in multiplet-like.

Table II. ^{13}C-NMR Data of Compounds (1-3)

C	1	2*	3*
2	163.75 s	163.61	71.0
3	123.39 s	121.93	32.4
4	144.47 d	138.58	32.3
4a	114.06 s	104.77	114.4
5	127.64 d	158.58	127.7
6	113.83 d	96.37	108.3
7	159.28 s	163.04	154.1
8	115.96 s	95.54	116.0
8a	153.65 s	156.75	154.7
9	22.59 t		23.0
10	122.34 d		124.3
11	132.70 s		130.6
12	18.06 q		17.9
13	25.90 q		25.9
1'	116.32 s	117.51	120.8
2'	154.89 s	154.86	153.7
3'	117.54 s	116.53	116.3
4'	157.58 s	157.53	155.3
5'	108.59 d	108.60	108.3
6'	129.19 d	129.15	125.2
7'	23.41 t	23.43	23.3
8'	124.26 d	124.28	123.9
9'	130.88 s	130.88	131.8
10'	17.95 q	17.95	17.9
11'	25.92 q	25.92	25.9
OMe		56.67	

Data are expressed as ppm from TMS in acetone-*d₆*
*; Data from T. Fukai *et al.* (ref. 7, 9)



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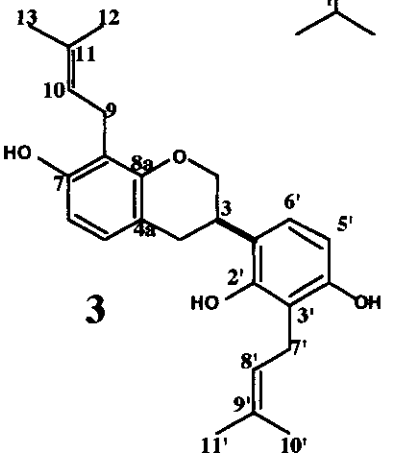

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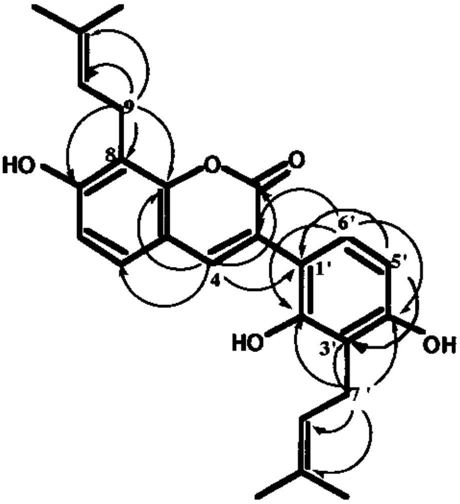

Figure 1. H-C Long Range Correlation of Compound (1) in HMBC

The HMBC spectrum showed the correlation of heterocyclic proton (δ 7.89, 1H, s) with carbonyl carbon (δ 163.75, s) and C_5(δ 127.64, d), C_{8a} (δ 153.65, s), $C_{1'}$ (δ 116.32, s), moreover the methylene protons (δ 3.57; 2H, d, *J*=7.32; H_9, δ 3.44; 2H, d, *J*=7.02; $H_{7'}$) with the C_7 (δ 159.28, s) and C_{8a} (δ 153.65, s), $C_{2'}$ (δ 154.89, s) and $C_{4'}$ (δ 157.58, s), respectively, led to 8, 3'-diprenyl-3-arylcoumarin (Figure 1). Chemical shifts of H_{10} and $H_{8'}$ were overlapped and resulted in multiplet-like signal (δ 5.32, m), however, C_{10} (δ 122.34, t) and $C_{8'}$ (δ 124.26, t) were assigned with the long-range correlation of H_9 (δ 3.57, 2H, d, J=7.32) and $H_{7'}$ (δ 3.44, 2H, d, J=7.02) . The HMBC spectra also showed the long range correlation of H_4 (δ 7.89, 1H, s) with aromatic carbon (δ 116.32, s, $C_{1'}$), $H_{7'}$ with aromatic carbon (δ 117.54, s, $C_{3'}$), $H_{5'}$ with two aromatic carbons (δ 116.32, s, $C_{1'}$; 117.54, s, $C_{3'}$), respectively (Figure 1). Thus chemical sifts of $C_{1'}$ and $C_{3'}$ were assigned as shown in Table II. From these spectral data, **1** was identified as a new compound, 8, 3' -diprenyl- 7, 2', 4'-trihydroxy - 3 -arylcoumarin, and was named licocoumarin A. (Figure 2)

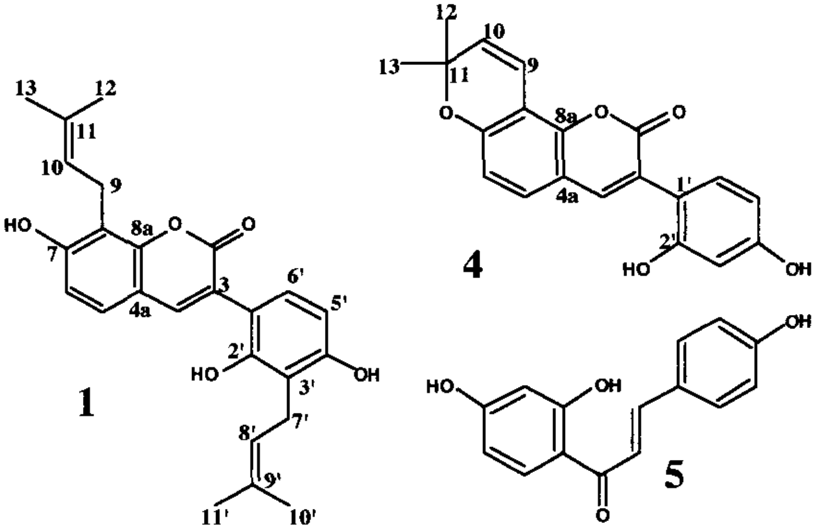

Figure 2. Structures of Compounds Isolated from Egyptian Licorice

EXPERIMENTAL

General Procedure

^{1}H-, ^{13}C-NMR and 2D NMR spectra were taken with JOEL-EX 270 and JOEL-JNM-LA 500 spectrometer in acetone-d_6. Chemical shifts are given in δ-values (ppm) with TMS as internal standard. MS were recorded using JMS-DX 302 Mass spectrometer. The UV spectrum were measured in methanol using Shimadzu UV-1600 spectrophotometer.

Materials

The thin crashed powder of Egyptian licorice was purchased from Tachibana Japan (Tokyo). 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.*[6] The HPLC profiles of specimen, *G. glabra* and experimental material 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 plants of the Egyptian licorice were identified as *G. glabra*.

Extraction and Isolation

The powdered licorice (4 kg) was extracted with ethyl acetate (AcOEt 10 L) three times for 12 h at rt. The AcOEt solution was concentrated and then dried *in vacuo* to give the dark brown resin (AcOEt extract 168.2 g). 80 g of AcOEt extract was separated into 10 fractions by silica gel column (800 g) chromatography with gradient mixture of *n*-hexane and AcOEt. The Fr. 5 (45.5 g) was carried out silica gel chromatography with gradient mixture of *n*-hexane and AcOEt, followed by HPLC with gradient mixture of acetonitrile and water to give two 3-arylcoumarins, **1** (10.3 mg), **4** (yellow amorphous solid, 22.9 mg),[7] and chalcone, **5** (yellow powder, 12.8 mg),[8] respectively.

Licocoumarin A (8,3'-diprenyl-7,2',4'-trihydroxy-3-arylcoumarin) (1)

A yellow amorphous solid, EI-MS *m/z* (rel. Int.): 407 $[M+1]^+$ (28), 406 $[M]^+$ (100), 389 (10), 363 (11), 351 (55), 350 (70), 307 (13), 295 (27), 294 (34), 267 (12), 266 (17), 44 (10). HR-MS *m/z* : 406.1785 $[M]^+$ ($C_{25}H_{26}O_5$ calcd 406.1789). UV λ_{max} (MeOH) nm: 252 (log ε 3.81), 352 (4.24) ^{1}H-NMR and ^{13}C-NMR spectra are shown in Tables I, II.

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