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The first prenylated biaurone, licoagrone from hairy root cultures of *Glycyrrhiza glabra*

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Abstract

A new prenylated biaurone, licoagrone, was isolated from the hairy root cultures of *Glycyrrhiza glabra* together with five known flavonoids, kanzonol D, afrormosin, odoratin, phaseol and echinatin. Their structures were elucidated on the basis of spectroscopic evidence. Licoagrone is the first example of a prenylated biaurone.

Keywords: *Glycyrrhiza glabra*; Leguminosae; Licorice; Hairy root; Flavonoids; Prenylated biaurone; Licoagrone

1. Introduction

Previously (Asada, Li, & Yoshikawa, 1998), we reported the isolation and structural determination of two new flavonoids, licoagrochalcone and licoagrocarpin, along with eight known flavonoids from *Glycyrrhiza glabra* hairy root cultures. In a continuing study of the hairy root cultures, a new prenylated biaurone, named licoagrone (**1**), was isolated together with five known flavonoids. This paper deals with the structural characterization of these flavonoids by spectroscopic evidence.

2. Results and discussion

The ethyl acetate extract was chromatographed on a silica gel column and purified by normal-phase HPLC to give a new flavonoid, licoagrone (**1**) and five known flavonoids, kanzonol D (**2**) (Fukai, Nishizawa, & Nomura, 1994), afrormosin (**3**) (Caballero, Smith, Fronczek, & Fischer, 1986), odoratin (**4**) (Hayashi & Thomson, 1974), phaseol (**5**) (Abe, Sato, & Sakamura, 1987) and echinatin (**6**) (Furuya, Matsumoto, & Hikichi, 1971).

Licoagrone (**1**) was obtained as an orange powder. The HR-FAB mass spectrum displayed $[M^+]$ at m/z 742.2773, which corresponded with the empirical formula $C_{45}H_{42}O_{10}$ (742.2778), indicating 25 degrees of unsaturation in the molecule. The IR spectrum showed absorption bands at 3430 and 1660 cm^{-1} due to hydroxyl and carbonyl groups, respectively. The ^1H NMR spectrum of **1** showed signals indicating three γ,γ-dimethylallyl groups, *ortho*-coupled aromatic protons at δ 6.79(d, $J=8.5$ Hz), 7.42(d, $J=8.5$ Hz), ABX-type aromatic protons at δ 6.67(d, $J=8.5$ Hz), 7.57 (dd, $J=8.5$, 2.5 Hz), 7.64(d, $J=2.5$ Hz), four isolated aromatic protons at δ 6.72, 7.17, 7.41, 7.84 and an olefinic proton at δ 7.01. The ^{13}C NMR spectrum showed 45 carbon signals; three carbonyl carbons (δ 182.7, 191.1 and 193.5), 24 aromatic carbons, seven of which have an oxygen function, 15 carbons due to three γ,γ-dimethylallyl groups and the residual 3 carbons (Table 1). The methylene carbon signal of one prenyl group was observed at δ 22.4, which indicated that both *ortho*-positions to the prenyl group were occupied by oxygenated substituents (Fukai & Nomura, 1989). The HMBC spectrum showed a correlation between the *ortho*-coupled aromatic proton at δ 7.42 (H-4) and the carbonyl carbon at δ 182.7 (C-3), which indicated that the proton was located at a *peri*-position to the carbonyl group. The same carbon was further correlated to an olefinic proton at δ 7.01 (H-10) which was correlated to two carbons at δ 120.2 (C-6′) and 130.0 (C-2′) on another aromatic ring. Moreover, the appearance of cross-peaks between the isolated aromatic protons at δ 7.84 (H-6′) and 7.17 (H-3′), which bound to the aromatic carbons at δ 120.2 through $^1J_{CH}$ and 130.0 through $^2J_{CH}$, respectively and two carbons (δ 146.3 and 147.1) carrying hydroxyl groups, suggested that two hydroxyl groups were 4′,5′-disubstituted. And then, the correlation peaks between two aromatic protons at δ 7.17

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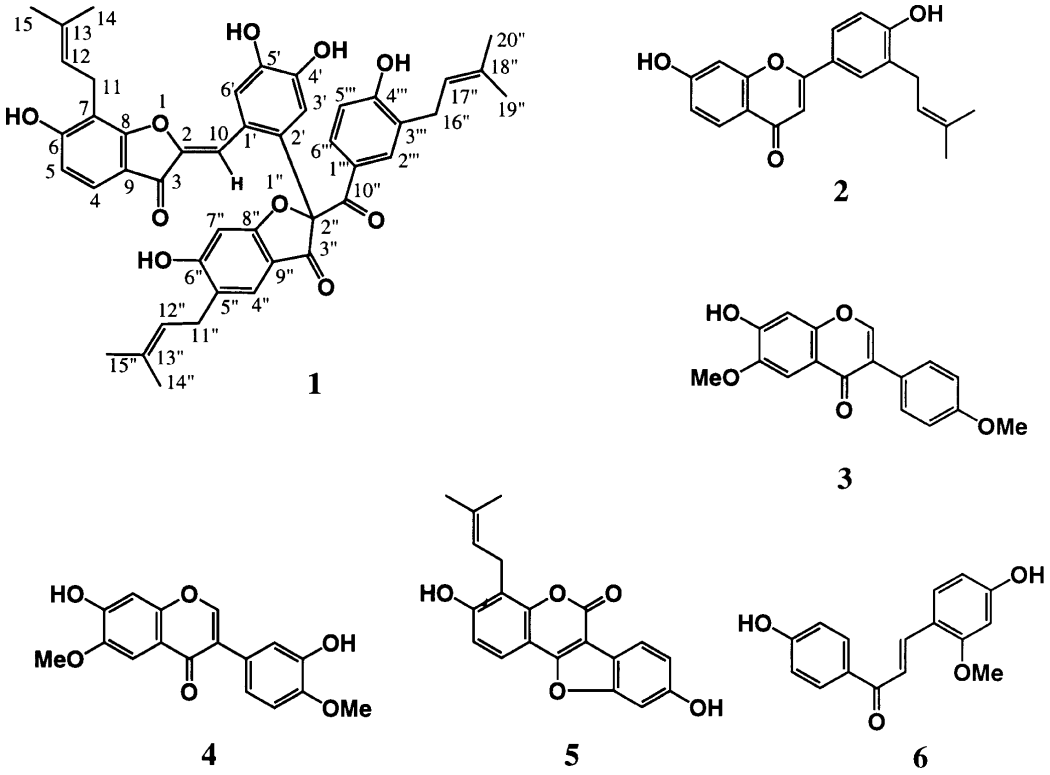

Structure 1.

Table 1
^{13}C NMR data for Licoagrone (**1**)

C-2	147.8	C-2″	97.7
C-3	182.7	C-3″	193.5
C-4	123.4	C-4″	125.4
C-5	112.8	C-5″	126.5
C-6	166.8	C-6″	166.3
C-7	113.1	C-7″	98.7
C-8	163.7	C-8″	173.1
C-9	114.8	C-9″	112.7
C-10	109.1	C-10″	191.1
C-1′	125.2	C-1‴	127.4
C-2′	130.0	C-2‴	133.2
C-3′	115.3	C-3‴	128.2
C-4′	147.1	C-4‴	160.2
C-5′	146.3	C-5‴	114.8
C-6′	120.2	C-6‴	131.0
C-11	22.4	C-11″	28.5
C-12	121.9	C-12″	122.6
C-13	133.0	C-13″	133.7
C-14	18.0	C-14″	17.8
C-15	25.9[a]	C-15″	25.9[a]
		C-16″	28.5
		C-17″	122.4
		C-18″	133.4
		C-19″	17.7
		C-20″	25.8[a]

[a]Assignments may be interchangeable.

(H-3′) and 7.84 (H-6′) and a quaternary carbon at δ 97.7 (C-2″) were observed. The location of the prenyl group was assigned to C-7 by a correlation observed between the methylene carbon at δ 22.4 (C-11) and the *ortho*-coupled aromatic proton at δ 6.79 (H-5). These correlations suggested the partial structure A in **1** (Fig. 1). A partial structure B was estimated by the cross-peaks showing that both aromatic protons at δ 7.41 (H-4″) and 6.72 (H-7″) correlated to three quaternary carbons at δ 166.3 (C-6″), 173.1 (C-8″) and 193.5 (C-3″) and the methylene carbon at δ 28.5 (C-11″) on the prenyl group. Moreover, correlations were observed between the proton at δ 7.64 (H-2‴) on a 1,3,4-trisubstituted aromatic ring and the carbonyl carbon at δ 191.1 (C-10″) and the methylene carbon at δ 28.5 (C-16″), suggested the partial structure C.

Taking account of the molecular formula and spectral data, it was suggested that **1** is a biflavonoid and partial structures B and C which form one flavonoid unit are bound with the asterisk carbon in the partial structure A and the asterisk carbon in Fig. 1 is the same carbon. Thus, the plane structure of licoagrone has been constructed as **1**. This presumption was supported by the EI mass spectrum. The EI mass spectrum showed the significant fragment ions at *m*/*z* 323 and 348, indicating that two

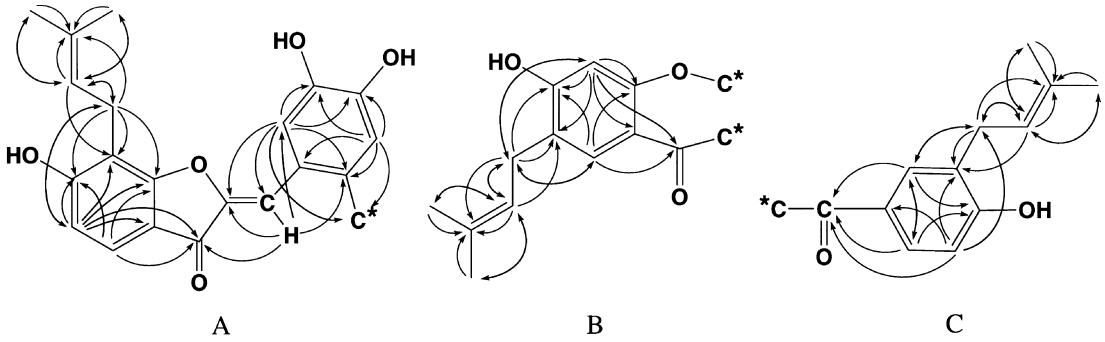

Fig. 1. Partial structures obtained from analysis of the HMBC spectrum of **1**.

aurone moieties are linked between C-2′ and C-2″ positions as shown in Fig. 2.

The stereochemistry of the double bond at C-10 was determined to be the Z-isomer owing to the chemical shift of C-10 (δ 109.1) (Pelter, Ward, & Heller, 1979). Licoagrone (**1**) was found to be racemic despite the asymmetric center at C-2″, since the optical rotation was zero and the CD spectrum exhibited no Cotton effect.

Thus, the structure of licoagrone was established as **1**.

In conclusion, we have isolated a new prenylated biaurone, licoagrone (**1**), along with five known flavonoids from the hairy root cultures of *G. glabra*. Biaurones rarely occur in the plant kingdom (Hahn et al., 1994) and the first prenylated aurone was isolated from *Antiaris toxicaria* (Hano, Mitsui, & Nomura, 1990). To our knowledge, licoagrone (**1**) having a unique dimerized structure is the first report of a prenylated biaurone. A possible route for the biosynthesis of **1** is shown in Fig. 3. This involves (i) initial phenol-oxidative coupling of a prenylated hispidol derivative with a prenylated suphuretin derivative, (ii) formation of a 2′-2″ bond and (iii) the oxidation of the hydroxyl group. It is considered to be a true natural product, since none of the putative monomeric precursors, hispidol or sulphuretin, were detected in any of the hairy root extracts.

3. Experimental

3.1. General

The OR were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm length cell. The IR spectra were recorded on a JASCO FT/IR-200. The EI-MS and HR-

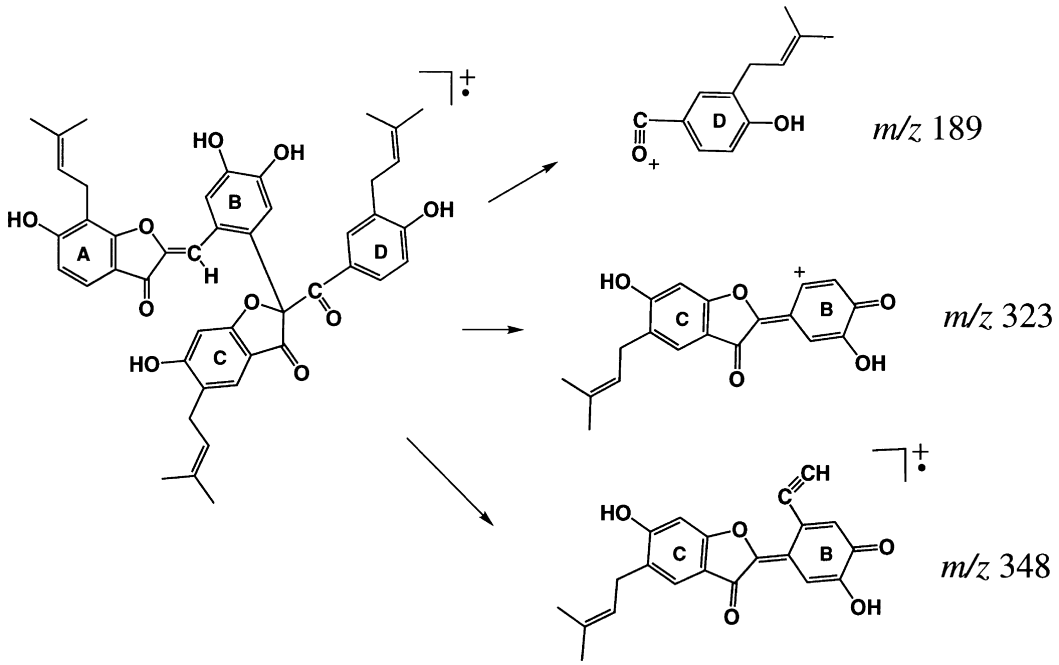

Fig. 2. Proposed EIMS fragmentation of **1**.

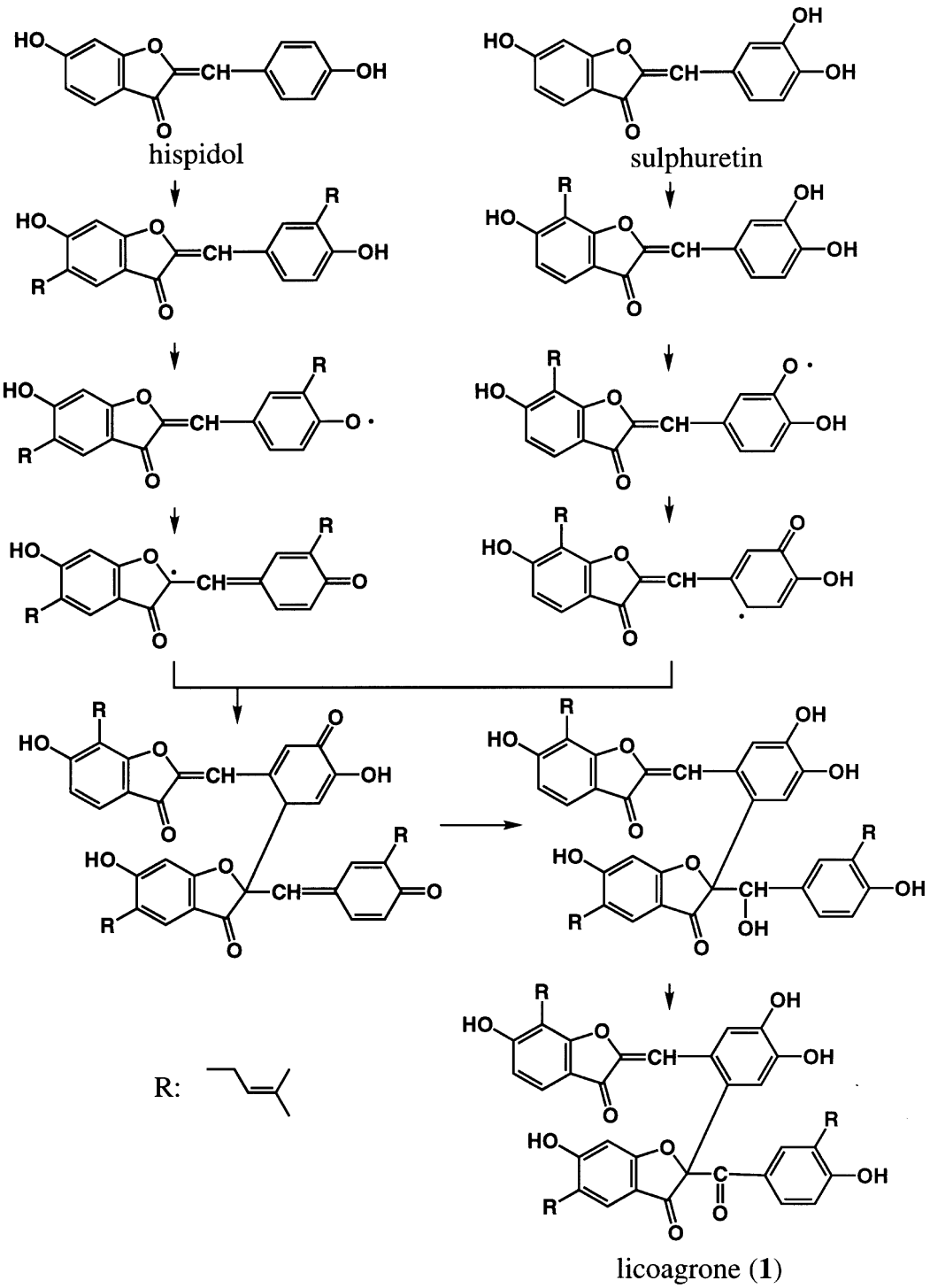

Fig. 3. Hypothetical biosynthetic pathway to compound **1**.

FAB-MS were taken on a JEOL JMS DX-300 spectrometer. The ^{1}H and ^{13}C NMR spectra were measured with a Varian XL-400 spectrometer. For HPLC, Waters model 510 HPLC system (column: μ-Bondasphere, 5 μm Si-100 Å, 19 × 150 mm) was used. CC was carried out using Wako-gel C-200. TLC was conducted on Kieselgel 60 F_{254} plates (Merck).

3.2. Mass culture of G. glabra hairy root

The mass culture conditions of *G. glabra* hairy root were described in a previous paper (Asada et al., 1998).

3.3. Separation procedures of flavonoids

The EtOAc fraction (50.66 g) obtained previously (Asada et al., 1998) from *G. glabra* hairy root cultures was subjected to column chromatography on silica gel (1500 g) and eluted with *n*-hexane:acetone (3:2) to give seven fractions, fr.1 (2.28 g), fr.2 (1.11 g), fr.3 (6.58 g), fr.4 (2.28 g), fr.5 (3.28 g), fr.6 (3.7 g) and fr.7 (11.19 g), respectively.

3.4. Isolation of **1** and **2**

Fr.6 was treated with a silica gel column ($CHCl_3$:MeOH = 9:1) and further separated by normal-phase HPLC ($CHCl_3$:MeOH = 98.5:1.5) to give two main fractions A and B. Fr. A was purified by repeated HPLC (*n*-hexane:$CHCl_3$:EtOH = 50:50:5.5; *n*-hexane:isopropanol = 87:13; *n*-hexane:EtOAc:EtOH = 70:30:2) to give **1** (11.7 mg) and fr. B was purified by HPLC (*n*-hexane: EtOH = 87:13; *n*-hexane: EtOAc:EtOH = 80:20:3) to give **2** (4.3 mg).

3.5. Isolation of **3** and **4**

Fr.5 was treated with a silica gel column ($CHCl_3$:MeOH = 9:1) and further separated by normal-phase HPLC ($CHCl_3$:MeOH = 99:1) to give two fractions C and D. Fr. C was purified by HPLC (*n*-hexane: EtOH = 88:12) to give **4** (3.3 mg). Fr. D was purified by HPLC (*n*-hexane:EtOH = 90:10) to give **3** (6.7 mg).

3.6. Isolation of **5** and **6**

Fr.4 was subjected to a silica gel column (*n*-hexane: acetone = 65:35) to give six fractions I–VI. Fr.III was further purified by repeated HPLC (*n*-hexane: isopropanol = 92:8; *n*-hexane:EtOAc:*n*-propanol = 85:15: 2) to give **5** (7.5 mg), and fr. VI was purified by HPLC (*n*-hexane:EtOH = 89:11; *n*-hexane:$CHCl_3$:EtOH = 10: 90:2; *n*-hexane:EtOAc:EtOH = 90:30:0.5) to give **6** (10.6 mg).

3.7. Licoagrone (**1**)

Orange powder, $[\alpha]_D^{24}$ ±0° (MeOH; *c* 1.01). UV λ_{max}^{MeOH} nm (log ε): 230 sh(4.55), 278(4.46), 324(4.41), 3.96(4.25). IR ν_{max}^{KBr} cm^{-1}: 3430, 1660, 1600. HR-FAB-MS: Calcd for $C_{45}H_{42}O_{10}$ (M^+), 742.2778; Found: 742.2773. EI-MS *m*/*z* (%): 348(28), 338(28), 324(23), 323(100), 189(9), 187(20), 174(10), 173(67), 150(14), 149(14), 137(15). ^{1}H NMR (400 MHz, acetone-d_6): δ 1.58 (3H, d, *J* = 0.5 Hz, H_3-19″), 1.64 (3H, d, *J* = 0.5 Hz, H_3-15), 1.66 (3H, d, *J* = 0.5 Hz, H_3-20″), 1.72 (3H, d, *J* = 0.5 Hz, H_3-14″), 1.76 (3H, d, *J* = 0.5 Hz, H_3-15″), 1.76 (3H, d, *J* = 0.5 Hz, H3-14), 3.12 (2H, d, *J* = 7.0 Hz, H-16″), 3.33 (2H, d, *J* = 7.0 Hz, H-11″), 3.44 (2H, d, *J* = 7.0 Hz, H-11), 5.10 (1H, m, H-17″), 5.31 (1H, m, H-12), 5.36 (1H, m, H-12″), 6.67 (1H, d, *J* = 8.5 Hz, H-5‴), 6.72 (1H, s, H-7″), 6.79 (1H, d, *J* = 8.5 Hz, H-5), 7.01 (1H, s, H-10), 7.17(1H, s, H-3′), 7.41 (1H, s, H-4″), 7.42 (1H, d, *J* = 8.5 Hz, H-4), 7.57 (1H, dd, *J* = 8.5, 2.5 Hz, H-6‴), 7.64 (1H, d, *J* = 2.5 Hz, H-2‴), 7.84 (1H, s, H-6′).

^{13}C NMR (100 MHz, acetone-d_6) δ: see Table 1.

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