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Minor flavonoids from licorice☆

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Abstract

Three minor flavonoids, licofuranocoumarin, isotrifoliol and glisoflavanone, were isolated from licorice (underground part of *Glycyrrhiza uralensis*), and their structures with 3-arylcoumarin, coumestan and isoflavanone skeletons were respectively elucidated on the basis of spectroscopic data.

Keywords: Leguminosae; *Glycyrrhiza uralensis*; Flavonoid; 3-Arylcoumarin; Coumestan; Isoflavanone

1. Introduction

Licorice, the roots and rhizomes of *Glycyrrhiza* species (Leguminosae), is one of the most frequently used natural medicines in Japan, and many research groups have investigated its pharmacologically active constituents. We have also reported the antibacterial effects of licorice phenolics on methicillin-resistant *Staphylococcus aureus* (MRSA). One of the phenolic compounds among them showed noticable reduction of the β-lactam resistance of MRSA (Hatano et al., 2000). In the course of the study on the bioactive phenolics, we isolated additional three flavonoids, licofuranocoumarin (**1**), isotrifoliol (**2**) and glisoflavanone (**3**), along with 9 known compounds from commercial licorice of which the source plant is *Glycyrrhiza uralensis* Fisch. (Hatano, et al., 1991). This paper deals with structure elucidation of these new flavonoids.

2. Results and discussion

Compounds **1–3**, orobol (Geiger et al., 1987), kaempferol (Stein and Zinsmeister, 1990), kaempferol 3-*O*-methyl ether (Valesi et al., 1972), 7-*O*-methylluteone (Tahara et al., 1989), topazolin (Tahara et al., 1987), isoglycycoumarin (Zeng et al., 1991), 3′-prenylnaringenin (Nkengfack et al., 1989), allolicoisoflavone B (Tahara et al., 1990) and licoflavonol (Saitoh, et al, 1976) were isolated from the ethyl acetate extract of licorice either by centrifugal partition chromatography (Okuda et al., 1990) or countercurrent distribution in combination with column chromatography, preparative HPLC and preparative layer chromatography as shown in the experimental section.

Licofuranocoumarin (**1**) was obtained as pale-yellow needles. The high-resolution ESIMS showed an $[M+H]^+$ ion peak corresponding to its molecular formula $C_{21}H_{20}O_7$. The UV spectrum of this compound is similar to that of glycycoumarin (**4**) (Zhu et al., 1984) and licopyranocoumarin (**5**) (Hatano et al., 1988), suggesting a 3-arylcoumarin structure[1] for **1**. The ^{1}H-NMR spectrum of **1** showed protons of the coumarin skeleton at δ 8.02 (1H, *s*, H-4) and δ 6.42 (1H, *s*, H-9), and those of the 2,4-dihydroxyphenyl group at δ 6.46 (1H, *d*, $J=2.5$ Hz, H-3′), δ 6.43 (1H, *dd*, $J=2.5$, 8.5 Hz, H-5′) and δ 7.17 (1H, *d*, $J=8$ Hz, H-6′). The spectrum also showed a methoxyl signal at δ 4.11 (3H, *s*), and signals attributable to a C_5 unit [δ 1.24, 1.28 (3H each, *s*, *gem*-dimethyl), 3.53 (2H, *m*, H-6) and 4.75 (1H, *dd*, $J=8$, 9.5 Hz, H-7)] structurally related to a γ,γ-dimethylallyl

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[1] 3-Arylcoumarins are regarded as members of isoflavanoids (Harborne, 1994).

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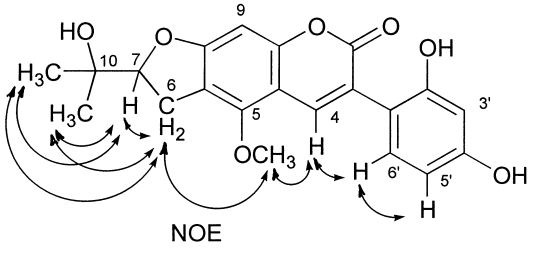
group. The NOESY spectrum showed correlations as indicated by arrows in formula **1**. The C-5 position of the methoxyl group on the coumarin skeleton was indicated by an NOE between the methoxyl signal and H-4 which was in turn correlated with H-6′ of the 2,4-dihydroxyphenyl group. The NOESY spectrum also revealed correlations, $OCH_3 \leftrightarrow CH_2 \leftrightarrow CH \leftrightarrow CH_3$, indicating the location at C-6 of the C_5 unit, and connectivity of protons of the C_5 unit. Formation of the dihydrofuran ring by the C_5 unit was indicated by a long-range C–H correlation between C-8a and H-7, which was observed in the HMBC spectrum. Correlations shown in the HMBC spectrum were summarized in Table 1. Based on these spectral data, structure of 6-(2,4-dihydroxyphenyl)-2-(1-hydroxy-1-methylethyl)-4-methoxy-2,3-dihydro-7*H*-furo[3,2-*g*][1]benzopyran-7-one (**1**) was assigned to licofuranocoumarin, although the absolute configuration at C-7 has not yet been determined.

Isotrifoliol (**2**) was obtained as pale-yellow needles. The high-resolution EIMS showed an M^+ ion peak corresponding to the molecular formula $C_{16}H_{10}O_6$ for this compound. The UV spectrum of **2** was similar to those of coumestans[2] such as glycyrol (**6**) (Saitoh and Shibata, 1969; Shiozawa et al., 1989). The ^{1}H-NMR spectrum (in acetone-d_6) showed two *meta*-coupled protons [δ 6.54 and 6.55 (1H each, *d*, $J=2$ Hz, H-2 and H-4)][3] and three protons forming an ABX system [δ



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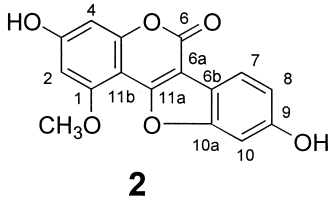

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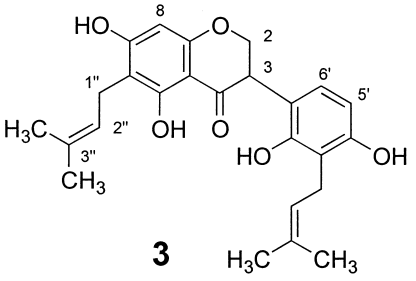

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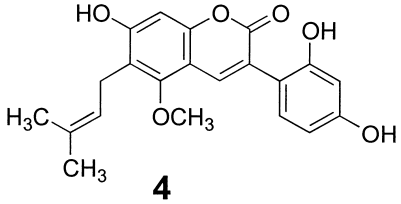

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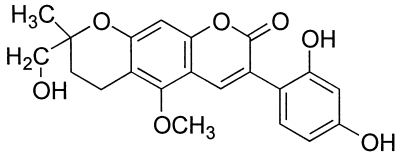

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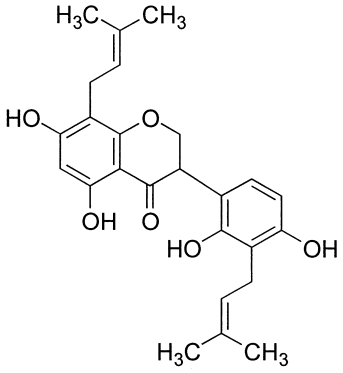

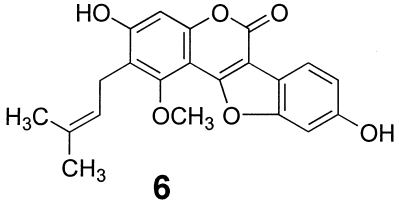

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7

[2] Coumestans form a group of isoflavonoids (Harborne, 1994).

[3] The numbering system is based on Harborne (1994).

Table 1
One-bond and long-range ^{1}H–^{13}C correlations observed in the HMQC and HMBC spectra of **1** (in acetone-d_6 at 27°C)

Carbon	δ_C	Correlated protons	
		Proton coupled one bond (δ_H)	Protons coupled via two or three bonds[a]
C-2	162.2		H-4
C-3	121.0		H-4, H-6′
C-4	138.3	8.02	
C-4a	107.0		H-9
C-5	153.4		H-4, H-6, OCH_3
C-5a	112.2		H-6, H-7, H-9
C-6	29.5	3.53	H-7
C-7	91.8	4.75	H-6, $CH_3\times2$
C-8a	166.0		H-6, H-7, H-9
C-9	92.2	6.42	H-4[b]
C-9a	156.4		H-4, H-9
C-10	71.4		H-6, $CH_3\times2$
C-1′	115.7		H-4, H-3′, H-5′
C-2′	157.1		H-3′, H-6′
C-3′	104.5	6.46	H-5′
C-4′	159.7		H-3′, H-5′, H-6′
C-5′	108.2	6.43	H-3′
C-6′	132.6	7.17	
CH_3	25.4	1.28	H-7
	26.0	1.24	H-7
OCH_3	59.9	4.11	

[a] *J* for the CCH or CCCH couplings were set at 4 Hz.
[b] Coupling via four bonds.

7.79 (1H, *d*, *J* = 8.5 Hz, H-7), 7.05 (1H, *dd*, *J* = 2, 8.5 Hz, H-8) and 7.18 (1H, *d*, *J* = 2 Hz, H-10)], along with a methoxyl signal [δ 3.95 (3H, *s*)]. The chemical shifts of the ABX protons were closely similar to those of **6** [δ 7.79 (1H, *d*, *J* = 8.5 Hz, H-7), 7.03 (1H, *dd*, *J* = 2, 8.5 Hz, H-8) and 7.22 (1H, *d*, *J* = 2 Hz, H-10) (in acetone-d_6)], indicating that the tri-substituted benzene ring of **2** has an identical substitution pattern than that of **6**. ^{13}C Chemical shifts of this benzene ring in **2** [δ 114.2 (C-6b), 120.2 (C-7), 113.4 (C-8), 156.4 (C-9), 98.2 (C-10), 155.7 (C-10a)] [in $(CD_3)_2SO$-$CDCl_3$, 1:1 by volume], which were also practically the same as those of **6** [δ 114.2 (C-6b), 120.0 (C-7), 113.6 (C-8), 156.6 (C-9), 98.1 (C-10), 155.9 (C-10a)], substantiated the substitution pattern for **2**. Irradiation of the methoxyl signal (δ 3.95) in the NOE difference spectrum [in $(CD_3)_2SO$–$CDCl_3$ (1:1)] showed an NOE with H-2 (δ 6.40), while the H-4 signal did not show an NOE. The location of the methoxyl group was therefore determined to be at C-1. Structure of 3,9-dihydroxy-1-methoxy-6*H*-benzofuro[3,2-*c*] [1] benzopyran-6-one (**2**) was thus assigned for isotrifoliol.

Racemic glisoflavanone (**3**) was obtained as colorless needles. The molecular formula $C_{25}H_{28}O_6$ was indicated by the high-resolution EIMS. The ^{1}H NMR spectrum showed signals assignable to protons of the A-ring [δ6.02 (1H, *s*)], B-ring [δ 6.40 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.96 (1H, *d*, *J* = 8.5 Hz, H-6′)] and C-ring [δ 4.59, 4.67 (1H each, *dd*, *J* = 6, 11 Hz, H-2), 4.08 (1H, br *t*, *J* = 6 Hz, H-3)] of an isoflavanone structure, along with signals due to two γ,γ-dimethylallyl groups [δ 1.61, 1.63, 1.73, 1.75 (3H each, br *s*, 2×*gem*-dimethyl), 3.21, 3.39 (2H each, *d*, *J* = 7.5 Hz, 2×H-1″), 5.21 (2H, *m*, 2×H-2″)]. The fragment ions at *m*/*z* 221 and 203 in the EIMS of **3** suggested that one of the γ,γ-dimethylallyl groups is on the A-ring and another is on the B-ring (Fig. 1). These data suggested that **3** is a regioisomer of 3′-(γ,γ-dimethylallyl)-kievitone (**7**) (O'Neill, et al., 1986), concerning the location of the γ,γ-dimethylallyl group on the A-ring. In fact, ^{1}H chemical shifts for **3** were similar to those of the corresponding protons of **7** except for the H-2 signals (δ 4.66 and 4.75 for **7**, in acetone-d_6). Structure **3**, i.e. 5,7-dihydroxy-3-[2,4-dihydroxy-3-(3-methyl-2-butenyl)phenyl]-6-(3-methyl-2-butenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one, was thus assigned for glisoflavanone. The ^{13}C NMR spectral data shown in the experimental section were in agreement with this interpretation.

3. Experimental

3.1. General

ESIMS were measured on a Micromass Autospec OA-Tof instrument with 50% aqueous MeOH containing 0.1% $AcONH_4$ as solvent. EIMS were measured on a VG-70 high-resolution mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^{1}H and 125.7 MHz for ^{13}C) in acetone-d_6 or acetone-d_6 containing D_2O, unless otherwise mentioned. Chemical shifts are given in δ (ppm) value based on those of the solvent signals (δ_H 2.04 and δ_C 29.8 for acetone-d_6).

3.2. Plant material

The source plant of licorice used in this study is *Glycyrrhiza uralensis*. This material was purchased from Tochimoto-tenkai-do, Osaka, Japan.

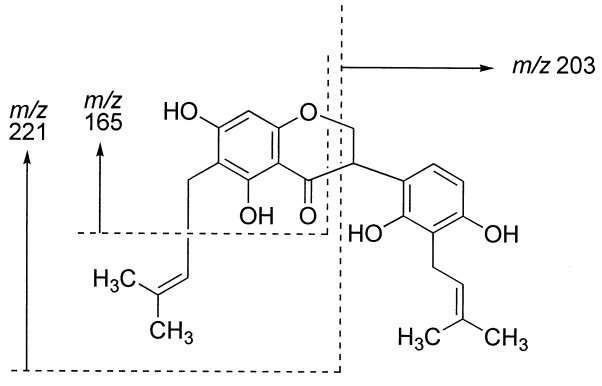

Fig. 1. Mass fragmentation in the EIMS of **3**.

3.3. Isolation of flavonoids from licorice

Licorice (2 kg) was extracted with *n*-hexane (6 l), and then with ethyl acetate (6 l × 3). A portion (27.9 g) of the ethyl acetate extract (99.3 g) was subjected to centrifugal partition chromatography ($CHCl_3$–MeOH–H_2O, 7:13:8; reversed-phase development), with the fractions chromatographed over MCI-gel CHP-20P (with H_2O–MeOH), and further purified by CC on YMC-gel ODS-AQ 120 S50 (H_2O–MeOH), preparative HPLC (YMC J-sphere ODS-H80 column; MeCN–H_2O–AcOH), or preparative layer chromatography (Si gel, $CHCl_3$–acetone–HCOOH) to give licofurano-coumarin (**1**) (0.016% from the ethyl acetate extract), orobol (0.006%), kaempferol (0.013%) and kaempferol 3-*O*-methyl ether (0.006%), along with compounds for which the isolation was reported previously (Hatano et al., 2000). In a separate experiment, the ethyl acetate extract (30 g) from licorice was separated by countercurrent distribution ($n=5$, $r=5$), and fractions were purified either by CC on MCI-gel CHP-20P or on Fuji gel ODS G3, with preparative HPLC and preparative layer chromatography to give isotrifoliol (**2**) (0.008% from the ethyl acetate extract) and 7-*O*-methylluteone (0.020%). Gliso flavanone (**3**) (0.025%), topazolin (0.19%), isogly cycoumarin (0.017%), 3′-prenylnarin-genin (0.005%), allolicoisoflavone B (0.017%) and licoflavonol (0.019%) were isolated from an ethyl acetate extract of licorice in an analogous way.

3.4. Licofuranocoumarin **1**

Pale-yellow needles, mp 242°C. $[\alpha]_D$−4.2° (EtOH; *c* 1). ESIMS *m*/*z*: 385 ($[M+H]^+$). High-resolution ESIMS *m*/*z*: 385.1262 ($[M+H]^+$). Calcd for $C_{21}H_{20}O_7+H$, *m*/*z*: 385.1287. UV λ_{max} (MeOH) nm (log ϵ): 212 (4.61), 254 (3.95), 354 (4.24). ^{1}H NMR spectral data: δ 1.24, 1.28 (3H each, *s*, *gem*-dimethyl), 3.53 (2H, *m*, H-6), 4.11 (3H, *s*, OCH_3), 4.75 (1H, *dd*, *J*=8, 9.5 Hz, H-7), 6.42 (1H, *s*, H-9), 6.43 (1H, *dd*, *J*=2.5, 8.5 Hz, H-5′), 6.46 (1H, *d*, *J*=2.5 Hz, H-3′), 7.17 (1H, *d*, *J*=8 Hz, H-6′), 8.02 (1H, *s*, H-4). ^{13}C-NMR spectral data: δ 25.4, 26.0 (*gem*-dimethyl), 29.5 (C-6), 59.9 (OCH_3), 71.4 (C-10), 91.8 (C-7), 92.2 (C-9), 104.5 (C-3′), 107.0 (C-4a), 108.2 (C-5′), 112.2 (C-5a), 115.7 (C-1′), 121.0 (C-3), 132.6 (C-6′), 138.3 (C-4), 153.4 (C-5), 156.4 (C-9a), 157.1 (C-2′), 159.7 (C-4′), 162.2 (C-2), 166.0 (C-8a).

3.5. Isotrifoliol **2**

Pale-yellow needles, mp > 300°C. EIMS *m*/*z* (relative intensity): 298 (M^+) (100%), 255 (40%), 149 (41%). High-resolution EIMS *m*/*z*: 298.0442 (M^+). Calcd for $C_{16}H_{10}O_6$, *m*/*z*: 298.0477. UV λ_{max} (EtOH) nm (log ϵ): 208 (4.42), 227 (sh), 249 (4.18), 348 (4.24), 361 (sh). ^{1}H NMR spectral data [$(CD_3)_2SO$–$CDCl_3$, 1:1 by volume]: δ 3.95 (3H, *s*, OCH_3), 6.40 (1H, *d*, *J*=2 Hz, H-2), 6.45 (1H, *d*, *J*=2 Hz, H-4), 6.85 (1H, *dd*, *J*=2, 8.5 Hz, H-8), 7.03 (1H, *d*, *J*=2 Hz, H-10), 7.64 (1H, *d*, *J*=8.5 Hz, H-7). ^{13}C NMR spectral data [$(CD_3)_2SO$-$CDCl_3$, 1:1]: δ 55.8 (OCH_3), 95.6 (C-2), 95.7 (C-4), 98.2 (C-10), 101.2 (C-6a), 108.7 (C-11b), 113.4 (C-8), 114.2 (C-6b), 120.0 (C-7), 153.5 (C-4), 155.1 (C-1), 155.7 (C-10a), 156.4 (C-9), 157.5 (C-6), 159.1 (C-11a), 161.4 (C-3).

3.6. Glisoflavanone **3**

Colorless needles, mp 131°C. $[\alpha]_D$ 0° (acetone; *c* 1). EIMS *m*/*z* (relative intensity): 424 (M^+) (48%), 221 (73%), 203 (15%), 165 (100%). High-resolution EIMS *m*/*z*: 424.1951 (M^+). Calcd for $C_{25}H_{28}O_6$, *m*/*z*: 424.1886. UV λ_{max} (MeOH) nm (log ϵ): 206 (4.46), 230 (sh), 295 (4.00). ^{1}H-NMR spectral data: δ 1.61, 1.63, 1.73, 1.75 (3H each, br *s*, 2 × *gem*-dimethyl), 3.21, 3.39 (2H each, *d*, *J*=7.5 Hz, 2 × H-1″), 4.08 (1H, br *t*, *J*=6 Hz, H-3), 4.59, 4.67 (1H each, *dd*, *J*=6, 11 Hz, H-2), 5.21 (2H, *m*, 2×H-2″), 6.02 (1H, *s*, H-6), 6.40 (1H, *d*, *J*=8.5 Hz, H-5′), 6.96 (1H, *d*, *J*=8.5 Hz, H-6′), 12.31 (1H, *s*, 5-OH). ^{13}C-NMR spectral data:δ 17.8, 17.9, 21.6, 23.6 (2×*gem*-dimethyl), 25.8 (2C, 2×C-1″), 46.6 (C-3), 70.9 (C-2), 95.3 (C-8), 102.6 (C-4a), 109.2 (C-7), 115.6 (C-1′), 117.0 (C-3′), 123.5, 123.8 (2×C-2″), 126.9 (C-6′). 131.2, 131.6 (2×C-3″), 154.8 (C-4′), 156.4 (C-2′), 162.0 (C-8a), 162.7 (C-5), 165.6 (C-7), 198.5 (C-4).

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