The Isolation and Structure Elucidation of Minor Isoflavonoids from Licorice of *Glycyrrhiza glabra* Origin

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Two new isoflavanone and one new 3-arylcoumarin derivatives, along with a known compound 3,4-didehydroglabridin, were isolated from commercially available licorice of *Glycyrrhiza glabra* origin, and their structures were elucidated on the basis of both the chemical and spectroscopic evidence.

Key words *Glycyrrhiza glabra*; licorice; 3-arylcoumarin; isoflavanone; Fabaceae

The root of *Glycyrrhiza glabra* L. (Fabaceae) is highly reputed for its medicinal properties as the crude drug licorice (liquorice) in Europe and its vicinity since ancient times. In Japan, it is utilized mainly for the extraction of the sweetening principle glycyrrhizin that is used as either a natural sweetener or pharmaceutical. The primary origin of licorice (kanzou in Japanese) used in Kampo medicine (Japanese traditional medicine) is *G. uralensis* that also contains glycyrrhizin, and the licorice of *G. glabra* origin has rarely found medicinal value in Kampo medicine. Though the main sweetening component glycyrrhizin has long been perceived as an active principle responsible for the pharmacological efficacy of licorice of both *G. glabra* and *G. uralensis* origin, a growing number of reports have proved that they are rich sources of phenolic constituents, in particular, isoflavonoids that have been found to exhibit a wide variety of biological activity.[1,2] We also reported earlier the isolation of a number of flavonoids and isoflavonoids from the dichloromethane soluble fraction of the extract of *G. glabra* root.[3—5] The hydrophobic fraction of licorice extract has recently found value as additives in cosmetics, and its development as either preservatives or supplements[6] is also underway, taking advantage of its strong antimicrobial and antioxidant property.[7,8] Therefore, chemical investigation of the hydrophobic fraction of licorice extract is still a work of necessity in order to evaluate the efficacy and safety of the extract. A recent growing interest in the isoflavonoid from viewpoints of health science is also one of the driving forces of continuing chemical research on licorice. Under these circumstances further chemical investigation was undertaken and resultantly led to the isolation of several minor constituents, three of which were new compounds. This paper describes the isolation and structure elucidation of two new isoflavanones and one new 3-arylcoumarin.

Results and Discussion

Several fractions obtained through the separation process of the dichloromethane extract of commercially available *G. glabra* root as reported previously[4] were subjected to a series of column chromatographic separation to yield new compounds **1**, **2** and **3**, for which names of glabroisoflavanones, A, B and glabrocoumarin, respectively, and a known isoflavene derivative 3,4-didehydroglabridin.

Glabroisoflavanone A (**1**)[9] was obtained in pure crystalline forms of mp 113—116 °C. It has a molecular formula of $C_{20}H_{18}O_5$ as determined by the high-resolution mass spectrometry. The IR spectrum showed an absorption band at 1661 cm^{-1} that is ascribed to the carbonyl group. The UV spectrum exhibited an absorption maximum at 265 nm. The ^{1}H-NMR spectrum revealed a set of resonances characteristic of a chromene ring [δ 1.44 (3H, s), 1.45 (3H, s), 5.75 (1H, d, J=10.0 Hz) and 6.64 (1H, d, J=10.0 Hz)] along with the AX [δ 6.49 (1H, d, J=8.8 Hz) and 7.70 (1H, d, J=8.8 Hz)] and ABX [δ 6.32 (1H, dd, J=8.4, 2.3 Hz), 6.43 (1H, d, J=2.3 Hz) and 6.93 (1H, d, J=8.4 Hz)] systems in the aromatic region. A doublet signal at δ 7.70 was assignable to 5-H that was deshielded by the carbonyl group. The occurrence of the chromene in A-ring was suggested by ^3J correlations between 4″-H/5-H and 7-C and between 5″-H/6-H and 8-C in the long range ^{13}C–^{1}H COSY. Two broad singlets at δ 8.23 and 8.54, which disappeared with the addition of D_2O, were also observed indicating the presence of two phenol hydroxyls. All of signals appearing at δ 4.16, 4.60 and 4.72 were exhibited as double doublet peaks. A signal at δ 4.16 shared

Table 1. ^{13}C-NMR[a] Data for Glabroisoflavanones A (**1**), B (**2**) and Glabrocoumarin (**3**)

Carbon	**1**[b]	**2**[c]	**3**[b]
C-2	71.92	70.20	160.63
C-3	47.69	45.62	122.60
C-4	191.67	193.08	142.23
C-5	130.00	129.10	130.13
C-6	111.43	111.70	113.66
C-7	159.78	160.68	161.38
C-8	110.04	109.10	102.91
C-9	158.70	157.97	156.27
C-10	114.36	112.86	113.51
C-1′	116.26	115.01	117.78
C-2′	157.16	156.67	152.57
C-3′	103.81	103.32	110.30
C-4′	158.77	160.57	154.10
C-5′	107.78	107.03	108.00
C-6′	131.36	127.70	131.48
C-4″	116.26	115.49	117.78
C-5″	128.84	128.90	129.40
C-6″	78.17	77.92	76.89
CH_3	28.23	28.28	27.97
	28.39	28.45	27.97
OCH_3	—	55.25	—

a) Spectra were measured at 100 MHz with TMS as internal standard. Assignments were based on ^{13}C–^{1}H COSY and long range ^{13}C–^{1}H COSY correlations. *b*) Measured in acetone-d_6. *c*) Measured in $CDCl_3$.

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a coupling constant of $J=10.3$ Hz with a signal at δ 4.72 and that of $J=5.5$ Hz with a signal at δ 4.60, whereas a signal at δ 4.60 shared a coupling constant of $J=11.0$ Hz with a signal at δ 4.72. Both chemical shifts and coupling constants of proton resonances in this signal system was consistent with the one occurring in the heterocyclic ring of isoflavanone skeleton. The ^{13}C-NMR spectrum exhibited the presence of methylene and methine carbon signals at δ 47.7 and 71.9, which were assignable to C-3 and C-2, respectively, in the heterocyclic ring of isoflavanone. The above spectroscopic evidence established the structure for glabroisoflavanone A as **1**. This structure was further substantiated by correlation data obtained from the long range ^{13}C–^{1}H COSY.

Glabroisoflavanone B (**2**) was obtained in pure crystalline forms of mp 161—162 °C. Its molecular formula was determined to be $C_{21}H_{20}O_5$ by the high-resolution mass spectrometry. The spectroscopic properties of glabroisoflavanone B in the IR, UV and NMR spectra were similar to those of glabroisoflavanone A indicating that both compounds were structurally related. The presence of a sharp singlet at δ 3.72 in the ^{1}H-NMR spectrum and an sp^3 carbon signal at δ 55.3 in the ^{13}C-NMR spectrum readily deduced that glabroisoflavanone B is a methylated form of glabroisoflavanone A. Significant nuclear Overhauser effects (NOEs) between a methoxyl signal at δ 3.72 and 3′-H/5′-H (17% and 23%, respectively) located a methoxyl group at the 4′-position. Correlation data observed in the long range ^{13}C–^{1}H COSY also supported the structure **2** for glabroisoflavanone B.

Though glabroisoflavanones A and B have an asymmetric carbon at the 3-position of the isoflavanone skeleton, both compounds were isolated in racemic forms. Many of naturally occurring isoflavanones are known to be racemic, and optically active examples are exceptional.[10] It is presumed that they undergo racemization during the extraction and purification procedures since the asymmetric carbon is a chemically active methine adjacent to the carbonyl group and is thus prone to racemization under mild condition. Since glabroisoflavanones A and B were obtained through many steps of the separation procedures, it is no wonder that both compounds are racemic. It is of interest to note that both glabroisoflavanones A and B correspond in the substitution pattern to glabiridin and 2′,4′-dihydroxy-[6″,6″-dimethylpyrano(2″,3″:7,8)-3-arylcoumarin, and their 4′-*O*-methyl derivatives, respectively, all of which were obtained from *G. glabra* root.[3—5] They are presumed to share common biosynthetic pathway undergoing oxygenation and/or hydrogenation to give rise to differentiation in the heterocyclic ring.

Glabrocoumarin (**3**) was obtained as slightly pale yellow fine needles of mp 254—256 °C, and its methanol solution gave a characteristic greenish yellow fluorescence. Its UV and IR spectra exhibited an absorption maximum at 341 nm, and an absorption band at 1686 cm^{-1} in the carbonyl region, respectively. These physicochemical and spectroscopic properties suggested that this compound is a 3-arylcoumarin derivative, a rare chemical species in the isoflavonoid family. The ^{1}H-NMR spectrum revealed the presence of a chromene ring [δ 1.36 (6H, s), 5.66 (1H, d, $J=9.9$ Hz) and 6.71 (1H, d, $J=9.9$ Hz)] along with the AX [δ 6.47 (1H, d, $J=8.4$ Hz) and 7.08 (1H, d, $J=8.4$ Hz)] and ABX [δ 6.77 (1H, d, $J=2.3$ Hz), 6.85 (1H, dd, $J=2.3$, 8.4 Hz) and 7.52 (1H, d, $J=8.4$ Hz)] systems in the aromatic region, and a characteristic singlet at

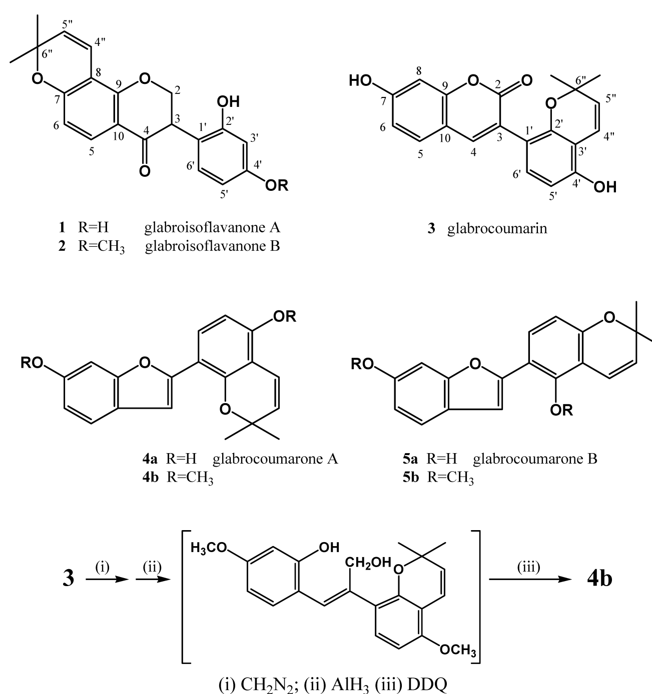

Fig. 1. The Scheme for Chemical Conversion of Glabrocoumarin (**3**) into the Glabrocoumarone A Dimethylether (**4b**)

δ 7.76 assignable to a proton at the 4-position of the 3-arylcoumarin skeleton. Correlations observed in the long range ^{13}C–^{1}H COSY suggested that the chromene ring occur in the B-ring rather than the A-ring. However, which of oxygen functionality the chromene ring cyclizes to could not be determined by the spectroscopic method. We reported earlier the isolation of two pyrano-2-arylbenzofurans glabrocoumarones A (**4a**) and B (**5a**) from *G. glabra* root.[4] It was reported by the first author that a dimethylated form of glycyrin, a 3-arylcoumarin isolated from *G. uralensis*, was converted into the corresponding 2-arylbenzofuran in reasonable yield.[11] If chemical conversion of glabrocoumarin dimethylether was achieved in the way described in the literature, the converted product would be identical to either **4b** or **5b**, *i.e.*, either a dimethylether of glabrocoumarones A or B,[4] respectively, which will determine the structure of glabrocoumarin unequivocally. Chemical conversion of glabrocoumarin was performed as shown in Fig. 1 eventually in one batch in order to minimize the loss of intermediates during workups. The product obtained from this conversion was found to be identical to glabrocoumarone A dimethylether (**4b**), and thus the structure for glabrocoumarin was established as **3**. Glabrocoumarin has the same substitution pattern as glabrene,[5] an isoflav-3-ene, but not glabrone, an isoflavone.[5] Both glabrene and glabrone, which also occurred in *G. glabra* root, were characterized as having the same substitution pattern.[8] However, the structure of glabrene was later revised as having the same substitution pattern as glabrocoumarin.[5] The structure for glabrene had been deduced solely from the Gibbs test that was a classical method once used to determine the occupancy of the *para* position of phenol, and in part from the presumption that both are biogenetically related.[12] The co-occurrence of biogenetically related compounds is quite common, but this example indicates that the biogenesis-based presumption may

lead to erroneous conclusion.

Experimental

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: proton and C-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra with a JEOL JNM GSX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer with tetramethylsilane (TMS) as internal standard; mass spectra (MS) with a JEOL SX-102A mass spectrometer; infrared (IR) spectra with a JASCO FT/IR-8000 infrared spectrometer; ultraviolet (UV) spectra with a Shimadzu UV-240 spectrometer and optical rotations with s JASCO DIP-370 polarimeter. Column chromatography was carried out with Wakogel C-200 or Merck Kieselgel 60, Sephadex LH-20 (Pharmacia) and RP-8 reversed-phase silica gel (Merck). Thin-layer chromatography (TLC) was conducted on a 0.25 mm precoated silica gel plate (60GF$_{254}$, Merck), and spots were detected by inspection under short (254 nm) or long (365 nm) wavelength UV lights, or by the colors developed with 10% H_2SO_4 spraying followed by heating on a hot plate.

Plant Material Commercially available licorice roots derived from *G. glabra* were obtained from central Asian countries through Maruzen Pharmaceutical Co., Ltd., Onomich, Japan.

Extraction and Isolation The dichloromethane extract (150 g) of *G. glabra* root was separated into eight fractions (Fr. I—VIII) on a silica gel column chromatography as reported earlier.[3] Fractionation of Fr. III (50.98 g) into fr. 1—fr. 24 was also described previously.[5] Combined fractions of fr. 11—13 (13.61 g) was further separated by silica gel ($CHCl_3$–acetone), Sephadex LH-20 (MeOH–$CHCl_3$ 3 : 1), and RP-8 reversed-phase silica gel (MeOH–H_2O) column chromatography to give glabrocoumarin (**3**; 69 mg) and glabroisoflavanone B (**2**; 14 mg). Combined fractions of fr. 14—18 (12.0 g) were also chromatographed in the same way as mentioned above to furnish glabroisoflavanone A (**1**; 26 mg) and 3,4-didehydroglabridin (17 mg). 3,4-Didehydroglabridin was identified in comparison with the spectral data of the authentic sample described in the literature.[13]

Glabroisoflavanone A (**1**): Colorless prisms from MeOH–H_2O, mp 113—116 °C. $[\alpha]_D^{25}$ ±0° (c=0.073, MeOH). IR (KBr) cm^{-1}: 3567, 3482, 1661, 1632, 1595, 1574, 1468. UV λ_{max} nm (log ε): 207 (4.38), 265 (4.48), 310 (3.82). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 1.44 (3H, s, –CH_3), 1.45 (3H, s, –CH_3), 4.16 (1H, dd, J=5.5, 10.3 Hz, 3-H$_{ax}$), 4.60 (1H, dd, J=5.5, 11.0 Hz, 2-H$_{eq}$), 4.72 (1H, dd, J=11.0, 10.3 Hz, 2-H$_{ax}$), 5.75 (1H, d, J=10.0 Hz, 5″-H), 6.32 (1H, dd, J=8.4, 2.3 Hz, 5′-H), 6.43 (1H, d, J=2.3 Hz, 3′-H), 6.49 (1H, d, J=8.8 Hz, 6-H), 6.64 (1H, d, J=10.0 Hz, 4″-H), 6.93 (1H, d, J=8.4 Hz, 6′-H), 7.70 (1H, d, J=8.8 Hz, 5-H), 8.23, 8.54 (1H each, br s, disappeared with the addition of D_2O, –OH). ^{13}C-NMR (100 MHz, acetone-d_6) δ: see Table 1. EI-MS m/z (rel. int., %): 338 (M^+, 31), 337 (M^+-CH_3, 40), 305 (67), 203 (72), 187 (100). HR-MS m/z: 338.1156 (Calcd for $C_{20}H_{18}O_5$: 338.1154).

Glabroisoflavanone B (**2**): Colorless prisms from MeOH–H_2O, mp 161—162 °C. $[\alpha]_D^{25}$ ±0° (c=0.036, MeOH). IR (KBr) cm^{-1}: 3385, 1665, 1628, 1574, 1441, 1373. UV λ_{max} nm (log ε): 207 (4.38), 265 (4.45), 310 (3.79). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.44 (3H, s, –CH_3), 1.47 (3H, s, –CH_3), 3.72 (3H, s, OCH_3), 3.95 (1H, dd, J=4.6, 4.2 Hz, 3-H$_{ax}$), 4.77 (1H, dd, J=4.6, 11.0 Hz, 2-H$_{eq}$), 4.95 (1H, dd, J=11.0, 4.2 Hz, 2-H$_{ax}$), 5.61 (1H, d, J=10.0 Hz, 5″-H), 6.45 (1H, dd, J=8.4, 2.5 Hz, 5′-H), 6.46 (1H, d, J=8.8 Hz, 6-H), 6.49 (1H, d, J=2.5 Hz, 3′-H), 6.65 (1H, d, J=10.0 Hz, 4″-H), 7.34 (1H, d, J=8.4 Hz, 6′-H), 7.73 (1H, d, J=8.8 Hz, 5-H), 8.40 (2H, br s, disappeared with the addition of D_2O, –OH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: see Table 1. EI-MS m/z (rel. int., %): 352 (M^+, 49), 337 (M^+-CH_3, 27), 334 (39), 319 (66), 203 (66), 187 (100). HR-MS m/z: 352.1319 (Calcd for $C_{21}H_{20}O_5$: 352.1311).

Glabrocoumarin (**3**): Slightly yellow needles from benzene–acetone, mp 254—256 °C. IR (KBr) cm^{-1}: 3360, 1686, 1596, 1460, 1437. UV λ_{max} nm (log ε): 206 (4.60), 286 (4.15), 341 (4.33). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 1.38 (6H, s, –CH_3), 5.66 (1H, d, J=9.9 Hz, 5″-H), 6.47 (1H, d, J=8.4 Hz, 5′-H), 6.71 (1H, d, J=9.9 Hz, 4″-H), 6.77 (1H, d, J=2.3 Hz, 8-H), 6.85 (1H, dd, J=8.4, 2.3 Hz, 6-H), 7.08 (1H, d, J=8.4 Hz, 6′-H), 7.52 (1H, d, J=8.4 Hz, 5-H), 7.76 (1H, s, 4-H), 9.0 (2H, br s, disappeared with the addition of D_2O, –OH). ^{13}C-NMR (100 MHz, acetone-d_6) δ: see Table 1. EI-MS m/z (rel. int., %): 336 (M^+, 47), 321 (M^+-CH_3, 100), 293 (M^+-CH_3-CO, 20). HR-MS m/z: 336.0995 (Calcd for $C_{20}H_{16}O_5$: 336.0998).

Conversion of Glabrocoumarin into Glabrocoumarone A Dimethylether (4b) To the ice-cold solution of Glabrocoumarin (**3**; 24 mg) in 5 ml of methanol was added a diazomethane solution in diethylether, the excess of which was quenched by the addition of formic acid. The mixture was evaporated to dryness *in vacuo*. The residue was dissolved in dry diethylether (10 ml), which was then added dropwise to the ice-cold and well-stirred solution (20 ml) of aluminum hydride prepared *in situ* from a 3 to 1 molar ratio of lithium aluminum hydride (38 mg) and aluminum chloride (44 mg) in dry diethylether. After the mixture was allowed to stand for 2 h, it was treated by the successive addition of 150 μl of H_2O, 50 μl of 15% sodium hydroxide solution and 150 μl of H_2O. The procedure of this step followed Micovic's method.[14] After 1 g of anhydrous sodium sulfate powder was added to the reaction mixture, the resulting precipitate was filtered off and washed with dry diethylether. The filtrate was combined and evaporated to dryness. The residue was dissolved in dry benzene (10 ml) and 16.2 mg (1.1 eq) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was added. The mixture was refluxed for 3 h, and the resulting precipitate was filtered off and the filtrate was concentrated to about 3 ml, which was loaded onto a short silica gel column on elution with benzene to furnish the reaction product in amorphous form (9.4 mg). It was identical to glabrocoumarone A dimethylether (**4b**) in ^{1}H- and ^{13}C-NMR spectra.

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