A Novel Biflavonoid from Roots of *Glycyrrhiza uralensis* Cultivated in China

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Received April 8, 2003; accepted May 7, 2003; published online July 15, 2003

A novel biflavonoid named licobichalcone was isolated from the roots of *Glycyrrhiza uralensis* cultivated in China, along with twelve known compounds, including five chalcones, two isoflavones, two flavanones, two flavones and one pterocarpan. Their structures were respectively elucidated on the basis of chemical and spectroscopic evidence.

Key words licorice; *Glycyrrhiza uralensis*; flavonoid; biflavonoid; licobichalcone

Licorice, the roots and rhizomes of some *Glycyrrhiza* species (Leguminosae) has been used by human being for at least 4000 years. Clinical studies of licorice make it one of the most thoroughly studied herbs, which includes antimutagenic activity,[1] anti-ulcer effect,[2] protective action for hepatotoxicity,[3] antitumor promoting activity,[4] anti-caries effect,[5] antimicrobial effect,[6] *etc*. Much of the recent research on licorice constituents has indicated the pharmacological importance of phenolic compounds. Until now, many studies on the flavonoid constituents of *Glycyrrhiza* species have been carried out, and about sixty phenolic compounds have been isolated from the underground parts of *G. uralensis*, one of the most important species of licorice.[7]

The natural resource of wild *Glycyrrhiza uralensis* is about exhaustion. So this plant is cultivated in a large scale in the Inner Mongolia area, China. Yet, there is still no report on chemical studies of the cultivated licorice. We report here the results of a phytochemical investigation of the phenolic constituents of the roots of *Glycyrrhiza uralensis*, which were cultivated in Inner Mongolia, China. A new biflavonoid named licobichalcone was isolated together with twelve known flavonoids, licochalcone B,[8] formononetin,[9] isoliquiritigenin,[10] liquiritin,[11] glabrone,[12] licochalcone A,[8] echinatin,[13] licoflavone A,[10] liquiritigenin,[10] 4′,7-dihydroxyflavone,[10] medicarpin 3-*O*-β-D-glucopyranoside,[14] and isoliquiritin.[10] The structures of known compounds were determined by analysis of the physical and spectroscopic evidence, and confirmed by comparing with the data of literatures.

Licobichalcone (**1**) was obtained as a yellow powder. Its molecular formula, $C_{32}H_{26}O_{10}$, was established by high resolution (HR)-FAB-MS spectrum. The IR spectrum showed absorption bands at 3211 and 1654 cm^{-1} due to hydroxyl and carbonyl groups, respectively. The ^{1}H-NMR spectrum of **1** (Table 1, CD_3OD) revealed the presence of four aromatic rings, namely rings A and C with the resonance for AA′XX′-type aromatic protons at δ 8.19 and 6.90, and δ 7.46 and 6.80, respectively, ring B with the resonance for *ortho*-coupled aromatic protons at δ 6.38 and 5.98, and ring E with the resonance for an isolated aromatic protons at δ 6.67. Furthermore, an isolated methine proton at δ 7.28, two coupled methine protons at δ 4.98 and 4.83, and two methoxyl groups at δ 3.90 and 3.32 were also observed. The ^{13}C-NMR spectrum showed 32 carbon signals (Table 1). All protonated carbons were then assigned by ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) experiment. Two carbonyl carbons at δ 199.0 and 197.6 were assigned to be linked to C-10 of ring A and C-10′ of ring C by observing the ^{1}H–^{13}C long-range correlations between H-11 (δ 8.19) and C-1 (δ 199.0), H-11′ (δ 7.46) and C-1′ (δ 197.6) respectively. Taking account the 20 degrees of unsaturation calculated from the empirical formula of **1**, it was suggested that **1** has at least another ring system with one more unsaturated double bond. The heteronuclear multiple bond connectivity (HMBC) experiment (Fig. 1) established the structure of ring D. Two methoxyl groups were assigned to be located in C-5 and C-6′ by observing of the correlations between their protons and chemical shift assigned carbons of C-5 and C-6′, respec-

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1**

Position	DEPT	δ_C	δ_H, *J* in Hz
1	C	199.0	
2	CH	50.5	4.83, d, 1.2
3	CH	35.8	4.98, d, 1.2
4	C	127.8	
5	C	147.0	
6	C	139.7	
7	C	146.6	
8	CH	111.4	6.38, d, 8.5
9	CH	120.1	5.98, d, 8.5
10	C	128.9	
11, 15	CH	132.9	8.19, d, 9.0
12, 14	CH	116.1	6.90, d, 9.0
13	C	163.7	
1′	C	197.6	
2′	C	123.2	
3′	CH	144.6	7.28, s
4′	C	126.4	
5′	C	133.1	
6′	C	147.1	
7′	C	142.5	
8′	C	146.6	
9′	CH	113.3	6.67, s
10′	C	130.7	
11′, 15′	CH	132.7	7.46, d, 8.7
12′, 14′	CH	115.9	6.80, d, 8.7
13′	C	162.7	
5-OCH_3	CH_3	60.6	3.32, s

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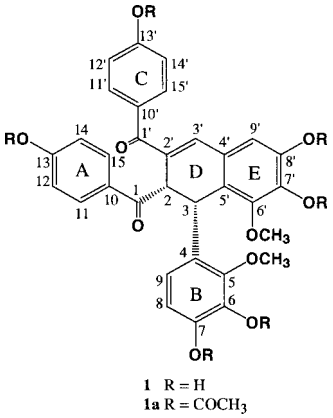

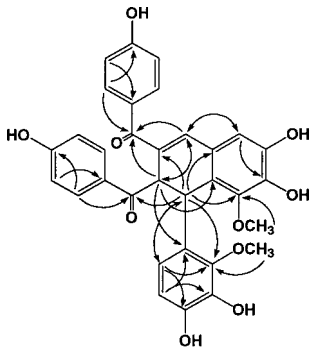

Fig. 1. Key ^{1}H–^{13}C long-Range Correlations by the HMBC Spectrum of **1**

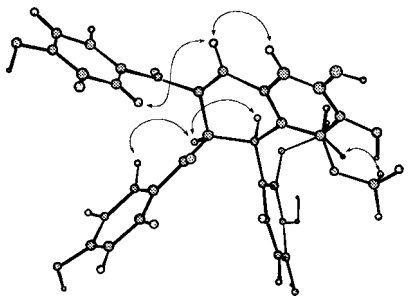

Fig. 2. Key NOEs Observed by NOESY Spectrum of **1**

tively. Per-acetylation of **1** gave **1a**, which showed 6 additional phenolic acetyl groups than **1**, supported the presence of six phenolic hydroxyls in **1**.

The relative stereochemistry between H-2 and H-3 was assigned by a combination of two-dimensional nuclear Overhauser effect spectroscopy (2-D NOESY) data, analysis of coupling constants, and molecular modeling studies performed on **1**. H-2 and H-3 were observed as doublets with a coupling constant of 1.2 Hz, indicating that the dihedral angle between H-2 and H-3 is about 60°, such small coupling indicates an axial/equatorial or equatorial/equatorial arrangement of these protons. In the case of H-2 and H-3 to be *trans*-configuration, molecular modeling studies performed on a Silicon graphics R5000 workstation using the Discover program within Insight II package,[15] suggested the existence of the H-2 and H-3 both pseudoaxial arrangements for conformational minimum. By this arrangement, a dihedral angle between H-2 and H-3 was predicted to be near 180 ° which requires a reasonable coupling. However, in the case of H-2 and H-3 to be *cis*-configuration, the same dihedral angle was measured 60.07 ° which is in agreement with the small coupling between H-2 and H-3. This assumption is supported by NOE correlations between H-2 and H-3. Therefore, the relative stereochemistry between H-2 and H-3 was proposed to be *cis*-configuration.

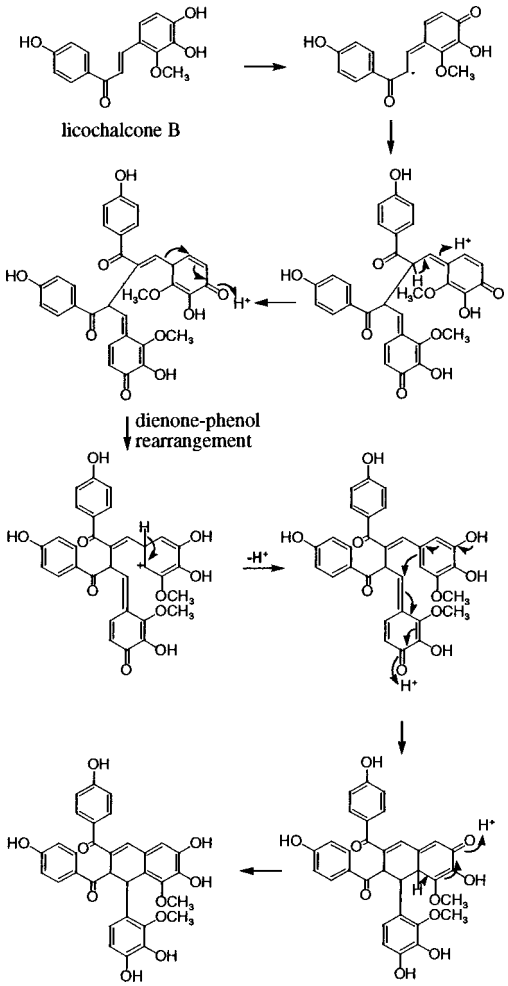

Fig. 3. Hypethetical Biosynthesis Pathway for the Formation of **1** from Licochalcone B

Licobichalcone (**1**) was found to be racemate, since the optical rotation was zero and the circular dichroism (CD) spectrum exhibited no Cotton effect. Racemic biflavonoids were also reported from the hairy root cultures of *G. glabra*, another important species of licorice.[15,16] We proposed here a possible route for the biosynthesis of **1** from licochalcone B, one main metabolite of *G. uralensis*, by radical reaction (Fig. 3).

Experimental

General Experimental Procedures UV spectra were obtained with a SHIMADZU UV-2201 spectrophotometer, whereas the IR spectra were measured with a BRUKER IFS 55 spectrometer. Electrospray ionization mass spectroscopy (ESI-MS) was taken on a LCQ mass system. High-resolution FAB-MS was taken on a JEOL JMS-AX505HA. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL ECP-500 spectrometer. Molecular modeling was carried out on a Silicon graphics R5000 workstation using the Discover program within Insight II package (version 97.2; Molecular Simulations, Inc.: San Diego, CA, U.S.A.).

Plant Material The roots of *Glycyrrhiza uralensis* F. were collected in April 2000 from Dongsheng of Inner Mongolia of China. Voucher speci-

mens are deposited in the Department of Phytochemistry, Shenyang Pharmaceutical University.

Extraction and Isolation The air-dried roots (5 kg) were extracted twice with hot water (30 l each). The extract fractions were combined, evaporated and freeze dried to gave the residue (1000 g). Partial of this residue (800 g) was partitioned between EtOAc and water. Removal of the solvent from EtOAc phase yielded the EtOAc soluble extract (45.5 g). The EtOAc soluble fraction was subjected to normal-phase silica gel column, and eluted with petroleum ether, followed by a gradient of EtOAc up to 100% and then with $CHCl_3$, followed by a gradient of MeOH to 50% to give sixteen fractions. Further separation of fr. 5 by repeated silica gel, Sephadex LH-20, and ODS column chromatography gave glabrone (35 mg) and formononetin (6 mg), of fr. 7 gave isoliquiritigenin (5 mg) and liquiritigenin (22 mg), of fr. 9 gave licochalcone A (48 mg), licochalcone B (50 mg), licoflavone A (39 mg) and echinatin (6 mg), of fr. 11 gave 4′,7-dihydroxyflavone (10 mg), medicarpin 3-*O*-*β*-D-glucopyranoside (5 mg) and licobichalcone (**1**, 60 mg), of fr. 12 gave isoliquiritin (15 mg), and of fr. 13 gave liquiritin (212 mg).

Licobichalcone (**1**): Yellow powder; $[\alpha]_D^{24}$ ±0° (c=0.7, MeOH); UV (MeOH) λ_{max} (log ε) 370 (4.14), 284 (4.37), 266 (4.37), 204 (4.77) nm; IR (KBr) ν_{max}, 3211, 1654, 1607, 1582, 1512, 1377, 1322 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD), given in Table 1; ESI-MS (positive) *m*/*z* 571.1 $[M+H]^+$; HR-FAB-MS (positive) *m*/*z* 571.1595 $[M+H]^+$ (Calcd for $C_{32}H_{27}O_{10}$, 571.1604).

Acetylation of 1 A mixture of **1** (12 mg), acetic anhydride (4 ml) and pyridine (2 ml) was left to stand overnight at room temperature, and then the solvent was removed. The residue was partitioned between water and $CHCl_3$. The $CHCl_3$ extract was subjected to ODS open column chromatograph, eluted with 50% MeOH to give **1a** (7 mg): ^{1}H-NMR (500 MHz, $CDCl_3$): δ: 8.18 (2H, d, J=8.7 Hz, H-11 and H-15), 7.65 (2H, d, J=8.7 Hz, H-11′ and H-15′), 7.37 (1H, s, H-3′), 7.22 (2H, d, J=8.7 Hz, H-12 and H-14), 7.15 (2H, d, J=8.7 Hz, H-12′ and H-14′), 7.07 (1H, s, H-9′), 6.80 (1H, d, J=8.7 Hz, H-8), 6.56 (1H, d, J=8.7 Hz, H-9), 5.07 (1H, br s, H-3), 4.98 (1H, d, J=0.9 Hz, H-2), 3.76, (3H, s, 6′-OCH_3), 3.36 (3H, s, 5-OCH_3), 2.23, 2.26, 2.27, 2.31, 2.32, 2.36 (each 3H, s, $OCOCH_3$×6); ^{13}C-NMR (125 MHz, $CDCl_3$): 196.1 (C-1), 194.4 (C-1′), 168.9, 168.8, 167.9, 167.8, 167.6, 167.2 (–OCO of –$OCOCH_3$×6), 154.5 (C-13), 153.7 (C-13′), 150.7 (C-5), 150.0 (C-6′), 143.0 (C-8′), 142.6 (C-7), 140.5 (C-3′), 137.6 (C-7′), 136.1 (C-6), 134.9 (C-10′), 134.4 (C-5′), 133.4 (C-10), 133.3 (C-4), 131.2 (C-4′), 131.0 (C-11, C-15), 130.9 (C-11′, 15′), 127.2 (C-2′), 125.7 (C-9), 121.7 (C-12, C-14), 121.6 (C-12′, C-14′), 119.2 (C-9′), 118.2 (C-8), 61.0 (5-OCH_3), 60.8 (6′-OCH_3), 48.1 (C-2), 33.8 (C-3), 21.2, 21.2, 20.8, 20.7, 20.5, 20.3 (–OCH_3 of –$OCOCH_3$×6); ESI-MS *m*/*z* 845 $[M+Na]^+$.

Acknowledgments The authors are grateful to Dr. Y. Asada, School of Pharmaceutical Sciences, Kitasato University, for valuable discussions on the possible biosynthesis of licobichalcone.

References

1) Zani F., Cuzzoni M. T., Daglia M., Benvenuti S., Vampa G., Mazza P., *Planta Med.*, **59**, 502—507 (1993).
2) Takagi K., Ishii Y., *Arzneim. Forsh.*, **17**, 1544—1547 (1967).
3) Nose M., Ito M., Kamimura K., Shimizu M., Ogihara Y., *Planta Med.*, **60**, 136—139 (1994).
4) Nishino H., Yoshioka K., Iwashima A., Takizawa H., Konishi S., Okamoto H., Okabe H., Shibata S., Fujiki H., Sugimura T., *Jpn. J. Cancer Res.*, **77**, 33—38 (1986).
5) Suido H., Makino T., Yamane Y., Naeshiro H., Jpn. Kokai Tokkyo Koho JP 04, 164021 [92, 164021] (cl.A61K7/26), 09 Jun 1992, Appl. 90/289447, 26 Oct. 1990; 5 pp. [*Chem. Abstr.*, **117**, 157447*h* (1992)].
6) Haraguchi H., Tanimoto K., Tamura Y., Mizutani K., Kinoshita T., *Phytochemistry*, **48**, 125—129 (1998).
7) Nomura T., Fukai T., "Fortschritte der Chemie Organischer Naturstoffe," Vol. 73, ed. by Herz W., Kirby G. W., Moore R. E., Steglich W., Tamm C., Springer-Verlag, New York, 1998, pp. 1—140.
8) Saitoh T., Shibata S., *Tetrahedron Lett.*, **50**, 4461—4462 (1975).
9) Hatano T., Kagawa H., Yasuhara T., Okuda T., *Chem. Pharm. Bull.*, **36**, 2090—2097 (1988).
10) Chang X. R., Xu Q. H., Zhu D. Y., Song G. Q., Xu R. S., *Acta Pharmaceutica Sinica*, **18**, 45—50 (1983).
11) Yang S. L., Liu Y. L., *Acta Botanica Sinica*, **30**, 176—182 (1988).
12) Kinoshita T., Saitoh T., Shibata S., *Chem. Pharm. Bull.*, **24**, 991—994 (1976).
13) Ayabe S., Kobayashi M., Hikichi M., Matsumoto K., Furuya T., *Phytochemistry*, **19**, 2179—2183 (1980).
14) Kitagawa I., Chen W. Z., Hori K., Harada E., Yasuda N., Yoshikawa M., Ren J. L., *Chem. Pharm. Bull.*, **42**, 1056—1062 (1994).
15) Insight II, version 97.2; Molecular Simulations, Inc.: San Diego, CA, U.S.A.
16) Asada Y., Li W., Yoshikawa T., *Phytochemistry*, **50**, 1015—1019 (1999).
17) Li W., Asada Y., Yoshikawa T., *Phytochemistry*, **55**, 447—456 (2000).