Antibacterial Compounds from Glycyrrhiza uralensis

Jian He,† Li Chen,† David Heber,‡ Wenyuan Shi,† and Qing-Yi Lu*†

School of Dentistry and Dental Research Institute, University of California, Los Angeles, California 90095, and Center for Human Nutrition, David Geffen School of Medicine, University of California, Los Angeles, California 90095

Received June 3, 2005

From the roots of Glycyrrhiza uralensis, two new pterocarpenes, glycyrrhizol A (1) and glycyrrhizol B (2), along with four known isoflavonoids, 5-O-methylglycryol (3), isoglycrylor (4), 6,8-diisoprenyl-5,7,4′-trihydroxyisoflavone (5), and gancaonin G (6), were isolated using a bioassay-guided fractionation method. The structures of the new compounds (1 and 2) were elucidated by spectroscopic data interpretation. The known compounds (3–6) were identified by comparison of their spectroscopic data with reported values in the literature. Glycyrrhizol A (1) and 6,8-diisoprenyl-5,7,4′-trihydroxyisoflavone (5) exhibited potent antibacterial activity against Streptococcus mutans with minimum inhibitory concentrations of 1 and 2 μg/mL, respectively, while glycyrrhizol B (2) and gancaonin G (6) showed more moderate activity.

The genus Glycyrrhiza consists of about 30 species,1 of which some have been used by humans for over 4000 years.2 Licorice is the name applied to the roots and stolons of Glycyrrhiza species (Fabaceae). Glycyrrhiza uralensis Fisch. ex DC. (Chinese name “Gancao”, or Chinese licorice) is one of the most frequently used traditional medicines in mainland China and in some other countries as well. Over the past decades, many research groups have investigated its chemical constituents3–8 and biological activities.1,9–13

Previous chemical studies have led to the identification of about 100 phenolic compounds, many of which are isoprenoid-substituted phenols.4,5 Most of these isoprenoid-substituted flavonoids are isoflavans with oxygen substituted at the C-5 position. Some of these flavonoids have shown inhibitory activities against bacterial growth.10–12

Streptococcus mutans (S. mutans) is an oral pathogen responsible for the initiation and progression of dental caries.14 There is an urgent need for new antimicrobial compounds that can inhibit S. mutans effectively.14,15 In our previous studies, we have screened over 1000 Chinese medicinal herbs for inhibitory activities against S. mutans and other pathogens.16,17 Recently, we have found that an ethanolic extract from the roots of Glycyrrhiza uralensis exhibited strong antibacterial activity against S. mutans. Following a bioassay-guided isolation procedure, two new pterocarpenes were identified, namely, glycyrrhizol A (1) and glycyrrhizol B (2), along with the previously known isoflavonoids 5-O-methylglycryol (3),4 isoglycrylor (4),6 6,8-diisoprenyl-5,7,4′-trihydroxyisoflavone (5),18 and gancaonin G (6).5 The antibacterial activity of compounds 1–6 was evaluated against S. mutans quantitatively.

Extraction of the air-dried ground roots of G. uralensis with 95% ethanol followed by solvent–solvent partitioning resulted in the localization of the active components in the CHCl3 fraction. Chromatography of this fraction on a Sephadex LH-20 column followed by column chromatography over silica gel and preparative TLC furnished six compounds (1–6). The structures of the known compounds (3–6) were determined by comparison of their spectroscopic data with reported values in the literature.6,8,18

Glycyrrhizol A (1) was obtained as a pale orange solid with a molecular formula of C26H28O5, as determined from its HRMS, APCIMS, and 13C NMR data. The molecular formula indicated 13 degrees of unsaturation within the molecule. Moreover, two 3,3-dimethylallyl substituents were observed from four sp2-hybridized carbons, along with two methylene and four methyl signals in the NMR spectra (1H, 13C NMR, HMBC, and HMQC, see Experimental Section). The UV absorptions at 338 and 354 nm, an oxygenated methylene at δ 5.45 (2H, s, C-2) in the 1H NMR spectrum and 65.0 (C-2), 106.7 (C-3), and 145.7 (C-4) were evident from the MS data and NMR spectra (see Experimental Section). The presence of two hydroxyls, one methoxy, and two 3,3-dimethylallyl substituents was evident from the MS data and NMR spectra (see Experimental Section). Three aromatic proton singlets at δ 7.10, 7.08, and 6.31 appeared in the 'H NMR spectrum, together with the biogenetically

---

* To whom correspondence should be addressed. Tel: (310) 825-3126. Fax: (310) 206-5264. E-mail: qylu@mednet.ucla.edu.
† School of Dentistry and Dental Research Institute.
‡ Center for Human Nutrition, David Geffen School of Medicine.
expected oxygenation at C-5, C-7, and C-4',17,19,21,22 indicating that the 3,3-dimethylallyl groups could be placed at C-6, C-8, or C-5'. This was resolved from the HMBC NMR spectrum, which showed correlations of the H-4 at δ 3.41 with C-5 (δ 153.0) and C-7 (δ 156.2); H-8 at δ 6.31 with C-4a (δ 104.6), C-6 (δ 114.3), C-7 (δ 156.2), and C-8a (δ 153.8); and H-2 at δ 5.45 with C-8a, allowing for the location of one 3,3-dimethylallyl group located at C-6. Additionally, the NOESY NMR correlations of the methoxy protons with H-4' and H-2'' and the HMBC correlation of the OCH₃ signal with C-5 were used to assign the substitution of this methoxy group at C-5. Finally, HMBC correlations between the methylene protons of H-4'' with C-4' and C-6' and the NOESY correlations between H-2 and H-6' and between H-6' and H-1' revealed the connectivity of the second 3,3-dimethylallyl group. Thus, on the basis of these spectroscopic data, the structure of glycyrrhizol A (1) as shown.

Figure 1. Selected HMBC correlations observed for 1 (from H to C).

Figure 2. Key NOE correlations observed for 1.

an analysis of the NOESY and HMBC spectra. A cross-peak shown in the NOESY spectrum between OCH₃ and H-6 and HMBC correlations observed between H-6 and C-7 and between H-8 and C-7, as well as the weak HMBC correlations of H-2 and C-8a and of H-8 and C-8a, suggested the substitution of the A ring, as shown. Accordingly, the structure of glycyrrhizol B (2) was assigned as 7-hydroxy-5-methoxy-2',2''-dimethyl-2H-pyran[3',4',5',6']-pterocarpane.

The antibacterial activity of all isolated compounds was tested against the oral Gram-positive bacterium S. mutans. The MIC (minimal inhibitory concentration) value determined for each compound was as follows: glycyrrhizol A (1), 1 μg/mL; glycyrrhizol B (2), 32 μg/mL; 5-O-methylglycyrrhizin (3), 500 μg/mL; isoglycyrrhizin (4), 500 μg/mL; 6,8-diisoprenyl-5,7,4'-trihydroxyisoflavone (5), 2 μg/mL; and ganciclovir (6), 125 μg/mL.

Licorice products are used worldwide as flavoring and sweetening agents in tobaccos, chewing gums, candies, beverages, and toothpaste. In some countries, Glycyrrhiza species including Glycyrrhiza uralensis is one of the oldest and most frequently employed traditional herbal medicines. Glycyrrhiza species produce a number of phytochemicals including essential oils, triterpenoids, alkaloids, phenols, polyamines, and polysaccharides, which may contribute to their many pharmaceutical effects such as anti-inflammatory, antiviral, antiulcer, and anticarcinogenic activities. In addition to these effects, flavonoids isolated from licorice were recently reported to have antimicrobial activities against methicillin-sensitive Staphylococcus aureus (MSSA), Micrococcus luteus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. In vitro inhibition of clarithromycin and amoxicillin-resistant Helicobacter pylori strains with licorice was also reported. Moreover, several isoflavones (flavonoids, pterocarpans, and pterocarpenes) from the plant were reported to have antibacterial activities against vancomycin-resistant enterococci (VRE) and methicillin-resistant S. aureus (MRSA). The present study provides further evidence that some of the isoflavones in the roots of G. uralensis possess antibacterial activities.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in CDCl₃ on a Bruker Avance-500 NMR spectrometer, with chemical shifts given in δ (ppm) with residual CDCl₃ as internal reference and coupling constants in Hz. ESIMS and APCIMS were performed on an IonSpec Ultima 7.0 T spectrometer. HREISMS were obtained on a VG Analytical Autospec spectrometer. Sephadex LH-20 (Sigma) was used for column chromatography. TLC was performed on silica gel 60 F₂₅₄ aluminum TLC sheets (E. Merck) and visualized under short (254 nm) and long (366 nm) wavelength UV light and by spraying.
with 1% anisaldehyde in H₂SO₄ followed by heating on a hot plate for 5 min. All solvents used were HPLC grade (Fishier).

**Plant Material.** Roots of *Glycyrrhiza uralensis* (3.1 kg) were air-dried and powdered. The air-dried and powdered roots of *G. uralensis* were prepared by the National Committee of Laboratory Standards (NCCLS) recommended minimum inhibitory concentration (MIC) protocol with modifications. Briefly, 2-fold dilution series were made from all tested antibacterial agents starting from 1000 µg/mL in a 96-well plate (Microtest 96 microtiter plate). *S. mutans* strain ATCC 25175 was grown at 37 °C under anaerobic conditions (10% H₂, 10% CO₂, 80% N₂) in brain heart infusion (BHI) broth media (Becton Dickinson, Sparks, MD). An aliquot of 50 µL of bacterial suspension at a concentration of 10⁶ colony-forming units/mL was added to 50 µL of antibacterial dilution. Chlorhexidine (0.12%) was used as positive control and the untreated suspension as negative control. The MIC is defined as the lowest concentration of the test agent that visibly inhibited bacterial growth after incubation at 37 °C for 16–20 h.

**Acknowledgment.** We thank Dr. L. Ma for the authentication of the plant collection. This study was supported by research grants from UCLA Center for Dietary Supplement Research: Botanicals (NIH grant AT00151) and from C3 Scientific Corporation and Biostar. L.C. was supported by a New Investigator Award (NIH grant AT00151).04.

**References and Notes**


24. Ingham, J. L.; Devick, P. M. Phytochemistry 1979, 18, 1711–1714.


