CYP3A4 Inhibitors Isolated from Licorice

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The extract of licorice (*Glycyrrhiza uralensis* FISHER, Leguminosae) showed CYP3A4 inhibitory activity with the IC_{50} value of 0.022 mg/ml. Bioassay-guided purification afforded nine compounds, 3-(*p*-hydroxyphenyl)propionic acid (1), isoliquiritigenin (2), (3*R*)-vestitol (3), licopyranocoumarin (4), 4-hydroxyguaiacol apioglucoside (5), liquiritin (6), liquiritigenin 7,4'-diglucoside (7), liquiritin apioside (8), and glucoliquiritin apioside (9). Among these compounds, 3, 7, and 5 showed potent CYP3A4 inhibitory activities with IC_{50} values of 3.6, 17, and 20 μ M, respectively. Glycyrrhizin (10), a main constituent of licorice, however, was inactive for CYP3A4 inhibition.

Key words *Glycyrrhiza uralensis*; licorice; CYP3A4 inhibitory activity

Cytochrome P450 (CYP) enzymes are responsible for metabolism of a wide range of endogenous compounds and xenobiotics, *e.g.* drug molecules, pollutants, and environmental compounds. Among members of the CYP enzyme family, CYP3A4 is the most abundant enzyme in human liver microsomes and intestinal epithelium; approximately 30% of the total CYP was suggested to be CYP3A4,¹⁾ and more than 50% of clinically used drugs are oxidized by CYP3A4.^{2,3)} It is well known that concomitant oral administration of several foods and herbs affects drug metabolism in humans by inhibiting CYP3A4 activity. In the course of our study on CYP inhibitors from foods, we have reported the isolation and structure elucidation of CYP inhibitors from grapefruit (*Citrus paradisi*) juice,^{4–6)} white pepper, *Piper nigrum*,^{7,8)} strawberry fruit, *Fragaria ananassa*,⁹⁾ and the commercially available black cohosh, *Cimicifuga racemosa*.¹⁰⁾ Licorice is prescribed in many Chinese traditional medicines and has been reported to contain flavonoids and triterpenoids.^{11–19)} Recently, we found that the extract of licorice (*Glycyrrhiza uralensis* FISHER, Leguminosae) showed potent CYP3A4 inhibitory activity with the IC_{50} value of 0.022 mg/ml. This paper reports the isolation, structure identification, and CYP inhibitory activity of the constituents of licorice.

MATERIALS AND METHODS

General Procedures Optical rotations were determined with a Horiba SEPA-300 high sensitive polarimeter. NMR spectra were recorded on a JEOL GSX500 NMR spectrometer. Mass spectra were measured on a JEOL SX-102 mass spectrometer.

Plant Materials The rhizomes of *G. uralensis* were purchased from Tochimoto Co., Ltd. (Osaka, Japan). A voucher specimen is deposited in the Laboratory of Pharmacognosy and Chemistry of Natural Products, Graduate School of Natural Science and Technology, Kanazawa University, Japan.

Isolation of Compounds 1–9 The rhizomes (0.5 kg) of *G. uralensis* were extracted with methanol (MeOH) (31×3) at room temperature. The extract was evaporated *in vacuo*, and the residue was partitioned between ethyl acetate (EtOAc) and water (H₂O). The aqueous fraction was then partitioned between butanol (BuOH) and water. The EtOAc soluble fraction (34.7 g) was subjected to column chromatography on silica gel with hexane/EtOAc followed by ODS col-

umn chromatography with MeOH/H₂O to afford 1–4 (Fig. 1). The BuOH soluble fraction (34.9 g) was subjected to column chromatography on ODS with MeOH/H₂O followed by ODS HPLC with MeOH/H₂O to afford 6 and 8. The aqueous fraction (31.3 g) was subjected to column chromatography on ODS with MeOH/H₂O followed by ODS HPLC with MeOH/H₂O to afford 5, 7, and 9.

Assay of CYP3A4 Inhibition CYP3A4 activity was measured based on nifedipine oxidation. Various amounts (0–10 μ M, final concentration) of samples in 1 μ l of dimethylsulfoxide (DMSO) were added to 192 μ l of 100 mM phosphate buffer (pH 7.4) containing 50 μ M nifedipine (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 5 mM glucose-6-phosphate (Oriental Yeast Co., Ltd., Tokyo, Japan), 0.5 mM β -NADP⁺ (Oriental Yeast Co., Ltd.), 0.5 mM MgCl₂, and 4.3 μ g/ml glucose-6-phosphate dehydrogenase (Oriental Yeast Co., Ltd.), and the mixture was incubated at 37 °C for 5 min. Human CYP3A4 (Gentest Co., Woburn, MA, U.S.A.) was also preincubated in 7 μ l of the buffer at 37 °C for 5 min and added to the above sample mixture. After incubation at 37 °C for 1 h, the reaction was quenched by the addition of 100 μ l of MeOH. After adding 3.7 μ g of 6-methoxycarbonyl-5-methyl-7-(2-nitrophenyl)-4,7-dihydrofuro[3,4-b]pyridin-1-(3*H*)-one in 1 μ l of DMSO as an internal standard, the reaction mixture was extracted with 1 ml of ether, and the ether layer was evaporated. The residue was dissolved in 100 μ l of MeOH, and an aliquot (20 μ l) was analyzed by reverse-phase HPLC (column, TSK-gel ODS-120T, 4.6 mm i.d.×150 mm; mobile phase, 64% MeOH–H₂O; flow rate, 1.0 ml/min; detection, UV 254 nm); retention times: 2.9 min for the internal standard, 4.0 min for the nifedipine metabolite (nifedipine pyridine), and 5.5 min for nifedipine. The value of IC_{50} , the concentration required for 50% inhibition of CYP3A4 activity, was calculated from the data of duplicate measurements.

RESULTS AND DISCUSSION

The rhizomes of *G. uralensis* were extracted with MeOH. The extract showed CYP3A4 inhibitory activity with the IC_{50} value of 0.022 mg/ml. The extract was then separated into EtOAc, BuOH, and H₂O soluble fractions. The three fractions were tested for CYP inhibitory activity and showed 97, 98, and 61% inhibition, respectively, at the concentration of

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Fig. 1. Compounds Isolated from *Glycyrrhiza uralensis*

0.25 mg/ml. The EtOAc soluble fraction of the extract was subjected to column chromatography on silica gel followed by ODS column chromatography to afford **1** (55.2 mg, $1.1 \times 10^{-2}\%$), **2** (13.4 mg, $2.7 \times 10^{-3}\%$),¹⁷⁾ **3** (4.7 mg, $9.4 \times 10^{-4}\%$),²⁰⁾ and **4** (238.0 mg, $4.8 \times 10^{-2}\%$).^{12,15)} The BuOH soluble fraction of the extract was subjected to ODS column chromatography followed by ODS HPLC to afford **6** (5.2 mg, $1.0 \times 10^{-3}\%$)¹⁴⁾ and **8** (22.9 mg, $4.6 \times 10^{-3}\%$).^{14,18)} The aqueous fraction of the extract was subjected to ODS column chromatography followed by ODS HPLC to afford **5** (2.0 mg, $4.0 \times 10^{-4}\%$),^{21,22)} **7** (45.2 mg, $9.0 \times 10^{-3}\%$),¹³⁾ and **9** (10.9 mg, $2.2 \times 10^{-3}\%$)¹⁸⁾ (Fig. 1). The structures were identified by comparison of their ¹H- and ¹³C-NMR, MS, and $[\alpha]_D$ data with the reported values. CYP3A4 inhibitory activities of compounds isolated from *G. uralensis* were measured using human CYP3A4 (Table 1). Among nine compounds isolated, **3**, **7**, and **5** showed potent CYP3A4 inhibitory activities with IC₅₀ values of 3.6, 17, and 20 μM, respectively. Glycyrrhizin (**10**) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), a main constituent of licorice, however, was a poor CYP3A4 inhibitor. Flavone glycosides containing sugar moieties at both C-7 and C-4' positions, **7** and **9**, showed more potent CYP3A4 inhibitory activities with IC₅₀ values of 17 and 87 μM, respectively, than the corresponding C-7 deglucosyl derivatives, **6** and **8** (IC₅₀, 57 and 655 μM, respectively). Compounds **8** and **9** correspond to apiosides of **6** and **7** at the C-2 position of the 4'-O-glucosyl moieties, respectively, and less hydrophilic glucosides **6** and **7** showed more potent CYP inhibitory activities than **8** and **9**, respectively. These data suggest that the hydrophilicity and bulkiness of flavone glycosides play key roles in their exhibition of inhibitory activity against CYP3A4. Although the CYP inhibitory activity of the licorice extract and glabridin, an isoflavan derivative of licorice, were investigated so far, this is the first report of the bioassay-guided isolation of CYP3A4 inhibitors from licorice.²³⁾ It is well known that CYP3A4 is involved in metabolism of a variety of endogeneous compounds and xenobiotics. Recently, three crystal structures of CYP3A4 that had been bound to the inhibitor metyrapone, bound to the substrate progesterone, and unliganded, were reported.²⁴⁾ Contrary to the previous expectation, the active site of CYP3A4 was revealed to be small with little conformational change, although CYP3A4 is involved in the metabolism of a structurally wide range of compounds. The study of the structure-activity relationship of CYP3A4 inhibitors could be helpful to understand the interaction between CYP3A4 and ligands. The fact that the IC₅₀ value of CYP3A4 inhibitory activity by the MeOH extract of licorice is 0.022 mg/ml implies that metabolism of nifedipin with a half-dose of one-day treatment is

Table 1. CYP3A4 Inhibitory Activity of Compounds Isolated from Licorice

Compd.	IC ₅₀ (μM)	Compd.	IC ₅₀ (μM)
1	48	6	57
2	29	7	17
3	3.6	8	655
4	32	9	87
5	20	10	^{b)}
Ketoconazole ^{a)}	0.11		

a) A typical CYP3A4 inhibitor. b) Compound **10** showed less than 50% inhibitory activity at the concentration of 1.2 mM.

inhibited by one-twentyfifth amount of the licorice extract contained in the traditional Chinese prescription, Kakkon-to, for one-day treatment. Thus, it is important to evaluate the IC₅₀ values of constituents in foods/herbs in order to avoid clinical risks due to interactions among orally administered drugs and concomitant foods/herbs.

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