Cytotoxic Allyl Retrochalcone from the Roots of Glycyrrhiza inflata

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Two known retrochalcones, licochalcone A (1) and licochalcone C (2), and one new retrochalcone, licochalcone E (4) were isolated by cytotoxicity-guided fractionation from the roots of Glycyrrhiza inflata along with an ordinary chalcone, isoliquiritigenin (3). The structure of the new retrochalcone was elucidated through a spectroscopic analysis.

Key words Glycyrrhiza inflata; licochalcone E; retrochalcone; cytotoxicity

Licorice is the roots and stolons of several Glycyrrhiza species (Leguminosae) that has been used worldwide since ancient times as a medicine and a sweetening agent. Traditionally they have been used in the Northeast Asia for the treatment of gastric and duodenal ulcers, bronchial asthma, inflammation, and other diseases. Much of the recent research on the constituents of licorice has resulted in finding the pharmacological importance of various phenolic compounds. Researchers identified the unusual phenolic compounds known as retrochalcones from Glycyrrhiza inflata, which are structurally distinguished from the normal chalcones by the lack of oxygen functionalities at C-2. Until now five retrochalcones, licochalcones A—D and echinatin, have been isolated and characterized from the roots of G. inflata. Licochalones A—D were shown to have various biochemical properties, e.g., antitumor, antiparasitic, antitileishmanial, antibacterial activities. They also displayed antioxidative and superoxide scavenging effects.

This paper describes the cytotoxicity-guided isolation and structure elucidation of a new retrochalcone 4.

The water extract of the roots of G. inflata was defatted with hexane and partitioned with methylene chloride, ethyl acetate, and n-butanol successively. Cytotoxicity-guided fractionation of the methylene chloride fraction led to an isolation of four compounds. Compounds 1—3 were identified as licochalcone A (1), licochalcone C (2), and isoliquiritigenin (3) by analysis of MS, 1H-, 13C-NMR, and IR spectra data of each compound and by comparison with the literature reports.

Licochalcone E (4), amorphous powder, with [α]D = −10.0° (c = 0.2, acetone), showed quite similar IR, MS, 1H-, and 13C-NMR spectra to those of licochalcone A (1) and licochalcone C (2). The high resolution (HR)-EI-MS spectrum of licochalcone E (4) showed the peak for [M]+ at m/z 338.1510 (Caled 338.1518), indicating a molecular formula of C21H22O4. The unique fragment peak at m/z 307 ([M−31]+) indicated that methoxyl group is located at C-2 position. IR spectrum showed the presence of a hydroxyl (3447 cm−1) and a conjugated carbonyl (1700, 1637 cm−1) group, and an aromatic (1585 cm−1) moiety. The 1H-NMR spectrum showed the presence of a methoxyl group [δ 3.83 (3H, s)], trans-olefinic protons [δ 7.62 (1H, d, J = 15.5 Hz, H-α)]. 13C-NMR spectrum showed the presence of a hydroxyl (3447 cm−1) and a conjugated carbonyl (1700, 1637 cm−1) group, and an aromatic (1585 cm−1) moiety. The 1H-NMR spectrum showed the presence of a methoxyl group [δ 3.83 (3H, s)], trans-olefinic protons [δ 7.62 (1H, d, J = 15.5 Hz, H-α)], and an α,β-dimethylallyl group [δ 1.34 (3H, d, J = 7 Hz, H-4')], 1.67 (3H, s, H-5'), 3.84 (1H, m, H-1'), 4.89 (2H, d, J = 16 Hz, H-3')].
values of compounds 1—4 were 57.0, 72.8, 96.8, and 45.2 μM, respectively. Licochalcone E (4) exhibited the most potent cytotoxic effect compared to the known antitumor agents, licochalcone A (1) and isoliquiritigenin (3).14,15

The total synthesis of licochalcone E (4) and the structure—activity relationship studies of allyl rhoforones on cytotoxicity are currently undergoing and the results will be published in due course.

Experimental

General Procedure  Optical rotation was measured on a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a Nicolet 520P spectrometer (Polaris/ICON). EI-MS spectra were obtained on a GC (HP6890N) spectrometer. HR-EI-MS spectrum was obtained on a GC (HP6890N)-1000 digital polarimeter. IR spectra were recorded on a Nicolet 520P spectrometer. Chemical shifts were expressed in ppm relative to TMS as the internal standard and coupling constants (J) were given in Hz. TLC was carried out on Merck silica gel F254 precoated aluminum sheets and RP-18F254 precoated glass plates. MPLC was carried out with silica gel 60 (230—400 mesh). Column chromatography was performed using Merck 60, 70—230 mesh silica gel.

Plant Material  The roots of Glycyrrhiza inflata were purchased from Chonnam Herb Association. A voucher specimen (CSH-005) was deposited in the College of Pharmacy, Chonnam National University, Gwangju, Korea.

Extraction and Isolation  Small scale isolation. The air-dried powdered Glycyrrhiza inflata roots (400 g) were extracted twice with boiling distilled water (1 l) for 2.5 h and the combined water extracts were defatted with n-hexane (300 ml). The aqueous layer was partitioned with methylene chloride (300 ml × 2), ethyl acetate (300 ml × 2), and n-butanol (300 ml × 2) successively. The methylene chloride fraction was subjected to silica gel chromatography using a n-hexane–ethyl acetate–methanol gradient system (2:1:0.1 to 100% MeOH) to provide 3 fractions (fractions 1 to 3). Fraction 1 was separated to 3 subfractions (fractions 1-1 to 1-3) by preparative TLC using a chloroform–methylene chloride (300 ml). The methylene chloride fraction was subjected to silica gel chromatography using RP18 to an analytically acceptable purity.

Compound 4: Amorphous powder. [%]D 25° = −10.0° (c=0.2, acetone). IR (neat) cm⁻¹: 3487, 1700, 1637, 1585, 1560, 1287, 1260, 1121, 1166. HR-EI-MS m/z: 338.1510 [M+] (Calcd for C17H18O2: 338.1518) EI-MS m/z: 338 [M⁺], 323, 307, 257 [M–OCH₃]⁺. 121. UV λmax (EtOH) nm (log ε): 222 (4.58), 271 (3.47), 286 (3.70), 364 (3.57), 377 (3.62). [1H]-NMR (500 MHz, acetone-d₆) δ: 1.34 (3H, d, J=7, H-4’), 1.67 (3H, s, H-5’), 3.83 (3H, s, OMe), 3.84 (1H, m, H-1’), 4.89 (2H, d, J=16, H-3’), 6.46 (6H, s, H-3’), 6.93 (2H, d, J=8.7, H-5’), 7.47 (1H, s, H-6’), 7.62 (1H, d, J=15.5, H-α), 7.96 (2H, d, J=8.7, H-2’, 6’), 8.00 (1H, d, J=15.5, H-β). [13C]-NMR (125 MHz, acetone-d₆) δ: 187.5 (C=O), 162.9 (C-4’), 159.7 (C-4), 158.8 (C-2), 149.2 (C-2’), 139.0 (C-β), 130.6 (C-2’, C-6’), 130.0 (C-1’), 128.3 (C-6), 124.3 (C-5), 118.1 (C-α), 115.5 (C-3’, C-5’), 114.9 (C-1), 109.1 (C-3’), 99.1 (C-3), 55.0 (OMe), 37.7 (C-1’), 21.5 (C-5’), 18.8 (C-4’).

MTT Assay  HT1080 cells (2×10⁴) were seeded in 96-wells and allowed to attach for 8—12 h. The media were changed to 0.5% FBS containing DMEM before reagents were added to the medium. Twenty-four hours after the addition of reagents, MTT was added to the cell culture medium with a final concentration of 0.5 mg/ml. After incubating for 4—6 h, the media were discarded. The remaining cells were lysed by addition of 100 μl dimethylsulfoxide (DMSO). Absorption was measured in a 96-well spectrophotometer using a 540 nm filter.

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References