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Licochalcone L, an undescribed retrochalcone from *Glycyrrhiza inflata* roots

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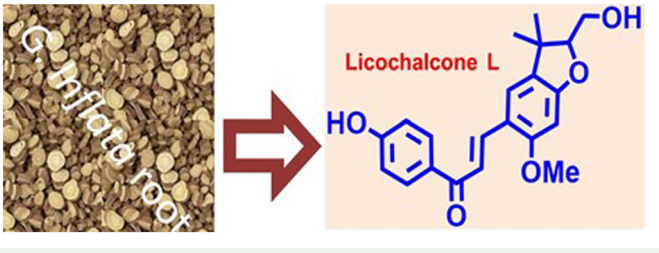
ABSTRACT

Glycyrrhiza inflata Batalin is among the three glycyrrhizin producing *Glycyrrhiza* species and can be distinguished from other species with regard to its retrochalcone contents. Seven retrochalcones, echinatin and licochalcones A, C, D, E, K, and L were isolated and characterized from the chloroform extract of *G. inflata* roots. Among the isolates, licochalcone L was found to be previously undescribed. Structure elucidation of these specialised metabolites was achieved through NMR and mass spectroscopic data analyses.



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Licorice/liquorice; *Glycyrrhiza inflata*; liquorice root; retrochalcone; licochalcone L; marker compounds

1. Introduction

Licorice/liquorice is the root of *Glycyrrhiza* species and has been described as the king of herbs by the Chinese. It has a long history of use as herbal medicine in Southern Europe and several parts of Asia such as China and India and its use as a medicine

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has been reported since 2100 BC in the Codex Hammurabi (Fenwick et al. 1990). The extreme taste of sweetness makes liquorice a popular candy, a flavoring agent, and an ingredient of various pharmaceutical products (Omar et al. 2012). Several notable *Glycyrrhiza* species such as *G. inflata* Bat., *G. glabra* L., and *G. uralensis* Fisch have been widely used for these purposes. Liquorice, an economically important medicinal plant, can be found in almost half of TCM prescriptions and is a part of many biological studies (Fenwick et al. 1990). Liquorice is reported to be effective in the treatment of asthma with coughing and unwarranted phlegm as well as in curing food and drug intoxication (Hocaoglu et al. 2011; Omar et al. 2012; Gong et al. 2015; Yang et al. 2017). Liquorice is currently harvested so that its main component glycyrrhizin can be used as a food additive and as an ingredient in tobacco, confectionery and pharmaceutical manufacturing (Fenwick et al. 1990; Haraguchi et al. 1998).

To date, over 500 chemical constituents, mainly triterpenoids and flavonoids, have been separated and identified from the *Glycyrrhiza* species. The chemical profile of liquorice can be affected by the source species as well as the geographical area. *G. inflata*, found mainly in Xinjiang, China, is one of three G*lycyrrhiza* species (*G. inflata*, *G. glabra,* and *G. uralensis*) recorded in the Chinese pharmacopoeia, and its chemical profile is not much different than other two species (Zhao et al. 2018). Nevertheless, more than 80 chemical constituents have been reported from *G. inflata,* mainly triterpenoid glycosides and flavonoids (Zheng et al. 2015; Lin et al. 2017). The chalcone core is composed of an α, β-unsaturated carbonyl system of three carbons lying between two aromatic rings. Chalcones are structurally one of the most diverse groups of flavonoids and are the biogenetic precursors of various heterocyclic compounds. Due to the significant contribution of chalcones and their derivatives in the pharmacological industry, a large number of procedures have been reported for their synthesis (Farooq and Ngaini 2019). *G. inflata* is a rich source of retrochalcones. Retrochalcones are chalcones having characteristic oxygen substitutional functional groups and structurally distinguished from typical chalcones by the lack of oxygenation at the second position of phenyl ring next to the carbonyl (Saitoh et al. 1975; Ayabe and Furuya 1981; 1982; Kajiyama et al. 1992; Lin et al. 2017). However, the primary specialized metabolite is glycyrrhizin, which is attributed to the sweet taste.

G. inflata has been part of several pharmacological studies, and various parts of the plant have shown different biological activities. For example, the roots of *G. inflata* have shown a relaxant activity in isolated mouse jejunum. Further investigation has demonstrated that licochalcone A and isoliquiritigenin were responsible for the activity, and they showed a relaxant effect in a concentration-dependent manner (Nagai et al. 2007). Besides, licochalcone A, isolated from the root of *G. inflata*, showed an apoptotic effect towards oral squamous cell carcinoma in HN22 and HSC4 cell lines. It decreased the cell viability in MTS assay in a dose- and time-dependent manner at micromolar concentration (Cho et al. 2014). Recently, anti-Alzheimer and neuroprotective activities of licochalcone B, another isolate of *G. inflata*, have been reported (Cao et al. 2020). As part of our continuing efforts to discern species-specific metabolites of broadly used plants in dietary supplements and botanical drug products under development in the United States, which can serve as 'chemical markers', the retrochalcones-targeted exploration of *G. inflata* roots was instigated. In this regard, seven

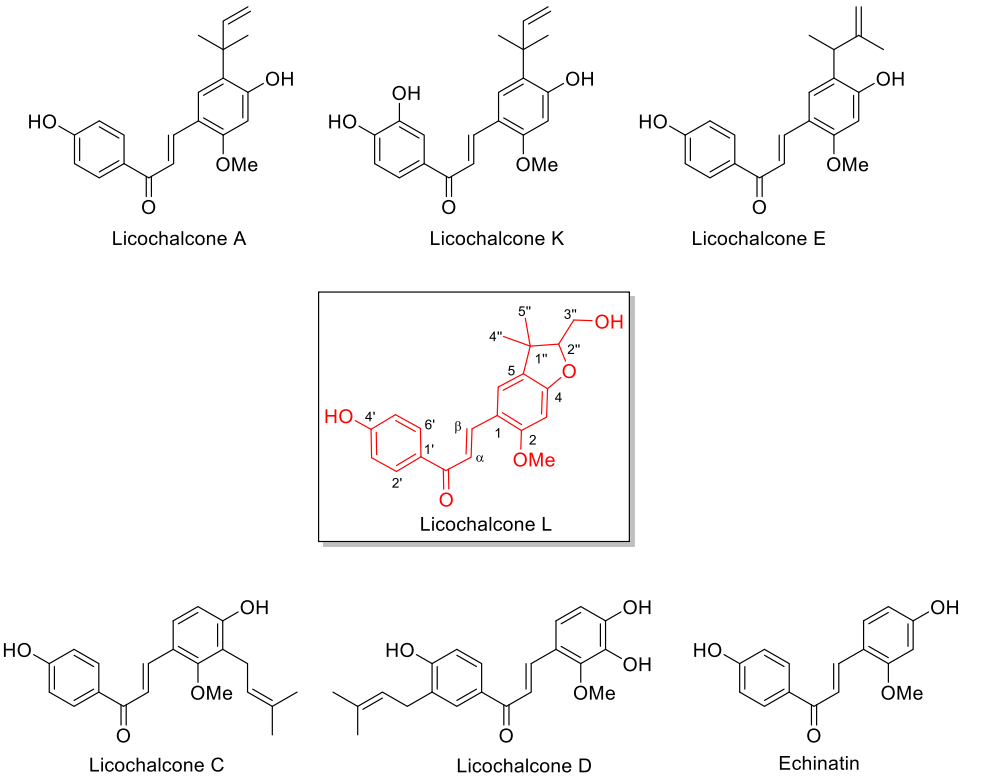

Figure 1. Structures of isolated retrochalcones.

retrochalcones were isolated and characterized by analyzing 1D- and 2D-NMR and HRESIMS data.

2. Results and discussion

To isolate retrochalcones, the chloroform was preferably selected as a choice of solvent for extracting *G. inflata* roots, which resulted in 12.3 g dried extract (2.7% yield). The unusual lower yield of chloroform extract indicated that the root is rich in polar compounds, which has also been supported by Li et al. (2016). Retrochalcones (Figure 1) were isolated from the chloroform extract using various chromatographic procedures. Previously described compounds were identified as echinatin and licochalcones A, C, D, E, and K by analyses of their NMR and mass spectral data, as well as by comparing their NMR data with those of reported in the literature (Kajiyama et al. 1992; Yoon et al. 2005; Lin et al. 2017). Licochalcone L was obtained as the yellow amorphous powder. Its molecular formula was established to be $C_{21}H_{22}O_5$ based on the $[M+H]^+$ ion peak at *m/z* 355.1554 (calcd for $C_{21}H_{23}O_5$, 355.1545) in the HRESIMS. The ^{1}H- and ^{13}C NMR data showed resonances for an aromatic AA′BB′ pattern [δ_H/δ_C (ppm) 8.02 (2H, d, $J=8.8$ Hz)/130.6 (CH-2′, 6′) and 6.95 (2H, d, $J=8.8$ Hz)/115.1 (CH-3′, 5′)], an α,β-unsaturated carbonyl functionality [δ_H/δ_C 8.12 (1H, d, $J=15.7$ Hz)/138.4 (CH-β), 7.71 (1H, d, $J=15.7$ Hz)/118.4 (CH- α), and δ_C 187.3 (CO)], and 1,2,4,5-tetrasubstituted phenyl ring [δ_H/δ_C 6.51 (1H, s, CH-3)/93.9, 6.73 (1H, s, CH-6)/121.9, δ_C 116.6 (C-

1), 160.1 (C-2), 162.0 (C-4), and 130.3 (C-5)] indicating the presence of a chalcone skeleton. In addition, the resonances for a methoxy group [δ_H/δ_C 3.91 (s, OMe)/55.4] and a prenyl group [δ_C 42.4 (C-1″), δ_H/δ_C 4.41 (1H, t, $J = 6.0$ Hz, CH-2″)/94.0, 3.86 (2H, m, CH_2-3″)/60.8, 1.43 (3H, s, CH_3-4″)/27.3, and 1.24 (3H, s, CH_3-5″)/22.4] were observed. The HMBC correlations (Figure S1, Supplementary material) of 1,4-disubstituted phenyl ring protons (H-2′/6′) with carbonyl and of olefin protons with carbons of pentasubstituted-phenyl ring demonstrated a retrochalcone skeleton where the oxo group was linked to phenyl ring lacking oxygenation at the second position in contradiction with normal chalcone (Kajiyama et al. 1992; Lin et al. 2017). The methoxy and prenyl groups were located at C-2 and C-5, respectively, based on the HMBC correlations (Figure S1, Supplementary material) as in licochalcone A (Kajiyama et al. 1992). The NMR spectroscopic data of licochalcone L were similar to those of licochalcone A, except for the resonances of a terminal olefin wherein the prenyl group of licochalcone A were exchanged with those of oxy-methine (δ_H/δ_C 4.41/94.0, CH-2″) and oxy-methylene (δ_H/δ_C 3.86/60.8, CH_2-3″) in licochalcone L, suggesting the epoxidation of the olefin moiety, followed by ring-opening of oxirane to form 5-*exo*-*tet*-cyclization to furnish substituted furano moiety. The pattern of furano moiety in licochalcone L was confirmed by ^{1}H-^{1}H-COSY couplings between oxy-methine (H-2″) and oxy-methylene (H_2-3″) protons as well as by HMBC correlations (Figure S1, Supplementary material) of oxy-methylene protons with C-1″ (δ_C 42.4) and C-5 (δ_C 130.3) and two methyl protons (CH_3-4″, 5″) with C-2″ (δ_C 94.0). An ether bridge between C-2″ and C-4 was evident from the HMBC correlation of oxy-methine proton (H-2″) and C-4 (δ_C 162.0) resulting in a dihydrofurano moiety which was in agreement with 11 degrees of unsaturation dictated by the molecular formula of licochalcone L. Ultimately, the structure of licochalcone L was elucidated as shown in Figure 1. It is worth mentioning that the structure of licochalcone L was previously mentioned as a metabolite in hepatic metabolism of licochalcone A which was tentatively identified based solely on the mass fragmentation pattern (Huang et al. 2017).

3. Experimental section

3.1. General experimental procedures

MS data were acquired on an Agilent Technologies 6230 ToF mass spectrometer. 1D- and 2D-NMR spectra were recorded on an Agilent DD2-500 NMR spectrometer. A Varian Cary 50 Bio UV-visible spectrophotometer was used to record UV-visible spectra. IR spectra were recorded on an Agilent Technologies Carry 630 FTIR. The specific rotation was measured on AUTOPOL IV Automatic Polarimeter (Rudolph, Hackettstown, NJ). Column chromatography (CC) was performed over flash silica gel (32-63μ, Dynamic Adsorbents Inc), reversed-phase C_{18} silica (Polar bond, J. T. Baker), and Sephadex LH-20 (Sigma). High-performance liquid chromatography was performed on JAI LaboACE LC-5060 system. Flash chromatography was performed using Isolera Four, Biotage, USA. Analytical TLC was performed on silica gel F_{254} aluminum sheet (20 × 20 cm, Fluka) or silica 60 RP-18 F_{254} S aluminum sheet (20 × 20 cm, Merck). Preparative TLC was performed on silica gel GF plate with UV_{254} (500 μm, 20 × 20 cm, Uniplate-Analtech). The detection was performed at UV-254 nm. Spots were visualised

by spraying with 0.5% vanillin (Sigma) solution in conc. H_2SO_4–EtOH (5:95) followed by heating. Analytical grade solvents (Fisher Chemicals) were used for extraction and purification.

3.2. Plant material

The roots of *G. inflata* were obtained from a commercial source. The authenticity of the plant material was confirmed by co-TLC of its methanolic extract with that of a reference plant material from the repository at the National Center for Natural Products Research, University of Mississippi (voucher # 21961). A sample specimen of the purchased material (# 9330) was deposited in the repository at the National Center for Natural Products Research, School of Pharmacy, University of Mississippi.

3.3. Extraction and isolation

The powder of *G. inflata* roots (450 g) was extracted with chloroform (2 L × 6 h) including 1 h sonication at room temperature and the process was repeated three times. The soluble part was concentrated to afford a residue (12.3 g, 2.7% yield), which was subjected to column chromatography (CC) over Sephadex LH-20 (30″ × 2″) using methanol as solvent. Based on the TLC profile, the resulting fractions (25 mL each) were pooled into four collective fractions (A-D). Fr. B (6.5 g) was chromatographed on Sephadex LH-20 (30″ × 2″) using methanol as the solvent. The resulting fractions (25 mL each) were pooled into five collective fractions (B1-B5). Eight fractions (B2a-B2h) were obtained from Fr. B2 (3.3 g) as a result of CC [silica gel (42″ × 2″), chloroform/methanol (19:1), fraction size 25 mL]. Licochalcone K (rt. 38 min., 5.5 mg) from Fr. B2f (64.3 mg) and licochalcone D (rt. 34 min., 54.7 mg) from combined Frs. B2g and B2h (222.0 mg) were purified by HPLC [silica gel column (6 μm, 100 Å, 300 mm × 19 mm), ethyl acetate/hexanes (from 10:90 to 50:50 in 50 min.), flow rate (10 mL per min.), UV 254 nm, and injection amount (about 25 mg)]. Fr. B2d (550.0 mg) was subjected to CC [silica gel (36″ × 0.8″), dichloromethane/methanol (49:1), fraction size 25 mL] to get nine fractions (B2d1-B2d9). Licochalcone A (20.0 mg) from Fr. B2d1 (25.1 mg) and licochalcone L (4.0 mg) from Fr. B2d9 were purified by preparative thin-layer chromatography (PTLC) [silica gel, hexanes/acetone (3:2) and hexanes/ethyl acetate (1:1), respectively]. Licochalcones C and E (13.8 mg each) were obtained from Fr. B2d2 (201.7 mg) by Biotage flash chromatography [SNAP25g silica gel cartridge, chloroform/ethyl acetate gradients (100:0 to 40:60), flow rate 75 mL/min., UV 254, and fraction size 20 mL] followed by PTLC [silica gel, hexanes/ethyl acetate (1:1)]. Echinatin (28.0 mg) was purified from Fr. B3 (1.6 g) by CC [silica gel (42″ × 1″), chloroform/methanol (49:1)].

3.3.1. Licochalcone L

Yellow powder. $[\alpha]^{22}_{D}$ +13.0 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε) 280 (3.08); IR ν_{max} 3267, 2961, 2922, 1640, 1610, 1584, 1485, 1451, 1281, 1213, 1164, 1026 cm^{-1}; HRESIMS *m/z* 355.1554 $[M+H]^+$ (calcd for $C_{21}H_{23}O_5$, 355.1545; ^1H NMR data (acetone-d_6, 500 MHz) δ_H 8.12 (1H, d, $J = 15.7$ Hz, H-β), 8.02 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 7.73 (1H,

s, H-6), 7.71 (1H, d, $J = 15.7$ Hz, H-α), 6.95 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 6.51 (1H, s, H-3), 4.41 (1H, t, $J = 6.0$ Hz, H-2″), 3.91 (3H, s, OMe), 3.86 (2H, m, H_2-3″), 1.43 (3H, s, H_3-4″), 1.24 (3H, s, H_3-5″) (Table S1); ^{13}C NMR data (acetone-d_6, 125 MHz) δ_C 187.3 (CO), 162.0, (C-4), 161.4 (C-4′), 160.1 (C-2), 138.4 (C-β), 130.9 (C-1′), 130.6 (C-2′, 6′), 130.3 (C-5), 121.9 (C-6), 118.4 (C-α), 116.6 (C-1), 115.1 (C-3′, 5′), 94.0 (C-2″), 93.9 (C-3), 60.8 (C-3″), 55.4 (OMe), 42.4 (C-1″), 27.3 (C-4″), 22.4 (C-5″) (Table S1, Supplementary material).

4. Conclusion

In an attempt to isolate the species-specific chemical markers of *G. inflata*, targeted isolation of retrochalcones was performed on the chloroform soluble part of the *G. inflata* roots. As a result, seven retrochalcones (echinatin, licochalcones A, C, D, E, K, and L) were isolated. Among the isolates, licochalcone L was found to be previously undescribed from any source.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Ayabe S, Furuya T. 1982. Studies on plant tissue cultures. Part 36. Biosynthesis of a retrochalcone, echinatin, and other flavonoids in the cultured cells of *Glycyrrhiza echinata*. A new route to a chalcone with transposed A- and B-rings. J Chem Soc, Perkin Trans. 1(11):2725–2734.

Ayabe S-L, Furuya T. 1981. Biosynthesis of a retrochalcone, echinatin: a feeding study with advanced precursors. Tetrahedron Lett. 22(22):2097–2098.

Cao Y, Xu W, Huang Y, Zeng X. 2020. Licochalcone B, a chalcone derivative from *Glycyrrhiza inflata*, as a multifunctional agent for the treatment of Alzheimer's disease. Nat Prod Res. 34(5):736–739.

Cho JJ, Chae J-I, Yoon G, Kim KH, Cho JH, Cho S-S, Cho YS, Shim J-H. 2014. Licochalcone A, a natural chalconoid isolated from *Glycyrrhiza inflata* root, induces apoptosis via Sp1 and Sp1 regulatory proteins in oral squamous cell carcinoma. Int J Oncol. 45(2):667–674.

Farooq S, Ngaini Z. 2019. Recent synthetic methodologies for chalcone synthesis (2013–2018). COCAT. 6(3):184–192.

Fenwick G, Lutomski J, Nieman C. 1990. Liquorice, *Glycyrrhiza glabra* L.—composition, uses and analysis. Food Chem. 38(2):119–143.

Gong H, Zhang B-k, Yan M, Fang P-f, Li H-d, Hu C-p, Yang Y, Cao P, Jiang P, Fan X-r. 2015. A protective mechanism of licorice (*Glycyrrhiza uralensis*): isoliquiritigenin stimulates detoxification system via Nrf2 activation. J Ethnopharmacol. 162:134–139.

Haraguchi H, Ishikawa H, Mizutani K, Tamura Y, Kinoshita T. 1998. Antioxidative and superoxide scavenging activities of retrochalcones in *Glycyrrhiza inflata*. Bioorg Med Chem. 6(3):339–347.

Hocaoglu AB, Karaman O, Erge DO, Erbil G, Yilmaz O, Bagriyanik A, Uzuner N. 2011. Glycyrrhizin and long-term histopathologic changes in a murine model of asthma. Curr Ther Res. 72(6): 250–261.

Huang L, Nikolic D, van Breemen RB. 2017. Hepatic metabolism of licochalcone A, a potential chemopreventive chalcone from licorice (*Glycyrrhiza inflata*), determined using liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 409(30):6937–6948.

Kajiyama K, Demizu S, Hiraga Y, Kinoshita K, Koyama K, Takahashi K, Tamura Y, Okada K, Kinoshita T. 1992. Two prenylated retrochalcones from *Glycyrrhiza inflata*. Phytochemistry. 31(9):3229–3232.

Li G, Nikolic D, Van Breemen RB. 2016. Identification and chemical standardization of licorice raw materials and dietary supplements using UHPLC-MS/MS. J Agric Food Chem. 64(42): 8062–8070.

Lin Y, Kuang Y, Li K, Wang S, Ji S, Chen K, Song W, Qiao X, Ye M. 2017. Nrf2 activators from *Glycyrrhiza inflata* and their hepatoprotective activities against CCl4-induced liver injury in mice. Bioorg Med Chem. 25(20):5522–5530.

Nagai H, He JX, Tani T, Akao T. 2007. Antispasmodic activity of licochalcone A, a species-specific ingredient of Glycyrrhiza inflata roots . J Pharm Pharmacol. 59(10):1421–1426.

Omar HR, Komarova I, El-Ghonemi M, Fathy A, Rashad R, Abdelmalak HD, Yerramadha MR, Ali Y, Helal E, Camporesi EM. 2012. Licorice abuse: time to send a warning message. Ther Adv Endocrinol Metab. 3(4):125–138.

Saitoh T, Shibata S, Sankawa U, Furuya T, Ayabe S. 1975. Biosynthesis of echinatin. New biosynthetical scheme of retrochalcone. Tetrahedron Lett. 16(50):4463–4466.

Yang R, Yuan B-C, Ma Y-S, Zhou S, Liu Y. 2017. The anti-inflammatory activity of licorice, a widely used Chinese herb. Pharm Biol. 55(1):5–18.

Yoon G, Do Jung Y, Cheon SH. 2005. Cytotoxic allyl retrochalcone from the roots of *Glycyrrhiza inflata*. Chem Pharm Bull. 53(6):694–695.

Zhao K, Li J, Zhang X, Chen Q, Liu M, Ao X, Gu Y, Liao D, Xu K, Ma M, et al. 2018. Actinobacteria associated with *Glycyrrhiza inflata* Bat. Are diverse and have plant growth promoting and antimicrobial activity. Sci Rep. 8(1):1–13.

Zheng Y-F, Wei J-H, Fang S-Q, Tang Y-P, Cheng H-B, Wang T-L, Li C-Y, Peng G-P. 2015. Hepatoprotective triterpene saponins from the roots of *Glycyrrhiza inflata*. Molecules. 20(4): 6273–6283.