Arch. Pharm. Res.
DOI 10.1007/s12272-013-0222-3

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Concise synthesis of licochalcone C and its regioisomer, licochalcone H

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Received: 30 May 2013 / Accepted: 22 July 2013

Abstract Licochalone C (**7a**) is a retrochalcone isolated from *Glycyrrhiza inflata*, which shows potent antioxidant properties and inhibition of bacterial growth and cellular respiration. Biological studies have suggested that licochalcone C attenuates the lipopolysaccharide and interferon-gamma induced inflammatory response by decreasing the expression and activity of inducible nitric oxide synthase and modulating the antioxidant network activity of superoxide dismutase, catalase, and glutathione peroxidase activity. Licochalcone C also inhibits NADH-cytochrome C reductase in the membrane fraction of *Micrococcus luteus*. Since pharmacological activity studies of licochalcone C are ongoing and the yield of the compound is poor from natural product, we report a concise four step synthesis of licochalcone C (**7a**) and its regioisomer, tentatively called licochalcone H (**7b**), by employing acid-mediated Claisen-Schmidt condensation as a key step with 6 and 20 % overall yield, respectively.

Keywords Licochalcone C · Licochalcone H · Acid-mediated Claisen-Schmidt condensation · Total synthesis

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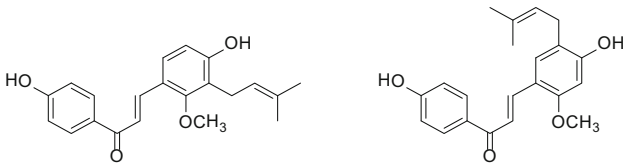
Introduction

Licorice is a popular food additive used worldwide as a sweetener in tobaccos, chewing gums, and candies, but it is also one of the most widely used medicinal herbs with anticancer (Park et al. 1998a; Yoon et al. 2007), antiparasitic (Nielsen et al. 1995a), antibacterial (Haraguchi et al. 1998b), superoxide-scavenging (Haraguchi et al. 1998a) and antioxidant activities (Nomura and Fukai 1998). Seven chalcones, licochalcones A–E, G and the closely related compound echinatin were isolated and identified from the root of *Glycyrrhiza inflata* (licorice) (Saitoh and Shibata 1975; Kajiyama et al. 1992; Yoon et al. 2005; Dao et al. 2011) except artificial synthetic licochalcone F (Na et al. 2009). Among these reported chalcones, licochalcone C (Fig. 1) exhibited a higher cytotoxicity compared to the analogous licochalcone B, which is active against *Staphylococcus aureus* with a minimum growth inhibitory concentration (MIC) of 6.25 μg/mL (Park et al. 1998b). Furthermore, licochalcone C has also been reported to show significant inhibitory activity against the PTP1B enzyme (Yoon et al. 2009). Recently, research revealed that licochalcone C attenuates the lipopolysaccharide and interferon-gamma-induced inflammatory response by significantly decreasing the expression and activity of inducible nitric oxide synthase via nuclear factor kappa B by influencing extracellular superoxide anion production and modulating the antioxidant network activity of superoxide dismutase, catalase, and glutathione peroxidase activity (Franceschelli et al. 2011).

Due to the demand of large amounts of licochalcone C for further biological activity studies and the low isolated yield from natural product (15 mg from 2 kg of powdered *Glycyrrhiza glabra*) (Franceschelli et al. 2011), chemical synthesis of licochalcone C was highly desirable. However,

Published online: 30 July 2013



7a, Licochalcone C **7b,** Licochalcone H

Fig. 1 Structures of licochalcone C (**7a**) and licochalcone H (**7b**)

only one report has described the synthesis of licochalcone C in the literature, but this synthetic procedure cannot be applied readily due to commercially unavailable starting material, tedious protecting group manipulation, and low yield (Nielsen et al. 1995b). Therefore, our goal was to search for a more convenient and efficient protocol for the synthesis of licochalcone C, attempting to overcome these shortcomings. Herein, we report a concise synthetic method for licochalcone C and its regioisomer, named as licochalcone H, prepared as a major product in the course of synthesis.

Materials and methods

Melting points were determined in capillary tubes using a capillary melting point apparatus and are not corrected. NMR (^{1}H, ^{13}C) spectra were recorded on Varian Unity Plus 300 spectrometer, which were performed using $CDCl_3$ or CD_3OD as a solvent at room temperature. Chemical shifts (δ) were expressed in ppm relative to tetramethylsilane used as an internal standard, coupling constant (J) values were estimated in hertz (Hz) and spin multiples were given as singlet (s), doublet (d), multiplet (m), and broad (br). The mass spectra (MS) were acquired in positive mode over 100:600 m/z range using a Varian 1200L triple quadrupole mass spectrometer equipped with electrospray ionization (ESI) source.

Unless stated otherwise, all solvents were purchased from OCI Company Ltd. (Seoul, Korea), reagents were all obtained from Alfa Aesar or Aldrich, and used without further purification. Silica gel plates (Merck F254) and silica gel 60 (Merck, 70–230 mesh) were used for analytical and column chromatography, respectively. Compounds were visualized by ultraviolet light.

Synthetic Method for Compounds 3a and 3b

Compound **1** (25 g) in 150 mL dioxane was added to a stirred fresh solution of 3-hydroxy-3-methyl-1-butene (20 g) and 12.5 mL boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) in 100 mL dioxane, and stirring was continued for 5 h at room temperature. Ether (250 mL) was added and resulting solution extracted with water (3 × 500 mL). The remaining organic layer dried over $MgSO_4$ before evaporation to dryness. The crystalline residue was extracted with hexane (5 × 50 mL), which dissolved the reaction products and left behind most unchanged starting material. After evaporation of the solvent *in vacuo* to afford the crude product, which was purified by flash chromatography (SiO_2, hexane:ethyl acetate = 10:1) to give **3a** (1.98 g, 18 % based on recovered compound **1**) and **3b** (4.18 g, 38 % based on recovered compound **1**) as a white solid as well as compound **1** (17.63 g).

Compound 3a

Mp 119–121 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 11.77 (s, 1H, CHO), 9.68 (s, br, 1H, 2-OH), 7.31 (d, J = 9.0 Hz, 1H, H-6), 6.48 (d, J = 9.0 Hz, 1H, H-5), 6.19 (s, br, 1H, 4-OH), 5.24–5.30 (m, 1H, H-2″), 3.44 (d, J = 6.0 Hz, 2H, H-1″), 1.84 (s, 3H, Me-5″), 1.77 (3H, s, Me-4″). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 194.66, 162.46, 161.71, 135.90, 133.51, 120.68, 115.02, 113.83, 108.89, 25.80, 21.38, 17.89. LC-EIMS: m/z = 205 $[M-H]^-$.

Compound 3b

Mp 121-123 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 11.26 (s, 1H, CHO), 9.69 (s, br, 1H, 2-OH), 7.24 (s, 1H, H-6), 6.37 (s, 1H, H-3), 5.95 (s, br, 1H, 4-OH), 5.27–5.33 (m, 1H, H-2″), 3.32 (d, J = 7.2 Hz, 2H, H-1″), 1.80 (s, 3H, Me-5″), 1.78(s, 3H, Me-4″). ^{13}C-NMR (75 MHz, CD_3OD): δ 195.59, 165.33, 163.96, 135.32, 133.78, 123.41, 123.05, 115.86, 102.75, 28.37, 26.09, 17.94.

Synthetic Method for Compounds 4a and 4b

To a stirred solution of **3a** or **3b** in acetone was added K_2CO_3 (2 eq), followed by MOMCl (1.2 eq) at room temperature, and the mixture was stirred at ambient temperature overnight. The reaction was quenched with water and extracted with EtOAc and the combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a crude oil, which was purified by silica gel chromatography (hexane–EtOAc 15:1) to give the desired product as a colorless oily liquid.

Compound 4a (81 %)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 11.47 (s, 1H, CHO), 9.72 (s, br, 1H, 2-OH), 7.35 (d, J = 9.0 Hz, 1H, H-6), 6.75 (d, J = 9.0 Hz, 1H, H-5), 5.28 (s, 2H, OCH_2 of MOM), 5.18–5.24 (m, 1H, H-2″), 3.48 (s, 3H, OCH_3 of MOM), 3.38 (d, J = 6.0 Hz, 2H, H-1″), 1.80 (s, 3H, Me-5″), 1.68

(s, 3H, Me-4″). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 194.88, 161.51, 161.00, 133.16, 132.02, 121.53, 117.93, 115.96, 105.78, 93.79, 56.27, 25.74, 21.60, 17.74. LC-EIMS: m/z = 249 $[M-H]^-$.

Compound 4b (85 %)

^{1}H-NMR (300 MHz, $CDCl_3$) δ 11.32 (1H, s, CHO), 9.68 (s, br, 1H, 2-OH), 7.24 (s, 1H, H-6), 6.61 (s, 1H, H-3), 5.25–5.30 (1H, m, H-2″), 5.24 (s, 2H, OCH_2 of MOM), 3.47 (s, 3H, OCH_3 of MOM), 3.26 (d, J = 6.9 Hz, 2H, H-1″), 1.75 (d, J = 1.2 Hz, 3H, Me-5″), 1.71 (d, J = 0.6 Hz, 3H, Me-4″). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 194.44, 162.45, 161.75, 133.46, 132.95, 122.85, 121.55, 114.92, 101.16, 93.79, 56.11, 27.53, 25.56, 17.50.

Synthetic Method for Compounds 5a and 5b

To a cooled solution (ice-water bath) of **4a** or **4b** in dry DMF was added NaH (1.5 eq) portionwise, and the mixture was stirred at the same temperature for 30 min. MeI (1.1 eq) was added to the mixture and it was stirred at room temperature for 12–16 h. The reaction mixture was then filtered and the filter cake was washed with EtOAc. The filtrate was concentrated under reduced pressure and the residue was dissolved in EtOAc, washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel) using hexane–EtOAc (10:1) as an eluent to give the desired product as a colorless oily liquid.

Compound 5a (80 %)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 10.22 (s, 1H, CHO), 7.72 (d, J = 9.0 Hz, 1H, H-6), 6.97 (d, J = 9.0 Hz, 1H, H-5), 5.27 (s, 2H, OCH_2 of MOM), 5.15–5.20 (m, 1H, H-2″), 3.88 (s, 3H, 2-OCH_3), 3.48 (s, 3H, OCH_3 of MOM), 3.40 (d, J = 9.0 Hz, 2H, H-1″), 1.80 (s, 3H, Me-5″),1.69 (s, 3H, Me-4″). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.69, 162.24, 161.01, 131.65, 128.10, 124.41, 123.31, 122.00, 109.62, 93.68, 63.99, 55.99, 25.46, 22.48, 17.60.

Compound 5b (92 %)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 10.28 (s, 1H, CHO), 7.61 (s, 1H, H-6), 6.68 (s, 1H, H-3), 5.28(s, 2H, OCH_2 of MOM), 5.22–5.27 (m, 1H, H-2″), 3.87 (s, 3H, 2-OCH_3), 3.49 (s, 3H, OCH_3 of MOM), 3.25 (d, J = 7.2 Hz, 2H, H-1″), 1.72 (d, J = 0.9 Hz, 3H, Me-5″), 1.70 (d, J = 0.6 Hz, 3H, Me-4″). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 188.06, 161.98, 161.07, 132.33, 128.81, 123.00, 121.75, 118.50, 96.86, 93.83, 55.94, 55.40, 27.63, 25.48, 17.42.

Synthetic Method for Licochalcone C (7a) and H (7b)

4-Hydroxyacetophenone (**6**) (1.1 eq) and intermediate **5a** or **5b** (1 eq) were dissolved in anhydrous EtOH, cooled by ice-water bath, then anhydrous 2.0 M HCl-EtOH (6 eq) was added slowly to the stirred solution. The mixture was continuously stirred for 15–20 h at 0 °C. When the starting martial **5a** or **5b** disappeared (monitored by TLC), then the mixture was kept stirring at room temperature for 8–12 h. HCl and EtOH solvent were removed under reduced pressure, the mixture was then extracted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$, then H_2O. The extracted organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Then the title compound licochalcone C (**7a**) and licochalcone H (**7b**) were obtained as a yellow oil after silica gel column chromatography using mixture of chloroform and methanol (50:1) as an eluent.

Licochalcone C (7a, 52 %)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.02(d, 1H, J = 15.9 Hz, H-β), 8.00 (d, 2H, J = 8.7 Hz, H-2′ and H-6′), 7.51 (d, 1H, J = 15.9 Hz, H-α), 7.50 (d, 1H, J = 8.7 Hz, H-6), 6.93 (d, 2H, J = 8.7 Hz, H-3′ and H-5′), 6.69 (d, 1H, J = 8.7 Hz, H-5), 5.78 (s, br, 1H, OH), 5.72 (s, br, 1H, OH), 5.22–5.26 (m, 1H, H-2″), 3.76 (s, 3H, OMe), 3.47 (d, 2H, J = 6.9 Hz, H-1″), 1.85 (s, 3H, Me-5″), 1.78 (s, 3H, Me-4″). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 189.10, 159.55, 159.04, 158.25, 139.70, 135.86, 131.63, 131.03, 127.72, 121.26, 120.89, 120.53, 115.32, 112.74, 62.56, 29.70, 25.80, 23.08, 17.99. LC-EIMS: m/z = 337 $[M-H]^-$.

Licochalcone H (7b, 67 %)

^{1}H-NMR (300 MHz, CD_3OD): δ 8.00 (d, J = 15.6 Hz, 1H, H-β), 7.95 (d, J = 8.7 Hz, 2H, H-2′ and H-6′), 7.56 (d, J = 15.6 Hz,1H, H-α), 7.39 (s, 1H, H-6), 6.88 (d, J = 8.7 Hz, 2H, H-3′ and H-5′), 6.46 (s, 1H, H-3), 5.29-5.34 (m, 1H, H-2″), 3.87 (s, 3H, OMe), 3.25 (d, J = 7.2 Hz, 2H, H_2-1″), 1.75 (s, 3H, Me-5″) 1.74 (s, 3H, Me-4″). ^{13}C-NMR (75 MHz, CD_3OD): δ 191.91, 163.68, 160.80, 160.55, 141.96, 133.09, 132.27, 132.22, 131.77, 131.63, 124.21, 122.48, 119.29, 116.46, 116.35, 99.68, 56.25, 28.76, 26.11, 18.04.

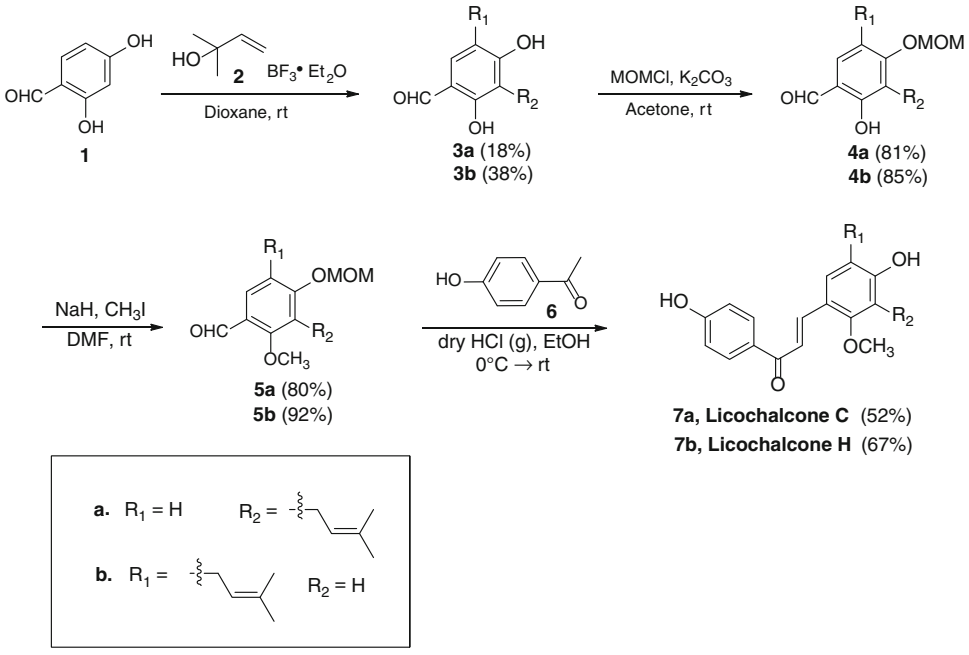
Results and discussion

The synthetic methods are depicted in Scheme 1. First, we prepared a key intermediate, 2,4-dihydroxy-3-(3-methylbut-2-en-1-yl)benzaldehyde (**3a**) from 2,4-dihydroxybenzaldehyde (**1**) and 3-hydroxy-3-methyl-1-butene (**2**). Lewis

acid-promoted Friedel–Crafts alkylation reaction in dioxane with $BF_3{\cdot}OEt_2$ as a catalyst was used to synthesize **3.** In this reaction, the electron-rich aromatic ring attacked the electrophile generated in the presence of the Lewis acid catalyst, $BF_3{\cdot}OEt_2$, and 3-hydroxy-3-methyl-1-butene (**2**), followed by aromatization of the benzene ring to produce the target compound **3a** in 18 % yield based on recovered 2,4-dihydroxybenzaldehyde (70 %). The other regioisomer **3b** was obtained as a major product (38 % based on recovered starting material), possibly due to preferable alkylation of sterically less demanding C-5 in Friedel–Crafts reaction. All attempts to increase the yield of **3a** by modifying reaction conditions (i.e. Lewis acid, solvent, reaction time and temperature, etc.) were unsuccessful. ^{1}H-nuclear magnetic resonance of **3a** revealed a doublet ($J = 9$ Hz) for a C-5 aromatic proton at 6.48 ppm and a doublet ($J = 9$ Hz) for a C-6 aromatic proton at 7.31 ppm that proved the substitution of the prenyl group at the C-3 position. Compound **3b** was confirmed by observing two singlet protons at 6.37 ppm and 7.24 ppm corresponding to the aromatic C-3 and C-6 protons, respectively.

The 4-hydroxyl group of **3a** and **3b** were selectively protected as methoxymethyl (MOM) ether to give compound **4a** and **4b** in 81 and 85 % yield, respectively. As expected, 2-hydroxy group remained intact in this reaction. Maybe the steric hindrance of the 2-OH group in compounds **4a** and/or hydrogen bond formation between the 2-hydroxyl and aldehyde group prevented methoxymethylation of 2-hydroxyl group. Nevertheless methylation on 2-OH group in **4** was successfully carried out using methyliodide and NaH in *N*, *N*-dimethylforamide (Nandy et al. 2008). Sterically more demanding **4a** gave lower yield than **4b**. Thus compound **5a** and **5b** were obtained in 80 and 92 % yield, respectively. Compound **5a** and **5b** were in hand, we studied the final condensation and deprotection of 4-hydroxyl group. Given the removal of the MOM group in the final step of the synthesis, we have adopted a strategy to perform Claisen-Schmidt condensation reaction under acidic condition. It was anticipated that Aldol condensation and the removal of protection group could be achieved simultaneously under acidic condition. It has been previously demonstrated that licochalcone A was synthesized by using acid-mediated Claisen-Schmidt condensation reaction (Kromann et al. 2004). The aldehydes **5a** and **5b** with 4-hydroxyacetophenone, **6** in the presence of dry HCl gas in ethanol at 0 °C afforded **7a** and **7b** in 52 and 67 % yield, respectively. In this reaction, only E isomer was formed with coupling constants of 15.9 and 15.6 Hz for **7a** and **7b**, respectively. All the spectral data of **7a** were fully consistent with those for the licochalcone C in the literature (Nielsen et al. 1995b).

In conclusion, a facile synthesis of licochalcone C and H using 2,4-dihydroxy-3-(3- methylbut-2-en-1-yl)benzaldehyde (**3a**) and 2,4-dihydroxy-5-(3-methylbut-2-en-1-yl) benzaldehyde (**3b**) as the key intermediate in four steps. This is an efficient synthesis for licochalcone C and its regioisomer, licochalcone H starting from readily available 2,4-dihydroxybenzaldehyde, and licochalcone H is reported for the first time. In addition, the one-step completion of an acid-mediated Claisen-Schmidt condensation and removal of the protecting group spares the need for a deprotection procedure. This approach will provide sufficient quantities of licochalcone C and H needed for further



Scheme 1 Synthetic method for licochalcone C and H

biological studies and could be applied for the synthesis of structurally diverse licochalcones.

Acknowledgments This work was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant No. 2012R1A1A2006613). We also thank the Korea Basic Science Institute (KBSI), Gwangju branch, for performing the NMR and analytical experiments.

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