A Concise Synthesis of Licochalcone E and Its *Regio*-Isomer, Licochalcone F

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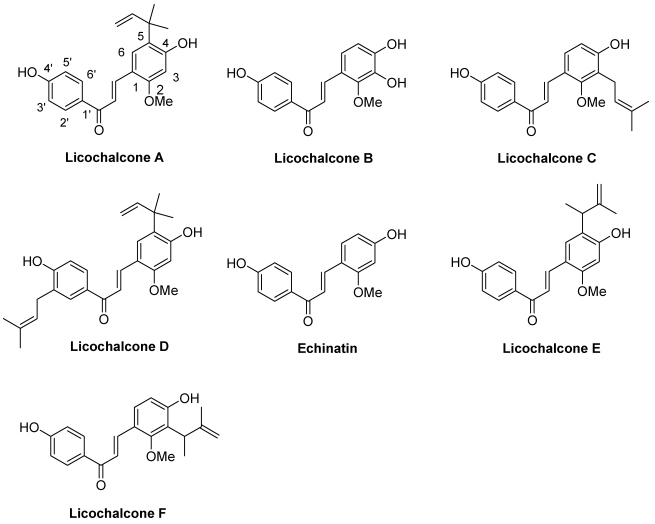
Licochalone E is one of the retrochalcones isolated from *Glycyrrhiza inflata* which shows potent cytotoxicty against human tumor cell lines. Biological studies suggested that topoisomerase I inhibition correlates with cytotoxic properties. Other research revealed that licochalcone E modulats the nuclear factor (NF)-kB and Bcl-2 families to induce endothelial cell apoptosis. Since licochalcone E has been isolated recently, synthetic information on this compound has not been reported yet. Therefore we report the concise synthesis of licochalcone E and its regioisomer, tentatively called licochalcone F, by employing Claisen rearrangement for key intermediate synthesis.

Key words retrochalcone; licochalcone E; licochalcone F; Claisen rearrangement

Retrochalcones,[1,2] which lack 2′- and 6′-hydroxy groups in the normal chalcone core, are an unusual phenolic compound family. *Glycyrrhiza inflata* is the major source of these compounds. Six retrochalcone compounds, licochalcone A—E and echinatin, have been isolated from *Glycyrrhiza inflata*. They have shown various pharmacological profiles including anticancer,[3,4] antiparasitic,[5] antibacterial,[2] antioxidative and superoxide-scavenging activities.[6] Among these compounds, licochalcone E[7] has recently been isolated and its biological study is in the initial stage. According to the biological activity reports, licochalone E has potent cytotoxic activity and this might be correlated with topoisomerase I inhibitory properties. It has also been reported that licochalcone E modulated the nuclear factor (NF)-kB and Bcl-2 families to induce endothelial cell apoptosis.[8] We, however, suspect that this compound needs more intense biological investigation to verify the exact biological targets and its mechanism of action. But, like other natural compounds isolated from plants, the isolation yield of licochalcone E is very low (5 mg from 1 kg of powdered *Glycyrrhiza inflata*)[4] and this is another bottleneck for the further biological study of this compound.



Since there are no reports on the synthesis of licochalcone E, we report here a concise synthetic method for licochalcone E and its *regio*-isomer, tentatively called licochalcone F (**1b**), prepared as by-product in the course of synthesis.

Results and Discussion

The key step employed for the synthesis was Claisen rearrangement in an *N*,*N*-dimethylaniline solvent system[9] at high temperature using *n*-butyric anhydride as the protective group of phenolic OH generated as a rearrangement product.

The synthetic methods are depicted in Chart 1. First, we prepared a key intermediate, 5-(1,2-dimethyl-2-propenyl)-4-hydroxy-2-methoxy benzaldehyde (**4a**) from 4-hydroxy-2-methoxybenzaldehyde (**1**). 4-Hydroxy-2-methoxybenzaldehyde was coupled with 1-bromo-*E*-2-methyl-2-butene[10] under K_2CO_3 basic condition in acetone to give an *O*-alkenylated compound (**2**). In the ^{1}H-NMR spectrum, we observed

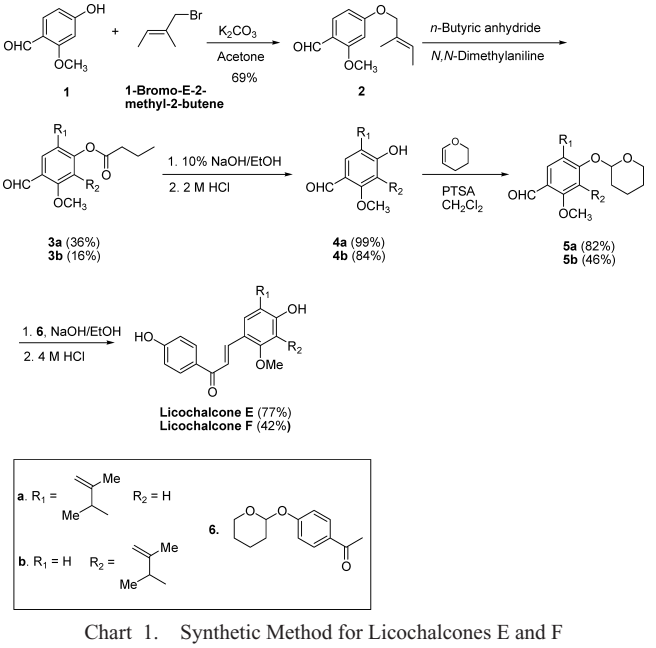


Chart 1. Synthetic Method for Licochalcones E and F

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a quartet peak of a C-3′ methine proton at δ 5.66 coupled with a C-4′ methyl group at δ 1.68 and a singlet peak of C-1′ methylene protons at δ 4.45. To achieve the rearrangement of the alkenyl group to the *ortho*-position, we employed two-step methods modified from those reported in the literature[9] including Claisen rearrangement as key process in *N*,*N*-dimethylaniline with *n*-butyric anhydride at 220—240 °C under nitrogen. We separated two compounds, **3a** and **3b**, in the first step. In both compounds, the methine proton signal of compound **2** at δ 5.66 disappeared and a new quartet (**3a**) or multiplet (**3b**) signal coupled with a methyl signal at δ 1.43 was generated. We also observed two sets of singlet signals corresponding to the protons attached to the terminal sp^2 carbon at δ 4.83 and 4.86 for **3a** and at δ 4.89 and 4.90 for **3b**. These observations confirmed that the double bond location between C-2′ and C-3′ of the butenyl group in compound **2** was shifted to the terminus of the propenyl group by the rearrangement process. Finally, compound **3a** was confirmed by observing two singlet protons at δ 6.77 and 7.66 corresponding to the C-3 and C-6 protons and other signals derived from the butyroyl group in the ^{1}H-NMR spectrum. This compound was isolated together with starting compound **2**. The other *regio*-isomer **3b** showed two doublet signals at δ 6.69 and 7.74 with coupling constant J=8.4 Hz, which is the conventional coupling pattern of *ortho* protons in the benzene ring system. Other ^{1}H- and ^{13}C-NMR data are similar to those of compound **3a**. Based on the spectral data, we assigned compound **3b** as a *regio*-isomer of compound **3a**. The isolation ratio of these two *regio*-isomers was approximately 2 : 1 (**3a** : **3b**).

After hydrolysis of **3a** and **3b** in 10% NaOH/EtOH, we obtained key intermediates **4a** and **4b** in 99% and 84% yields, respectively. Before coupling **4a** and **4b** with 4-tetrahydropyranyloxyacetophenone (**6**), we protected the 4-hydroxyl group of **4a** and **4b** to increase the product formation. According to the literature[9] and our laboratory experience, performing the condensation reaction without protection of the hydroxyl group reduces the reaction yield. Compounds **5a** and **5b** were prepared after protection of **4a** and **4b** with THP and PTSA in CH_2Cl_2. Even 72 h reaction time at 50 °C was not sufficient to complete this reaction, and we separated the protected and starting compounds. We suspected that this slow protection process was generated by the 1,2-dimethyl-2-propenyl group located at the *ortho*-position, leading to the steric hindrance of the OH group in compounds **4a** and **4b**. The final condensation of **5a** or **5b** with 4-tetrahydropyranyloxyacetophenone (**6**) was conducted with NaOH in EtOH to give the desired compounds licochalcone E or F, respectively. The spectral data of licochalcone E were consistent with those in the literature.[7] All the spectral data for licochalcone F were similar to those of licochalcone E. In the ^{1}H-NMR spectrum, two doublet signals were coupled with each other with coupling constant J=15.6 Hz, which confirms the *trans*-structure of licochalcone F.[11]

Conclusion

In summary, we successfully synthesized licochalcones E and F using 5-(1,2-dimethyl-2-propenyl)-4-hydroxy-2-methoxy benzaldehyde (**4a**) as the key intermediate in five steps. This is an efficient synthesis for licochalcone E of natural resources and its *regio*-isomer, licochalcone F. Licochalcone F is reported for the first time. This result will provide a tool to secure sufficient quantities of licochalcones E and F necessary for further biological study to elucidate the mechanism of action of these compounds. Pharmacological activity studies of these compounds are ongoing, and the results will be reported in future.

Experimental

The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. TLC plates were Kieselgel 60 F_{254} (art A715, Merck), and for column chromatography Silica gel 60 (0.040—0.063 mm ASTM, Merck) was used. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian NMR AS 400 MHz instrument. Chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane as an internal standard, and coupling constants (J values) are in Hertz. Mass spectral investigations were performed on a GC:7890A MS:5975C MSD (Agilent, U.S.A.) mass spectrometer equipped with an electron ionization (EI) source at the Catholic University of Daegu, Gyeongsan, Korea. Melting points were measured on a Barnstead International MEL-TEMP 1202D instrument without correction.

Synthesis of Compound 2 1-Bromo-*E*-2-methyl-2-butene in the acetone (10 ml) was added to the reaction mixture of 4-hydroxy-2-methoxybenzaldehyde (0.40 g, 2.63 mmol) and K_2CO_3 (0.57 g, 4.14 mmol) in the acetone (20 ml). The reaction mixture was refluxed for 8 h and cooled to room temperature. After filtration of solids, the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: EtOAc : *n*-hexane=1 : 5) to give a colorless oil (0.4 g, 66.1%).

Compound **2**: *Rf*: 0.60 (EtOAc : *n*-hexane=1 : 3), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.68 (d, J=6.8 Hz, 3H), 1.74 (s, 3H), 3.89 (s, 3H), 4.45 (s, 2H), 5.66 (q, J=6.8 Hz, 1H), 6.48 (d, J=2.0 Hz, 1H), 6.54 (dd, J=2.0, 8.8 Hz, 1H), 7.78 (d, J=8.8 Hz, 1H), 10.28 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.5, 13.8, 55.8, 74.7, 98.8, 106.7, 19.1, 124.7, 130.9, 131.2, 163.8, 165.9, 188.6 ppm; EI-MS (*m*/*z*) $[M]^+$ 220.1.

Synthesis of Compounds 3a and 3b The reaction mixture of compound **2** (0.76 g, 3.28 mmol) in *N*,*N*-dimethylaniline (7 ml) and *n*-butyric anhydride (3 ml) was kept at 220—240 °C for 3 h under nitrogen. After cooling to room temperature, the reaction mixture was poured into d-HCl and extracted with ethyl acetate. The organic layer was separated, washed with saturated NaCl and dried with anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: EtOAc : *n*-hexane=1 : 5) to give compounds **3a** (0.35 g, 35.5%) and **3b** (0.16 g, 15.7%) as oil, respectively.

Compound **3a**: *Rf*: 0.56 (EtOAc : *n*-hexane=1 : 3), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.05 (t, J=7.6 Hz, 3H), 1.33 (d, J=7.2 Hz, 3H), 1.57 (s, 3H), 1.87 (hexet, J=7.6 Hz, 2H), 2.57 (t, J=7.6 Hz, 2H), 3.47 (q, J=7.2 Hz, 1H), 3.89 (s, 3H), 4.83 (s, 1H), 4.86 (s, 1H), 6.69 (s, 1H), 7.74 (s, 1H), 10.39 (s, 1H); EI-MS (*m*/*z*) $[M]^+$ 290.1.

Compound **3b**: *Rf*: 0.74 (EtOAc : *n*-hexane=1 : 3), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.03 (t, J=7.6 Hz, 3H), 1.46 (d, J=7.2 Hz, 3H), 1.61 (s, 3H), 1.74 (hexet, J=7.6 Hz, 2H), 2.49 (dt, J=2.8, 7.6 Hz, 2H), 3.87—3.90 (m, 1H), 3.91 (s, 3H), 4.89 (s, 1H), 4.90 (s, 1H), 6.92 (d, J=8.4 Hz, 1H), 7.76 (d, J=8.4 Hz, 1H), 10.31 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.9, 17.7, 18.3 (*2), 22.9, 36.2, 65.3, 109.7, 120.7, 127.3, 128.0, 131.9, 147.1, 155.5, 163.1, 171.3, 189.4 ppm; EI-MS (*m*/*z*) $[M]^+$ 290.1.

General Synthetic Method for Compounds 4a and 4b The reaction mixture in 10% NaOH and EtOH was stirred at 110 °C for 3 h. The solvent was reduced under reduced pressure, and water was added to the residue. The aqueous layer was washed with ether and acidified with 2 N HCl. The resulting cloudy aqueous solution was extracted with ether and the organic layer was washed with saturated $NaHCO_3$. After drying with anhydrous Na_2SO_4, the solvent was removed and dried under a vacuum to give the desired products.

Compound **4a** (Orange Solid, 99%): mp 126 °C; *Rf*: 0.21 (EtOAc : *n*-hexane=1 : 3), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.43 (d, J=7.2 Hz, 3H), 1.65 (s, 3H), 3.55 (q, J=7.2 Hz, 1H), 3.87 (s, 3H), 5.06 (s, 1H), 5.12 (s, 1H), 6.77 (s, 1H), 7.66 (s, 1H), 10.28 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 18.6, 20.8, 41.9, 55.9, 99.9, 112.3, 118.7, 122.5, 129.6, 149.9, 162.5, 163.0, 188.9 ppm; EI-MS (*m*/*z*) $[M]^+$ 220.1.

Compound **4b** (Semi-solid, 84%): *Rf*: 0.38 (EtOAc : *n*-hexane=1 : 3), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.43 (d, J=6.8 Hz, 3H), 1.73 (s, 3H), 3.90 (s, 3H), 3.97 (q, J=6.8 Hz, 1H), 5.20 (dd, J=1.2, 3.2 Hz, 1H), 5.21 (s, 1H), 6.67 (d, J=8.4 Hz, 1H), 7.76 (d, J=8.4 Hz, 1H), 10.28 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.5, 23.1, 36.0, 65.0, 112.6, 114.2, 122.8, 123.3,

129.7, 149.7, 162.8, 163.0, 189.3 ppm; EI-MS (*m*/*z*) $[M]^+$ 220.1.

General Synthetic Method for Compounds 5a and 5b A solution of compound **4a** or **4b** (1 eq), pyridinium *p*-toluenesulfonate (catalytic amount), and 3,4-dihydro-2*H*-pyran (2 eq) in CH_2Cl_2 was stirred at 30 °C for 72 h. The reaction mixture was diluted with CH_2Cl_2. The solution was washed with 1 M Na_2CO_3, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was applied to silica gel column chromatography (eluent: EtOAc : *n*-hexane = 1 : 5) to give the desired products as oil in diastereomeric mixture.

Compound **5a** (82.0%): *Rf*: 0.42 (EtOAc : *n*-hexane = 1 : 3), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.33 (1.35) (d, J=7.2 Hz, 3H), 1.59 (1.62) (s, 3H), 1.69 (1.74) (m, 2H), 1.88—1.92 (m, 2H), 1.96—2.02 (m, 2H), 3.66—3.74 (m, 1H), 3.75—3.79 (m, 1H), 3.80—3.86 (m, 1H), 3.89 (s, 3H), 4.80 (s, 1H), 4.83 (4.84) (s, 1H), 5.58 (d, J=10.8 Hz, 1H), 6.77 (s, 1H), 7.66 (7.67) (s, 1H), 10.31 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 18.4 (18.5), 19.3 (19.4), 21.9 (22.1), 25.3, 30.3 (30.4), 38.7 (38.8), 55.8 (55.9), 61.8 (62.0), 96.1 (96.4), 97.7 (97.8), 110.2 (110.3), 118.7 (118.8), 126.7 (126.8), 127.7 (127.8), 148.7 (148.8), 161.3 (161.4), 162.5, 188.8 ppm; EI-MS (*m*/*z*) $[M-DHP]^+$ 220.1.

Compound **5b** (45.8%): *Rf*: 0.58 (EtOAc : *n*-hexane = 1 : 3), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.44 (1.48) (d, J=7.2 Hz, 3H), 1.61 (1.65) (s, 3H), 1.82—1.92 (m, 4H), 1.96—2.02 (m, 2H), 3.61—3.66 (m, 1H), 3.76—3.88 (m, 1H), 3.87 (3.88) (s, 3H), 3.95—4.03 (m, 1H), 4.81 (4.83) (s, 1H), 4.84 (4.95) (s, 1H), 5.43—5.44 (m, 1H), 7.01 (7.03) (d, J=8.4 Hz, 1H), 7.73 (d, J=8.4 Hz, 1H), 10.22 (10.23) (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.3 (17.5), 18.4 (18.6), 22.9, 25.3, 30.1 (30.3), 35.8 (35.9), 61.9 (62.0), 64.8 (64.9), 96.1 (96.7), 108.8 (109.0), 110.8 (111.0), 123.4 (123.5), 127.3 (127.4), 129.0 (129.3), 147.6 (147.7), 162.2 (162.5), 162.8 (162.9), 189.5 ppm; EI-MS (*m*/*z*) $[M-DHP]^+$ 220.1.

General Synthetic Method for Licochalcones E and F A solution of **6** (1 eq), compound **5a** or **5b** (1 eq), and NaOH (1.5 eq) in EtOH was stirred at 50 °C for 17 h. To this solution was added 4 M HCl and this mixture was stirred for an additional 20 min. After adding water, the reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water and saturated NaCl successively. After drying with anhydrous Na_2SO_4, the solvent was removed under reduced pressure. The residue was applied to silica gel column chromatography (eluent: EtOAc : *n*-hexane = 1 : 3 to 3 : 2) to give desired products.

Licochalcone E (77.4%): *Rf*: 0.28 (EtOAc : *n*-hexane = 1 : 1), ^{1}H-NMR (400 MHz, acetone-d_6) δ: 1.34 (d, J=7.0 Hz, 3H), 1.66 (s, 3H), 3.79 (q, J=7.0 Hz, 1H), 3.87 (s, 3H), 4.87 (s, 1H), 4.89 (s, 1H), 6.59 (s, 1H), 6.94 (d, J=7.2 Hz, 2H), 7.51 (s, 1H), 7.63 (d, J=15.6 Hz, 1H), 7.98 (d, J=7.2 Hz, 2H), 8.00 (d, J=15.6 Hz, 1H); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 18.9, 21.6, 38.0, 55.3, 99.1, 109.5, 115.4 (*2), 115.9, 118.9, 124.3, 128.7, 130.8 (*2), 131.1, 139.1, 149.2, 158.6, 159.1, 161.6, 187.7 ppm; EI-MS (*m*/*z*) $[M]^+$ 338.2, $[M-CH_3O]^+$ 307.2.

Licochalcone F (42.1%): mp 165—167 °C; *Rf*: 0.28 (EtOAc : *n*-hexane = 1 : 1), ^{1}H-NMR (400 MHz, acetone-d_6) δ: 1.49 (d, J=7.2 Hz, 3H), 1.68 (s, 3H), 3.74 (s, 3H), 3.98 (q, J=7.2 Hz, 1H), 4.85 (d, J=1.4 Hz, 1H), 4.86 (d, J=1.4 Hz, 1H), 6.71 (d, J=8.7 Hz, 1H), 6.96 (d, J=8.8 Hz, 2H), 7.68 (d, J=15.6 Hz, 1H), 7.69 (d, J=8.7 Hz, 1H), 7.99 (d, J=15.6 Hz, 1H), 8.05 (d, J=8.8 Hz, 2H); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 16.8, 22.2, 36.3, 62.5, 109.0, 112.8, 115.5 (*2), 119.9, 120.7, 125.1, 127.2, 130.9, 131.0 (*2), 138.7, 148.1, 159.5, 160.1, 161.8, 187.6 ppm; EI-MS (*m*/*z*) $[M]^+$ 338.2, $[M-CH_3O]^+$ 307.2.

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