DOI 10.5012/bkcs.2010.31.04.1085

Semisynthesis of Licochalcone E and Biological Evaluation as Vasorelaxant Agents

Goo Yoon, Min-Ho Oak,† Jung-Ok Lee,† and Seung Hoon Cheon‡,*

Korea Institute of Science and Technology, Gangneung Institute, Gangneung 210-340, Korea
†Research and Development Center, Hanwha Pharma. Co., Ltd., Chuncheon 200-921, Korea
*‡College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Korea. *E-mail: shcheon@chonnam.ac.kr*
Received January 11, 2010, Accepted February 16, 2010

Key Words: Semisynthesis, Licochalcone A, Licochalcone E, Enolene rearrangement, Vasorelaxant effect

Licochalcones A-E, isolated and characterized from the roots of *G. inflata*, are unusual allyl retrochalcones that are structurally distinguished from normal chalcones by the lack of oxygen functionalities at C-2′ and C-6′.[1-4] Although licochalcones A-D reportedly exhibit various biological activities,[5-15] the search for the biological activities of licochalcone E is only in the initial stage of intensive studies due to its recent discovery. Licochalcone E exhibits topoisomerase 1 inhibition[13] and induces endothelial cell apoptosis by modulating NF-*k*B and the Bcl-2 family.[14] It is also reported that licochalcones A and E inhibit protein tyrosine phosphatase 1B.[15]

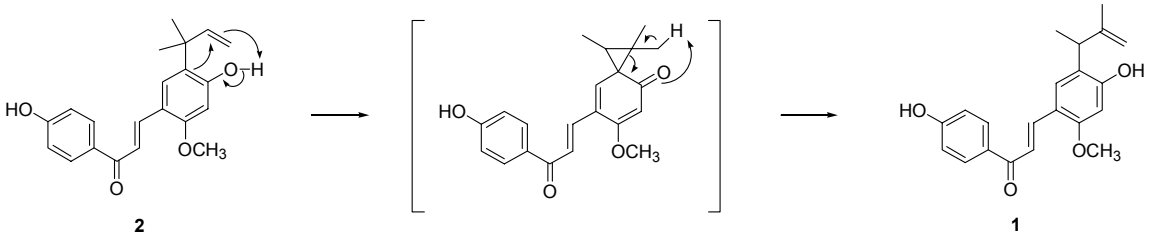
Because of very low isolation yield (5 mg from 1 kg of powdered *G. inflata*),[13] biological studies of licochalcone E have been limited, like those of many other natural products. Thus, to obtain large quantities of licochalcone E for biological activity studies and animal experiments, licochalcone E was synthesized from 4-hydroxy-2-methoxy benzaldehyde[16] and 2,4-dihydroxy benzaldehyde.[17] Recently, we completed the enantioselective total synthesis of (-)-licochalcone E.[18] In this report, we described a one-step semisynthetic method that can be applied to the conversion of the relatively abundant licochalcone A to the rare licochalcone E using enolene rearrangement and their vasorelaxant effects.

We thought that the α,α-dimethylallyl of licochalcone A (**2**) could be transformed into the α,β-dimethylallyl of licochalcone E (**1**) by enolene rearrangement based on the precedents of similar reactions in the literature.[19,20] A homodienyl [1,5]-sigmatropic hydrogen shift of **2** formed a spirocyclopropane intermediate which underwent a [1,5]-homosigmatropic rearrangement to give **1** as shown in Scheme 1. A solvent with a high boiling point is required in order to perform enolene rearrangement for facile transformation. An optimization study to assess the effects of solvent and reaction time for the conversion of **2** to **1** is summarized in Table 1. Refluxing licochalcone A (**2**) in *N*,*N*-dimethylaniline (DMA, bp 193 °C) for 1 hr gave licochalcone E (**1**) in 66% yield. Although DMA provided a good yield, it was impossible to prevent the formation of small amount of inseparable by-products under this reaction condition. In contrast, refluxing licochalcone A (**2**) in *N*,*N*-diethylaniline (DEA, bp 217 °C) for 30 min afforded licochalcone E (**1**) in 77% yield without contamination of inseparable by-products. The spectral data of licochalcone E were consistent with those in the litera-

Table 1. Effect of solvents and time on the equilibrium of **1** and **2**

Entry	Solvent	Time (h)	Ratio 2:1[a]	Yield (%)
1	DMA	1	0:100	66[b]
2	DMA	2	0:100	52[b]
3	DMA	3	0:100	23[b]
4	DEA	0.5	0:100	77[c]
5	DEA	1	0:100	64[c]
6	DMF	12	76:24	50[d]
7	Xylenes	20	27:73	29[d]
8	MeCN	12	62:38	33[d]
9	DMSO	1	dec[e]	-
10	EtOH	12	dec	-
11	Dioxane	9	trace	-
12	DME	6	trace	-
13	Toluene	9	trace	-
14	Decalin	1	dec	-
15	Water	3	NR[f]	-

[a]Ration determined *via* integration of allylic protons of **2** and **1** in the ^{1}H-NMR spectrum of the crude reaction mixture. [b]Combined yield with inseparable by-products. [c]Isolated yield of purified product. [d]Combined yield with **2** and **1**. [e]Decomposition. [f]No reaction.



Scheme 1

Table 2. Optimization of reaction conditions by microwave irradiation

Entry	Solvent	MW (°C)	Time	Ratio 2:1[a]	Yield (%)
1	DEA	200	30 min	6:94	64[b]
2	Xylenes	180	2 h	31:69	23[b]
3	DMA	200	20 min	23:77	39[c]
4	MeCN	110	2 h	trace	-
5	DME	150	2 h	trace	-
6	Dioxane	120	2 h	trace	-
7	Toluene	140	2 h	trace	-

[a]Ration determined *via* integration of allylic protons of **2** and **1** in the ^{1}H-NMR spectrum of the crude reaction mixture. [b]Isolated yield of purified product. [c]Combined yield with inseparable by-products.

ture.[4,13] This result may indicate that the proper reaction temperature for this rearrangement is approximately the boiling point of DEA. Reaction time also played an important role in the yield and purity of the product. Generally more by-products were formed at the cost of licochalcone E with a longer reaction time in both DMA and DEA. Obviously, prolonged heating at high temperature did not increase the conversion, but rather destroyed licochalcone E. Other solvents tested in the rearrangement reaction are listed in Table 1. Refluxing licochalcone E in *N*,*N*-dimethylformamide (DMF) or acetonitrile (MeCN) for 12 h, or xylenes for 20 h resulted in the formation of a mixture of licochalcone A (**2**) and licochalcone E (**1**) in a ratio of 76:24, 62:38, or 27:73, respectively. The by-products were also increased in these solvents when the reaction mixture was heated for an extended period of time. Refluxing the reaction mixture for 6 ~ 9 h in dioxane, 1,2-dimethoxyethane (DME), or toluene yielded only trace amounts of licochalcone E (**1**). No product was detected when the rearrangement was carried out in solvents such as dimethylsulfoxide (DMSO), ethanol (EtOH), decalin, and water.

Next, we explored microwave irradiation as a heating source for more effective optimization than conventional heating since it's known that microwave induced facile rearrangement. Under microwave irradiation at 200 °C for 30 min using DEA as a solvent, licochalcone E (**1**) was obtained in 64% yield, but the product contained 6% licochalcone A. Prolonged reaction time gave lower yield of the product. In DMA, the yield was remarkably reduced compared to that with conventional heating. Under microwave irradiation, yields were decreased compared to those under conventional heating in the case of xylenes, MeCN, dioxane, DME, and toluene (Table 2). Based on these results, we concluded that conventional heating is better than microwave irradiation in enolene rearrangement of licochalcone A to licochalcone E.

Cardiovascular diseases are the major cause of death in developed countries. Because of long-term drug therapy and many side effects of drugs, traditional herbal remedies are increasingly considered as safe and effective alternatives to modern synthetic drugs, even in western industrialized countries.[21-25] During our previous research efforts aimed at the discovery of new cardiovascular protective agents, hundreds of different compounds isolated from medicinal plants used in oriental medicine were screened for their vasorelaxant activity. Among them, licochalcones A and E isolated from *G. inflata* exhibited potent vasorelaxant activity. Licochalcones A and E induced concentration-dependent relaxation in rat artery rings with endothelium. The EC_{50} values of the vasorelaxing effects of licochalcones A and E were 41.1 μM and 50.1 μM, respectively. The present study has demonstrated for the first time that licochalcones A and E are powerful endothelium-dependent vasodilators and could serve as a new scaffold for finding other potent vasorelaxant agents.

In conclusion, a facile one-step chemical transformation of licochalcone E has been accomplished with 77% yield from licochalcone A, which can be isolated in abundance from *G. inflata*. Utilization of this concise chemical transformation of licochalcone E could provide enough material for biological studies to elucidate the mechanism of action of licochalcone E. Licochalcones A and E were evaluated for their vasorelaxant effect and they exhibited potent vasodilatory activity. This result suggests that allyl retrochalcone could be developed as a candidate for anti-hypertensive medicines. A study of asymmetric synthesis of licochalcone E using Claisen rearrangement and intensive studies of the mechanisms of action and animal experiments of vascular reactivity of allyl retrochalcones are ongoing, and the results will be published in the future.

Experimental Section

Isolation of licochalcone A (2). The isolation of licochalcone A from *G. inflata* has been previously described. Licochalcone A was identified according to its spectroscopic properties.[4,13]

Semisynthesis of licochalcone E (1) by heating. Oxygen free N_2 was passed for 1 hr through a solution of licochalcone A (50 mg) in each solvent (5 mL). The solution was refluxed for the time specified in Table 1. After cooling to room temperature, the reaction mixture was evaporated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane:EtOAc = 2:1) to give licochalcone A and/or licochalcone E. Ratio was determined *via* integration of allylic protons of licochalcone A and licochalcone E in the ^{1}H-NMR spectrum of the crude reaction mixture.

Semisynthesis of licochalcone E (1) by microwave irradiation. Microwave reactions were carried out in a monomode microwave apparatus (CEM Discover®). To a glass vessel was added licochalcone A (15 mg) in each solvent (0.6 mL). An initial microwave irradiation of 300 W was used, with the temperature being ramped from rt to the desired temperature. Once the desired temperature was reached, the reaction mixture was held at this temperature with stirring for the appropriate time. The reaction mixture was then cooled to room temperature. The crude product was purified by flash column chromatography (*n*-hexane:EtOAc = 2:1) to give licochalcone A and/or licochalcone E. Ratio was determined *via* integration of allylic protons of licochalcone A and licochalcone E in the ^{1}H-NMR spectrum of the crude reaction mixture.

Animals. Male Sprague-Dawley (SD) rats (7 ~ 10 weeks old, weighing 250 ~ 300 g) were purchased from Orient BIO Inc., Korea, and were used in these studies. Animals were housed in colony cages under standard laboratory conditions (12:12 h light/dark cycle) and had free access to standard commercial

diet and water. The study conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Institutional Animal Care and Utilization Committee for Gyeonggi BioCenter, Suwon, Republic of Korea.

Vascular reactivity study. Aortas were cleaned of connective tissue and cut into rings (3 ~ 4 mm in length). As indicated, the endothelium was removed by rubbing the intimal surface of rings with a pair of forceps. Rings were suspended in organ baths containing oxygenated (95% O_2; 5% CO_2) Krebs bicarbonate solution (in mM: NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 1.25, $NaHCO_3$ 25 and D-glucose 11, pH 7.4, 37 ^oC) for the determination of changes in isometric tension. Following equilibration for 90 min under a resting tension of 2 g, rings were contracted with phenylephrine (1 μM) to about 80% of the maximal contraction reached by increasing concentrations of phenylephrine. After washout and a 30-min equilibration period, rings were contracted again with phenylephrine (1 μM) and the relaxation in acetylcholine (10 μM) was determined. After washout and a 30-min equilibration period, rings were again contracted with phenylephrine (1 μM) before concentration-relaxation curves were constructed for licochalcone E and licochalcone A.

Acknowledgments. This work was supported by a National Research Foundation of Korea Grant funded by the Korean Government 2009-0075257. We thank the Korea Basic Science Institute (KBSI), Gwangju branch, for performing the NMR experiments.

References

1. Furuya, T.; Matsumoto, K.; Hikichi, M. *Tetrahedron Lett.* **1971**, *12*, 2567.
2. Saitoh, T.; Shibata, S. *Tetrahedron Lett.* **1975**, *16*, 4461.
3. Kajiyama, K.; Demizu, S.; Hiraga, Y.; Kinoshita, K.; Koyama, K.; Takahash, K.; Tamura, Y.; Okada, K.; Kinoshita, T. *Phytochemistry* **1992**, *31*, 3229.
4. Yoon, G.; Jung, Y. D.; Cheon, S. H. *Chem. Pharm. Bull.* **2005**, *53*, 694.
5. Park, E. J.; Park, H. R.; Lee, J. S.; Kim, J. W. *Planta Med.* **1998**, *64*, 464.
6. Shibata, S.; Inoue, H.; Iwata, S.; Ma, R.; Yu, L.; Ueyama, H.; Takayasu, J.; Hasegawa, T.; Tokuda, H.; Nishino, A.; Nishino, H.; Iwashima, A. *Planta Med.* **1991**, *57*, 221.
7. Nomura, T.; Fukai, T.; Hano, Y. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science: London, 2003; Vol. 28, pp 199-256.
8. Friis-Moller, A.; Chen, M.; Fuursted, K.; Christensen, S. B.; Kharazmi, A. *Planta Med.* **2002**, *68*, 416.
9. Nielsen, S. F.; Chen, M.; Theander, T. G.; Kharazmi, A.; Christensen, S. B. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 449.
10. Nielsen, S. F.; Christensen, S. B.; Cruciani, G.; Kharazmi, A.; Liljefors, T. *J. Med. Chem.* **1998**, *41*, 4819.
11. Haraguchi, H.; Tanimoto, K.; Tamura, Y.; Mizutani, K.; Kinoshita, T. *Phytochemistry* **1998**, *48*, 125.
12. Haraguchi, H.; Ishikawa, H.; Mizutani, K.; Tamura, Y.; Kinoshita, T. *Bioorg. Med. Chem.* **1998**, *6*, 339.
13. Yoon, G.; Kang, B. Y.; Cheon, S. H. *Arch Pharm. Res.* **2007**, *30*, 313.
14. Chang, H. J.; Yoon, G.; Park, J. S.; Kim, M. H.; Baek, M. K.; Kim, N. H.; Shin, B. A.; Ahn, B. W.; Cheon, S. H.; Jung, Y. D. *Biol. Pharm. Bull.* **2007**, *30*, 2290.
15. Yoon, G.; Lee, W.; Kim, S. N.; Cheon, S. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5155.
16. Na, Y.; Cha, J. H.; Yoon, H. G.; Kwon, Y. *Chem. Pharm. Bull.* **2009**, *57*, 607.
17. Yoon, G.; Liu, Z.; Jeong, H. J.; Cheon, S. H. *Bull. Korean Chem. Soc.* **2009**, *30*, 2959.
18. Liu, Z.; Yoon, G.; Cheon, S. H. *Tetrahedron* Submitted.
19. Roberts, R. M.; Landolt, R. G.; Greene, R. N.; Heyer, E. W. *J. Am. Chem. Soc.* **1967**, *89*, 1404. This rearrangement was named as enolene rearrangement for the first time in ref. 19, previously it was recognized as one component of the abnormal Claisen rearrangement (cf. ref. 20). This rearrangement could also be considered as Conia ene reaction and retro-Conia ene reaction as suggested by one referee (for a review see: Conia, J. M.; Le Perchec, P. *Synthesis* **1975**, 1-19).
20. Marvell, E. N.; Anderson, D. R.; Ong, J. *J. Org. Chem.* **1962**, *27*, 1109.
21. Feletou, M.; Vanhoutte, P. M. *Am. J. Physiol. Heart Circ. Physiol.* **2006**, *291*, H985.
22. Stoclet, J. C.; Chataigneau, T.; Ndiaye, M.; Oak, M. H.; El Bedoui, J.; Chataigneau, M.; Schini-Kerth, V. B. *Eur. J. Pharmacol.* **2004**, *500*, 299.
23. Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. *Lancet* **1993**, *341*, 454.
24. Fitzpatrick, D. F.; Fleming, R. C.; Bing, B.; Maggi, D. A.; O'Malley, R. M. *J. Agric. Food Chem.* **2000**, *48*, 6384.
25. Sarr, M.; Chataigneau, M.; Martins, S.; Schott, C.; El Bedoui, J.; Oak, M. H.; Muller, B.; Chataigneau, T.; Schini-Kerth, V. B. *Cardiovasc. Res.* **2006**, *71*, 794.