Facile Purification of Honokiol and Its Antiviral and Cytotoxic Properties

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A separation of honokiol **1** from the closely structurally related magnolol **2** was developed. Honokiol demonstrated weak activity against HIV-1 in human lymphocytes.

Introduction

Simple biphenyl neolignans, honokiol **1** and magnolol **2**, are the main constituents of the stem bark of *Magnolia abovata* thumb and *Magnolia officinalis* rhed, which have been used in traditional Chinese medicine for thousands of years for the treatment of anxiety and stroke. These natural products are also reported to have various biological activities, including antioxidative and antidepressant properties.[1,2] They inhibit intracellular calcium mobilization in platelets[3] and relax vascular smooth muscles by inhibiting calcium influx through voltage-gated calcium channels.[4] Moreover, recent studies in our laboratory indicated that honokiol induced apoptosis in tumor cells and inhibited angiogenesis through blocking the phosphorylation of vascular endothelial growth factor receptor 2 (VEGFR 2).[5] Honokiol also exhibited direct antitumor activity through tumor necrosis factor apoptosis-inducing ligand (TRAIL/Apo2L) signaling.[5] Thus, the combination of both anti-angiogenic and antitumor activities distinguishes honokiol from many other natural products. Because of their important biological properties, large quantities of pure materials were needed for further preclinical and clinical studies. However, the syntheses of pure honokiol and magnolol are not very efficient, requiring multistep strategies with low to good global yields.[6] Currently, the extraction of honokiol is achieved from magnolia trees but always as a mixture with magnolol. Because of the structural resemblance, these two compounds are difficult to separate by conventional thin-layer chromatography or column chromatography. Thus, the purification requires the use of either special chromatographic conditions or electromigration,[7] significantly adding to the cost of preparing honokiol or magnolol and are thus not readily adaptable to large-scale experiments.

Honokiol differs from magnolol only in the position of a hydroxyl group, but in the case of magnolol, the hydroxyl groups appear like a protectable diol. This spacial arrangement provided an opportunity for a selective chemical modification allowing facile separation.

Discussion

The reaction of a mixture of **1** and **2** with 2,2-dimethoxypropane under catalytic acidic conditions readily resulted in the selective formation of **3**, whereas honokiol was unchanged under these conditions because both hydroxyl groups are spatially too far to be protected. The resulting mixture of **1** and **3** could be readily separated by simple and economical flash chromatography on silica gel giving honokiol in good yield with 96.8% purity as determined by HPLC. The purity of honokiol can be raised to 99.8% by repeating the reaction under the same conditions on the partially purified honokiol. Finally, the deprotection of **3** under acidic conditions affords highly pure (99.8%) magnolol in good yield with no detectable honokiol as determined by HPLC.

The purified honokiol and magnolol were evaluated for their cytotoxicity in normal and cancer cells. Both compounds demonstrate moderate cytotoxicity in a human peripheral blood mononuclear (PBM) cells, African green monkey kidney (Vero) cells, and human T-cell lymphoma (CEM) cells. The median cytotoxic concentration (CC_{50}) in these cells were 16.1, 22.5, 10.9 μM, respectively, for honokiol and 38.6, 50.6, 99.5 μM, respectively, for magnolol. Honokiol and magnolol were also evaluated against HIV-1 in human PBM cells and found to have a median effective concentration (EC_{50}) of 3.3 and 69.3 μM, respectively. Thus, the selectivity index of honokiol was approximately five. (The selectivity index is generally defined as the ratio between CC_{50} and EC_{50} values.) The antiviral[8] and cytotoxicity[9] assays were performed as previously described.

This work provides a simple and economical method for the separation of **1** and **2**. Whereas our method produced honokiol in one step in a 91% yield, two previously published syntheses were completed in three[6a] to nine[6b] steps with yields of 15 and 26%.

Experimental Section

Commercially available chemicals and solvents were reagent-grade and used as received except for *p*-TSA, which was coevaporated with toluene prior to use. Extracts of a mixture of honokiol and magnolol (58/33) obtained from magnolia trees was purchased from Conba Pharmaceutical (Zhejiang, China). The reactions were monitored by thin-layer chromatography (TLC) analysis using silica-gel plates (Kieselgel 60 F_{254}, E. Merck). The compounds were visualized by UV irradiation and/or spraying with 20% H_2SO_4 in EtOH, followed by charring at 150 °C. Column chromatography was performed on 60 M silica gel (0.040−0.063 mm, E. Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Mercury 300 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C using internal $CHCl_3$ as the reference (7.26 ppm for ^{1}H and 77.23 ppm for ^{13}C); signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). High-Resolution Mass spectra (HRMS) were performed by the Mass Spectrometry Center of Emory University. The nomenclature used for the compounds reported in this article is in accordance with the IUPAC rules.

Protection of Magnolol in a Mixture of Honokiol and Magnolol. A solution of a mixture of honokiol and magnolol (58:33 as determined by HPLC, 8 g) in 2,2-dimethoxypropane (48 mL) was stirred overnight in the presence of a catalytic amount of *p*-TSA. After neutralization of the solution with $NaHCO_3$, the

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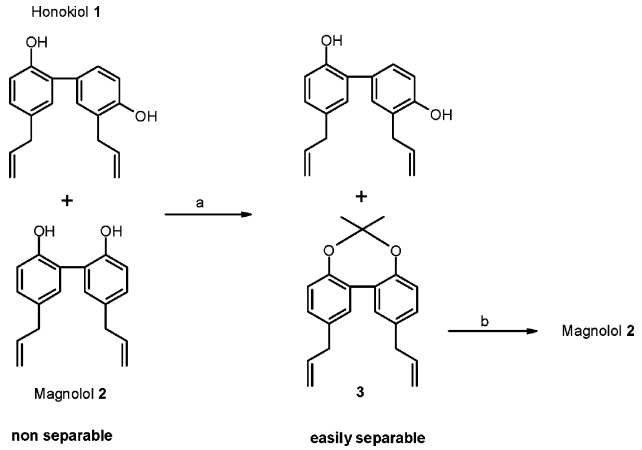
Scheme 1[a]



[a] (a) 2,2-dimethoxypropane, *p*-toluene sulfonic acid, rt; (b) MeOH, 1 M HCl, reflux.

mixture was diluted with EtOAc (150 mL) and washed with H_2O and brine, and then, the organic layer was dried over $MgSO_4$, filtered, and evaporated. The crude product was separated by flash chromatography on silica gel (hexane/EtOAc, 85:15) to give protected magnolol **3** (2.8 g) and honokiol **1** (4.64 g).

5,5′-Diallyl-[1,1′-biphenyl]-2,2′-dimethylacetal (3) (Protected Magnolol). ^{1}H NMR ($CDCl_3$) δ 1.64 (s, 6H, $CH_3 \times 2$), 3.44 (d, 4H, $J = 6.6$ Hz, $CH_2 \times 2$), 5.08−5.15 (m, 4H, $CH_2 \times 2$), 5.81−6.12 (m, 2H, $CH \times 2$), 7.02 (d, 2H, $J = 8$ Hz, $CH_{ar} \times 2$), 7.14 (dd, 2H, $J = 1.8$, 8 Hz, $CH_{ar} \times 2$), 7.31 (d, 2H, $J = 1.8$ Hz, $CH_{ar} \times 2$); ^{13}C NMR ($CDCl_3$) δ 25.3, 40.0, 115.4, 116.1, 123.3, 128.4, 128.8, 133.2, 136.8, 137.5, 150.1. HRMS: $C_{21}H_{23}O_2$ [M + H^+] calcd, *m*/*z* 307.16926; found, *m*/*z* 307.16925.

Preparation of Magnolol from 3. A solution of protected magnolol **3** (2.8 g) in MeOH (75 mL) and 1 M HCl (1 mL) was heated overnight under reflux. After evaporation of the solvent, the crude compound was diluted in EtOAc (100 mL) and washed with H_2O and brine. The organic layer was then dried with $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (hexane/EtOAc, 85:15) to give magnolol **3** (2.3 g) in 95% yield.

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Supporting Information Available: HPLC and NMR data of compounds, characterization and evaluation of their purity. This material is available free of charge via Internet at http://pubs.acs.org.

References

(1) Lo, Y. C.; Teng, C. M.; Chen, C. F.; Chen, C. C.; Hong, C. Y. Magnolol and honokiol isolated from Magnolia officinalis protect rat heart mitochondria against lipid peroxidation. *Biochem. Pharmacol*. **1994**, *47*, 549−553.

(2) Nakazawa, T.; Yasuda, T.; Ohsawa, K. Metabolites of orally administered Magnolia officinalis extract in rats and man and its antidepressant-like effects in mice. *J. Pharm. Pharmacol*. **2003**, *55*, 1583−1591.

(3) Teng, C. M.; Chen, C. C.; Ko, F. N.; Lee, L. G.; Huang, T. F.; Chen, Y. P.; Hsu, H. Y. Two antiplatelet agents from Magnolia officinalis. *Thromb. Res*. **1988**, *50*, 757−765.

(4) Teng, C. M.; Yu, S. M.; Chen, C. C.; Huang, Y. L.; Huang, T. F. EDRF-release and Ca+(+)-channel blockade by magnolol, an antiplatelet agent isolated from Chinese herb Magnolia officinalis, in rat thoracic aorta. *Life Sci*. **1990**, *47*, 1153−1161.

(5) Bai, X.; Cerimele, F.; Ushio-Fukai, M.; Waqas, M.; Campbell, P. M.; Govindarajan, B.; Der, C. J.; Battle, T.; Frank, D. A.; Ye, K.; Murad, E.; Dubiel, W., Soff, G.; Arbiser, J. L. Honokiol, a small molecular weight natural product, inhibits angiogenesis in vitro and tumor growth in vivo. *J. Biol. Chem*. **2003**, *278*, 35501−3557.

(6) (a) Takeya, T.; Okubo, T.; Tobinaga, S. Synthesis of unsymmetrical biphenyl lignans, honokiol and related coumpounds, utilizing quinol-acetates as reactive intermediates. *Chem. Pharm. Bull*. **1986**, *34*, 2066−2075. (b) Esumi, T.; Makado, G.; Zhai, H.; Shimizu, Y.; Mitsumoto, Y.; Fukuyama, Y. Efficient synthesis and structure−activity relationship of honokiol, a neurotrophic biphenyl-type neolignan. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 2621−2625. (c) Gu, W.; She, X.; Pan, X.; Yang, T.-K. Enantioselective syntheses of (*S*)- and (*R*)-8,9-dihydroxydihydromagnolol. *Tetrahedron: Asymmetry* **1998**, *9*, 1377−1380.

(7) (a) For a recent review see: Slanina, J.; Glatz, Z. *J*. Separation procedures applicable to lignan analysis. *Chromatogr., B* **2004**, *812*, 215−229 (b) Wang, X.; Wang, Y., Geng, Y.; Li, F.; Zheng, C. Isolation and purification of honokiol and magnolol from cortex Magnoliae officinalis by high-speed counter-current chromatography. *J. Chromatogr., A* **2004**, *1036*, 171−175.

(8) Schinazi, R. F.; Sommadossi, J. P.; Saalman, V.; Cannon, D. L.; Xie, M.-W.; Hart, G. C.; Hahn, E. F. Activities of 3′-azido-3′-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. *Antimicrob. Agents Chemother*. **1990**, *34*, 1061−1067.

(9) Stuyver, L. J.; Lostia, S.; Adams, M.; Mathew, J.; Pai, B. S.; Grier, J.; Tharnish, P.; Choi, Y.; Chong, Y.; Choo, H.; Chu, C. K.; Otto, M. J.; Schinazi, R. F. Antiviral activities and cellular toxicities of modified 2′,3′-dideoxy-2′,3′-didehydrocytidine analogues. *Antimicrob. Agents Chemother*. **2002**, *46*, 3854−3860.

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