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Facile Purification of Honokiol and its Antiviral and Cytotoxic Properties

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Abstract

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

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 as a traditional Chinese medicine for thousands of years for treatment of anxiety and stroke. These natural products are also reported to have various biological activities including anti-oxidative and antidepressant properties^{1,2} They inhibit intracellular calcium mobilization in platelets³, 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 phosphorylation of vascular endothelial growth factor receptor 2 (VEGFR 2).⁵ Honokiol also exhibited a direct antitumor activity through tumor necrosis factor apoptosis-inducing ligand (TRAIL/Apo2L) signaling.⁵ Thus, the combination of both anti-angiogenic and anti-tumor 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, syntheses of pure honokiol and magnolol are not very efficient, requiring multisteps 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; the purification requiring either use of special chromatographic conditions or electromigration⁷ adding significantly to the cost of preparing honokiol or magnolol and are thus not readily adaptable to large scale.

Honokiol differs from magnolol only on 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 a facile separation.

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, since both hydroxyl groups are spatially too far to be protected. The

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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>.

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, deprotection of **3** under acidic condition affords highly pure (99.8%) magnolol in good yield with no detectable honokiol as determined by HPLC.

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 respectively 16.1, 22.5, 10.9 μ M for honokiol and 38.6, 50.6, 99.5 μ M 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 μ M 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 antiviral⁸ and cytotoxicity⁹ 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 *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 F₂₅₄, E. Merck). Compounds were visualized by UV irradiation and/or spraying with 20% H₂SO₄ in EtOH, followed by charring at 150 °C. Column chromatography was performed on silica gel 60 M (0.040-0.063 mm, E. Merck). ¹H and ¹³C NMR spectra were recorded on a Mercury 300 spectrometer at 300 MHz for ¹H and 75 MHz for ¹³C, respectively, using internal CHCl₃ as reference (7.26 ppm for ¹H and 77.23 ppm for ¹³C); 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 paper 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 presence of a catalytic amount of *p*-TSA. After neutralization of the solution with NaHCO₃, the mixture was diluted with EtOAc (150 mL) and washed with H₂O and brine then the organic layer was dried over MgSO₄, 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): ¹H-NMR (CDCl₃) δ 1.64 (s, 6H, CH₃ x 2), 3.44 (d, 4H, *J* = 6.6 Hz, CH₂ x 2), 5.08-5.15 (m, 4H, CH₂ x 2), 5.81-6.12 (m, 2H, CH x 2), 7.02 (d, 2H, *J* = 8 Hz, CH_{ar} x 2), 7.14 (dd, 2H, *J* = 1.8, 8 Hz, CH_{ar} x 2), 7.31 (d, 2H, *J* = 1.8 Hz, CH_{ar} x 2); ¹³C-NMR (CDCl₃) δ 25.3, 40.0, 115.4, 116.1, 123.3, 128.4, 128.8, 133.2, 136.8, 137.5, 150.1. HRMS: C₂₁H₂₃O₂ [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 under reflux overnight. After evaporation of the solvent, the crude compound was diluted in EtOAc (100 mL) and washed with H₂O and brine. The organic layer was then dried with MgSO₄ 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1.

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