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Configurational assignments of conformationally restricted bis-monoterpene hydroquinones: Utility in exploration of endangered plants

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ABSTRACT

Background: Endangered plant species are an important resource for new chemistry. *Lindera melissifolia* is native to the Southeastern U.S. and scarcely populates the edges of lakes and ponds. Quantum mechanics (QM) used in combination with NMR/ECD is a powerful tool for the assignment of absolute configuration in lieu of X-ray crystallography.
Methods: The EtOAc extract of *L. melissifolia* was subject to chromatographic analysis by VLC and HPLC. Spin–spin coupling constant (SSCC) were calculated using DFT at the MPW1PW91/6-31G(d,p) level for all staggered rotamers. ECD calculations employed Amber* force fields followed by PM6 semi-empirical optimizations. Hetero- and homo-nuclear coupling constants were extracted from 1D ^{1}H, E.COSY and HETLOC experiments.
Results: Two meroterpenoids, melissifolianes A (1) and B (2) were purified and their 2-D structures elucidated using NMR and HRESIMS. The relative configuration of 1 was established using the combination of NOE-based distance restraints and the comparisons of experimental and calculated SSCCs. The comparison of calculated and experimental ECD assigned the absolute configuration of 1. The relative configuration of a racemic mixture, melissifoliane B (2) was established utilizing *J*-based analysis combined with QM and NMR techniques.**Conclusion** Our study of the *Lindera melissifolia* metabolome exemplifies how new chemistry remains undiscovered among the numerous endangered plant species and demonstrates how analysis by ECD and NMR combined with various QM calculations is a sensible approach to support the stereochemical assignment of molecules with conformationally restricted conformations.
General significance: QM–NMR/ECD combined approaches are of utility for unambiguous assignment of 3-D structures, especially with limited plant material and when a molecule is conformationally restricted. Conservation of an endangered plant species can be supported through identification of its new chemistry and utilization of that chemistry for commercial purposes.

1. Introduction

Endangered plant species are an underutilized resource for new chemistry. These discoveries ironically hold the potential for development into a commercial application that would justify the conservation of the plant. This is evidenced by a recent phylogenic analysis which shows drug producing plant families are concentrated around families which contain endangered species [1]. However, the challenge associated with such extraordinarily limited resources can prohibit the full elucidation of new structures through X-ray crystallography or NMR spectroscopy alone. The discovery of new bis-monoterpene hydroquinones from a U.S. endangered plant provides a unique opportunity in this regard. Owing to the conformationally restricted assemblies, the hydroquinones help demonstrate how combined quantum mechanical calculations with NMR and ECD studies can unambiguously assign three dimensional structures in cases where such detail might otherwise be lost due to limited resources.

Worldwide, at least 13% of the known flora is endangered or threatened [2] and the USDA verifies that 780 plant species in the U.S. and its territories are endangered or threatened [3]. Although several reports reveal the potential natural products from U.S. endangered species may provide [4], these reports are infrequent relative to

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the number of endangered U.S. species. Representative species of most of the U.S. endangered plant genera are distributed throughout the world. In some cases, the species have been investigated in regard to their unusual chemistry and traditional uses [4–7].

The endangered status of *Lindera melissifolia* (Walt.) Blume (Lauraceae), found in the Southeastern U.S., prompted our study of the plant as an example of how endangered U.S. plants can yield novel chemistry that could potentially be lost due to extinction. Commonly called pondberry, *L. melissifolia* is an aromatic and rhizomatous shrub that inhabits the edges of lakes and ponds. The essential oil from this plant possesses significant insect repellency [8]. The ethyl acetate extraction of pondberry drupes led to the discovery of two new bis-monoterpene hydroquinones named melissifolianes A (1) and B (2) (Fig. 1). The structures of the melissifolianes combine a common 2-(hydroquinone) acetic acid ester moiety flanked uniquely by two monoterpene units in contrast to a single monoterpene moiety as reported in *Magnolia denudata* [9]. The limited amounts of the melissifolianes negated assignment of their three dimensional structure by chemical methods or screening appropriate conditions for crystallization. However the conformationally restricted state associated with the monoterpene-linked acetic ester moiety provided the opportunity to employ computational methods to verify configuration. Herein, we report the use of NMR and ECD analysis coupled with quantum mechanical (QM) calculations for the establishment of configuration and conformation of natural products like the melissifolianes which contain conformationally restricted moieties.

2. Materials and methods

2.1. General procedures

NMR spectra were obtained using a Bruker Avance 400 MHz spectrometer referenced by residual dichloromethane and chloroform signals. Homo- and hetero-nuclear coupling constants were measured using ^{1}H-specturm, E.COSY (Exclusive COSY) and HETLOC (HETeronuclear LOng-range Coupling). Optical rotations were measured on a Rudolph Autopol V polarimeter. UV–vis spectra were recorded on an Agilent 1100 series diode array and multiple wavelength detectors (DAD). The LC–MS analyses were performed using an Agilent 1100 HPLC system with a Phenomenex Luna 5 μm C8(2) column (4.6 × 150 mm), an MeOH/H_2O (0.1% HCOOH) gradient solvent system and a Bruker Daltonics microTOF mass spectrometer or an Astec Chirobiotic R column (4.6 × 100 mm), 55% MeOH/H_2O isocratic condition (20 mM NH_4Ac, 35 °C) for the chiral separation. HRESIMS spectra were measured using the LC–MS system with electrospray ionization. Column chromatography was conducted using silica gel 60 (40–63 μm particle size) and RP-18 (40–63 μm particle size). Precoated TLC silica gel 60 F254 plates (Merck) were used for TLC. HPLC was performed on a Waters System equipped with a Waters 2487 dual absorbance detector. Experimental CD data was acquired at 5 °C using an Olis CD Spectrophotometer. The concentration of the sample was 500 μg/ml in MeCN and the path length was 5 mm.

2.2. Extraction and purification

Drupes (5 kg) of *L. melissifolia* were harvested in the Fall of 2009 at the Flooding Research Facility in Sharkey County, MS (Fig. S1-1). The dried ripe drupes of *L. melissifolia* were coarsely ground and extracted with EtOAc, and the extract was dried under reduced pressure to give 100 g of crude extract. The extract was fractionated on silica gel eluted sequentially by hexanes–EtOAc (100:0, 80:20, 50:50 and 0:100) and then EtOAc–MeOH (80:20, 50:50 and 0:100) to afford 10 fractions, respectively. Fractions 6–9 exhibited significant anti-infective activities and the active fractions were further chromatographed by normal phase [Phenomenex Luna Si, 10 × 250 mm, 5 μm, flow rate 5 mL/min] with a gradient elution of hexanes–EtOAc (1:0 to 8:2 over 120 min) and, finally, by reverse phase [Waters C_{18} 20 × 250 mm, 10 μm, flow rate 10 mL/min, Phenomenex C_{18} 5 × 250 mm, 5 μm, flow rate 1 mL/min] using a gradient elution of H_2O–MeOH (1:1 to 100% methanol over 110 min) to obtain the pure compounds.

2.2.1. Melissifoliane A (1)

Melissifoliane A (1, RT ≈ 46 min), (*R*)-methyl-2-{3-[(*E*)-3,7-dimethylocta-2,6-dien-1-yl]-2,5-dihydroxyphenyl}-2-[(1*S*,2*S*,5*S*)-2-hydroxy-5-isopropyl-2-methylcyclohex-3-en-1-yl]acetate, a pale yellowish oil, had the molecular formula $C_{29}H_{42}O_5$ deduced from HRESIMS (obsd. $[M + Na]^+ - H_2O$ at *m/z* 475.2842, theor. $[M + Na]^+ - H_2O$ = 475.2819 and $[2\,M + Na]^+ - H_2O$ at *m/z* 927.5725, theor. $[2\,M + Na]^+ - H_2O$ = 927.5751). The specific rotation ($[\alpha]_D^{23}$) was established as + 54.0 (*c* 0.2, MeCN). For ^{1}H and ^{13}C data, see Table 1.

2.2.2. Melissifoliane B (2)

Melissifoliane B (2, RT ≈ 45 min), (*E*)-methyl-2-{3-[(*E*)-3,7-dimethylocta-2,6-dien-1-yl]-2,5-dihydroxyphenyl}-4-hydroxy-5,9-dimethyldeca-5,8-dienoate, a pale yellowish oil, had the molecular formula $C_{29}H_{42}O_5$ as deduced from HRESIMS results (obsd. $[M + Na]^+ - H_2O$ at *m/z* 475.2852, theor. $[M + Na]^+ - H_2O$ = 475. 2819 and $[2\,M + Na]^+ - H_2O$ at *m/z* 925.5530, theor. $[2\,M + Na]^+ - H_2O$ =927.5751). For ^{1}H and ^{13}C data, see Table 1.

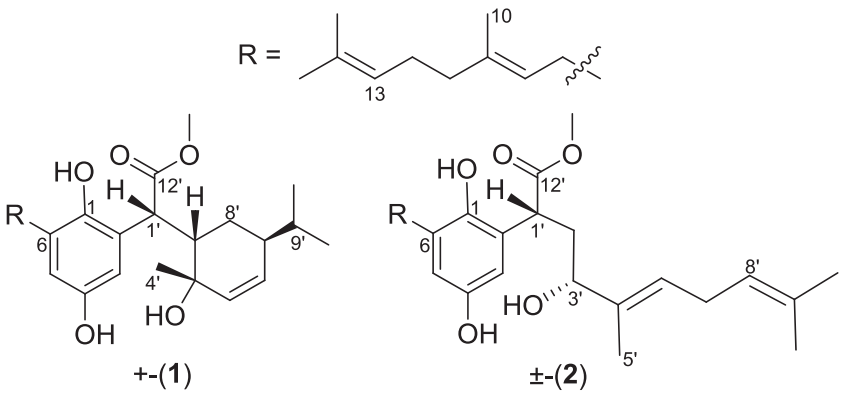

Fig. 1. Structure of melissifolianes A (1) and B (2).