ORIGINAL ARTICLE

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Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

Myrrhalindenane C, a New Eudesmane Sesquiterpenoid From *Lindera Myrrha* Roots

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(Received March 24, 2022; Revised July 12, 2022; Accepted July 14, 2022)

Abstract: Phytochemical investigation of *Lindera myrrha* roots growing in Vietnam afforded a new eudesmane sesquiterpenoid, myrrhalindenane C (**1**), along with seven known compounds, *rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**2**), 1-*O*-(4-hydroxy-2,6-dimethoxyphenoxy)-6-*O*-[*rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoyl]-β-D-glucopyranose (**3**), curcumin (**4**), demethoxycurcumin (**5**), bisdemethoxycurcumin (**6**), (1*E*,6*E*)-1,7-bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (**7**), and sanjoseolide (**8**). The structures were determined by analysis of their MS and NMR data as well as by comparison with literature values. All compounds were evaluated for antimicrobial activity against antibiotic-resistant, pathogenic bacteria *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*.

Keywords: Lauraceae, *Lindera myrrha*, myrrhalindenane C, eudesmane, antimicrobial activity.

1. Introduction

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.343.2203.2396
Available online: July 20, 2022

The genus *Lindera,* which belongs to the Lauraceae family, has economic applications in eastern Asia and America [1]. Many *Lindera* plants are associated with ethnopharmacological claims, including treatment of urinary system diseases, inflammatory-related health hazards, antispasmodic properties, as well as beneficial effects in gastric ulcer, cholera, and beriberi [2, 3]. Previous phytochemical investigation of *Lindera* species reported a wealth of skeletons which include mainly sesquiterpenes (lindenanes, eudesmanes, and germacranes) and aporphine alkaloids [2]. *Lindera myrrha* (Lour.) Merr. is a native species in Vietnam. Chemical data of these Vietnamese plants are scarce. The first phytochemical investigation, conducted in 1994, indicated the isolation of several newly-reported aporphine alkaloids [4]. More recently, lindermyrrhin and myrrhalindenanes A-B were further described from this bio-source [5, 6]. The current report describes the isolation and structural elucidation of one new compound named myrrhalindenane C, together with seven known compounds, *rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-penta-dienoic acid (**2**) [7], 1-*O*-(4-hydroxy-2,6-dimethoxyphenoxy)-6-*O*-[*rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoyl]-β-D-glucopyranose (**3**) [8], curcumin (**4**) [9], demethoxycurcumin (**5**) [9], bisdemethoxycurcumin (**6**) [9], (1*E*,6*E*)-1,7-bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (**7**) [10], and sanjoseolide (**8**) [11] (Figure 1). Additionally, all isolated compounds were evaluated for antimicrobial activity against the antibiotic-resistant, pathogenic bacteria *Enterococcus faecium, Staphylococcus aureus*, and *Acinetobacter baumannii*.

2. Materials and Methods

2.1. General Experimental Procedures

NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) with TMS as internal standard. HRESIMS was recorded using a MicrOTOF–Q mass spectrometer on an LC-Agilent 1100 LC-MSD Trap spectrometer. Thin-layer chromatography (TLC) was carried out using precoated silica gel 60 F_{254} or 60 RP–18 F_{254S} (Merck). Spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Himedia).

2.2. Plant Material

The roots of *L. myrrha* were collected from Cu Chi District, Ho Chi Minh City, Vietnam, in July, 2019. The botanical sample was authenticated by Dr. Le Van Minh, Research Center of Ginseng and Materia Medical, Ho Chi Minh City, Vietnam. A voucher specimen (No UP-B05) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Education and in Institute of Tropical Biology, Vietnam Academy of Science and Technology (VNM-Dung0025).

2.3. Extraction and Isolation

Dried and ground roots of *Lindera myrrha* (5.2 kg) were extracted by maceration with EtOAc (3 × 10 L) at ambient temperature. The filtrated solution was evaporated to dryness under reduced pressure to obtain a crude extract (145 g). The extract was applied to Sephadex LH-20 gel column chromatography (CC), eluted with MeOH to afford fractions EA1-EA5. Fraction EA5 (23.3 g) was re-chromatographed by Sephadex LH-20 gel CC to afford fractions EA5.1-4. Fraction EA5.4 (6.7 g) was applied to normal phase silica gel CC, and isocratically eluted with a solvent system of *n*-hexane-EtOAc (5:1, v/v) to afford fractions D1-D5. Fraction D5 (677 mg) was applied to a C_{18} reversed-phase (RP) CC eluted with MeOH/H_2O (5:1, v/v) to yield subfractions D5.1- D5.3. Subsequently, subfraction D5.1 (135 mg) was purified by semi-preparative HPLC to obtain compounds **4** (2.1 mg), **5** (2.5 mg), **6** (2.3

mg), **7** (2.7 mg), and **8** (1.6 mg). Subfraction D5.3 was applied to a C_{18}-RP CC eluted with MeOH/H_2O (3:2, v/v) to obtain compounds **1** (1.9 mg), **2** (4.7 mg), and **3** (1.5 mg).

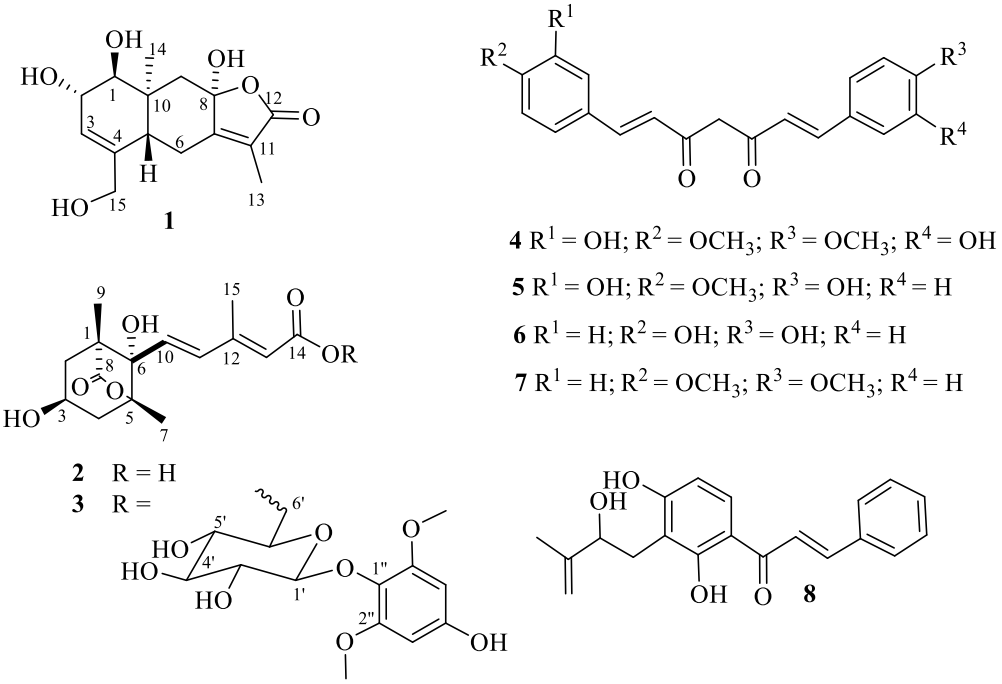

Figure 1. Chemical structures of compounds **1-8**

*Myrrhalindenane C (**1**)*: colorless gum. UV (MeOH), λ_{max} (log ε) 207 (4.58) nm; IR ν_{max} (neat): 3480, 1709, 1701, 1260, 1101 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm, *J*/Hz): 7.12 (1H, s, 8-OH), 5.57 (1H, brs, H-3), 4.85 (1H, d, *J* = 4.0 Hz, 1-OH), 4.80 (1H, d, *J* = 4.5 Hz, 2-OH), 4.75 (1H, t, J = 5.5 Hz, 15-OH), 4.01 (1H, dd, *J* = 13.5, 5.5 Hz, H-15a), 3.90 (1H, dd, *J* = 13.5, 5.5 Hz, H-15b), 3.84 (1H, m, H-2), 3.21 (1H, d, *J* = 4.0 Hz, H-1), 2.96 (1H, d, *J* = 10.0 Hz, H-6a), 2.21 (1H, d, *J* = 13.5 Hz, H-5), 2.18 (1H, dd, *J* = 10.0, 13.5 Hz, H-6b), 1.88 (1H, d, *J* = 13.5 Hz, H-9a), 1.86 (1H, d, *J* = 13.5 Hz, H-9b), 1.73 (3H, s, H-13), 1.03 (3H, s, H-14). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 7.9 (C-13), 16.1 (C-14), 22.3 (C-6), 36.8 (C-10), 39.9 (C-5), 45.4 (C-9), 62.8 (C-15), 70.4 (C-2), 76.6 (C-1), 104.6 (C-8), 120.4 (C-11), 122.9 (C-3), 137.7 (C-4), 161.6 (C-7), 171.8 (C-12). HR-ESI-MS *m/z* 295.1191 $[M-H]^-$ (calcd. for $C_{15}H_{19}O_6$, 295.1182).

2.4. Semi-Preparative HPLC Method

The selected fraction was applied to preparative HPLC LC-8A Shimadzu, equipped with an SPD-20A detector, following a published method with modification [12]. Samples were injected separately. The mobile phase used (ACN) as solvent A and (H_2O) as solvent B, with an isocratic mode of 60% A and 40% for 60 min. The flow rate was 5 mL/min, and 1 mL of each sample was injected. The detection wavelength was 254 nm. A Luna C18 column (Phenomenex, 150 mm × 10 mm. i.d., 5 μm) and a C18 guard column (Phenomenex, Torrance, CA, USA) were used.

2.5. Antimicrobial Activity Assay

Evaluation of the antibacterial activity of all the isolated compounds followed a previous procedure [13]. The agar well diffusion method was used to evaluate the antibacterial activity of the isolated compounds on antibiotic-resistant, pathogenic bacteria *Enterococcus faecium, Staphylococcus aureus,* and *Acinetobacter baumannii*. Three bacterial pathogens were cultured in nutrient broth at 37ºC for 18 hours. The cultures were diluted with sterile 0.9% NaCl to obtain bacterial solutions of 1.5×10^8 CFU/ml. This solution with the volume of 100 μL was spread on a Mueller-Hinton agar plate. Holes

with a diameter of 8 mm were punched aseptically to create wells on the surface of the Mueller-Hinton agar. The compounds were then dissolved in DMSO. Following this, the amount of 50 µg of each compound solution was inserted into the wells. The plates were incubated at 37ºC for 16-18 hours and the antibacterial activity of each compound was recorded by measuring the diameters of the inhibition zones surrounding the wells. DMSO was used as a control. The positive control, apramycin showed the inhibition zone of 21 mm, 20 mm, and 21 mm against *Enterococcus faecium, Staphylococcus aureus,* and *Acinetobacter baumannii*, respectively.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1**, colorless gum, had the molecular formula of $C_{15}H_{20}O_6$ based on HRESI mass data. The ^{1}H NMR spectrum, in accordance with the HSQC spectrum, showed the presence of four hydroxy groups, one olefin proton [δ 5.57 (1H, brs, H-3)], one oxygenated methylene [δ 4.01 (1H, dd, *J* = 13.5, 5.5 Hz, H-15a), 3.90 (1H, dd, *J* = 13.5, 5.5 Hz, H-15b)], two oxymethines [δ 3.84 (1H, m, H-2) and 3.21 (1H, d, *J* = 4.0 Hz, H-1)], one methine, two methyls (δ 1.73 and 1.03), and two methylenes. The ^{13}C NMR spectrum revealed one lactone carbonyl carbon, four olefinic carbons, one hemiketal carbon, two oxygenated methines, and one oxygenated methylene. At a higher magnetic field, there was the presence of one methine, two methylenes, two methyls, and a quaternary carbon. The above spectroscopic data indicated that **1** had a eudesmane-type sesquiterpene lactone [6, 14-17]. The HMBC correlations of the methyl at δ 1.73 to carbons at δ 171.8 (C-12), 161.6 (C-7), and 120.4 (C-11) and of the hemiacetal proton at δ 7.12 to carbons at δ 104.6 (C-8) and C-7, indicated the presence of the lactone ring (so-called C-ring) Figure 2. Moreover, HMBC cross-peaks of the methylenes at δ 2.96 (H-6a), 1.88 (H-9a), and 1.86 (H-9b) to both C-7 and C-8 defined the connectivity through C-6-C-9. The methyl at δ 1.03 gave HMBC correlations to C-5, C-9, and C-10, confirmed the structure of the B-ring. This group also gave a HMBC cross-peak to an oxymethine carbon at δ 76.6 (C-1), indicating the position of the 1-OH group. The oxymethylene at δ 4.01 and 3.90 gave HMBC correlations to carbons at 122.9 (C-3), 137.7 (C-4), and C-5 while the hydroxy protons at δ 4.80 had the HMBC correlations to carbons C-1 and C-3. These correlations, along with the HMBC cross-peaks of H-1 with carbons C-2, C-3, and C-5 indicated the position of 2-OH, which further confirmed the structure of the A-ring. NMR data of **1** were highly similar to those of eudebeiolide J [14], except for the replacement of the oxymethylene H_2-15 of **1** for the methyl H_3-15 of eudebeiolide J (Table S1) [14]. Altogether, the chemical structure of **1** was elucidated as shown, namely myrrhalindenane C.

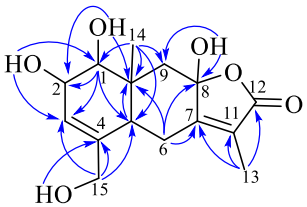

Figure 2. Key HMBC correlations of **1**

The stereochemistry of **1** was determined by *J*-coupling analysis, NOESY correlations, and comparison with the literature. At first, proton H-5 had a large coupling constant (*J* = 13.5 Hz). This indicated its axial position, which was similar to those of previously reported eudesmane sesquiterpenes [6]. Next, the coupling constant of H-1 and H-2 indicated their stereochemistry. Particularly, the null coupling constant of H-1 and H-2 indicated that both protons are in *trans* orientation. This phenomenon can be found in previously-reported eudesmanolides from *Mikania campunulata* [15] or artemisargin A isolated from *Artemisia argyi* [17]. In contrast, in the case of eudbeiolide J, the coupling constant of H-1 and H-2 was 4.0 Hz, assigning that both H-1 and H-2 were *syn*-facial. Finally, the NOESY correlation of H_3-14 and H-1 indicated an equal orientation. This methyl also gave the NOESY cross peak to H-6b,

while proton H-5 had a strong NOESY correlation to H-6a, indicating the *trans* orientation of H-5 and H_3-14. Based on the abovementioned spectroscopic information, the relative stereochemistry of **1** was defined except for the C-8 chiral center. The undefined C-8 center led to 4 possible stereoisomers of **1** (Figure S10). ECD spectrum of **1** was recorded to define the absolute stereochemistry of **1**. ECD spectra of **1** and eudbeiolide J [14] gave two similar characteristic Cotton effects at -220 and -241 nm (Figure S9), indicating that both compounds have a similar stereochemistry at C-8. Thus, the absolute configuration of **1** was proposed as 1*S*,2*R*,5*R*,8*R*,10*S*.

To the best of our knowledge, compounds **1**-**6** and **8** were found for the first time in the *Lindera* genus.

3.2. Antimicrobial Activity

The compounds displayed antimicrobial activity against antibiotic-resistant, pathogenic bacteria *Enterococcus faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii*. Compound **8** showed strong activity against *S. aureus* with a MIC value of 8 µg/mL. This compound showed moderate activity against *Enterococcus faecium* and *Acinetobacter baumannii* with the inhibition zones of 13 and 11 mm at the concentration of 50 µg/mL but it is inactive against the other strain. Other compounds failed to reveal any activity.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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