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Two new phenolic compounds from the seeds of Machilus yunnanensis

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\textbf{ABSTRACT}

Chemical constituents investigation on the seeds of Machilus yunnanensis led to two new phenolic compounds 8-O-acetyl-phenylethanoid-4-O-β-D-glucopyranoside (1) and (E)-2,3-bis(4-hydroxyphenyl)acrylaldehyde (2), together with 16 known compounds. Their structures were elucidated on the basis of spectroscopic data analysis (IR, MS, 1D, and 2D NMR). Meanwhile, compounds 1–3, 6–13, 17, and 18 were evaluated for vasorelaxant effects on the rat endothelium-intact thoracic aorta rings precontracted with phenylephrine (PE) or KCl. The bioassay results showed that compound 17 had significant vasorelaxant effect on the endothelium-intact thoracic aorta rings precontracted with KCl.

\section{1. Introduction}

\textit{Machilus yunnanensis} (Lauraceae) is a deep rooted tree, mainly distributed in Yunnan and Sichuan provinces of China \cite{1}. In China, it has been used for the treatments of skin swelling, sore, mumps, burn, bone fractures, and rheumatism \cite{2}. Previous studies on the plants of \textit{Machilus} led to the isolation of different compounds, such as alkaloids \cite{3}, triterpenoid glycosides \cite{4}, butyrolactones \cite{5}, sesquiterpenoids \cite{6}, and lignans \cite{7}. However, no phytochemical or biological activities literature on \textit{M. yunnanensis} was available. We made a phytochemical investigation and isolated two new phenolic compounds 8-O-acetyl-phenylethanoid-4-O-β-D-glucopyranoside (1) and (E)-2,3-bis(4-hydroxyphenyl)acrylaldehyde (2) (Figure 1), along with 16 known compounds from the 70% aqueous acetone extract of \textit{M. yunnanensis} seeds. Their structures were established by extensive spectroscopic data analysis and comparison with those of literature values. In addition, previous literature reported that meso-dihydroguaiaretic acid isolated from a species of \textit{Machilus} genus, \textit{M. thunbergii}, had aggregation activity and vasorelaxant effect \cite{8}. Therefore, compounds 1–3, 6–13, 17, and 18 were evaluated for their vasorelaxant effects on the rat endothelium-intact thoracic aorta rings precontracted with PE or KCl, but only compound 17

\textbf{KEYWORDS}

Lauraceae; \textit{Machilus yunnanensis}; phenolic compounds; vasorelaxant effect

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showed vasorelaxant effect. Herein, we describe the isolation, structure elucidation, and bioassays of these isolated compounds.

2. Results and discussion

Compound 1 ([α]D21.6 – 28.2) was obtained as a colorless oil. The molecular formula C16H22O8 was deduced by HREIMS at m/z 365.1205 [M + Na]+. The UV absorption maxima at 202, 219, and 271 nm revealed an aromatic ring moiety. The IR showed absorption bands at 3418 (hydroxy), 1729 (ester), 1614, and 1511 cm⁻¹ (aromatic ring). The 1H NMR spectral data (Table 1) showed one methyl at δH 2.03 (3H, s), two methenes at δH 2.91 (2H, t, J = 6.9 Hz), and 4.25 (2H, t, J = 6.9 Hz), a set of AA'BB'-type aromatic resonances at δH 7.06 (2H, d, J = 8.6 Hz) and 7.18 (2H, d, J = 8.6 Hz), one anomeric proton at δH 4.92 (1H, d, J = 7.5 Hz), and a series of sugar signals at δH 3.38–3.94. The 13C NMR spectral data (Table 1) displayed one methyl (δC 20.9), two methenes (δC 35.3, 66.5), one ester (δC 173.1), six aromatic carbons (δC 117.9 × 2, 131.0 × 2, 133.3, 158.0), and one glucopyranosyl unit (δC 102.5, 75.0, 78.1, 71.5, 78.2, and 62.6). The 1H and 13C NMR spectral data of 1 were very similar to 3 (icariside D2) except for the presence of acetyl group (δC 173.1 and 20.9) in 1, which suggested 1 was the analog of 3. Acid hydrolysis of 1 liberated a sugar identified as D-glucose by TLC (CHCl₃/MeOH; 2:1; Rf 0.26) with an authentic sample [9], and [α]D21.6 + 68.83 of the liberated sugar further confirmed that. The β anomeric configuration for the glucose of 1 was
determined by its coupling constant \((J = 7.5 \text{ Hz})\). The arrangements of the methyl, ester, methenes, aromatic ring, and \(\beta\)-D-glucose were determined by analysis of the proton coupling patterns, HMQC, HMBC data (Figure 2). In the HMBC spectrum, the correlations from H-7 \((\delta_H 2.91, \text{ t})\) to C-1 \((\delta_C 133.3)\), C-2, 6 \((\delta_C 131.0 \times 2)\), and C-8 \((\delta_C 66.5)\); H-8 \((\delta_H 4.25, \text{ t})\) to C-1 \((\delta_C 133.3)\), C-7 \((\delta_C 35.3)\), and carbonyl \((\delta_C 173.1)\); protons of methyl \((\delta_H 2.03, \text{ s})\) to carbonyl \((\delta_C 173.1)\) proved these links successively. Hence, compound 1 was elucidated as 8-\(O\)-acetyl-phenylethanoid-4-\(O\)-\(\beta\)-D-glucopyranoside.

Compound 2 ([\(\alpha\])_D^{21.6} − 3.2) was obtained as a yellowish amorphous powder. The molecular formula \(\text{C}_{15}\text{H}_{12}\text{O}_{3}\) was determined by HREIMS at \(m/z\ 263.0678 [M + Na]^+\). The UV absorption maxima at 202, 231, 329 nm indicated a conjugated system. The IR spectrum showed absorption bands at 3411 (hydroxy), 1661 (\(\alpha\),\(\beta\)-unsaturated carbonyl), 1600 and 1512 cm\(^{-1}\) (aromatic ring). The \(^1\)H NMR spectral data (Table 1) displayed a set of AA'BB'-type aromatic resonances at \(\delta_H 6.66 (2\text{H}, \text{ d}, J = 8.7 \text{ Hz}), 6.86 (2\text{H}, \text{ d}, J = 8.4 \text{ Hz}), 6.99 (2\text{H}, \text{ d}, J = 8.4 \text{ Hz}), \) and 7.17 (2\text{H}, \text{ d}, \(J = 8.7 \text{ Hz})\), revealing two \(p\)-substituted aromatic rings. Moreover, one conjugated olefinic proton and one aldehyde proton were shown at \(\delta_H 7.40 (1\text{H}, \text{ s})\) and 9.60 (1\text{H}, \text{ s}), respectively. The \(^{13}\)CNMR spectral data (Table 1) displayed 12 aromatic carbons \((\delta_C 116.4 \times 2, 116.7 \times 2, 126.1, 127.0, 131.8 \times 2, 134.2 \times 2, 158.5, 161.5)\), one double bond \((\delta_C 140.5, 153.0)\), and one aldehyde group \((\delta_C 196.6)\). In the HMBC spectrum, the correlations from H-3 \((\delta_H 7.40, \text{ s})\) to C-1' \((\delta_C 127.0)\), C-2'', 6'' \((\delta_C 134.2 \times 2)\), and C-1 \((\delta_C 196.6)\); H-1 \((\delta_H 9.60, \text{ s})\) to C-1'' \((\delta_C 126.1)\), and C-2 \((\delta_C 140.5)\); H-2'', 6'' \((\delta_H 6.99, \text{ d})\) to C-2 \((\delta_C 140.5)\) indicated the two \(p\)-substituted aromatic rings were connected to the double bond in C-2 and C-3, respectively, and the aldehyde group was substituted at C-2 in the double bond. The arrangements of the two aromatic rings, double bond, and aldehyde group were determined by analysis of the proton coupling patterns, HMQC, and HMBC data (Figure 2). Furthermore, the \textit{cis}-configuration of the double bond was proved by the correlations from H-3 \((\delta_H 7.40, \text{ s})\) to H-2', 6' \((\delta_H 7.17, \text{ d})\), and H-1 \((\delta_H 9.60, \text{ s})\) in the ROESY spectrum (Figure 3). Thus, compound 2 was characterized as \((E)-2,3\text{-bis(4-hydroxyphenyl)acrylaldehyde}.

Sixteen known compounds (3–18) were identified on the basis of comparing of their NMR spectral data with those in the literature as icariside D\(_2\) (3) [10], 3-[4-(\(\beta\)-D-glucopyranosyloxy)phenyl]propionic acid (4) [11], \(p\)-2-ethylphenyl-\(\beta\)-D-glucopyranoside (5) [12], gastrodin (6) [13], koaburasidemoethyl ether (7) [14], phenylethyl-\(\beta\)-D-glucopyranoside (8) [15], piceatannol-4'-\(O\)-\(\beta\)-D-glucopyranoside (16) [16], 4,4'-dihydroxychalcone (10) [17], tyrosylhydroxylphenyl acetate (11) [18], methyl 4-hydroxyphenyl acetate (12) [19], \(p\)-hydroxybenzoic acid (13) [10], (1'S)-1'-((4-hydroxyphenyl)ethane-1',2'-diol (14) [20], hydroxytyrosol (15) [21], 4-(2-hydroxyethyl)-1,3-benzenediol (16) [22], clovane-2\(\beta\), 9\(\alpha\)-diol (17) [23], (−)-oleuropeic acid (18) [21]. All above compounds were reported for the first time.
Compounds 1–3, 6–13, 17, and 18 were tested for their vasorelaxant effects on the rat endothelium-intact thoracic aorta rings precontracted with phenylephrine (PE) or KCl. The results showed that compound 17 had significant vasorelaxant effect on the rat endothelium-intact aortic rings precontracted with KCl (Figure 4). Other compounds were inactive in current assay.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020C digital polarimeter (JASCO, Tokyo, Japan). UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Tensor 27 spectrometer (Bruker
Optics, Ettlingen, Germany) with KBr pellets. ESI-MS were recorded on an Agilent 6530 Q spectrometer (Agilent, Palo Alto, CA, USA). HREIMS were measured using a Waters Auto Premier P776 spectrometer (Waters, Milford, MA, USA). 1D and 2D NMR were performed on Bruker AVANCE III-600 spectrometers with TMS as an internal standard (Bruker Optics, Karlsruhe, Germany). Column chromatography was performed over silica gel (100–200 or 200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China), MCI gel (CHP 20P, 75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). Semi-preparative HPLC was performed on an Agilent 1260 apparatus (Agilent, Santa Clara, CA, USA) equipped with a UV detector and a Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. Thin-layer chromatography was carried out on silicagel plates GF254 (Qingdao Haiyang Chemical Inc.) using various solvent systems, and spots were visualized by spraying with 10% H2SO4 in EtOH followed by heating.

3.2. Plant material

The air-dried seeds of M. yunnanensis (6.0 kg) used in this study were collected from Kunming, Yunnan province, China, in September 2013. The plant was identified by Prof. Xun Gong at Kunming Institute of Botany, Chinese Academy of Sciences (voucher no. KIB-2013114).

3.3. Extraction and isolation

The air-dried seeds of Machilus yunnanensis seeds (6.0 kg) was extracted with 70% aqueous acetone (48 h × 3) at room temperature and concentrated under vacuum to give residue, which was partitioned between H2O and EtOAc. The EtOAc extract (120 g) was chromatographed over a silica gel column (CHCl3/MeOH: 1:0–0:1) to afford six fractions A–F. Fr. C (12 g) was chromatographed on MCI (MeOH/H2O, 10:95–100:0) to give four fractions C1–C4. Fr. C2 (6 g) was subjected to Sephadex LH-20 (MeOH) to provide four major fractions C2-1–C2-4. Fr. C2-3 (2.5 g) was separated into four subfractions C2-3-1–C2-3-4 by repeated silica gel columns (CHCl3/MeOH; 8:1). Fr. C2-2-3 (36 mg) was further purified by HPLC (MeOH/H2O, 35:65, flow rate: 3 ml/min) to afford 1 (13.0 mg, tR 8.2 min) and 3 (10.6 mg, tR 11.2 min). Compounds 2 (2.6 mg, tR 9.6 min), 10 (1.5 mg, tR 14.8 min), and 11 (4.8 mg, tR 14.8 min) were obtained from C2-3-3 (16 mg). Fr. C2-2-3 (57 mg) was subjected to HPLC (MeOH/H2O, 40:60, flow rate: 3 ml/min) to produce 9 (12 mg, tR 8.2 min), 13 (5.0 mg, tR 10.3 min), and 8 (1.2 mg, tR 12.7 min). Fr. C3 (3 g) was subjected to repeated silica gel columns (CHCl3/MeOH, 15:1–1:1) to yield 4 (4.6 mg). Fr. B (2.8 g) was dealt with the same steps just as C, then B2-3-3 (368 mg) yielded 17 (12 mg), 18 (6 mg), and 12 (9.6 mg) by silica gel column (CHCl3/acetone, 20:1–2:1). Compounds 14 (11 mg, tR 11.3 min), 15 (8 mg, tR 12.5 min), and 16 (7.5 mg, tR 14.2 min) were obtained from B2-3-2 (480 mg) by HPLC (MeOH/H2O; 60:40; flow rate: 3 ml/min). Compounds 5 (1.8 mg, tR 6.4 min), 6 (12 mg, tR 8.2 min), and 7 (8.8 mg, tR 11.9 min) were afforded from D2-3-3 (29 mg) by HPLC (MeOH/H2O; 25:75; flow rate: 3 ml/min).

3.3.1. 8-O-Acetyl-phenylethanoid-4-O-β-D-glucopyranoside (1)

Colorless oil; [α]D21.6 − 28.2 (c 0.43, MeOH); UV (MeOH) λmax (log ε): 271 (2.65), 219 (3.58), 202 (3.54) nm; IR (KBr) νmax 3418, 2925, 2854, 1729, 1614, 1511, 1384, 1235, 1072, and...
1039 cm\(^{-1}\); For \(^1\)H and \(^{13}\)C NMR spectral data, see Table 1; ESI-MS: \(m/z\) 365 [M + Na]+; HREIMS: \(m/z\) 365.1205 [M + Na]+ (calcd for C\(_{16}\)H\(_{22}\)O\(_8\)Na, 365.1207).

### 3.3.2. (E)-2,3-Bis(4-hydroxyphenyl)acrylaldehyde (2)

Yellowish amorphous powder; \([\alpha]_D^{21.6} - 3.2\) (c 0.52, MeOH); UV (MeOH) \(\lambda_{\text{max}}\) (log \(\varepsilon\)): 329 (4.05), 231 (3.96), 202 (4.09) nm; IR (KBr) \(\nu_{\text{max}}\): 3411, 2929, 2855, 1661, 1600, 1512, 1383, 1269, 1225, 1172, 1086, 837 and 557 cm\(^{-1}\); For \(^1\)H and \(^{13}\)C NMR spectral data, see Table 1; ESI-MS: \(m/z\) 263 [M + Na]+; HREIMS: \(m/z\) 263.0678 [M + Na]+ (calcd for C\(_{15}\)H\(_{12}\)O\(_3\)Na, 263.0679).

### 3.3.3. Acid hydrolysis of 1

Compound 1 (8.0 mg) was hydrolyzed with 2 M HCl/dioxane (1:1, 4 ml) under reflux for 8 h, then partitioned between H\(_2\)O and CHCl\(_3\) (2 ml \(\times\) 3). The aqueous layer was further neutralized with 2 M NaOH and dried to liberate a glucose identified by TLC (CHCl\(_3\)–MeOH; 2:1; \(R_f\) 0.26) with the authentic sample.

### 3.4. Bioassay for vasorelaxant effect on the rat endothelium-intact thoracic aorta rings

Vasorelaxant effects of 100 \(\mu\)mol/L of selected compounds were evaluated on the rat endothelium-intact thoracic aorta rings precontracted with PE or KCl. Rat aortic rings were prepared according to the literature [24]. Aortic rings were mounted on stainless steel hooks in organ baths containing 37 \(^\circ\)C. Krebs solution continuously bubbled with 95% O\(_2\) and 5% CO\(_2\), then equilibrated for 60 min under a resting tension of 1.5 g. After equilibration, the vessels were exposed to 1 \(\mu\)mol/L PE, followed by 10 \(\mu\)mol/L acetylcholine (Ach) to check functional endothelial integrity, and more than 80% relaxation of the rings was considered to be an endothelium-intact ring. Endothelium-intact rings precontracted with 1 \(\mu\)mol/L PE or 60 \(\mu\)mol/L KCl were treated with different compounds or DMSO for 30 min, and the changes in tension of aortic rings were recorded. The vasorelaxant effect was calculated as a percentage of the relaxation in response to PE or KCl on the aortic rings.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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### References
