Two New Monoterpenes from the Fruits of *Illicium lanceolatum*

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Received: 5 August 2013; in revised form: 1 September 2013 / Accepted: 4 September 2013 / Published: 26 September 2013

**Abstract:** Two new monoterpenes, $\alpha$-mentha-1(7),8-dien-2-O-$\beta$-D-glucoside (1) and trans-2,4-dihydroxy-2,4-dimethyl-trans-1-acetic acid $\gamma$-lactone (2) were isolated from the fruits of *Illicium lanceolatum* along with trans-2,4-dihydroxy-2,4-dimethyl-cis-1-acetic acid $\gamma$-lactone (3), (1R,2R,4R)-8-$\alpha$-menthen-1,2-diol (4), trans-sobrerol (5), (1S,2S,4R)-$\alpha$-menthane-1,2,8-triol (6) and (1S, 2S, 4R, 8R)-$\alpha$-menthane-1,2,9-triol (7). The structures of the isolates were confirmed by spectroscopic analysis and they showed no inhibitory effects on the *in vitro* growth of microbial organisms (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*) at less than 1.0 mg/mL.

**Keywords:** *Illicium lanceolatum*; monoterpenes; anti-microbial activity

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**1. Introduction**

The *Illicium* (Illiciaceae) genus consists of aromatic evergreen trees that are distributed primarily in southwestern China and the southeast of America [1]. Investigations on the chemical constituents of *Illicium* have led to the isolation of monoterpenoids, sesquiterpenoids, phenylpropanoids, lignans and flavonoids, some of which exhibited anti-bacteria, neurotoxic and neurotrophic activities [2]. *Illicium lanceolatum* is a medicinal plant of the genus *Illicium* with the Chinese name ‘Mangcao’ or ‘Hongduhui’. Its roots and leaves have anti-inflammatory and analgesic activities and have been used to treat bruises, internal injuries and back pain [3]. Previous investigations of *I. lanceolatum* have
resulted in the isolation of sesquiterpenes, phenylpropanoids, lignans and flavones [4,5]. As part of investigations on the genus *Illicium* to seek more novel bioactive compounds, we carried out an extensive chemical study on *I. lanceolatum*, which led to the isolation of seven monoterpenic compounds (Figure 1). Among them, 2 and 3 are a pair of stereoisomers, the latter being a known compound synthesized by Wolinsky in 1966 [6], however, the complete NMR data has not been reported to date. In this paper, we report the isolation and structure elucidation of two new monoterpenes, *p*-mentha-1(7), 8-dien-2-*O*-β-D-glucoside (1) and trans-2,4-dihydroxy-2,4-dimethyl-trans-1-acetic acid γ-lactone (2) from the fruits of *I. lanceolatum*. The spectroscopic data of trans-2,4-dihydroxy-2,4-dimethyl-cis-1-acetic acid γ-lactone (3) is also reported.

![Figure 1. Structures of compounds 1–7.](image)

### 2. Results and Discussion

Compound 1 was obtained as a white amorphous powder with the molecular formula C_{16}H_{26}O_{6} according to HRESIMS. The pseudo-molecular ion at 337.1622 [M + Na]^+ (calcd. for C_{16}H_{26}O_{6}Na, 337.1627), suggests the presence of four degrees of unsaturation. The IR spectrum showed a strong absorption band due to hydroxyl (3,423 cm\(^{-1}\)) groups. The \(^1\)H-NMR spectrum of 1 showed the presence of one methyl group at δ\(_H\) 1.71 (3H, s); one anomeric proton signal of β-glucopyranosyl moiety at δ\(_H\) 4.40 (1H, d, J = 7.8 Hz), indicating the existence of a β-D-linkage sugar moiety in compound 1; one exocyclic methylene protons at δ\(_H\) 4.77 and 5.27 (each s) (Table 1). The \(^13\)C-NMR spectrum of 1 displayed 16 carbon signals grouped by DEPT experiment into one methyl, six methylene, seven methine and two quaternary carbons, which consisted of a set of β-D-glucopyranosyl unit and a monoterpenoid unit signals (Table 1). Analysis of the \(^1\)H–\(^1\)H COSY (Figure 2) and HMBC spectra of 1 led to the fragment -CH(2)-CH(3)-CH(4)-CH(5)-CH(6) in its structure. The planar structural skeleton of 1 was further established on the basis of HMBC spectral data (Figure 2) in which the correlations between H-7 with C-1, C-2, C-6, H-9 with C-4, C-8, C-10, and H-10 with C-4, C-8 were
displayed. The β-D-glucopyranosyl unit linked at C-2 was further supported by the correlations H-1'/C-2 and H-2/C-1' in the HMBC spectrum (Figure 2). The relative configuration of 1 was determined from its NOESY spectrum (Figure 3) in which the correlation between H-2 and H-4 was found, indicating that H-2 and H-4 were in α orientation. Therefore, the structure of 1 was elucidated as Figure 1 and named as p-mentha-1(7), 8-dien-2-O-β-D-glucoside (1).

Table 1. 1H- (400 MHz) and 13C-NMR (100 MHz) data (CD3OD) of compound 1. Chemical shifts δ in ppm relative to TMS, J in Hz.

| Position | δC  | δH  | Position | δC  | δH  |
|----------|-----|-----|----------|-----|-----|
| 1        | 147.9 | -  | 8        | 148.7 | -  |
| 2        | 76.7 | 4.29 (m) | 9 | 108.1 | 4.70 (s) |
| 3        | 40.5 | 2.16–2.19 (m) | 10 | 19.6 | 1.71 (s) |
| 4        | 44.1 | 2.24–2.28 (m) | 1' | 101.8 | 4.40 (d, 7.8) |
| 5        | 33.7 | 2.39–2.44 (m) | 2' | 74.1 | 3.23–3.28 (m) |
| 6        | 32.9 | 1.78–1.81 (m) | 3' | 77.5 | 3.27–3.36 (m) |
| 7        | 104.9 | 5.27 (s) | 4' | 70.3 | 3.26–3.34 (m) |
|          |      | 4.77 (s) | 5' | 76.5 | 3.22–3.27 (m) |
|          |      |       | 6' | 61.4 | 3.87 (dd, 12.0, 2.0) |
|          |      |       |    |      | 3.65 (dd, 11.9, 5.6) |

Figure 2. Key 1H-1H COSY and HMBC correlations of compounds 1–2.

Figure 3. Key NOE correlations in the NOESY spectrum of compounds 1–2.
Compound 2 was isolated as a white powder. The HRESIMS of 2 showed a pseudo-molecular ion at m/z 207.0999 [M + Na]+ (calcd. for C_{10}H_{16}O_{3}Na, 207.0997), consistent with the molecular formula C_{10}H_{16}O_{3}. The IR spectrum suggested the presence of hydroxyl (3,447 cm\(^{-1}\)) and carbonyl (1,746 cm\(^{-1}\)) groups. The \(^{13}\)C-NMR and DEPT spectra displayed 10 carbon signals, including two methyl, four methylene, one methine and three quaternary carbon signals (Table 2). \(^1\)H- and \(^{13}\)C-NMR spectra showed two methyl groups at \(\delta_H\) 1.32 (3H, s)/\(\delta_c\) 33.4, \(\delta_H\) 1.48 (3H, s)/\(\delta_c\) 19.5 and the characteristic signals of a \(\gamma\)-lactone moiety at \(\delta_H\) 2.37–2.42 (dd, \(J = 8.4, 16.5\) Hz, H-8a), 2.43–2.49 (dd, \(J = 8.4, 16.4\) Hz, H-8b) and \(\delta_c\) 86.2 (s, C-2), 33.3 (t, C-8), 176.6 (s, C-9) (Table 2). The \(^1\)H–\(^1\)H COSY spectrum showed cross-peaks at H-5/H-6, H-6/H-1 and H-1/H-8, indicating the presence of a structural fragment CH\(_2\)(5)-CH\(_2\)(6)-CH(1)-CH\(_2\)(8) as shown with bold line in Figure 2. The planar structural skeleton of 2 was further established on the basis of its HMBC spectrum (Figure 2), in which \(^1\)H–\(^{13}\)C long-range correlation signals were observed at H-7/C-3, C-4, C-5; H-10/C-1, C-2, C-3; H-8/C-1, C-2, C-6, C-9 and H-1/C-6, C-8. This NMR data were very similar to that of trans-2,4-dihydroxy-2,4-dimethyl-cis-1-acetic acid \(\gamma\)-lactone (3) synthesized by Wolinsky in 1966 [6], and the molecular formulas of the two compounds were the same, which indicated that compounds 2 and 3 were likely stereoisomers of each other. The correlation between CH\(_3\)-7 and CH\(_3\)-10 observed in the NOESY spectrum (Figure 3), in addition to the absence of correlation between H-1 with CH\(_3\)-10 disclosed that H-1 is in the \(\beta\) configuration, whereas CH\(_3\)-7 and CH\(_3\)-10 are in the \(\alpha\) configuration. Therefore, H-1 and CH\(_3\)-10 of compound 2 was determined to have a trans relationship and named as trans-2,4-dihydroxy-2,4-dimethyl-trans-1-acetic acid \(\gamma\)-lactone. From a biogenetic point of view, compounds 2 and 3 perhaps came from the same precursor, (1\(R\), 2\(R\), 4\(R\))-8-\(p\)-menthen-1,2-diol (4), according to the reference [6]. In this literature, Wolinsky and Chan reported that compound 3 came from 3-isopropenyl-6-oxoheptanoic acid which is the oxidation product of compound 4.

**Table 2.** \(^1\)H- (400 MHz) and \(^{13}\)C-NMR (100 MHz) data (CDCl\(_3\)) of compounds 2 and 3.

| Position | \(\delta_c\) | \(\delta_h\) | \(\delta_c\) | \(\delta_h\) |
|---------|-------------|-------------|-------------|-------------|
| 1       | 47.2        | 2.01–2.07 (dddd, 3,3, 7,8, 11,8, 15,2) | 39.8        | 2.36–2.41 (m) |
| 2       | 86.2        | -           | 86.1        | -           |
| 3       | 48.9        | 2.12 (d, 13,1.) | 46.3        | 1.89 (d, 14,5) |
|         | 71.3        | 1.80 (d, 13,1.) | 69.5        | -           |
| 4       | 40.4        | 1.81–1.86 (m) | 33.2        | 1.56–1.60 (m) |
|         | 1.53–1.60 (m) |           | 1.48–1.55 (m) |           |
| 5       | 21.3        | 1.70–1.74 (m) | 21.7        | 2.01–2.07 (m) |
|         | 1.60–1.67 (m) |           | 1.49–1.53 (m) |           |
| 6       | 33.4        | 1.32 (s)    | 31.1        | 1.30 (s)   |
| 7       | 33.3        | 2.43–2.49 (dd, 8,4, 16,4) | 34.0        | 2.53–2.60 (dd, 7,9, 16,8) |
|         | 2.37–2.42 (dd, 8,4, 16,5) |           | 2.40–2.48 (dd, 7,9, 16,8) |           |
| 8       | 176.6       | -           | 176.3       | -           |
| 9       | 19.5        | 1.48 (s)    | 27.4        | 1.57 (s)   |
| 10      |             |             |             |             |
Compounds 3–7 were identified as trans-2,4-dihydroxy-2,4-dimethyl-cis-1-acetic acid γ-lactone (3) [6], (1R, 2R, 4R)-8-p-menthen-1,2-diol [7], trans-sobrerol (5) [8], (1S, 2S, 4R)-p-menthan-1,2,8-triol (6) [9], (1S, 2S, 4R, 8R)-p-menthane-1,2,9-triol (7) [10], respectively, by comparison of their data (MS and NMR) with those in the literature.

The isolates were preliminarily evaluated against the test organisms (Escherichia coli, Staphylococcus aureus and Bacillus subtilis) in vitro. A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) [11], however, no compound was bacteriocidal against all three bacteria at less than 1.0 mg/mL.

3. Experimental

3.1. General

Optical rotations were determined on a Perkin-Elmer model 341 and Polar 3001. IR (KBr) spectra were recorded on a PE-1710 FT-IR spectrometer. 1D and 2D NMR spectra were recorded on Bruker DPX-400 NMR with TMS as internal standard. HR-ESI-MS spectra were run on a Waters-Q-Toft MS instrument. Silica gel (200–300 mesh) for column chromatography was obtained from Qingdao Meigao Chemical Company (Qingdao, China). MCI gel (10 μm) was purchased from Merck Chemicals Ltd. (Nottingham, UK). Sephadex LH-20 (20–150 μm) was purchased from Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden.

3.2. Plant Material

The fruits of Illicium lanceolatum were collected in the Tianmu Mountains, Jiangsu Province, China, in September 2011, and identified by Associate Professor Dr. Mengqi Liu from the School of Pharmaceutical Science, Henan University of Traditional Chinese Medicine. A voucher specimen (2011-09-01) was deposited in the School of Pharmaceutical Science, Zhengzhou University.

3.3. Extraction and Isolation

The powdered fruits of I. lanceolatum (5.0 kg) were extracted with 95% EtOH (20 L) under reflux for three times, 2 h each time. The extract was concentrated under reduced pressure to give a residue. The residue was dissolved in H2O and then extracted successively with CHCl3 (each 2 L) and n-BuOH (each 2 L). The CHCl3 fraction (90 g) was subjected to silica gel chromatography (1,000 g, 200–300 mesh) and eluted with a CHCl3/MeOH (100:0, 90:10, 80:20, v/v, each 8 L) gradient to afford eight fractions (Fr.s.1–8). Fr.3 (15 g) was further separated to obtain six sub-fractions (Fr.s.3a–f). Fr.3b (1.0 g) was subjected to silica gel column (40 g, 200–300 mesh) with an eluent of petroleum ether/Me2CO (80:20) to yield compounds 3 (10 mg) and 4 (30 mg). Fr.3c (2.0 g) was chromatographed through a silica gel column (80 g, 200–300 mesh) eluted with petroleum ether/Me2CO (90:10, 80:20, 70:30, v/v, each 1.0 L) to yield compounds 2 (20 mg) and 5 (35 mg). Fr.5 (8.0 g) was subjected to silica gel chromatography (100 g, 200–300 mesh) eluted with a CHCl3/Me2CO (100:0, 90:10, 80:20, v/v, each 2 L) gradient to afford six fractions (Fr.s.5a–f). Sub-fractions (5a–f) were respectively chromatographed through MCI (MeOH/Water: 10:90–70:30), Sephadex LH-20 (MeOH), and then further separated by silica gel
chromatography with petroleum ether/Me₂CO (70:30) and CHCl₃/Me₂CO (80:20) to furnish compounds 1 (15 mg), 6 (5 mg) and 7 (15 mg).

*p*-Mentha-1(7), 8-dien-2-O-β-D-glucoside (1). White amorphous powder; [α]D25 +4.7 (c 0.102, MeOH); IR (KBr) v_max 3423, 2923, 1643, 1437, 1074, 1020 cm⁻¹; ¹H- and ¹³C-NMR spectral data, see Table 1; HRESIMS: m/z 337.1622 (calcd. for C₁₆H₂₆O₆Na, 337.1627).

trans-2,4-Dihydroxy-2,4-dimethyl-trans-1-acetic acid γ-lactone (2). White amorphous powder; [α]D25 +133.3 (c 0.105, MeOH); IR (KBr) v_max 3446, 2972, 2940, 1746, 1378, 1268, 1105 cm⁻¹; ¹H- and ¹³C-NMR spectral data, see Table 2; HRESIMS: m/z 207.0999 (calcd. for C₁₀H₁₆O₃Na, 207.0997).

trans-2,4-Dihydroxy-2,4-dimethyl-cis-1-acetic acid γ-lactone (3). White amorphous powder; [α]D25 +54.5 (c 0.101, MeOH); IR (KBr) v_max 3473, 2924, 2852, 1735, 1382, 1269, 1100 cm⁻¹; ¹H- and ¹³C-NMR spectral data, see Table 2; HRESIMS: m/z 207.0994 (calcd. for C₁₀H₁₆O₃Na, 207.0997).

4. Conclusions

This work was part of a series of investigations on anti-microbial compounds obtained from plants of the genus *Illicium*. Compounds 1 and 2 were found to be new monoterpenes, and the other five compounds were found for the first time in *I. lanceolatum*. The compounds 1–7 showed no inhibitory effects on the growth of the tested microbial organisms at less than 1.0 mg/mL in vitro.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/18/10/11866/s1.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC No. 81172961) and the External Cooperation Program of Chinese Academy of Sciences (P2009-KF10). The authors are grateful to Bing Zhao, School of Pharmaceutical Science, Zhengzhou University, for measurements of all spectra.

Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability*: Samples of the compounds 1–7 are available from the authors.

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