Methyl Pothoscandensate, a New ent-18(4 → 3)-Abeokaurane from Pothos scandens

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Methyl pothoscandensate (1), a new molecular skeleton of ent-18(4 → 3)-abeokaurane, along with eight known compounds was isolated from the whole plant of Pothos scandens. The structure of the new compound was established by spectroscopic techniques and confirmed by single-crystal X-ray diffraction. The inhibitory activity of selected compounds against porcine respiratory and reproductive syndrome virus (PRRSV) was measured by the cytopathic effect (CPE) method. Compound 1 showed weak effect on PRRSV with an IC₅₀ value of 40.3 ± 8.3 μM (TI = 15.7).

Introduction. – The genus Pothos is a medicinally important member in the plant family Araceae, consisting of ca. 75 species. There are five naturally occurring species found in China, mainly distributed in the south and southwest of Yunnan Province [1]. Some species of this genus have been used in treatment of traumatic injuries, fractures, and inflammation in Chinese traditional medicinal systems. Furthermore, boiled water decoctions of P. scandens leaves are used as tea by the Dai people [2]. Though this genus includes many individuals used as medicinal herbs, there have been very few chemical investigations on Pothos. As a result, we conducted the investigation of the chemical constituents of P. scandens. Repeated column chromatography and recrystallization of the AcOEt extract of the whole plant yielded a novel diterpenoid 1 (Fig. 1), as well as eight known compounds, N-trans-cinnamoyltyramine (= (2E)-N-[2-(4-hydroxyphenyl)ethyl]-3-phenylprop-2-enamide) [3], N-trans-feruloyltyramine (= (2E)-3-(4-hydroxy-3-methoxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl]prop-2-enamide) [4], N-trans-p-cumaroyltyramine (= (2E)-3-(4-hydroxyphenyl)-N-[2-(4-hydroxyphenyl)ethyl]prop-2-enamide) [5], (–)-serotobenine (= rel-(2R,2aR)-2,2a,4,5,6,8-hexahydro-2-(4-hydroxy-3-methoxyphenyl)-3H-furo[2,3,4-kl]pyrrolo[4,3,2-fg][3]bentatocin-3-one) [6], (3β)-ent-kaurane-3,16,17-triol (2) [7], (+)-syringaresinol (= 4,4’-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyldibis[2,6-dimethoxyphenyl]]) [8], (3β)-ent-kaurane-3,16,17-triol 3-β-d-glucopyranoside) [9], and (2R)-2-hydroxy-2-phenylacetonitrile 2-[(O-β-d-xylpyranosyl-(1 → 6)β-d-glucopyranoside] [10]. Herein we report the isolation and structure elucidation of 1, and the results of the bioassay.
Results and Discussion. – Compound 1 was isolated as colorless needle crystals and its molecular formula was established as C_{21}H_{32}O_{4} based on the HR-ESI-MS \((m/z 349.2381 ([M + H]^+\text{, calc. 349.2378})), which requires 6 degrees of unsaturation. The \(^{13}\text{C}-\text{NMR and DEPT spectrum of 1 (Table) showed 21 C-atom signals including those of three Me, nine CH}_{2}, and three CH groups and six quaternary C-atoms, of which the signals of an ester C-atom (\(\delta(C) 170.2\)), two olefine C-atoms (\(\delta(C) 145.4\) and 123.7), two O-bearing C-atoms (\(\delta(C) 81.8\) and 66.3), and one MeO group (\(\delta(C) 51.2\)) were detected. By comparing the NMR data with those of (3\(\beta\)-ent-kaurane-3,16,17-triol (2; see Scheme below) [7], 1 was determined to be an ent-kaurane-type compound.

Analysis of \(^1\text{H},^1\text{H}-\text{COSY data of 1 (Fig. 2) established the two segments CH}_{2}(1)\text{CH}_{2}(2) (a) and CH}_{3}(12)\text{CH}(13)\text{CH}_{2}(14) (b) (Fig. 2). In the HMBC spectrum, the correlations CH}_{2}(14)/C(9) (\(\delta(C) 53.1\)), C(12) (\(\delta(C) 25.9\)), C(15) (\(\delta(C) 53.0\)), and C(16) (\(\delta(C) 81.8\)), CH}_{3}(11)/C(8) and C(13), and H–C(13)/(11), revealed the presence of a bicyclo[3.2.1]octane system (rings C and D). Based on the HMBCs CH}_{2}(1)/C(20) and C(3), CH}_{2}(2)/C(10), H–C(5)/C(1), CH}_{2}(6)/C(8) and C(10), CH}_{2}(7)/C(15), H–C(9) to C(1) and C(5), Me(19)/C(3), C(4), and C(5), and Me(20)/C(1), C(5), C(9), and C(10), rings A and B were assigned. The two OH groups were located at

![Fig. 1. Methyl pothoscandensate (1), isolated from Pothos scandens](image1)

![Fig. 2. \(^1\text{H},^1\text{H}-\text{COSY (---)} and key HMBC (H–C) features of 1](image2)

**Table. \(^1\text{H- and }^{13}\text{C-NMR Data (400 und 100 MHz; CDCl}_{3}\text{) of Compound 1.} \delta in ppm, J in Hz.**

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta(H))</th>
<th>(\delta(C))</th>
<th>Position</th>
<th>(\delta(H))</th>
<th>(\delta(C))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH}_{2}(1)</td>
<td>1.86–1.89, 1.05–1.08 (2m)</td>
<td>35.6 (CH}_{3}</td>
<td>CH}_{2}(12)</td>
<td>1.62–1.65, 1.54–1.57 (2m)</td>
<td>25.9 (CH}_{3}</td>
</tr>
<tr>
<td>CH}_{2}(2)</td>
<td>2.32–2.36 (m)</td>
<td>24.2 (CH}_{3}</td>
<td>H–C(13)</td>
<td>2.07 (br. s)</td>
<td>45.3 (CH)</td>
</tr>
<tr>
<td>C(3)</td>
<td>123.7 (C)</td>
<td></td>
<td>H–C(14)</td>
<td>2.01 (d, (J = 11.9))</td>
<td>37.6 (CH}_{3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H–C(14)</td>
<td>1.63–1.66 (m)</td>
<td></td>
</tr>
<tr>
<td>C(4)</td>
<td>145.4 (C)</td>
<td></td>
<td>CH}_{2}(15)</td>
<td>1.58–1.61, 1.48–1.51 (2m)</td>
<td>53.0 (CH}_{3}</td>
</tr>
<tr>
<td>H–C(5)</td>
<td>1.86–1.88 (m)</td>
<td></td>
<td>50.2 (CH)</td>
<td>C(16)</td>
<td>81.8 (C)</td>
</tr>
<tr>
<td>CH}_{2}(6)</td>
<td>1.86–1.89, 1.39–1.42 (2m)</td>
<td>22.0 (CH}_{3}</td>
<td>CH}_{2}(17)</td>
<td>3.81, 3.69 (2d, (J = 10.9))</td>
<td>66.3 (CH}_{3}</td>
</tr>
<tr>
<td>CH}_{2}(7)</td>
<td>1.67–1.69, 1.54–1.57 (2m)</td>
<td>41.0 (CH}_{3}</td>
<td>C(18)</td>
<td></td>
<td>170.2 (C)</td>
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<tr>
<td>C(8)</td>
<td>44.1 (C)</td>
<td></td>
<td>Me(19)</td>
<td>1.92 (s)</td>
<td>18.1 (Me)</td>
</tr>
<tr>
<td>H–C(9)</td>
<td>1.05–1.08 (m)</td>
<td>53.1 (CH)</td>
<td>Me(20)</td>
<td>0.89 (s)</td>
<td>14.3 (Me)</td>
</tr>
<tr>
<td>C(10)</td>
<td>37.1 (C)</td>
<td></td>
<td>(MeO)</td>
<td>3.71 (s)</td>
<td>51.2 (Me)</td>
</tr>
<tr>
<td>CH}_{2}(11)</td>
<td>1.66–1.69, 1.56–1.59 (2m)</td>
<td>18.5 (CH}_{3}</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
C(16) and C(17), respectively, by the correlations CH$_2$(14)/C(16), H–C(13)/C(11) and CH$_2$(17)/C(13) and C(15). Although the correlations between CH$_2$(2) and C(18) were not observed in the HMBC spectrum, the methoxycarbonyl group should be at the quaternary C-atom (C(3)). At last, the planar structure of 1 was deduced as shown in Fig. 2. The partial relative configuration of 1 was deduced by the analysis of ROESY correlations (Fig. 3). The key ROESY correlations H–C(5)/H–C(9) showed that the two H-atoms were cofacial, and were arbitrarily assigned to be β-oriented, while Me(20) was assigned the α-orientation. The correlation of CH$_2$(14)/Me(20) and H–C(13)/CH$_2$(14) confirmed H–C(13) to be α-oriented. The entire structure of 1 was finally determined by a single-crystal X-ray diffraction analysis (Fig. 4) [11]. Thus, 1 was elucidated as 16,17-dihydroxy-18(4→3)-abeo-ent-kaur-3-en-18-oic acid methyl ester and given the trivial name methyl pothoscandensate.

A possible biogenetic pathway of 1 is proposed as shown in the Scheme. Compound 2 would be transformed into iii by Wagner–Meerwein rearrangements. Firstly, the OH group would leave from 2 to generate the cation intermediate i under acidic conditions.
Afterwards, one of the Me groups at C(4) would migrate to C(3) forming the intermediate ii, followed by H-atom elimination to give the key intermediate iii. Then, iii could be finally transformed to 1 by selectively oxidizing the allylic Me group to the acid followed by esterification.

Scheme. Proposed Biogenetic Pathway of 1

The inhibitory activity of methyl pothoscandensate (1), N-trans-feruloylttyramine, N-trans-p-cumaroylttyramine, (−)-serotenoline, (3β)-ent-kaurane-3β,16,17-triol (2), (+)-syringaresinol, and (2R)-2-hydroxy-2-phenylacetonitrile 2-[O-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] against porcine respiratory and reproductive syndrome virus (PRRSV) was measured by the cytopathic effect (CPE) method [12]. Compound 1 showed a weak inhibitory effect on PRRSV with an IC₅₀ value of 40.3 ± 8.3 μM (TI (therapeutic index) = 15.7) compared with tilmicosin phosphate (IC₅₀ = 225.1 ± 27.4 μM, TI = 3.8). The other tested compounds were inactive (IC₅₀ > 200 μM). Furthermore, by the real-time fluorescent quantitative reverse transcription-polymerase chain reaction (FQ RT-PCR) [13–15], the relative expression ratio of PRRSV ORF 7 and NSP9 genes was tested. ORF 7 and NSP9 mRNA relative expression level was significantly reduced by compound 1 at the concentration of 100 μM or more (P < 0.001; Fig. 5).

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Experimental Part

General. Column chromatography (CC): MCI gel (70–150 μm; Mitsubishi Chemical Corporation), C₈ silica gel (SiO₂, 40–75 μm; Fuji Silitia Chemical Ltd.), Sephadex LH-20 gel (GE Healthcare Bio-Sciences AB), and silica gel (SiO₂, 80–100, 20–300, and 300–400 mesh; Qingdao Meiguo Chemical Co.). TLC: pre-coated SiO₂, F₂₅₄₄ plates (Qingdao Meiguo Chemical Co.); spots were detected under UV light (254 and 365 nm), and by spraying with 5% aq. H₂SO₄ in EtOH, followed by heating. M.p.: X-ray meling-point apparatus (Yingyu Yuhua Apparatus Factory, Gongyi, P.R. China). Optical rotations: Jasco-DIP-370 automatic digital polarimeter. UV Spectra: Shimadzu-210A spectrophotometer; KBr pellets; ν in cm⁻¹. IR Spectra: Bruker-AM-400 and-DRX-500 spectrometers; δ in ppm rel. to MeSi as internal standard, J in Hz. ESI-MS and HR-ESI-MS: API-Qstar-Pulsar-I instrument; in m/z.

Plant Material. The whole plant of Pothos scandens (8.3 kg) was collected from Xishuangbanna of Yunnan Province, P.R. China, in October 2010, and identified by Dr. Guang-Wan Hu, Kunming Institute of Botany. A voucher specimen (No. LHX-0091) was deposited with the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany.

Extraction and Isolation. The air-dried powder of the plant material (8.3 kg) was exhaustively extracted with MeOH a total of 3 times, and the extract (0.79 kg) was suspended in H₂O and partitioned into three fractions with petroleum ether (A, 100 g), AcOEt (B, 165 g), and H₂O (C, 200 g). Subsequently, Fr. B was subjected to CC (SiO₂, CHCl₃/MeOH 1:0 → 0:1); Frs. 1–6. Fr. 2 (CHCl₃/MeOH 15:1) was separated by CC (RP-18, MeOH/H₂O 2:8 → 9:1); Frs. 2.1–2.3. Fr. 2.1 was fractionated by CC (Sephadex LH-20, MeOH); SiO₂, CHCl₃/MeOH 10:1): 2 (40.5 mg). Fr. 2.2 was fractionated by CC (Sephadex LH-20, MeOH; SiO₂, CHCl₃/MeOH 15:1): (+)-syringaresinol (13.4 mg). Fr. 3 (CHCl₃/MeOH 10:1) was separated by CC (RP-18, MeOH/H₂O 3:7 → 9:1); Frs. 3.1–3.6. Fr. 3.1 was fractionated by CC (Sephadex LH-20, MeOH; silica gel, CHCl₃/MeOH 10:1): (--)serotobenine (11.0 mg). Fr. 3.2 was fractionated by CC (Sephadex LH-20, MeOH; SiO₂, petroleum ether/Me₂CO 3:1, CHCl₃/MeOH 10:1): N-trans-ferruloyltamrine (29.0 mg) and N-trans-p-cumaroyltyramine (113.0 mg). Fr. 3.3 was fractionated by CC (Sephadex LH-20, MeOH; SiO₂, CHCl₃/MeOH 10:1): N-trans-cinnamoyltyramine (4.2 mg). Fr. 3.4 was fractionated by CC (Sephadex LH-20, MeOH; SiO₂, petroleum ether/Me₂CO 5:1): 1 (64.5 mg). Fr. 5 (CHCl₃/MeOH 3:1) was separated by CC (RP-18, MeOH/H₂O 6:4 → 9:1); Frs. 5.1–5.3. Fr. 5.2 was fractionated by CC (Sephadex LH-20, MeOH; SiO₂, CHCl₃/MeOH 5:1); (2R)-2-hydroxy-2-phenylacetonitrile 2-[O-β-D-xylopyranosyl(1→6)-β-D-glucopyranoside] (15.0 mg). Fr. 5.3 was fractionated by CC (Sephadex LH-20, MeOH; SiO₂, CHCl₃/MeOH 4:1): (3β)-ent-kaurane-3,16,17-triol 3-(β-D-glucopyranoside) (32.0 mg).

X-Ray Crystallographic Analysis of 1. C₉₆H₁₅₁O₁₁ (2 × C₂₃H₁₉O₅), M, 696.93, colorless needle crystal, size 0.03 × 0.12 × 0.78 mm³, monoclinic, space group P2₁; a = 9.319(7), b = 7.234(6), c = 28.78(2) Å, α = γ = 90.00, β = 90.180(12); V = 1878(3) Å³, T 296(2) K, Z = 2, μMo = 1.232 g/cm³; F(000) = 760, 18137 reflections in −12 ≤ h ≤ 12, −9 ≤ k ≤ 9, −37 ≤ l ≤ 7, measured in the range 0.73 ≤ θ ≤ 28.97°, completeness θmax = 94.7%, Rint = 0.153. Final R indices: R₁ = 0.1257 and wR₂ = 0.2601, Flack parameter −2(4), largest difference peak and hole = 0.347 and −0.297 e Å⁻³. The intensity data for 1 were collected on a Bruker APEX DUO diffractometer with graphite-monochromated MoKα radiation. The structure of 1 was solved by direct methods (SHELXS97 [11]), expanded with difference Fourier techniques, and refined by the program and full-matrix least-squares calculations. The non-H-atoms were refined anisotropically, and H-atoms were fixed at calculated positions. CCDC-848942 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif.

Yunnan Province, P. R. China [19]. The tissue culture medium infective dose (cytopathic effect (CPE) inhibition assay [12]. The YN-1 strain of PRRSV was isolated from local pigs in protocols [16–18]. The antiviral activity of tested compounds against PRRSV was evaluated by the of the Chinese Academy of Sciences, Shanghai) was measured according to previously described phate (positive control; Hubei Hengshuo Chemical Co., Ltd [119x672] M.p. 102 – 103 genes was determined by real-time RT-PCR [13 – 15]. Briefly, after 4 d of incubation, total virus RNA of was defined as 50% inhibited concentration (IC50) of 500 viral particles with twofold serial dilutions of the compounds were added to each test well, and the plates were re-incubated for 4 d to allow development of a cytopathologic effect (CPE) if any. A noninfection control was made in the absence of natural products, and tilmicosin phosphate was used for drug control. The concentration reducing CPE by 50% with respect to virus control was estimated from graphic plots and was defined as 50% inhibited concentration (IC50). The therapeutic index (TI) was calculated from the ratio IC50/CC50.

PRRSV mRNA Expression Inhibition Assay. The mRNA expression of PRRSV ORF 7 and NSP9 was determined by real-time RT-PCR [13 – 15]. Briefly, after 4 d of incubation, total virus RNA of both administration and control groups was isolated by means of RNAiso™ Plus (TaKaRa Biotechnology, Dalian, P. R. China), dissolved in 30 µl of RNase-free H2O (TaKaRa), and then stored at –80°C. According to the GenBank data base, accession No. PRU87392, primers were selected and designed from conserved regions based on the ORF7 and NSP9 sequences by using Primer5.0 and Oligo6.0 software. A 330 base pair fragment of the PRRSV ORF7 gene was amplified by using the following primers: forward primer was 5′-AATGGCCAGCCAGTCAATCA-3′ and reverse primer was 5′-TCATGTGAGGTTGATGCTG-3′. A 162 base pair fragment of the PRRSV NSP9 gene was amplified by using the following primers: forward primer was 5′-CACTAAAGAGAAGGTCGGC-3′ and reverse primer was 5′-GATATGTCTCTCAAAAACCTTATTC-3′. A 130 base pair fragment of the beta-actin gene was amplified by using the following primers: forward primer was 5′-ATCCAGGTCTGTGCTCC-3′ and reverse primer was 5′-GAGGATCTTCATGAGGTAGTCG-3′. cDNAs were synthesized with the PrimeScript® RT reagent kit (TaKaRa) with 10 µl of reaction mixtures containing 4.5 µl of RNase free dH2O, 2 µl of 5 × PrimeScript® buffer, 0.5 µl of PrimeScript® RT enzyme mix I, 0.5 µl of random 6 mers (100 µm), 0.5 µl of oligo dT primer (50 µm), and 2 µl of total RNA. The reaction programme was as follows: 37°C for 15 min and 85°C for 5 s. The PCR reaction mixture (25 µl) contained 12.5 µl of SYBR® Premix Ex Taq™/II (TaKaRa), 0.5 µl of PCR forward primer (10 µm), 0.5 µl of PCR reverse primer (10 µm), 9.5 µl of dH2O, and 2 µl of cDNA. The reactions were carried out in an iQ5 real-time PCR system (Bio-Rad Co., Ltd.). The reaction programme was as follows: one cycle at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, 60°C for 30 s.

Statistical Analyses. All experiments were performed in three replications. Continuous variables, expressed as mean ± s.d., were compared by using one-way ANOVA. Statistical analyses were conducted with SPSS 17.0.


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