Two New Constituents from *Torricellia tiliifolia* Stem Barks

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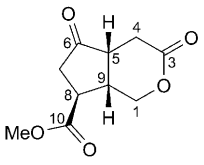
A new iridoid, torrilliolide (**1**), and a new pyridine alkaloid, torricelline (**2**), were isolated from the stem barks of *Torricellia tiliifolia*. Their structures were determined by analysis of their spectroscopic data. Both compounds exhibited cytotoxicity against *Spodoptera litura* cell line.

Introduction. – *Torricellia tiliifolia* DC., belonging to the family Torricelliaceae, is distributed in southwest of China and north of India. Its barks are used for alleviating bruises, swelling, and pain from traumatic injury including falls, sprains, fractures, and contusions in Chinese medicine [1]. During our search for pesticidal plants growing in southwest of China, the MeOH extract of this plant was found to possess a potent activity against *Musca domestica vicina* MACQUART, *Tribolium castaneum* (HERBST), *Spodoptera litura* FABRICIUS, *Culex pipiens quinquefasciatus* SAY, and *Ostrinia furnacalis* (GUENÈE) [2]. The chemical constituents of this plant were, therefore, investigated, and a new iridoid, torrilliolide (**1**), and a new pyridine alkaloid, torricelline (**2**), were isolated. Here, we report the isolation, structure elucidation, and cytotoxicity of these new compounds.



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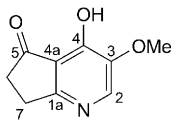

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Results and Discussion. – Compound **1**, obtained as colorless needles, had the molecular formula $C_{10}H_{12}O_5$, as determined from the HR-EI-MS and ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectra (see *Exper. Part*) in combination with HSQC spectrum revealed the presence of a MeO group (δ(H) 3.79; δ(C) 52.7), three CH_2 groups, of which one was O-bearing (δ(H) 4.31–4.33, 4.43–4.46 (2*m*, CH_2(1)); δ(C) 68.4 C(1)), three CH groups, and three quaternary C-atoms, including two ester CO groups (δ(C) 170.2 (C(3)), 173.4 (C(10))) and a ketone CO group (δ(C) 213.3 (C(6))). Analysis of the ^{1}H,^{1}H-COSY spectrum showed connectivities of C(1) with C(9), C(9) with C(8), C(8) with C(7), C(9) with C(5), and C(5) with C(4). In the HMBC spectrum, correlations were observed from CH_2(1), CH_2(4), and H–C(5) to C(3), suggesting a

connectivity of C(1) to C(3) *via* an ester linkage, and C(3) to C(4) to form a six-membered lactone ring. Long-range correlations were also observed of H–C(9), H–C(8), CH_2(7), and the MeO H-atoms to C(3), and from CH_2(4), H–C(5), H–C(9), H–C(8), and CH_2(7) to C(6), indicating that the MeO group was attached to C(10), which was further attached to C(8), and the ketone CO group, *i.e.*, C(6), was connected to both C(5) and C(7). Therefore, **1** was deduced to be an iridoid with a planar structure as shown in the *Formulae* shown above. To determine the relative configuration, a single crystal of **1** was subjected to X-ray diffraction analysis using MoK_α radiation. The result (*Fig.*) showed that H–C(5) and H–C(9) had a mutual *cis*-relationship and a *trans*-relationship to H–C(8). Thus, the structure of **1**, trivially named torrilliolide, was established as shown.

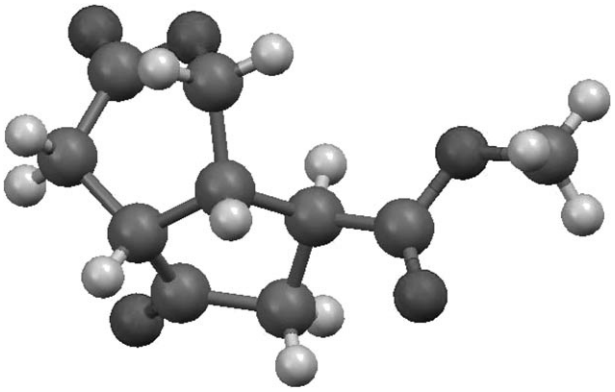

Figure. *X-Ray crystal structure of* **1**

Compound **2** was obtained as a white amorphous powder. Its molecular formula was determined as $C_9H_9NO_3$ from HR-EI-MS (at *m*/*z* 179.0579 (calc. 179.0577)). The ^{1}H-NMR spectrum displayed signals indicating the presence of two CH_2 groups (δ(H) 2.74–2.76 (*m*, CH_2(7)), 2.85–2.86 (*m*, CH_2(6))), a MeO (δ(H) 3.70 (*s*, MeO–C(3))) and an aromatic CH group (δ(H) 7.66 (*s*, H–C(2))), and a chelated OH group (δ(H) 12.02 (br. *s*, HO–C(4))). The ^{13}C-NMR spectrum exhibited (see *Exper. Part*) resonances for nine C-atoms including those of a MeO (δ(C) 50.8 (MeO–C(3))) and a CO group (δ(C) 193.7 C(5)), two CH_2 groups, a sp^2-CH group, and four sp^2 quaternary C-atoms. In the HMBC spectrum, correlations from H–C(2) to C(3) (δ(C) 163.2), C(4) (δ(C) 123.9), and C(1a) (δ(C) 160.9), from the MeO H-atoms to C(3), and from both CH_2(6) and CH_2(7) to C(1a), C(4a) (δ(C) 109.7), and C(5) were observed. These findings, in combination with the presence of a chelated OH group, revealed a structure of 6,7-dihydro-5*H*-cyclopenta[*b*]pyridin-5-one with a OH group attached to C(4) and a MeO group attached to C(3). Therefore, the structure of **2**, named torricelline, was determined as shown.

The cytotoxicity of **1** and **2** against *Spodoptera litura* cells were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method. Both

compounds were found to be cytotoxic with LC_{50} values of 67.79 and 43.52 μg/ml, respectively, which were comparable with that of rotenone ($LC_{50} = 69.62$ μg/ml).

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Amersham*), silica gel (SiO_2; 100–200 mesh, *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China), and reversed-phase (RP) silica gel (*Merck*, 10 μm). Optical rotations: *Perkin-Elmer 341* digital polarimeter. ^{1}H- (600 MHz), ^{13}C- (150 MHz), and 2D-NMR: *Bruker DRX-600* instrument using TMS as an internal reference. ESI-MS: *API 2000* LC/MS/MS (*Applied Biosystems*). HR-ESI-MS: *Thermo MAT 95 XP* mass spectrometer.

Plant Material: The stem barks of *Torricellia tiliifolia* were collected from Enshi County, Hubei Province, P. R. China, in July 2005. The plant material was identified by Prof. *Bing-Tao Li* at South China Agricultural University, Guangzhou, P. R. China. Herbarium specimens were deposited with the Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agricultural University, P. R. China.

Extraction and Isolation: The powder of dry stem barks of *T. tiliifolia* (30 kg) was extracted with MeOH (3 × 100 l) at r.t. for 48 h. The MeOH extract (1628 g) was suspended in H_2O and successively partitioned with petroleum ether (PE; 3 × 3000 ml) and AcOEt (3 × 3000 ml). The AcOEt-soluble fraction (450 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH of increasing polarity, 100 : 0 to 50 : 50) to give 116 fractions. *Frs. 20–23* were further separated by CC (SiO_2; sequentially PE/acetone 90 : 10 to 30 : 70 and $CHCl_3$/AcOEt 97 : 3 to 50 : 50), and then separated by CC (*Sephadex LH-20*; acetone), followed by CC repeated (SiO_2; PE/AcOEt 90 : 10 to 30 : 70) again to obtain **1** (18.7 mg). *Frs. 58–65* subjected to CC (SiO_2; PE/acetone of increasing polarity, 70 : 30 to 30 : 70, and then $CHCl_3$/MeOH 90 : 10 to 50 : 50), and the fractions were washed with acetone and THF to afford **2** (15.3 mg).

Torrilliolide (= *Methyl (4a*R*,7*S*,7a*S*)-Octahydro-3,5-dioxocyclopenta[*c*]pyran-7-carboxylate*; **1**): White needle crystals. $[\alpha]_D = +54.2$ ($c = 0.16$, acetone). ^{1}H-NMR ($CDCl_3$, 600 MHz): 2.59–2.63, 2.79–2.82 (2*m*, CH_2(7)); 2.67–2.71, 2.76–2.79 (2*m*, CH_2(4)); 2.98–3.01 (*m*, H–C(5)); 3.02–3.04 (*m*, H–C(8)); 3.05–3.08 (*m*, H–C(9)); 3.79 (*s*, MeO–C(10)). 4.31–4.33, 4.43–4.46 (2*m*, CH_2(1)). ^{13}C-NMR ($CDCl_3$, 150 MHz): 28.5 (C(4)); 36.9 (C(9)); 40.4 (C(8)); 40.5 (C(7)); 41.8 (C(5)); 52.7 (*Me*O–C(10)); 68.4 (C(1)); 170.2 (C(3)); 173.4 (C(10)); 213.0 (C(6)). HR-EI-MS: 212.0679 ($C_{10}H_{12}O_5^+$; calc. 212.0681).

Torricelline (= *6,7-Dihydro-4-hydroxy-3-methoxy-5*H*-cyclopenta[*b*]pyridin-5-one*; **2**): White powder. $[\alpha]_D = -2.0$ ($c = 0.028$, MeOH). ^{1}H-NMR ((D$_6$)DMSO, 600 MHz): 2.74–2.76 (*m*, CH_2(7)); 2.85–2.86 (*m*, CH_2(6)); 3.70 (*s*, MeO–C(3)); 7.66 (*s*, H–C(2)). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz): 16.2 (C(3)); 20.1 (C(6)); 41.4 (C(7)); 50.8 (*Me*O–C(3)); 109.7 (C(4a)); 123.9 (C(4)); 132.4 (C(2)); 160.9 (C(1a)); 193.7 (C(5)). HR-EI-MS: 179.0579 ($C_9H_9O_3N^+$; calc. 179.0577).

Cell Lines. Spodoptera litura (*SL*) cells were from a continuous line of oocytes maintained at the Laboratory of Insect Toxicology at South China Agricultural University, and the culturing method followed that of *Zhao et al.* [3].

Cytotoxicity Assay: SL Cell suspensions were seeded at a density of 4×10^4 cells/ml in 96-well plates and pre-incubated for 24 h. Test samples were dissolved in small amounts of DMSO and diluted in the appropriate culture medium (final concentration of DMSO $< 0.5\%$). After removal of pre-incubated culture medium, medium containing various concentration of test compound were added and further incubated for 48 h. DMSO (final concentration $< 0.5\%$) alone was used as a control. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyldihydro-2*H*-tetrazolium bromide (MTT) colorimetric assay [4][5].

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