

## A NEW HOPANTENIC ACID DERIVATIVE FROM THE RHIZOMES OF *Heterosmilax japonica*

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A new hopantenic acid derivative, (R)-methyl-4-(2,4-dihydroxy-3,3-dimethylbutanamido)butanoate (**1**), was isolated from the rhizomes of *Heterosmilax japonica* Kunth, together with three known compounds, (3S)-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid (**2**), daucosterol (**3**), and ethyl- $\beta$ -D-xylobioside (**4**). The structure of **1** was determined by extensive spectroscopic analyses, including 1D and 2D NMR and HR-ESI-MS. Compounds **2** and **4** are new for the genus *Heterosmilax*.

**Keywords:** *Heterosmilax japonica*, chemical constituents, alkaloids.

*Heterosmilax japonica* Kunth (Liliaceae), a climbing flowering shrub, is widely distributed in the Southern Province of China [1]. The rhizomes of *H. japonica* have been used in Traditional Chinese Medicine to relieve fever and for detoxification and detumescence [2]. Previous chemical investigations on the rhizomes of *H. japonica* Kunth have demonstrated the presence of several types of compounds, including flavonoids, anthraquinones, stilbenes, fatty acids, and alkanes [2–4]. In our research for structurally diverse and biologically active molecules, a new hopantenic acid derivative (**1**) was identified from the rhizomes of *H. japonica*, as well as three known compounds **2–4**. This paper describes the isolation and structural determination of these isolates.

Compound **1** was isolated as an amorphous powder with the molecular formula C<sub>11</sub>H<sub>21</sub>O<sub>5</sub>N, determined from the HR-ESI-MS at *m/z* 248.1486 [M + H]<sup>+</sup> (calcd for C<sub>11</sub>H<sub>22</sub>O<sub>5</sub>N, 248.1492). The <sup>1</sup>H NMR spectrum of **1** showed an oxygen-connected methine at  $\delta$  3.69 (1H, d, J = 5.5 Hz, H-2'), two heteroatoms bearing methylenes at  $\delta$  3.29 (1H, m, H-4'a), 3.17 (1H, dd, J = 10.4, 5.5 Hz, H-4'b), 3.11 (1H, m, H-4a), and 3.05 (1H, m, H-4b), two other methylenes at  $\delta$  2.30 (2H, t, J = 7.5 Hz, H-2) and 1.66 (2H, m, H-3), and three methyl signals at  $\delta$  3.58 (3H, s), 0.80 (3H, s, H-5'), and 0.79 (3H, s, H-6'). The <sup>13</sup>C NMR of **1** coupled with HSQC data classified 11 carbon signals into ester carbon signal at  $\delta$  173.0 and amide carbonyl at  $\delta$  173.1, two oxygen-connected carbons at  $\delta$  75.2 and 68.0, a methoxy signals at  $\delta$  51.3, a quaternary carbon signal at  $\delta$  39.0, a nitrogen-connected carbon at  $\delta$  37.4, two methylene signals at  $\delta$  30.7 and 24.6, and two methyl signals at  $\delta$  21.0 and 20.4. The <sup>1</sup>H–<sup>1</sup>H COSY experiment indicated the connectivities from C-2 to C-4 (Fig. 1). In the HMBC of **1**, the correlations from H-2 ( $\delta$  2.30) and H-3 ( $\delta$  1.66) to C-1 ( $\delta$  173.0) revealed the existence of a  $\gamma$ -amino butyric acid moiety. The HMBC correlations of H-5', H-6'/C-2', C-3', and C-4', H-4'/C-2', H-2'/C-1' revealed the existence of a pantoyl moiety. The pantoyl moiety was connected with  $\gamma$ -aminobutyric acid through an amide bond as suggested by HMBC correlations from H-4 ( $\delta$  3.11, 3.05) to C-1' ( $\delta$  173.1). The HMBC correlation of methoxy ( $\delta$  3.58) with C-1 ( $\delta$  173.0) was also observed. The absolute configuration of C-2' was determined to be *R* by comparison of the optical rotation value of **1** with that of hopantenic acid, a central nervous system depressant drug approved in Russia. Thus, the structure of **1** was elucidated as shown in Fig. 1, with the trivial name methyl hopantenate.

The known compounds were identified as (3S)-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid (**2**) [5], daucosterol (**3**) [3], and ethyl- $\beta$ -D-xylobioside (**4**) [6] by comparison of the physiochemical property with those reported in the literature. Compounds **2** and **4** are reported from the genus *Heterosmilax* for the first time.

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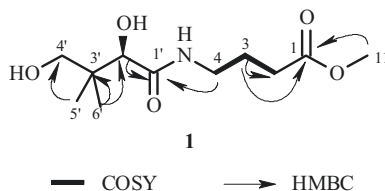


Fig. 1. Structure and key  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations of **1**.

## EXPERIMENTAL

**General Experimental Procedures.** Optical rotation was measured with a PerkinElmer 341 polarimeter. IR spectrum was obtained on a Shimadzu IRAffinity-1 using KBr pellets,  $\text{cm}^{-1}$ ; NMR was recorded on a Bruker AV-600 spectrometer with TMS as internal standard, J in Hz; HR-ESI-MS spectrum was measured on a Triple TOF<sup>TM</sup> 5600 plus (AB SCIEX, Foster City, CA, USA) hybrid triple quadrupole time-of-flight mass spectrometer equipped with ESI source. Column chromatography (CC) was performed on Silica gel (200–300 mesh, Qingdao Marine Chemical), MCI gel CHP 20P (Fiji), Sephadex LH-20 (Pharmacia), and Toyopearl HW 40F (TOSOH), or ODS (Merck).

**Plant Material.** The rhizomes of *H. japonica* Kunth were collected from Yulin City, Guangxi Province, China, in October 2015. They were identified by co-author Shuyuan Li. A voucher specimen (No. TBX-GZ-20151007) of this collection was deposited in the Chinese Medicine Laboratory, School of Traditional Chinese Medicine, Guangdong Pharmaceutical University.

**Extraction and Isolation.** The rhizomes (3.0 kg) of *H. japonica* Kunth were ground and homogenized in 70% aqueous ethanol at room temperature three times per week. The combined extracts were concentrated and then partitioned with petroleum ether, ethyl acetate, and *n*-butanol successively. The *n*-butanol soluble fraction (26.8 g) was subjected to MCI gel CHP 20P CC with an increasing gradient of MeOH–H<sub>2</sub>O (0:100–95:5) to yield five fractions 1–5. Fraction 2 (3.19 g) was applied to silica gel CC (DCM–MeOH, 40:1–4:1), ODS CC (MeOH–H<sub>2</sub>O, 30%), and Toyopearl HW 40F CC (MeOH–H<sub>2</sub>O, 40%) to yield **1** (2.0 mg) and **2** (3.0 mg). Fraction 3 (1.60 g) was applied to silica gel CC (DCM–MeOH, 4:1) and Sephadex LH-20 CC (DCM–MeOH, 1:1) to yield **4** (6.0 mg). Fraction 5 (0.26 g) was applied to silica gel CC (DCM–MeOH, 10:1) and Sephadex LH-20 CC (DCM–MeOH, 1:1) to yield **3** (3.0 mg).

**(R)-Methyl-4-(2,4-dihydroxy-3,3-dimethyl-butanamido)butanoate (1).** Amorphous powder;  $[\alpha]_{\text{D}}^{25} +0.41^\circ$  (*c* 0.7, MeOH). IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3331 (OH), 2922, 2833 (CH), 1653 (C=O), 1402, 1020 (CH).  $^1\text{H}$  NMR (600 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm, J/Hz): 7.77 (1H, br.s, NH), 5.32 (1H, d, J = 5.5, 2'-OH), 4.47 (1H, t, J = 5.5, 4'-OH), 3.69 (1H, d, J = 5.5, H-2'), 3.58 (3H, s, OCH<sub>3</sub>), 3.29 (1H, m, H-4'a), 3.17 (1H, dd, J = 10.4, 5.5, H-4'b), 3.11 (1H, m, H-4a), 3.05 (1H, m, H-4b), 2.30 (2H, t, J = 7.5, H-2), 1.66 (2H, m, H-3), 0.80 (3H, s, H-5'), 0.79 (3H, s, H-6').  $^{13}\text{C}$  NMR (150 MHz, DMSO-*d*<sub>6</sub>,  $\delta$ , ppm): 173.1 (C-1'), 173.0 (C-1), 75.2 (C-2'), 68.0 (C-4'), 51.3 (OCH<sub>3</sub>), 39.0 (C-3'), 37.4 (C-4), 30.7 (C-2), 24.6 (C-3), 21.0 (C-5'), 20.4 (C-6'). HR-ESI-MS *m/z* 248.1486 [M + H]<sup>+</sup> (calcd for C<sub>11</sub>H<sub>22</sub>O<sub>5</sub>N, 248.1492).

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## REFERENCES

1. J. Tang and F. Wang, *Flora of China*, Science Press, Beijing, 2004.
2. L. Qiao, J. Yuan, H. Cheng, X. Chen, and K. Bi, *Zhongyaocai*, **30**, 1242 (2007).
3. J. Yu, S. Zhang, and L. Liu, *Zhongguo Yaoxue Zazhi*, **40**, 19 (2005).
4. T. Wang, S. Li, and X. Guo, *Zhongyaocai*, **30**, 1091 (2007).
5. F.-X. Wang, A.-J. Deng, M. Li, J.-F. Wei, H.-L. Qin, and A.-P. Wang, *Molecules*, **18**, 418 (2013).
6. Y. Yan, Q. Lv, X. Dong, Y. Li, and Y. Cheng, *Tianran Chanwu Yanjiu Yu Kaifa*, **24**, 1712 (2012).