SHORT REPORT

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New Sulfureous Diketopiperazine from Roots of *Moringa oleifera*

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Abstract: Phytochemical investigation of the roots of *Moringa oleifera* led to the isolation of a new sulfureous diketopiperazine, 7-epi lasiodipline D **(1)** together with its diastereomer, lasiodipline D **(2)**, and two other known compounds. Structures of the isolated compounds were elucidated by HRESIMS, NMR, and ECD data. For the first time, sulfureous diketopiperazine derivatives **(1-3)** were obtained from the roots of *M. oleifera*.

Keywords: *Moringa oleifera*; Moringaceae; diketopiperazine; 7-epi lasiodipline D; lasiodipline D; lasiodipline C.
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1. Plant Source

Moringa oleifera Lam., also known as the 'drumstick' or 'horseradish tree', is a small deciduous tree of the Moringaceae family. The plant is extensively grown in Asia and Africa, and its leaves, seeds, and roots are commonly utilised for both culinary and medicinal purposes [1-3]. In the current study, roots of *M. oleifera* were collected in July 2022 at Quang Ninh province, Vietnam, and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (HN0000076382) was kept in the Herbarium of the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

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New sulfureous diketopiperazine from roots of *Moringa oleifera*

2. Previous Study

Moringa oleifera has been extensively studied for its biological activities such as antihypertensive, antitumor, antioxidant, antimicrobial, and anti-inflammatory activities [4-10]. Phytochemical investigation has revealed the presence of numerous secondary metabolites, including phenolics, flavonoids, glycosides, and others in *M. oleifera* [2, 5, 8, 11, 12]. While considerable research has been conducted on the chemical constituents in the leaves and seeds, the exploration of the root of *M. oleifera* remains relatively limited [13].

3. Present Study

This paper presented the isolation and structure elucidation of a new sulfureous diketopiperazine (**1**) and two known analogues, together with an amino acid (**4**) from the root of *M. oleifera***.**The dry powder of *M. oleifera* roots (2kg) was extracted with methanol (5L × 4 times) at room temperature. The combined extracts were evaporated under the vacuum condition to obtain the methanol residue (191 g). Next, the residue was suspended in water (2L) and successively partitioned in hexane and ethyl acetate. The organic layers were concentrated to give 38.9 g and 45.5 g of hexane (Hex) and ethyl acetate (EA) residues, respectively. The EA residue was separated on a silica gel chromatography column (CC) with gradient mixtures of CH_2Cl_2–MeOH (1/0-0/1, v/v) to yield twelve fractions: E1-E12. The fraction E7 (125 mg) was separated on a silica gel CC with CH_2Cl_2–MeOH (30/1, v/v) elution, followed by preparative HPLC using a YMC-Pack ODS-A column (250×20 mm, 5 μm) with the solvent gradient 30-65% MeOH in water in 90 min and at the flowrate 4 mL/min to yield compound **1** (1.3 mg) and compound **2** (1.8 mg). The fraction E11 (418 mg) was separated on a silica gel CC with EA–MeOH (30/1, v/v) elution to obtain subfractions E11.1-E11.5. The subfraction E11.2 (122 mg) was purified by preparative HPLC using a YMC-Pack ODS-A column (250×20 mm, 5 μm) with the solvent gradient 20-40% MeOH in water in 90 min and at the flowrate 4 mL/min to afford compound **3** (3.9 mg). The subfraction E11.3 (82 mg) was separated on an RP C18 CC eluted with MeOH 50% in water to obtain compound **4** (7.4 mg).

*7-epilasiodipline D (**1**)*: colorless solid; $[\alpha]_D^{25}$ +12.6 (MeOH, *c* 0.2). HR-ESI-MS: *m/z* 350.0638 $[M + H]^+$ (calcd 350.0633 for $C_{15}H_{16}N_3O_3S_2$, Δ 1.40 ppm), *m/z* 699.1047 $[2M+H]^+$, *m/z* 721.0947 $[2M+Na]^+$, *m/z* 680.4792 $[2M-H_2O+H]^+$. ^{1}H NMR (600 MHz, CD_3OD) δ_H (ppm): 4.60 (1H, s, H-7), 7.56 (1H, s, H-9), 7.39 (1H, d, *J* = 7.8 Hz, H-11), 7.15 (1H, td, *J* = 7.8, 1.2 Hz, H-12), 7.08 (1H, td, *J* = 7.8, 1.2 Hz, H-13), 7.59 (1H, d, *J* = 7.8 Hz, H-14), 1.90 (3H, s, 2-Me), 3.24 (3H, s, 3-NMe). ^{13}C NMR (150 MHz, CD_3OD) δ_C (ppm): 169.1 (C-1), 72.3 (C-2), 173.0 (C-4), 85.6 (C-5), 47.1 (C-7), 107.5 (C-8), 125.5 (C-9), 112.5 (C-11), 123.0 (C-12), 120.6 (C-13), 118.8 (C-14), 128.8 (C-15), 137.4 (C-16), 20.8 (2-Me), 29.0 (3-NMe).

*Lasiodipline D (**2**)*: colorless solid; $[\alpha]_D^{25}$ +205.7 (MeOH, *c* 0.2). HR-ESI-MS: *m/z* 350.0627 $[M+H]^+$ (calcd 350.0633 for $C_{15}H_{16}N_3O_3S_2$, Δ −1.74 ppm), *m/z* 699.1172 $[2M+H]^+$, *m/z* 721.0942 $[2M+Na]^+$, *m/z* 680.4712 $[2M-H_2O+H]^+$. ^{1}H NMR (600 MHz, CD_3OD) δ_H (ppm): 4.30 (1H, s, H-7), 7.75 (1H, brs, H-9), 7.39 (1H, d, *J* = 7.8 Hz, H-11), 7.14 (1H, td, *J* = 7.8, 1.2 Hz, H-12), 7.07 (1H, td, *J* = 7.8, 1.2 Hz, H-13), 7.67 (1H, d, *J* = 7.8 Hz, H-14), 1.93 (3H, s, 2-Me), 3.17 (3H, s, 3-NMe). ^{13}C NMR (150 MHz, CD_3OD) δ_C (ppm): 167.7 (C-1), 71.8 (C-2), 174.3 (C-4), 86.4 (C-5), 40.1 (C-7), 110.0 (C-8), 124.4 (C-9), 112.2 (C-11), 122.7 (C-12), 120.3 (C-13), 119.6 (C-14), 128.8 (C-15), 136.8 (C-16), 20.7 (2-Me), 29.4 (3-NMe).

NO Production Inhibition Assay: The effects of samples on the NO production in LPS-stimulated RAW 264.7 macrophage cells were examined, using the method as described previously [14]. The NO inhibition of each compound was tested at the concentration of 10 and 50 μM. Cardamonin was used as a positive control.

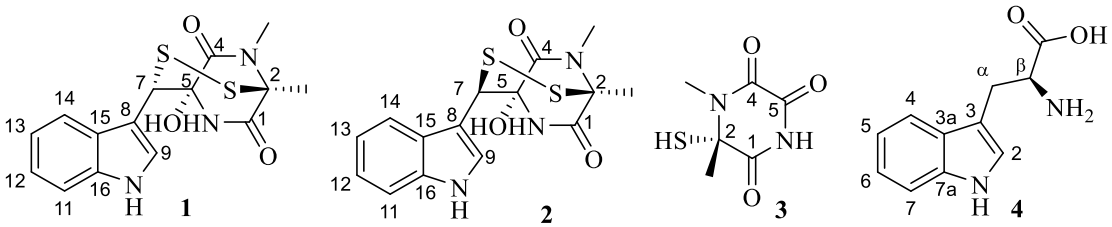

Figure 1. Structures of the isolated compounds from roots of *M. oleifera*

Compound **1** was isolated as a colourless solid. The HR-ESI-MS spectrum (positive mode) of **1** showed the ion at *m/z* 350.0638 $[M + H]^+$ (calcd 350.0633 for $C_{15}H_{16}N_3O_3S_2$, mass tolerance at 1.40 ppm), together with ions at *m/z* 699.1047 $[2M+H]^+$, *m/z* 721.0947 $[2M+Na]^+$, and *m/z* 680.4792, which confirmed the molecular formula $C_{15}H_{15}N_3O_3S_2$ for **1**. ^{1}H NMR spectrum of the compound showed the signals of four aromatic protons of an olefinic proton signal at δ_H 7.56, 7.39, 7.15, 7.08, and 7.59, as well as signals of N- and C-bonded methyl singlets (δ_H 3.24 and 1.90), and a singlet at δ_H 4.60 which revealed an S-bonded CH group. ^{13}C and HSQC spectra of the compound also exhibited signals of indole nucleus carbon at δ_C 107.5, 125.5, 112.5, 123.0, 120.6, 118.6, 127.1, and 134.7, together with signals of two amide carbonyl at δ_C 169.1 and 173.0. These observations indicated that compound **1** was probably a cyclic dipeptide of tryptophan and alanine. Besides, high chemical shifts of a quaternary carbon at δ_C 72.3 (C-2), 85.6 (C-5), and a CH group at δ_C 47.1 (C-7), together with the calculated molecular formula ($C_{15}H_{15}N_3O_3S_2$) revealed the presence of the 5-hydroxy group and 2,7-disulfide bridge. The planar structure of compound **1** was confirmed by HMBC correlations of H-7 (δ_H 4.60) with C-4 (δ_C 173.0) and C-9 (δ_C 125.5), of 2-Me protons (δ_H 1.90) with C-1 (δ_C 169.1), as well as of 3-NMe (δ_H 3.24) with C-2 (δ_C 72.3) and C-4 (δ_C 173.0) (Figure S7). NMR data of compound 1 were highly similar to those of compound 2, while the HRMS data illustrated the same molecular formula of them ($C_{15}H_{16}N_3O_3S_2$). Moreover, both two compounds had positive optical rotation values. These revealed that 1 and 2 were two diastereomers. While the spectroscopy data of **2** were in agreement with those of a previous study and could be determined as lasiodipine D (Table S1), a reported sulfureous diketopiperazine from the fungus *Lasiodiplodia pseudotheobromae* F2 [15], the data of compound **1** showed some difference in the chemical shift of C-7 (H-7) to the reference. Since the ECD curves of these two compounds were quite similar (Figure 2), the ^{1}H and ^{13}C NMR spectra of those two compounds were re-scrutinized to determine the absolute configuration of compound **1**. As shown in Table S2, the signal of C-7 was sharply shifted by 7 ppm downfield from those of compound **2** (lasiodipline D). Also, a similar observation could be found in the ^{1}H NMR data (Table S1). In contrast, the chemical shifts of C-5, C-2, and 2-Me were mostly similar between these compounds. While the stereochemistry of compound **2** was (2*S*,5*S*,7*R*), the absolute configuration of compound **1** could be identified as (2*S*,5*S*,7*S*). Thus, this was a new compound namely 7-epi lasiodipline D.

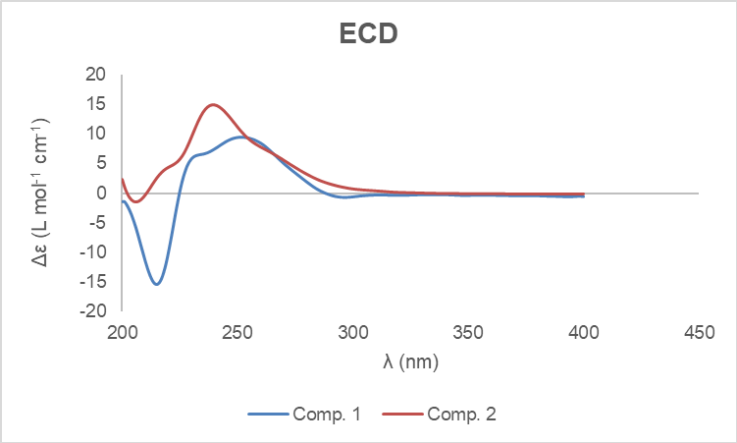

Figure 2. Comparison of the ECD spectra of compounds **1** and **2**

Structures of compounds **(3)** and **(4)** were elucidated as lasiodipline C and L-tryptophan by comparing their NMR data to those of reported studies [15, 16]. Previous studies investigated phenolics, flavonoids, and glycosides from *M. oleifera* [2, 5, 8, 11, 12]. However, these compounds were mostly determined in the leaves and seeds of the plant, meanwhile, the chemical constituents of the root of *M. oleifera* were rarely reported. Moreover, sulfureous diketopiperazines were only found in the bacteria [15], thus, this was the first time these compounds were identified from a plant.

The inhibitory effect of compounds **1**, **2**, and **3** on NO production was evaluated in LPS-induced RAW 264.7 cells. These compounds showed weak inhibition of NO production (IC_{50} > 50 µM) in comparison to Cardamonin. Previous studies indicated that sulfureous diketopiperazines might exhibit antibacterial and anti-tumour effects [15, 17, 18]. Thus, the bioactivities of these compounds should be further evaluated. Moreover, while lasiodipline D was previously isolated from a microorganism, this study presented for the first time the isolation of the compound and its diastereomer, a new compound, from a plant. Since just a few phytochemical studies on the roots of *Moringa oleifera* were conducted, these were promising results for other chemical investigations of this medicinal plant material.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

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