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Two folate-derived analogues from an aqueous decoction of *Uncaria rhynchophylla*

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[ABSTRACT] Two new folate-derived analogues, named uncarophyllofolic acids A (**1**) and B (**2**), respectively, were isolated from the *Uncaria rhynchophylla* hook bearing stem (Gouteng in Chinese). The distinct stereochemical structures of **1** and **2** were determined by spectroscopic data analysis in combination with acidic hydrolysis and Marfey's derivatization, along with comparison of their specific rotation and Cotton effect (CE) data with those of the biogenetically related known derivatives as well as theoretical calculations of electronic circular dichroism (ECD) spectra. A plausible biosynthetic pathway of **1** and **2**, associating to folate metabolism and the previously reported orychophragines A−C from *Orychophragmus violaceus*, is discussed.

[KEY WORDS] Rubiaceae; *Uncaria rhynchophylla*; Folate derivative; Uncarophyllofolic acid; Folate metabolism.

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Introduction

Hook-bearing stems of the Rubiaceous plant *Uncaria rhynchophylla* (Miq.) Jacks. are a major material of Chinese herbal medicine Gouteng, which is used alone or in many prescriptions and preparations for the treatment of hypertension, dizziness, headache, preeclampsia as well as infant convulsions [1-2]. Due to medicinal applications of the plant genus *Uncaria* in China and Japan as well as Peru, Guayana, and Brazil, all 34 known species in the world have chemically and/or pharmacologically been investigated since 1900 [3-4]. From various extracts of different parts of these plants, around 300 chemical constituents have been reported, covering indole alkaloids, triterpenoids, flavonoids, lignanoids, cumarins, steroids, iridoids, phenolic acids, and lipid acids [3-18]. Pharmacological and biological studies revealed that the extracts and some chemical constituents from *U. rhynchophylla* had a wide spectrum of effects and bioactivities, especially focusing on central neural systems and the major alkaloidal chemical constituents rhynchophylline and its analogues [3-4, 9, 19-26]. Although the herbal medicine Gouteng is practically utilized by decocting with water and the decoction showed sedative and hypotensive effects corresponding to the clinically treating diseases, the chemical constituents were isolated predominantly from the hydroethanolic and methanolic extracts. Especially the major bioactive rhynchophylline showed weaker effects than the water decoctions [1]. Hence, we studied the decoction of Gouteng as part of a program to search for unknown components containing in the practically used water extract of several popular Chinese herbal medicines and to assay their activities [26-39]. Previously we reported five components of this plant from the decoction of Gouteng [40], including four new rhynchophylline derivatives and one known *β*-carboline alkaloid. In this paper, described are isolation and structural determination of two unprecedented folate-derived metabolites, uncarophyllofolic acids A (**1**) and B (**2**) (Fig. 1). Owing to the structural architectures sharing heterocyclic, *p*-aminobenzoyl and glutamic acid moieties in a sequential connection, the two compounds are proposed biogenetically to be derived from B vitamin folates.

Results and Discussion

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ −36.0 (*c* 0.05, H_2O), of which the molecular

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These authors have no conflict of interest to declare.
Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

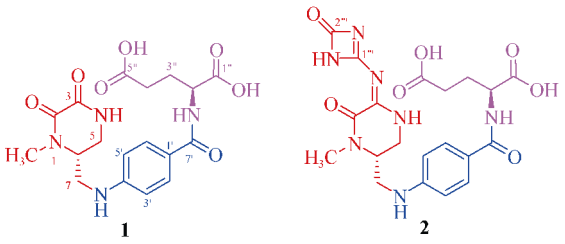

Fig. 1 Structures of compounds 1 and 2

formula was determined as $C_{18}H_{22}O_7N_4$ from HRESI-MS at *m/z* 405.1416 $[M - H]^-$ (Calcd. for $C_{18}H_{21}O_7N_4$, 405.1416). The presence of hydroxy and/or amino (3352 cm^{-1}), amide carbonyl (1668 cm^{-1}), and aromatic ring (1607 and 1522 cm^{-1}) functionalities in the molecule was elucidated by the corresponding characteristic absorption bands in the IR spectrum of **1**. Diagnostic resonances for a *p*-substituted benzoyl unit at δ_H 7.70 (2H, dd, *J* = 8.4 and 1.8 Hz, H-2′ and H-6′) and 6.77 (2H, br d, *J* = 8.4 Hz, H-3′ and H-5′) and a heteroatom-bearing methyl group at δ_H 3.13 (s, 1-CH_3) were observed in the ^{1}H NMR spectrum of **1** in D_2O, in addition to two heteroatom-bearing methylenes, two heteroatom-bearing methines, and two aliphatic methylenes between δ_H 4.54 and 2.14 (partially overlapping multiplets). In the ^{13}C NMR and DEPT spectra of **1**, 18 carbon resonances were detectable, corresponding to structural units bearing the aforementioned protons as well as five carboxylic carbons at δ_C 178.2 (C-1″ and C-5″), 170.9 (C-7′), 159.6 (C-2), and 159.4 (C-3). These spectroscopic data completely differed that reported for the chemical constituents from the genus [3-18], indicating **1** had an abnormal chemical structure which was further elucidated by the 2D NMR experimental data.

Analyzing the HSQC spectrum of **1**, the proton and corresponding proton-bearing carbon resonances in the NMR spectra were unequivocally assigned. The homonuclear vicinal coupling structural fragments (bold lines) in the molecule (Fig. 2) were readily deduced from the cross-peaks H_2-5/H-6/H_2-7, H-2′/H-3′ (overlapping with H-4′/H-6′), and H_2-2″/H_2-3″/H_2-4″ in the ^{1}H-^{1}H COSY spectrum of **1**. A 6-aminomethyl-1-methylpiperazine-2, 3-dione moiety was established by the multiple bond heteronuclear correlation peaks from the methyl protons (1-CH_3) to C-2 and C-6, from H_2-5 to C-3, and from H-6 to C-2 in the HMBC spectrum, in combination with the chemical shift values of these proton and carbon signals. The *p*-substituted benzoyl moiety was confirmed by the HMBC correlation peaks from H-2′ (H-6′) to C-4′ and C-7′ and from H-3′ (H-5′) to C-1′, while a glutamic acid moiety was elucidated by the HMBC correlation cross-peaks from H-2″ and H_2-3″ to C-1″ and from H_2-3″ and H_2-4″ to C-5″, together with their chemical shift values. Furthermore, considering the molecular formula and chemical shift values, the HMBC correlation cross-peak from H-2″ to C-7′ linked the carboxylic carbon of the benzoyl moiety to C-2″ of the glutamic acid moiety *via* an amide bond, and the HMBC correlation cross-peaks from H_2-7 to C-4′ connected C-4′ to C-7 *via* the remaining nitrogen atom. The linkages were further verified by the ^{1}H-^{1}H COSY correlations H-4/H_2-5, 4′-N*H*/H_2-7 and 2″-N*H*/H-2″ as well as the HMBC correlations from H-4 to C-2, C-3, C-5, and C-6 and from 4′-N*H* to C-3′ and C-5′ in the 2D NMR spectra of **1** in DMSO-d_6. Thus, **1** was deduced to have an architectural structure sequentially assembling by 1-methylpiperazine-2, 3-dione-6-yl-methylenyl, 4′-aminobenzoyl, and glutamic acid *via* their nitrogen linkers. Because the two chiral centers in the molecule are far away from each other, no useful information for assigning stereochemistry was observed in the NOESY spectrum of **1**. The 2″*S*-configuration of the glutamic acid moiety in **1** was subsequently determined by acid hydrolysis, followed by Marfey's derivatization and HPLC analysis [41], indicating liberation of L-glutamic acid in the hydrolysate. Because our attempts failed to obtain a suitable single crystal for X-ray crystallographic analysis, electronic circular dichroism (ECD) spectra of only two possible 1*R* and 1*S* stereoisomers were theoretically calculated by using CONFLEX and Gaussian MMFF94 conformational search protocols and reoptimizing the conformers at different base sets (see Experimental section). However, the calculated ECD spectra of both the 1*R* and 1*S* stereoisomers did not match with the measured CD spectrum of **1** (Fig. 3), indicating the theoretically predicted conformers could not represent the real ones of the flexible structures of the stereoisomers in solution. Fortunately, the X-ray crystal structures of orychophragines A–C from *Orychophragmus violaceus* were recently reported [42], especially orychophragine A has the same structural moieties as that of **1** except for replacement of a methoxy unit in the former by the glutamic acid unit in **1**. Biogenetically **1** and orychophragines A–C would be derived from metabolism of folates, and the natural form of the chiral folates is thought to be mainly the 6*S* diastereoisomers [43], which is completely supported by a single crystal X-ray diffraction of orychophragines A–C [42]. With an authentic sample donated kindly by Prof. DONG Jun-Xing (Department of Pharmaceutical Chemistry, Beijing Institute of Radiation Medicine), the measured CD spectrum of orychophragine A was similar to that of **1** (Fig. 3), while the specific rotation value of **1** was in well agreement with adducting the measured $[\alpha]_D^{20}$ values of orychophragine A [$[\alpha]_D^{20}$−14.8 (*c* 0.25, H_2O)] and *p*-aminobenzoyl-L-glutamic acid [$[\alpha]_D^{20}$−17.1 (*c* 0.46, 1 mol·L^{-1} HCl, H_2O), a sample purchased from InnoChem Technology Co., Ltd., Beijing, China] [44]. These data were supportive for assignment of the 6*S* configuration of **1**. Thus, the structure of compound **1** was determined and named uncarophyllofolic acid A.

Compound **2** has the molecular formula $C_{20}H_{23}O_7N_7$ deduced from HRESI-MS at *m/z* 474.1729 $[M + H]^+$ (Calcd. for $C_{20}H_{24}O_7N_7$, 474.1732) and 472.1586 $[M - H]^-$ (Calcd. for $C_{20}H_{22}O_7N_7$, 472.1575). The NMR spectroscopic data of **2** in D_2O were similar to those of **1** (Table 1). Analyzing 2D

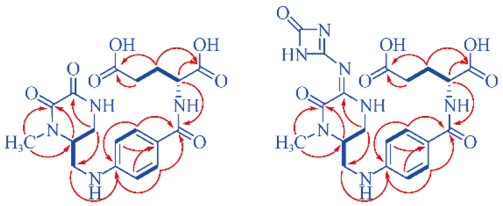

Fig. 2 ^{1}H-^{1}H COSY (bold lines) and HMBC (arrows, from ^{1}H to ^{13}C) correlations of compounds 1 and 2

NMR experimental data revealed that **2** shared the sequentially connecting three structural moieties with **1** (Fig. 2). However, as compared with those of **1**, H-5a ($\Delta\delta_H$ +0.62), H-5b ($\Delta\delta_H$ +0.42), and H-6 and C-5 in **2** were deshielded notably by $\Delta\delta_H$ +0.26 and $\Delta\delta_C$ +3.6, respectively, in contrast, C-3 was shielded by $\Delta\delta_C$ −5.8. These differences, along with the molecular composition, revealed the presence of an additional C_2HON_3 unit at C-3 in **2**. Based on the chemical shift value, a carbon-nitrogen double bond was deduced to be formed between the sp^2 hybridized C-3 (δ_C 153.6) and one nitrogen atom of the C_2HON_3 unit, meanwhile, a carbonimidisocyanatidic or 1‴, 3‴-diazet-2‴(1‴*H*)-one moiety consisting of the remaining atoms was elucidated to match the molecular formula of **2**. Because the IR spectrum did not exhibit a diagnostic absorption (2200−2300 cm^{-1}) for isocyanate [45] in the carbonimidisocyanatidic moiety, whereas a very strong band assignable to overlapping carbon-nitrogen double bond and amide vibrations was observed, the 1‴, 3‴-diazet-2‴(1‴*H*)-one moiety was determined to locate at the C-3 nitrogen in **2**. Based on the specific rotation value and CD data, along with plausible biogenetical formation from the folate metabolism (Scheme 1), the 6*S* configuration of **2** with a 3*E* geometry was assigned to be identical to that of the natural folate precursors. In this case, the assignment was supported by consistence of the theoretically calculated ECD and experimental CD spectra (Fig. 3). Thus, compound **2** was determined to be a 4a-hydroxy-5-methyltetrhydrofolic acid derived product with an opening pyrimidine ring and designated as uncarophyllofolic acid B.

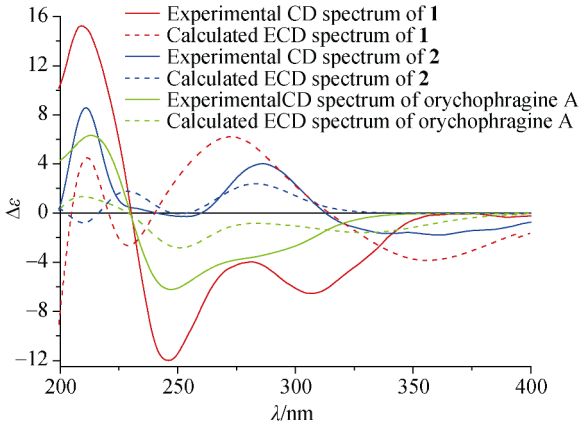

Fig. 3 The experimental CD (full lines) and calculated ECD (dashed lines) spectra of 1, 2, and orychophragine A (the experimental CD spectrum of 2 was magnified by 10 times)

Table 1 The NMR spectroscopic data (δ) for compounds 1 and 2[a]

Position	**1** (D_2O)		**1** (DMSO-d_6)		**2** (D_2O)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		159.6		157.3		158.7
3		159.4		157.3		153.6
4			8.43 d (4.8)			
5a	3.88 brd (13.8)	40.7	3.63 m	39.1	4.50 d (14.4)	44.3
5b	3.52 brd (13.8)		3.28 m		3.94 dd (14.4, 4.2)	
6	3.87 m	57.1	3.62 m	55.4	4.13 t (4.2)	56.0
7a	3.67 m	42.8	3.37 m	42.0	3.82 d (15.6)	41.9
7b	3.67 m		3.37 m		3.62 dd (15.6, 3.0)	
1-*CH*$_3$	3.13 s	35.3	2.98 s	33.9	3.25 s	34.9
1′		121.4		121.1		122.7
2′/6′	7.70 dd (8.4, 1.8)	130.0	7.67 brd (8.4)	129.1	7.59 d (8.4)	129.4
3′/5′	6.77 brd (8.4, 8.4)	112.3	7.62 brd (8.4)	110.7	6.54 d (8.4)	111.7
4′		151.9		150.8		150.0
7′		170.9		166.4		170.5
4′-N*H*			6.40 t (6.0)			
1″		178.2		173.8		/[b]
2″	4.54 m	53.8	4.33 m	51.8	4.35 m	56.5
3″a	2.32 m	26.7	2.01 m	26.2	2.19 m	29.0

Continued

Position	**1** (D_2O)		**1** (DMSO-d_6)		**2** (D_2O)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
3″b	2.14 m		1.92 m		2.06 m	
4″	2.55 t (7.2)	31.2	2.31 m	30.3	2.35 m	34.9
5″		178.2		174.0		/[b]
2″-N*H*			8.09 brs			
1‴						/[b]
2‴						/[b]

[a] Chemical shift values (δ) were measured at 600 MHz for ^{1}H and at 150 MHz for ^{13}C, respectively; Proton coupling constants (*J*) in Hz are given in parentheses; The assignments were based on DEPT, ^{1}H-^{1}H COSY, HSQC, and HMBC experiments; [b] Not observed

Compounds **1** and **2** have the unprecedented structural architectures in synthetic or natural products. Especially **1** and **2** share the sequentially linking heterocyclic, *p*-aminobenzoyl and glutamic acid moieties with the B vitamin folates, differing only in the heterocyclic ring moieties. Therefore, compounds **1** and **2** are reasonably proposed to be derived from a post metabolic modification of the folates, and their biosynthetic pathways are postulated as illustrated in Scheme 1. Enzyme-catalyzed sequential methylation and hydrogenation of folic acid (**3**) generate 5-methyltetrahydrofolic acid (**4**), which is subsequently oxidized to yield another folate species 4a-hydroxy-5-methyltetrahydrofolic acid (**5**). Dehydrogenation of **5** accompanying by a carbon bond (C-4−C-4a) cleavage gives an intermediate **6**, which undergoes either oxidative degradation releasing a carbamimidoyl isocyanate molecule to produce **1** or dehydrogenation to generate **7** in turn, either which is intramolecularly cyclized to yield **2** or oxidatively degradated to give **1**. Also **1** may be afforded by oxidative degradation of **2**. Additionally, **1** would be produced by oxidative deamination of **5**, flowed by spontaneous or sequential dehydrogenated carbon bond (C-4−C-4a) cleavage, hydrolysis, and further deamination through **8**, **9**, and **10**. Notably, the reported orychophragines A−C from *Orychophragmus violaceus* [42] might be biosynthesized in the same pathways. Orychophragine A would be afforded by hydrolysis of **1** liberating glutamic acid and methylation of the newly formed carboxylic group, while the isocyanate unit in **8** undergoes intramolecular cyclization between isocyanate and methylpiperazinone amino groups, followed by hydrolytic release of glutamic acid and further methylation, to yield orychophragines B and C (not shown in Scheme 1), though the reported pathway is not excluded [42].

Although compound **1** was inactive in the preliminary *in vitro* assays including the neuroprotective activity against serum deprivation-induced PC12 cell damage [46] and H_2O_2 or L-glutamate-induced SK-N-SH cell injury [47-48], and inhibition activity against production of TNF-*α* from RAW264.7 cell [49] and **2** was not tested due to limitation of the sample amount, isolation and structural characterization of the two compounds, along with the previously reported orychophragines A−C, unravel seemly a novel metabolic pathway of the B vitamin folates in the plants. Additionally, because the folic acid with high biological activity plays an important role in the proper functioning of the human body and has both direct and indirect effects on the metabolism of the body's cells [50-51], the roles and functions of the folate metabolized derivatives **1** and **2** in the clinical application of Gouteng are deserved for further studies in future when enough sample amount available by chemical synthesis.

Experimental

General experimental procedures

Optical rotations were recorded on a Rudolph Research Autopol V polarimeter (Rudolph, NJ, USA). UV spectra were measured on a V-650 spectrometer (JASCO, Tokyo, Japan). CD spectra were recorded on a JASCO J-815 CD spectrometer (JASCO, Tokyo, Japan). IR spectra were obtained on a Nicolet 5700 FT-IR microscope transmission instrument (Thermo Electron Corporation, Madison, WI, USA). HRESI-MS data were measured on a Q Exactive Focus Mass Spectrometry (Thermo Fisher Scientific, MA, USA). NMR spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C, respectively, on an Inova SYS 600 (Varian Associates Inc., Palo Alto, CA, USA) spectrometer in D_2O or DMSO-d_6 with the residual solvent peak of HDO (δ_H 4.800) or the methyl peak of CH_3OH (δ_C 49.500) and DMSO (δ_C 39.500) as references. Column chromatography (CC) was performed with macroporous adsorbent resin (HP-20, Mitsubishi Chemical Inc., Kawasaki, Japan), MCI gel (CHP 20P, 75−150 μm, Mitsubishi Chemical Inc., Tokyo, Japan), silica gel (200−300 mesh, Qingdao Marine Chemical Inc. Qingdao, China), Toyopearl HW-40F gel or HW-40S (Tosoh Corporation Bioscience Davison, Tokyo, Japan), or Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on a system consisting of an Agilent ChemStation for LC system, an Agilent 1200 pump, and an Agilent 1100 single-wavelength absorbance detector (Agilent Technologies, Ltd.) or a Smartline RI detector (Knauer, Berlin, Germany) detector, using Grace C_{18} (W.R.Grace & Co., Maryland, USA) (250 mm × 10 mm i.d., 5 μm). TLC was conducted on precoated silica gel GF_{254} plates. Spots were visualized under UV light (254 or 365 nm) or by spraying with 7% H_2SO_4 in

95% EtOH followed by heating. Unless otherwise noted, all chemicals were obtained from commercially available sources and were used without further purification.

Plant material

The air-dried hooking stems of *Uncaria rhynchophylla* (Miq.) Jacks. were purchased in An-Guo of Hebei province, which were collected in August 2016 from Hengdong county, Hunan Province, China. Plant identity was verified by Prof. MA Lin (Institute of Materia Medica, Beijing, China). A voucher specimen (No. ID-S-2753) was deposited at the herbarium of Natural Medicinal Chemistry, Institute of Materia Medica.

Extraction and isolation

The plant material (97 kg) was powdered and decocted with H_2O for 30 min (3 × 300 L). The H_2O extract was concentrated to 230 L under reduced pressure, subjected to column chromatography over a macroporous adsorbent resin (HP-20, 75 L) column (25 cm × 200 cm), and eluted successively with H_2O (300 L), 50% EtOH (300 L), and 95% EtOH (200 L) to afford corresponding fractions A−C. After removal of the solvent, fraction B (2.6 kg) was chromatographed over MCI gel (CHP 20P, 15 kg) successively eluting with H_2O (20 L), 30% EtOH (20 L), 50% EtOH (20 L), 95% EtOH (18 L), and $(CH_3)_2CO$ (5 L) to give fraction B1−B5. Fraction B1 (680 g) was chromatographed over reversed phase silica gel (C_{18}, 1.0 kg) with a gradient elution increasing CH_3OH in H_2O (0%−100%, *V*/*V*) to afford subfractions B1-1−B1-14 based on TLC analysis. Fraction B1-2 (30 g) was separated by CC over Toyopearl HW-40F gel eluting with H_2O to yield B1-2-1−B1-2-3. Fraction B1-2-2 (3.8 g) was further isolated by CC Toyopearl HW-40S gel eluting with H_2O to yield B1-2-2-1−B1-2-2-3. Separation of B1-2-2-2 (0.99 g) by CC over Sephadex LH-20 (H_2O) obtained B1-2-2-2-1−B1-2-2-2-4, of which B1-2-2-2-2 (0.58 g) was fractionated by reversed phase HPLC using Grace C_{18} column (10% CH_3CN in H_2O, containing 0.2% AcOH, 2.0 mL·min^{-1}) to yield B1-2-2-2-2-1−B1-2-2-2-2-3. HPLC isolation of B1-2-2-2-2-1 (320 mg, 6% CH_3CN in H_2O, containing 0.2% AcOH, 2.0 mL·min^{-1}) afforded **2** (0.6 mg, t_R = 44 min), while HPLC purification of B1-2-2-2-2-2 (80 mg, 10% CH_3CN in H_2O, containing 0.2% AcOH, 2.0 mL·min^{-1}) yielded **1** (1.0 mg, t_R = 32 min).

*Uncarophyllofolic acid A (**1**)*

White amorphous powder (H_2O); $[\alpha]_D^{20}$ −36.0 (*c* 0.05, H_2O); UV (H_2O) λ_{max} (log ε) 215.4 (sh, 2.90), 295.2 (2.83) nm; CD (H_2O) λ_{max} ($\Delta\varepsilon$) 209 (+15.24), 246 (−12.00), 281 (−4.00), 307 (−6.54); IR ν_{max} 3352, 2932, 1668, 1607, 1574, 1522, 1449, 1408, 1336, 1275, 1188, 1109, 840, 769, 617 cm^{-1}; negative HRESI-MS *m/z* 405.1416 $[M - H]^-$ (Calcd. for $C_{18}H_{21}N_4O_7S$, 405.1416). ^1H and ^{13}C NMR spectral data are shown in Table 1.

*Uncarophyllofolic acid B (**2**)*

White amorphous powder (H_2O); $[\alpha]_D^{20}$ −11.7 (*c* 0.06, H_2O); UV (H_2O) λ_{max} (log ε) 206.4 (sh, 3.28), 276.2 (2.68) nm; CD (H_2O) λ_{max} ($\Delta\varepsilon$) 211 (+0.86), 254 (−0.02), 286 (+0.40); IR ν_{max} 3384, 2989, 1685, 1441, 1206, 1143, 1009, 932, 879, 845, 803, 776, 725, 602, 529 cm^{-1}; positive HRESI-MS *m/z* 474.1734 $[M + H]^+$ (Calcd. for $C_{20}H_{24}O_7N_7$, 474.1732), negative HRESI-MS *m/z* 472.1586 $[M - H]^-$ (Calcd. for $C_{20}H_{22}O_7N_7$, 472.1575). ^1H and ^{13}C NMR spectral data are shown in Table 1.

*Marfey's analysis of **1***

Compound **1** (0.1 mg) was hydrolyzed with 6 mol·L^{-1} HCl (0.5 mL) in a sealed glass bomb at 120 °C for 12 h, then the reaction mixture was freeze-dried. The residue was dissolved in 10 μL H_2O, and (1-fluoro-2, 4-dinitrophenyl)-5- L-alanine amide (FDAA) in acetone (1%, 15 μL) and 1 mol·L^{-1} $NaHCO_3$ (6 μL) were added to react at 40 °C for 1 h. The reaction solution was cooled, neutralized with 2 mol·L^{-1} HCl (3 μL), and filtrated. The authentic L- or D-glutamic acid (1.0 mg) was individually dissolved in water (100 μL) with addition of 1% FDAA solution (150 μL) and 1 mol·L^{-1} $NaHCO_3$ (60 μL). The mixtures were reacted at 40 °C for 1 h, and neutralized with 2 mol·L^{-1} HCl (30 μL). The FDAA-amino acid derivatives from the hydrolysate as well as L- and D-glutamic acids were analyzed using UPLC: ACQUITY UPLC BEH C_{18} column (1.7 μm, 2.1 mm × 100 mm); flow rate, 0.2 mL·min^{-1}; UV detection at 340 nm; column temperature, 30 °C; gradient elution of increasing CH_3OH in H_2O from 35% to 45% over a period of 12 min. Comparison of retention times of the FDAA derivatives determined liberation L-glutamic acid from hydrolysis of **1**.

*ECD Calculations of **1**, **2**, and orychophragine A*

Conformational analysis was conducted by Monte Carlo searching with the MMFF94 molecular mechanics force field using Gaussian 16 program package (Gaussian, Inc., available from: https: //www. gaussian.com). The lowest-energy conformers having relative energies within 3 kcal·mol^{-1} were optimized using DFT at the B3LYP/6-31 + G(d, p), APFD/6-31 + G(d, p), BMW1PW91/6-31 + G(d, p), or CAM-B3LYP/6-31 + G(d, p) level with the solvent effects considered using the dielectric constant of H_2O (ε 78.36) *via* conductor-like polarizable continuum model (CPCM). The energies, oscillator strengths, and rotational strengths of the excitations were calculated using the TDDFT methodology at the B3LYP/6-311 + G(d, p), APFD/6-311 + G(d, p), BMW1PW91/6-311 + G(d, p), or CAM-B3LYP/6-311 + G(d, p) level. The optimized conformers showed relative Gibbs free energies (ΔG) under 3 kcal·mol^{-1} were used for ECD and UV spectra simulation by the Gaussian function (σ = 0.30 eV). To obtain the final ECD and UV spectra, the simulated spectra of the lowest energy conformers were averaged on the basis of the Boltzmann distribution theory and their relative Gibbs free energy (ΔG).

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