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Peganumine A, a β-Carboline Dimer with a New Octacyclic Scaffold from *Peganum harmala*

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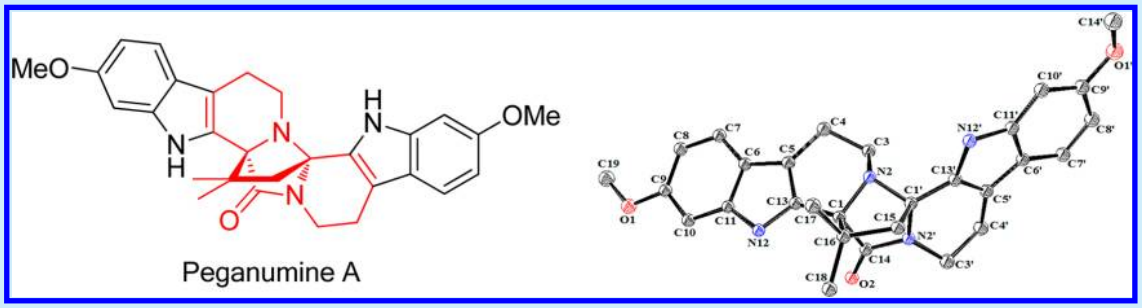
Supporting Information



ABSTRACT: Peganumine A (**1**), a new dimeric β-carboline alkaloid characterized by a unique 3,9-diazatetracyclo-[6.5.2.0^{1,9}.0^{3,8}]pentadec-2-one scaffold, was isolated from the seeds of *Peganum harmala*. The structure including the absolute configuration was determined by spectroscopic data, X-ray crystallography, ECD calculation, and CD exciton chirality approaches. Compound **1** showed moderate cytotoxic activity against MCF-7, PC-3, and HepG2 cells and selective effects on HL-60 cells with an IC_{50} value of 5.8 μM.

β-Carboline alkaloids are a large group of natural indole alkaloids which are widespread in nature, including various plants, foodstuffs, marine creatures, insects, mammalians, and human tissues and body fluids.[1] During the past few years, numerous simple and complicated β-carboline alkaloids with a saturated or unsaturated tricyclic ring system have been isolated and synthetised.[2] This family of compounds have attracted great attention for their unique structures and diverse biological activities,[3] such as antitumor,[4] antimicrobial,[4a,5] insecticidal,[4a,d,6] antimalarial,[4a,d] antinociception,[7] myeloperoxidase inhibitory,[8] antioxidant,[9] anti-inflammatory,[9] and analgesic effects.[4e]

Peganum harmala L. (Zygophyllaceae) is a perennial plant which is not only native to eastern Iran and western India but also found in different regions of USA and China, which is rich in β-carboline alkaloids.[4d,10] In our course to explore the antitumor active natural products, a novel β-carboline alkaloid, peganumine A (**1**), with a new polycyclic scaffold was isolated from the seeds of *P. harmala*. Moreover, the signature C_2 bridge and the five-membered γ-lactam are also novel motifs in natural products. To the best of our knowledge, this unique β-carboline dimer has no counterpart in the literature. We herein reported the isolation, structural elucidation, biosynthetic consideration, and antitumor activity of this compound.

The seeds of *P. harmala* L. (15.4 kg) were extracted under reflux with 95% ethanol (2 × 2 h × 100 L) and 75% ethanol (1 × 2 h × 100 L), respectively. The combined EtOH extracts were concentrated in vacuo to yield a residue (1.9 kg), which was suspended in water (13 L) and adjusted to pH 3 with 5% HCl. The acidic mixture was partitioned with CH_2Cl_2 (6 × 13 L), and the aqueous layer was then basified to pH 10 with 3 N NaOH, followed by exhaustive extraction with CH_2Cl_2 (6 × 13 L) to yield the crude alkaloids (420.2 g). The crude alkaloids were separated by a silica gel chromatography column (CC) using CH_2Cl_2/MeOH (1:0 → 0:1) as eluent, to give nine fractions (Fr. A–Fr. I). Fraction B, eluted with CH_2Cl_2/MeOH (100:1), was chromatographed on silica gel (CH_2Cl_2/acetone 1:0 → 0:1) to yield six subfractions (Fr. B1–Fr. B6). Fr. B3 was then separated by ODS CC, eluted with MeOH–H_2O (70:30), and was purified by preparative HPLC on a YMC C-18 column using MeOH–H_2O (80:20) as the mobile phase to yield **1** (3.5 mg).

Peganumine A (**1**)[11] was obtained as a white amorphous powder. The molecular formula of $C_{29}H_{30}N_4O_3$ with 17

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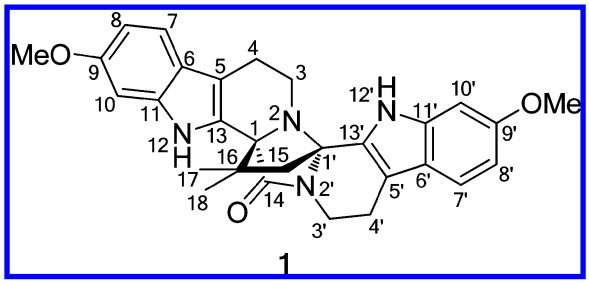

degrees of unsaturation was determined by HRESIMS at m/z 483.2390 $[M + H]^+$ (calcd 483.2391). The UV spectrum of **1** showed absorption maxima at 229, 270, and 297 nm, suggesting the existence of the β-carboline chromophore.[12] The ^{1}H NMR spectrum (Table 1) showed signals assigned to six aromatic protons, two methoxyls [δ_H 3.78 (3H, s) and 3.77 (3H, s)], two methyls [δ_H 1.38 (3H, s) and 1.15 (3H, s)], and two broad NH singlets (δ_H 11.25 and 10.77) and five methylenes, which were confirmed by HSQC experiment. Additionally, protons for two aromatic AMX spin systems (δ_H 7.38, 1H, d, J = 8.6 Hz; 6.70, 1H, dd, J = 8.6, 1.8 Hz; 6.87, 1H, d, J = 1.8 Hz; 7.24, 1H, d, J = 8.6 Hz; 6.63, 1H, dd, J = 8.6, 1.6 Hz; 6.93, 1H, d, J = 1.6 Hz) were observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum (Table 1) displayed 29 resonances that were classified by HSQC experiment as one amide carbonyl (δ_C 171.4), 16 aromatic carbons, two methoxyls, two methyls, five methylenes, and three sp^3 quaternary carbons. These data accounted for 9 out of 17 degrees of unsaturation, indicating the presence of an octacyclic skeleton of **1**.

Table 1. ^{1}H and ^{13}C NMR Data for 1 in DMSO-d_6[a]

no.	δ_H (mult, J, Hz)	δ_C	HMBC (^{1}H → ^{13}C)
1		77.4	
3α	2.34 (1H, dd, 10.9, 4.9)	40.0	1
3β	2.45 (1H, dd, 10.9, 4.9)		1, 5
4α	2.63 (1H, dd, 11.0, 4.9)	21.0	5, 13
4β	2.64 (1H, dd, 11.0, 4.9)		5, 13
5		109.5	
6		120.5	
7	7.24 (1H, d, 8.6)	118.2	5, 9, 11
8	6.63 (1H, dd, 8.6, 1.6)	108.3	6, 9, 10
9		155.4	
10	6.93 (1H, d, 1.6)	94.9	6, 8, 9, 11
11		137.6	
13		127.3	
14		171.4	
15α	1.88 (1H, d, 10.9)	50.4	1′, 16, 17
15β	2.30 (1H, d, 10.9)		1′, 16, 18
16		40.0	
17	1.15 (3H, s)	26.8	1, 15, 16, 18
18	1.38 (3H, s)	26.0	1, 15, 16, 17
1′		78.8	
3′α	4.00 (1H, dd, 12.6, 5.7)	35.6	1′, 5′
3′β	3.09 (1H, td, 12.6, 4.4)		
4′α	2.70 (1H, ddd, 15.1, 12.6, 5.7)	20.9	3′, 5′
4′β	2.90 (1H, dd, 15.1, 4.4)		5′, 13′
5′		111.3	
6′		120.4	
7′	7.38 (1H, d, 8.6)	119.0	5′, 9′, 11′
8′	6.70 (1H, dd, 8.6, 1.8)	109.1	
9′		156.1	
10′	6.87 (1H, d, 1.8)	94.7	6′, 8′, 9′, 11′
11′		137.5	
13′		125.7	
12-NH	11.25 (1H, br.s)		5, 6, 11, 13
12′-NH	10.77 (1H, br.s)		5′, 6′, 11′, 13′
9-OCH$_3$	3.78 (3H, s)	55.2	9
9′-OCH$_3$	3.77 (3H, s)	55.2	9′

[a]600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. Data were assigned based on the HSQC, HMBC, ^{1}H−^{1}H COSY, and NOESY experiments.

Comprehensive analyses of 1D and 2D NMR spectra, especially the HMBC experiment (Figure 1), indicated the existence of a β-carboline dimer in the molecule. The key HMBC correlations of two NH singlets with C-5, C-6, C-11, and C-13, and C-5′, C-6′, C-11′, and C-13′, respectively, suggested the existence of two indole units. In addition, the ^{1}H−^{1}H COSY and HSQC data of **1** defined the two identical moieties of −CH_2CH_2N− fragments which were attached to the two indole units at C-5 and C-5′, respectively, supported by the HMBC correlations of H-3β with C-5 (δ_C 109.5) and H_2-4 with C-13 (δ_C 127.3), and of H-3′α with C-5′ (δ_C 111.3) and H-4′β with C-13′ (δ_C 125.7), respectively. The above evidence, together with the HMBC correlations from H_2-3 to C-1 (δ_C 77.4) and H-3′α to C-1′ (δ_C 78.8), assigned the presence of two β-carboline skeletons. Furthermore, the HMBC correlations from H_2-15 (δ_H 1.88, 1H, d; 2.30, 1H, d) to C-16 (δ_C 40.0) and two methyl carbons (δ_C 26.0, 26.8), and from two methyl protons to C-15 (δ_C 50.4) and C-16, led to the identification of the partial structural fragment of −$(CH_3)_2CCH_2$−, which was connected to C-1 and C-1′ according to the correlations of the two methyl protons with C-1 (δ_C 77.4) and of H_2-15 with C-1′ (δ_C 78.8). With a remaining amide carbonyl signal at δ 171.4 in the ^{13}C NMR spectrum and the molecular formula of **1** taken into consideration, it is readily deduced that the amide carbonyl bridged N-2′, C-1′, N-2, C-1, and C-14 (δ_C 171.4) to form a five-membered γ-lactam. Therefore, the planar structure of **1** with a novel octacyclic skeleton was established.

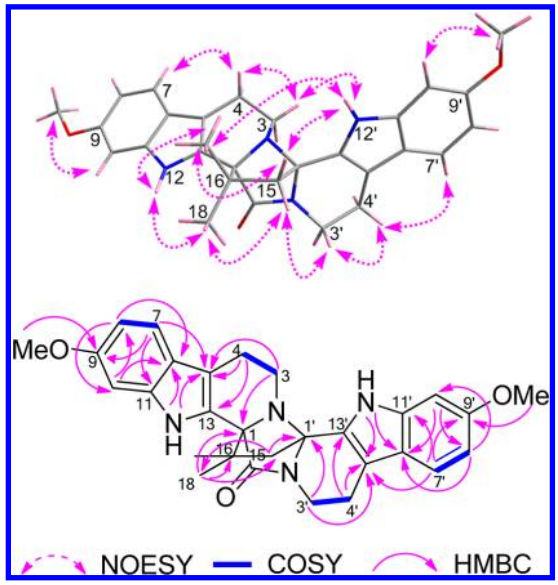

Figure 1. Selected 2D NMR correlations for peganumine A (**1**).

The relative configuration of **1** was elucidated by analysis of the NOESY spectrum as shown in Figure 1. Moreover, a single-crystal X-ray diffraction study (Figure 2)[13] unambiguously

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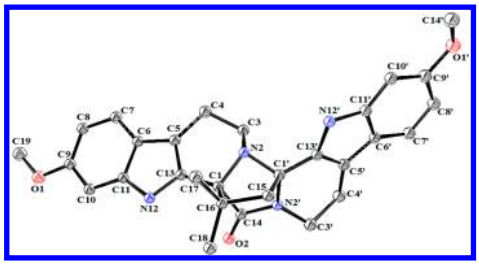


Figure 2. X-ray crystallographic structure of **1**.

confirmed the expected planar structure and the relative stereochemistry of **1**. The absolute configuration of compound **1** was established by a CD exciton chirality method.[14] The UV spectrum of **1** exhibited a strong absorption at 229 nm (log ε 4.24) attributable to the two indole rings. Consistent with this UV maximum, the ECD spectrum of **1** showed a positive Cotton effect at 226 nm ($\Delta\varepsilon$ + 13.0) and a negative Cotton effect at 207 nm ($\Delta\varepsilon$ − 9.6) due to the transition interaction between two identical indole chromophores, indicating a positive chirality for **1**. The positive chirality suggested that the transition dipole moments of the two chromophores were oriented in a clockwise manner (Figure 3) and, thus,

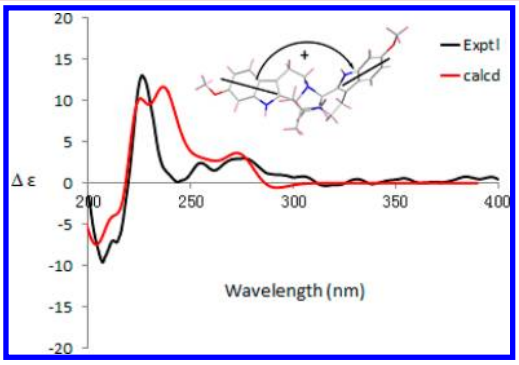


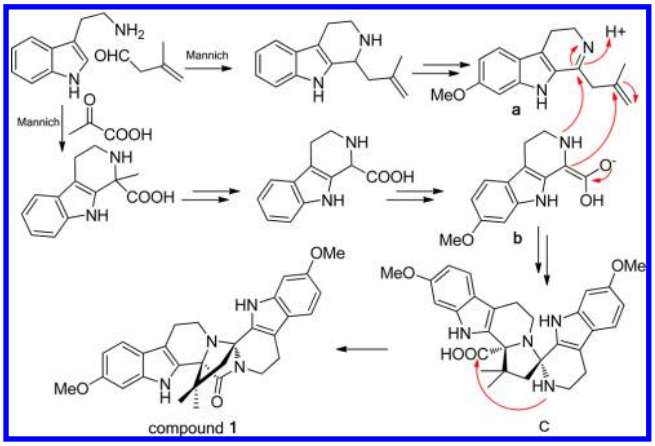
Figure 3. Experimental and suitable calculated ECD spectra of compound **1**. Arrows denote the electric transition dipole of the two chromophores of peganumine A (**1**).

established the configuration of C-1 and C-1′ all as *S*. Additionally, a comparison was made between the experimental and calculated ECD spectra (Figure 3). The calculated ECD curve matched well with the experimental one, assigning unambiguously the absolute configuration of compound **1**.

A hypothetical biosynthetic pathway for peganumine A (**1**) was postulated (Scheme 1). The important intermediate **a** was presumed to be synthesized via a Mannich/Pictet−Spengler-type reaction and coupled with the other β-carboline intermediate **b** by undergoing a Claisen-like reaction.[15] Subsequently, a condensation reaction between NH-2 and C-1′ [16] and the intramolecular dehydration of intermediate **c** led to the five-membered γ-lactam motif.

Peganumine A (**1**) was evaluated for its cytotoxic effects against HL-60, MCF-7, PC-3, and HepG2 cancer cell lines using the trypan blue method[17] and the MTT method[18] with 5-fluorouracil (5-FU) as a positive control. Compound **1** showed significant cytotoxicity against HL-60, MCF-7, PC-3, and HepG2 cell lines with IC_{50} values of 5.8, 38.5, 40.2, and 55.4 μM, respectively. The molecule exhibited significant cytotoxic effects and may be a potential anticancer lead compound.

Scheme 1. Hypothetical Biosynthetic Pathway for 1



■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, 1D and 2D NMR, HRESIMS, CD, UV spectra, X-ray crystal structure (CIF), and details of the quantum chemical ECD calculations for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(11) Peganumine A (**1**): amorphous powder, $[\alpha]_D^{20}$ + 5.6 (*c* 0.15, MeOH); UV (MeOH) λmax (log ε) 229 (4.24), 270 (0.89), and 297 (1.01) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 226 (+13.0), 207 (−9.6) nm; for ^{13}C and ^{1}H NMR data, see Table 1; (+)-HR-ESI-MS *m*/*z* 483.2390 $[M + H]^+$ (calcd for $C_{29}H_{31}N_4O_3$, 483.2391).

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(13) The crystals of **1** are triclinic, belonging to space group *P*-1, with a = 8.3324(17) Å, b = 11.148(2) Å, c = 14.586(3) Å, α = 109.23(3)°, β = 99.14(3)°, γ = 103.70(3)°, V = 1200.7(4) Å^3, D_x = 1.335 mg/m^3, and Z = 2. The final R_1 was 0.0447 [$I > 2\sigma(I)$], and wR_2 was 0.1126 (all data). The crystallographic data for the structure of **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC No. 1010344.

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