

Psychotripine: A New Trimeric Pyrroloindoline Derivative from *Psychotria pilifera*

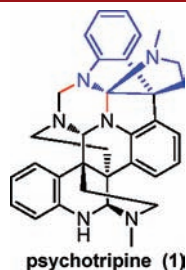
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ABSTRACT



Psychotripine, a trimeric pyrroloindoline derivative with an unprecedented hendecacyclic system bearing a hexahydro-1,3,5-triazine unit, was isolated from the leaves of *Psychotria pilifera*. The structure was elucidated on the basis of spectroscopic and quantum theory. A possible biogenesis was also postulated.

Alkaloids of *Psychotria* are a class of structurally complex and polymeric natural compounds which have been attractive subjects of natural products and synthetic chemistry for decades.¹ And the potent cytotoxic activity of

polyindoline alkaloids have also been reported.² In our continuing chemical and pharmacological studies on indole alkaloids possessing cytotoxicity,³ phytochemical research on the alkaloidal constituents of *Psychotria pilifera* Hutchinson resulted in the isolation of a novel alkaloid, psychotripine, possessing an unprecedented skeleton with 11 rings bearing a hexahydro-1,3,5-triazine unit. Psychotripine was likely derived biosynthetically from a structurally related alkaloid, calycosidine,^{1g} via the formation of C-8a/N-8' and N₁-CH₃/N-8 bonds. We report herein its isolation and structural elucidation.

The dried and powdered leaves of *P. pilifera* (8 kg)⁴ were extracted with MeOH (30 L × 3) under reflux conditions, and the solvent was evaporated *in vacuo*. The residue was dissolved in 0.3% HCl, and the solution was subsequently basified to pH 9–10, using ammonia. The basic solution

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(1) (a) Lebsack, A. D.; Link, J. T.; Overman, L. E.; Stearns, B. A. *J. Am. Chem. Soc.* **2002**, *124*, 9008–9009. (b) Kodanko, J. J.; Overman, L. E. *Angew. Chem., Int. Ed.* **2003**, *42*, 2528–2531. (c) Takayama, H.; Mori, I.; Kitajima, M.; Aimi, N.; Lajis, N. H. *Org. Lett.* **2004**, *6*, 2945–2948. (d) Matsuda, Y.; Kitajima, M.; Takayama, H. *Org. Lett.* **2008**, *10*, 125–128. (e) Lajis, N. H.; Mahmud, Z.; Toia, R. F. *Planta Med.* **1993**, *59*, 383–384. (f) Verotta, L.; Pilati, T.; Tato, M.; Elisabetsky, E.; Amador, T. A.; Nunes, D. S. *J. Nat. Prod.* **1998**, *61*, 392–396. (g) Libot, F.; Miet, C.; Kunesch, N.; Poisson, J. E. *J. Nat. Prod.* **1987**, *50*, 468–473. (h) Roth, A.; Kuballa, B.; Cabalion, P.; Anton, R. *Planta Med.* **1985**, *51*, 289.

(2) (a) Roth, A.; Kuballa, B.; Bounthan, C.; Cabalion, P.; Sevenet, T.; Beck, J. P.; Anton, R. *Planta Med.* **1986**, *52*, 450–453. (b) Adjibade, Y.; Kuballa, B.; Cabalion, P.; Jung, M. L.; Beck, J. P.; Anton, R. *Planta Med.* **1989**, *55*, 567–568.

(3) (a) Feng, T.; Li, Y.; Liu, Y. P.; Cai, X. H.; Wang, Y. Y.; Luo, X. D. *Org. Lett.* **2010**, *12*, 968–971. (b) Feng, T.; Li, Y.; Wang, Y. Y.; Cai, X. H.; Liu, Y. P.; Luo, X. D. *J. Nat. Prod.* **2010**, *73*, 1075–1079. (c) Feng, T.; Cai, X. H.; Liu, Y. P.; Li, Y.; Wang, Y. Y.; Luo, X. D. *J. Nat. Prod.* **2010**, *73*, 22–26. (d) Feng, T.; Li, Y.; Cai, X. H.; Gong, X.; Liu, Y. P.; Zhang, R. T.; Zhang, X. Y.; Tan, Q. G.; Luo, X. D. *J. Nat. Prod.* **2009**, *72*, 1836–1841.

(4) Leaves of *P. pilifera* collected in Apr. 2009 in Mengna of Yunnan Province, P. R. China, and identified by Mr. Jing-Yun Cui, Xishuangbanna Tropical Plant Garden. Voucher specimen (No. Cui20090427) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Table 1. ^1H and ^{13}C NMR Data of Psychotripine (**1**)^a

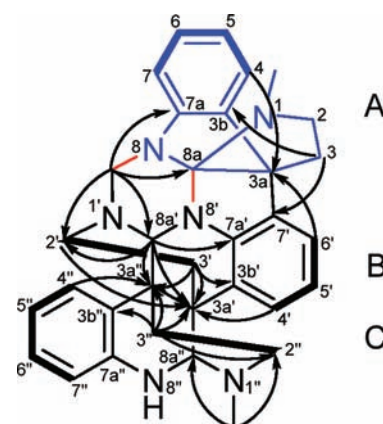
units	A		B		C	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
2	54.9 t	2.95, m	42.3 t	2.30, m 2.21, dt (12.1, 3.8)	45.9 t	2.57, overlap 2.34, overlap
3	36.3 t	2.56, overlap	33.1 t	2.05, m 0.80, dd (13.1, 2.8)	33.7 t	2.55, overlap 1.41, dd (14.1, 4.1)
3a	69.1 s		37.0 s		38.4 s	
3b	133.8 s		122.0 s		122.3 s	
4	122.9 d	7.27, d (7.4)	123.7 d	6.85, d (7.5)	125.4 d	7.15, d (7.9)
5	119.3 d	6.70, t (7.4)	122.0 d	6.79, overlap	117.8 d	6.77, overlap
6	128.2 d	7.01, t (7.8)	121.1 d	7.07, overlap	127.6 d	7.08, overlap
7	107.7 d	6.48, d (7.8)	130.9 s		112.5 d	6.62, d (7.9)
7a	152.2 s		148.8 s		144.4 s	
8a	106.9 s		69.7 d	5.07, s	69.4 d	4.33, brs
$\text{N}_1\text{-CH}_3$	36.4 q	2.79, s			41.8 q	2.39, s
$\text{N}_1\text{-CH}_2$			68.0 t	4.93, d (13.8) 4.56, d (13.8)		

^aData were measured in $\text{CDCl}_3 + \text{CD}_3\text{OD}$ at 600 MHz (^1H) and 150 MHz (^{13}C). Chemical shifts (δ) are in ppm relative to TMS.

was partitioned with EtOAc, affording aqueous and EtOAc phases (total alkaloids). The total alkaloid fraction (98 g) was subjected to column chromatography over silica gel eluting with $\text{CHCl}_3\text{-MeOH}$ [from CHCl_3 to MeOH] to afford seven fractions (I–VII). Fraction I (9.8 g) was further chromatographed using $\text{CHCl}_3\text{-MeOH}$ (20:1) as eluent and then purified by Sephadex LH-20 CC (MeOH) to give psychotripine (5 mg).

The molecular formula of compound **1** was obtained as $\text{C}_{33}\text{H}_{34}\text{N}_6$ by HRESIMS (m/z at 515.2914 [$\text{M} + \text{H}$]⁺), which indicated 20 degrees of unsaturation. The UV and IR spectra showed absorption bands at 307 and 245 nm, and bands at 3428, 2922, and 1630 cm^{-1} , respectively, which were consistent with polymeric indole alkaloids.⁶ The ^{13}C and DEPT NMR spectra (Table 1) displayed 18 aromatic carbons and 15 sp^3 carbons, with the assumption of a trimeric *N*-methyl-pyrroloindoline derivative for **1**. Three characteristic quaternary carbons are present (δ_{C} 69.1, 37.0, 38.4), with assumption that **1** was composed of one calycanthine moiety (units B and C in Figure 1)^{6b} and one *N*-methyl-pyrroloindoline (unit A in Figure 1).^{1c}

In the ^1H and ^{13}C NMR spectra of **1** (Table 1), the characteristic proton signals at δ_{H} 5.07 (1H, s, H-8a', unit B) and δ_{H} 4.33 (1H, brs, H-8a'', unit C), together with key carbon signals at δ_{C} 42.3 (C-2'), 33.1 (C-3'), 37.0 (C-3a'), 122.0 (C-3b'), 69.7 (C-8a') for unit B, and δ_{C} 45.9 (C-2''), 33.7 (C-3''), 38.4 (C-3a''), 122.3 (C-3b''), 69.4 (C-8a''), 41.8 ($\text{N}_1\text{-CH}_3$) for unit C, were consistent with those of calycanthine (dimeric *N*-methyl-pyrroloindoline) and different from those of the dimeric *N*-methyl-pyrroloindoline,

**Figure 1.** $^1\text{H}\text{-}^1\text{H}$ COSY (bold bonds) and key HMBC correlations (arrows) of **1**.

chimonanthine.^{6b} In addition, the HMBC correlations (Figure 1) of δ_{H} 6.85 (H-4'), 5.07 (H-8a'), 2.30 (H-2'), 2.05 (H-3'), and 1.41 (H-3'') with δ_{C} 37.0 (C-3a'), and of δ_{H} 7.15 (H-4''), 5.07 (H-8a''), 2.55 (H-3''), and 0.80 (H-3'') with δ_{C} 38.4 (C-3a'') indicated the linkages of C-2'/C-3'/C-3a'/C-3b'/C-4', and C-3''/C-3a''/C-3b''/C-4'', together with C-8a'/C-3a''/C-3a'. The linkages were also supported by the correlations of δ_{H} 2.05 (H-3') with δ_{C} 122.0 (C-3b') and of δ_{H} 2.55 (H-3'') with δ_{C} 122.3 (C-3b'') in the HMBC spectrum.

Furthermore, the correlations of δ_{H} 5.07 (H-8a') with δ_{C} 148.8 (C-7a') and 42.3 (C-2'), of δ_{H} 2.55 (H-3'') and 2.39 ($\text{N}_1\text{-CH}_3$) with δ_{C} 45.9 (C-2''), and of δ_{H} 2.39 ($\text{N}_1\text{-CH}_3$) with δ_{C} 69.4 (C-8a'') suggested the linkages of C-7a'/N-8'/C-8a'/N-1'/C-2' and C-3''/C-2''/N-1''/C-8a''. The above data incontrovertibly established a substituted calycanthine moiety. The remainder ^1H and ^{13}C NMR data of **1** were similar to those of the *N*-methyl-pyrroloindoline

(5) Amorphous powder; $[\alpha]_{\text{D}}^{26} -84.2^\circ$ (c 0.15, CHCl_3); UV (CHCl_3) λ_{max} (log ϵ) 307 (3.31), 245 (3.76) nm; IR (KBr) ν_{max} 3428, 2922, 1630 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1, respectively; HRESIMS m/z 515.2914 (calcd for $\text{C}_{33}\text{H}_{35}\text{N}_6$ [$\text{M} + \text{H}$]⁺, 515.2923).

(6) (a) Jannic, V.; Gueritte, F.; Laprevote, O.; Serani, L.; Martin, M. T.; Sevenet, T.; Potier, P. *J. Nat. Prod.* **1999**, *62*, 838–843. (b) Adjibade, Y.; Weniger, B.; Quirion, J. C.; Kuballa, B.; Cabalion, P.; Anton, R. *Phytochemistry* **1992**, *31*, 317–319.

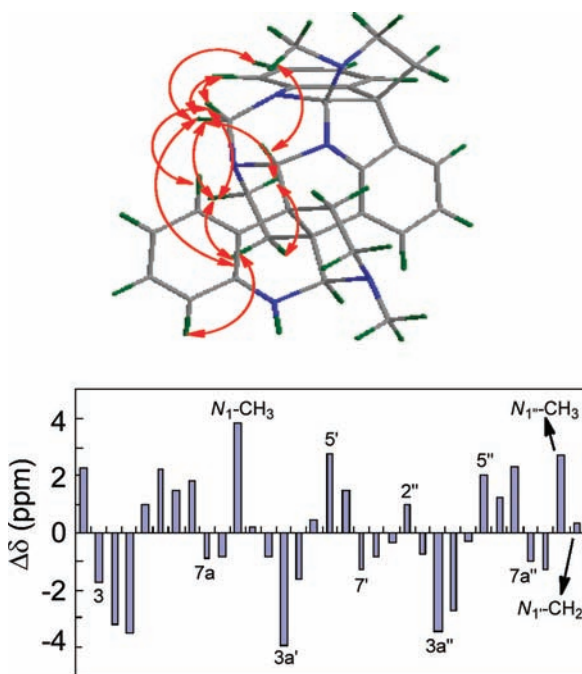


Figure 2. Key ROESY correlations of **1**, and its relative errors between the computed and recorded ^{13}C NMR data.

unit,^{1c} except a downfield quaternary carbon signal at δ_{C} 106.9 (C-8a) presented in **1**, instead of a methine signal at *ca.* δ_{C} 87.

The ^1H , ^{13}C , and ^1H - ^1H COSY spectra (Figure 1) suggested unsubstituted aromatic rings in units A, B, and C. The HMBC correlations of δ_{H} 7.27 (H-4) and 7.07 (H-6') with δ_{C} 69.1 (C-3a), and of δ_{H} 2.56 (H-3) with both δ_{C} 133.8 (C-3b) and 130.9 (C-7') established the linkage between C-3a and C-7'. The conjunct calycanthine and *N*-methyl-pyrroloindoline moieties accounted for by 18 degrees of unsaturation, and the remaining degrees of unsaturation required **1** forming two additional rings. The correlations of δ_{H} 4.93, 4.56 (N_1 -CH₂) with δ_{C} 152.2 (C-7a), 106.9 (C-8a), 69.7 (C-8a'), and 42.3 (C-2') revealed the connection of N-8 with N_1 -CH₂ (red bond in Figure 1). The downfield quaternary carbon signal of C-8a (δ_{C} 106.9) suggested an additional chemical bond between C-8a and N-8' (red bond in Figure 1) was formed to meet the degrees of unsaturation.

(7) (a) Frisch, M. J. et al. *Gaussian 03*, revision D.01; Gaussian, Inc.: Wallingford, CT, 2005 (see the Supporting Information for the complete reference). (b) Zhu, H. J. *Modern Organic Stereochemistry*; Science Presses: Beijing, 2009. (c) Pu, J. X.; Huang, S. X.; Ren, J.; Xiao, W. L.; Li, L. M.; Li, R. T.; Li, L. B.; Liao, T. G.; Lou, L. G.; Zhu, H. J.; Sun, H. D. *J. Nat. Prod.* **2007**, *70*, 1706–1711.

(8) (a) Taniguchi, T.; Martin, C.; Monde, K.; Nakanishi, K.; Berova, N.; Overman, L. *J. Nat. Prod.* **2009**, *72*, 430–432. (b) Stephens, P. J.; Pan, J. J.; Devlin, F. J.; Urbanova, M.; Julinek, O.; Hajicek, J. *Chirality* **2008**, *20*, 454–470. (c) Zhao, S. D.; Shen, L.; Luo, D. Q.; Zhu, H. J. *Curr. Org. Chem.* **2011**, *15*, 1843–1862.

(9) (a) Zhang, Y. L.; Ge, H. M.; Zhao, W.; Dong, H.; Xu, Q.; Li, S. H.; Li, J.; Zhang, J.; Song, Y. C.; Tan, R. X. *Angew. Chem., Int. Ed.* **2008**, *47*, 5823–5826. (b) Specht, K. M.; Nam, J.; Ho, D. M.; Berova, N.; Kondru, R. K.; Beratan, D. N.; Wipf, P.; Pascal, R. A., Jr; Kahne, D. *J. Am. Chem. Soc.* **2001**, *123*, 8961–8966.

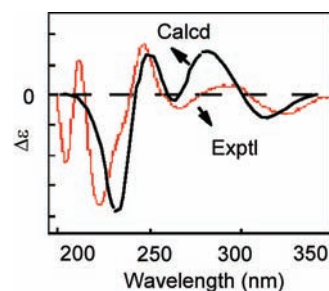


Figure 3. Computed and observed ECD.

The relative configuration of **1** was established by analysis of key correlations in the ROESY spectrum (Figure 2). The ROESY correlations of H-3'/H-2', H-3'/ N_1 -CH₂, H-2'/ N_1 -CH₂, N_1 -CH₂/H-7, N_1 -CH₂/H-6 indicated that C-3', C-2', N-1', N_1 -CH₂, N-8, and the benzene ring in unit A were located at one side, whereas N-1, C-2, and C-3 were at the other side, which were also supported by the NOE correlation of N_1 -CH₃/H-8a'. In addition, the ROESY correlations of H-3'/H-7'' and N_1 -CH₂/H-4'' suggested N-1'', C-2'', and C-3'' were oriented on the same side of N-1. Furthermore, chemical shifts of ^{13}C NMR were computed at the B3LYP/6-311++G(2d,p)//B3LYP/6-31+G(d) level, which is one of the most widely used quantum methods.⁷ The calculated ^{13}C NMR data were then compared to those of experiment to produce the relative errors. The relative errors (< 5.0 ppm) were good in accordance with the relative configurations deduced by ROESY correlations (Figure 2).

The absolute configuration of **1** was also assigned using the quantum method. After a conformational search, two conformations with low energy were found and the B3LYP/6-31+G(d)-optimized conformations were used in optical rotation (OR) computations at the B3LYP/6-311++G(2d,p) level.⁸

The calculated OR value for the absolute configuration of (3aR,8aR,3a'R,8a'R,3a''S,8a''R) was -97.2, which is close to the experimental value of -84.2. The result suggested a reliable absolute configuration assignment for **1**. In addition, its electronic circular dichroism (ECD) was investigated at the B3LYP/6-31+G(d,p) level.^{8c,9} The half-width of 0.2 eV was used in its ECD simulations. Both ECDs had agreement. The recorded and the computed CD

(10) Picon, S.; Dau, E. T. H.; Martin, M. T.; Retailleau, P.; Zaparucha, A.; Al-Mourabit, A. *Org. Lett.* **2009**, *11*, 2523–2526.

(11) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

(12) Cytotoxicity assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO₂ at 37 °C. The cytotoxicity assay was performed according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method in 96-well microplates. Briefly, 100 μL of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and the cell growth curve was graphed.

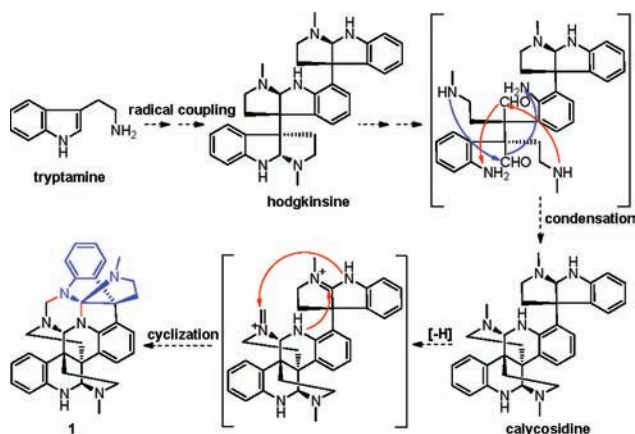


Figure 4. Plausible biogenic pathway to **1**.

curves are illustrated in Figure 3. Based on all of the evidence from ^{13}C NMR, 2D NMR, OR, and ECD, it is suggested that **1** has an absolute configuration of (3a*R*,8a*R*,3a'*R*,8a'*R*,3a''*S*,8a''*R*).

The biosynthetic origin of **1** could be tracked back to the tryptamine (Figure 4). In brief, three *N*-methyl-tryptamine units were condensed to hodgkinsine through radical

coupling. Then, calycosidine was biosynthesized after a cascade of chemical reactions, including C-8a'/N-1' and C-8a''/N-1'' bond cleavage, retroamination, amination, dehydration, and nucleophilic addition reactions. Finally, C-8a/N-8' and *N*_{1'}-CH₃/N-8 bonds were formed to yield **1** via a nucleophilic addition reaction, which might undergo an imine ion intermediate.¹⁰

Compound **1** was evaluated for cytotoxicity against five human cancer cell lines, HL-60 (promyelotic leukemia), SMMC-7721 (hepatocellular carcinoma), A-549 (lung adenocarcinoma), MCF-7 (breast cancer), and SW480 (colon cancer), using the MTT method reported previously¹¹ with minor revisions.¹² Unfortunately, it did not show potential activity ($\text{IC}_{50} > 40 \mu\text{M}$).

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Supporting Information Available. NMR, MS, and CD spectra and computational methods for configuration determination of psychotropine (**1**). This material is available free of charge via the Internet at <http://pubs.acs.org>.