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Calophyline A, a New Rearranged Monoterpenoid Indole Alkaloid from *Winchia calophylla*

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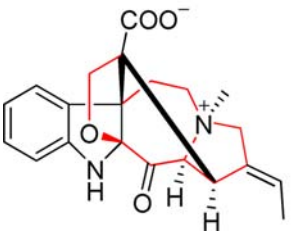
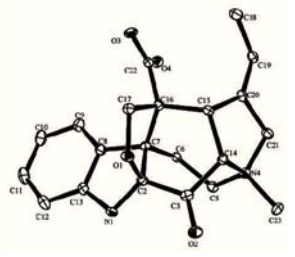
ABSTRACT





Calophyline A (1), a novel unprecedented rearranged monoterpenoid indole alkaloid, along with a new natural product *N*-methyl aspidodasycarpine (2) and six known analogues, was isolated from the trunk barks of *Winchia calophylla*. The structure of compound 1 was elucidated on the basis of spectroscopic data and then confirmed by a single-crystal X-ray crystallographic analysis. A hypothetical biogenetic pathway for compound 1 was proposed. All isolated compounds were evaluated for their in vitro cytotoxicity against a small panel of human cancer cell lines.

Monoterpenoid indole alkaloids are characteristic of complex frameworks and diverse biological activities (especially anticancer activity), which have been attracting great interest from biogenetic, synthetic, and biological points of view.[1] Well-known examples are vinblastine and vincristine, which have been used for cancer chemotherapy.[2] The fascinating properties of this class of alkaloids are still prompting chemists and biologists to search for biogenetically interesting and novel skeleton structures with higher effective and lower toxic antitumor activity from medicinal plants.

Winchia calophylla A. DC. (Apocynaceae), natively abundant in Yunnan Province and Hainan island (China), represents one of two species belonging to the genus *Winchia*.[3] The trunk barks and leaves of this plant were used in traditional medicine to treat cough, asthma, and acute and chronic bronchitis.[4] Previous phytochemical studies on this medicinal plant have led to the isolation of alkaloids and nonalkaloid compounds; however, no new carbon skeleton has been found from those compounds.[5] This work resulted in the isolation of eight indole alkaloids from the trunk barks of *W. calophylla*.

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(1) (a) Yonekura-Sakakibara, K.; Saito, K. *Nat. Prod. Rep.* **2009**, *26*, 1466–1487. (b) Zhou, X.; Xiao, T.; Iwama, Y.; Qin, Y. *Angew. Chem. Int. Ed.* **2012**, *51*, 4909–4912. (c) Zhang, M.; Huang, X. P.; Shen, L. Q.; Qin, Y. *J. Am. Chem. Soc.* **2009**, *131*, 6013–6020. (d) Cai, X. H.; Bao, M. F.; Zhang, Y.; Zeng, C. X.; Liu, Y. P.; Luo, X. D. *Org. Lett.* **2011**, *13*, 3568–3571.

(2) (a) Van der Heijden, R.; Jacobs, D. I.; Snoeijer, W.; Hallard, D.; Verpoorte, R. *Curr. Med. Chem.* **2004**, *11*, 607–628. (b) Ramírez, A.; García-Rubio, S. *Curr. Med. Chem.* **2003**, *10*, 1891–1915. (c) Leonard, J. *Nat. Prod. Rep.* **1999**, *16*, 319–338.

(3) Guangdong Farming and Forestry College. *Chinese Flora (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, 1980; Vol. 63, pp 95–97.

(4) State Administration of Traditional Chinese Medicine. *Chinese Herb (Zhonghua Bencao)*; Shanghai Science and Technology Press: Shanghai, 1999; Vol. 6, p 320.

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Calophyline A (**1**)[6] is a novel compound, possessing an unprecedented 7/5 ring system by means of rearrangement (Figure 1). Furthermore, the nonindole part of the molecule forms a cagelike structure after the cyclization of C-2 and C-17 via an ether oxygen atom. *N*-Methyl aspidodasycarpine (**2**) is a new natural product. Herein, we report the structure elucidation and plausible biogenetic pathway of compound **1**, NMR data of compound **2**, and the isolation and biological evaluation of all compounds.

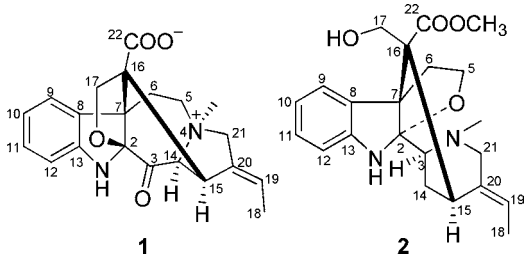


Figure 1. Structures of compounds **1** and **2**.

The air-dried and powdered trunk barks of *W. calophylla* (1.4 kg) were extracted with 95% EtOH at room temperature and evaporated in vacuo. The crude extract was treated with 2 N HCl (pH = 3) and then partitioned with EtOAc. The aqueous layer was basified with saturated Na_2CO_3 (aq) to pH = 10 and extracted with $CHCl_3$ and *n*-BuOH, respectively. The $CHCl_3$ fraction (35.2 g) and *n*-BuOH fraction (49.7 g) were separated continuously by column chromatography over silica gel, RP-18, Sephadex LH-20, and macroporous resin D-101 to obtain calophyline A (**1**, 8 mg), *N*-methyl aspidodasycarpine[7] (**2**, 6 mg), N_b-demethylechitamine[5b] (**3**, 1.6 g), echitaminic acid[8] (**4**, 1.1 g), N_b-demethylechitamine *N*-oxide[5b] (**5**, 5 mg), N_b-demethylalstogustine *N*-oxide[8] (**6**, 2 mg), alstilobanine C[9] (**7**, 6 mg), and undulifoline[9] (**8**, 5 mg).

Calophyline A (**1**) was obtained as a pale yellow powder and recrystallized in methanol to afford colorless cubic crystals. The molecular formula of **1** was established as $C_{21}H_{22}N_2O_4$ based on the accurate molecular ion peaks at *m/z* 367.1658 $[M + H]^+$ (calcd 367.1652) and 389.1473 $[M + Na]^+$ (calcd 389.1472) in the HRESIMS. Characteristic ^1H and ^{13}C NMR signals at δ [7.36 (1H, d, J = 7.4 Hz, H-9), 7.07 (1H, t, J = 7.6 Hz, H-11), 6.79 (1H, d, J = 7.8 Hz, H-12), 6.75 (1H, t, J = 7.4 Hz, H-10); 147.2 (C-13), 133.4 (C-8), 128.4 (C-11), 123.3 (C-9), 120.5 (C-10), 111.6 (C-12)] suggested the presence of a dihydroindole unsubstituted on the benzene ring (Table 1). The IR absorptions

Table 1. ^1H and ^{13}C NMR Data of **1** and **2**[a]

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
2		101.3 (s)		100.4 (s)
3		188.6 (s)	4.36 (m)	69.4 (d)
5	3.20 (m)	61.9 (t)	4.21 (d, 14.9)	65.2 (t)
	3.58 (m)		4.40 (d, 15.7)	
6	1.57 (d, 14.9)	27.2 (t)	2.02 (m)	41.6 (t)
	4.10 (m)		2.24 (m)	
7		57.5 (s)		56.2 (s)
8		133.4 (s)		130.3 (s)
9	7.36 (d, 7.4)	123.3 (d)	7.73 (d, 7.7)	127.2 (d)
10	6.75 (t, 7.4)	120.5 (d)	6.75 (overlap)	120.0 (d)
11	7.07 (t, 7.6)	128.4 (d)	7.10 (t, 7.5)	129.4 (d)
12	6.79 (d, 7.8)	111.6 (d)	6.75 (overlap)	111.1 (d)
13		147.2 (s)		147.9 (s)
14	4.38 (d, 7.4)	78.2 (d)	1.52 (dd, 14.6, 5.6)	31.4 (t)
			2.58 (m)	
15	3.97 (d, 7.4)	50.0 (d)	3.86 (d, 5.1)	35.0 (d)
16		60.3 (s)		62.4 (s)
17	3.81 (d, 7.8)	80.6 (t)	3.16 (dd, 15.4, 5.0)	65.2 (t)
	3.99 (d, 7.8)		3.74 (overlap)	
18	1.81 (d, 7.0)	14.8 (q)	1.78 (d, 5.4)	15.4 (q)
19	5.75 (m)	125.9 (d)	5.73 (m)	129.3 (d)
20		131.2 (s)		133.0 (s)
21	4.27 (d, 15.7)	67.8 (t)	3.35 (m)	61.1 (t)
	4.57 (d, 15.7)		3.60 (dd, 11.8, 8.5)	
22		172.9 (s)		173.6 (s)
N-Me	3.30 (s)	54.1 (q)	3.28 (s)	50.1 (q)
OMe			3.73 (s)	52.4 (q)
OH			5.20 (d, 5.0)	

[a] ^1H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. **1** in MeOH-d_4, **2** in DMSO-d_6, δ in ppm, J in Hz.

of the amino group (3418 cm^{-1}) and aromatic ring (1605 and 1463 cm^{-1}) and the UV maxima at 238 and 295 nm further confirmed the above conclusion. In the HSQC spectrum, a pair of AB doublets of the isolated methylene at δ 3.81 and 3.99 (H_2-17) attached to a carbon at δ 80.6 suggested that this methylene is adjacent to both an oxygen atom and a quaternary carbon. Furthermore, in the HMBC spectrum, H_2-17 showed correlations to 50.0 (C-15), 57.5 (C-7), and 172.9 (C-22), hence indicating an akuammiline-type alkaloid similar to compound **2**.[7] An N^+-methyl could be deduced from the characteristic shifts at δ 3.30 (3H, s) and δ 54.1, and the HMBC correlations of

(5) (a) Gan, L. S.; Yang, S. P.; Wu, Y.; Ding, J.; Yue, J. M. *J. Nat. Prod.* **2006**, *69*, 18–22. (b) Chen, W. M.; Zhang, P. L.; Rücker, G. *Planta Med.* **1988**, *54*, 480–481. (c) Li, C. M.; Zhang, X. M.; Zhou, Y. L.; Huang, L. Y.; Tao, G. D. *Yaoxue Xuebao* **1993**, *28*, 512–515. (d) Zhu, W. M.; He, H. P.; Fan, L. M.; Shen, Y. M.; Zhou, J.; Hao, X. J. *J. Integr. Plant Biol.* **2005**, *47*, 892–896. (e) Zhu, W. M.; Shen, Y. M.; Hong, X.; Zuo, G. Y.; Yang, X. S.; Hao, X. J. *Acta Bot. Sin.* **2002**, *44*, 354–358. (f) Zhu, W. M.; Wang, B. G.; Kang, W. Y.; Hong, X.; Zhou, J.; Hao, X. J. *Chin. Chem. Lett.* **2003**, *14*, 1029–1032. (g) Zhu, W. M.; He, H. P.; Wang, Y. H.; Hao, X. J. *Acta Bot. Yunnan.* **2004**, *26*, 683–686. (h) Zhu, W. M.; Lu, C. H.; Wang, Y.; Zhou, J.; Hao, X. J. *J. Asian Nat. Prod. Res.* **2004**, *6*, 193–198.

(6) Calophyline A (**1**): colorless cubic crystals (methanol); mp 201.5–202.6 °C; $[\alpha]^{20}_D$ + 28.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (3.32), 295 (3.76) nm; IR (KBr) ν_{max} 3418, 2965, 1747, 1683, 1605, 1463, 1378, 1203, 1181, 1154, 1137, 1076, 987, 762 cm^{-1}; ^1H and ^{13}C NMR data: see Table 1; HRESIMS *m/z* $[M + H]^+$ 367.1658 (calcd for $C_{21}H_{23}N_2O_4$, 367.1652), $[M + Na]^+$ 389.1473 (calcd for $C_{21}H_{22}N_2NaO_4$, 389.1472).

(7) Joule, J. A.; Ohashi, M.; Gilbert, B.; Djerassi, C. *Tetrahedron* **1965**, *21*, 1717–1734.

(8) Salim, A. A.; Garson, M. J.; Craik, D. J. *J. Nat. Prod.* **2004**, *67*, 1591–1594.

(9) Koyama, K.; Hirasawa, Y.; Zaima, K.; Hoe, T. C.; Chan, K. -L.; Morita, H. *Bioorg. Med. Chem.* **2008**, *16*, 6483–6488.

δ 3.30 with δ 61.9 (C-5), 78.2 (C-14), and 67.8 (C-21), respectively. Besides, HMBC correlations of H-14 with N^+-methyl suggested that C-14 was directly connected to the N^+-methyl to form an unprecedented 7/5 ring system. A downfield signal in the ^{13}C NMR spectrum at δ 188.6 indicated the presence of one carbonyl functionality, which was consistent with the absorption of a carbonyl (1747 cm^{-1}) group in the IR spectrum. This carbonyl group was placed at C-3 by an HMBC cross-peak from H-14 (δ 4.38, d, J = 7.4 Hz) to C-3. The ^{1}H NMR signals of a methyl group at δ 1.81 (3H, d, J = 7.0 Hz, H-18) and an olefinic proton at δ 5.75 (1H, m, H-19) and the HMBC correlations of H-18 with C-20 (δ 131.2) and C-19 (δ 125.9) allowed the assignment of an ethylidene side chain. According to the 12 degrees of unsaturation, one more ring was required for the structure of **1**. Meanwhile, the HMBC correlations between H_2-17 and 101.3 (C-2) revealed that C-2 and C-17 were linked by an ether oxygen atom. The last two methylene protons (δ 4.10, 1.57) were assigned as H_2-6 on the basis of the HMBC correlations from H_2-6 to C-7 (57.5), C-16 (60.3), and C-2 (101.3). With all of the protons accounted for, the carboxyl group at δ 172.9 (C-22) is in the form of COO^-. This was confirmed by the unusual downfield shift of H-6 (δ 4.10) due to deshielded effect of COO^-. Therefore compound **1** is an inner salt. The nonindole part of the molecule forms a cagelike structure.

The relative stereochemistries of N-4, C-14, and C-15 were determined to be 4*S*, 14*S*, and 15*S* from the NOESY cross-peaks between H-15/H-14, H-14/N^+-Me, and H-21/N^+-Me. NOESY correlations seen for H-15/Me-18 and H-19/H-21 indicated that the geometry of the 19,20-double bond is *E* (Figure 2). The relative configuration of all the chiral centers and the final structure of **1** were determined on the basis of X-ray single crystallographic analysis (Figure 3).[10] Thus, the structure of **1** was elucidated as represented, and the trivial name, calophyline A, was given to this compound.

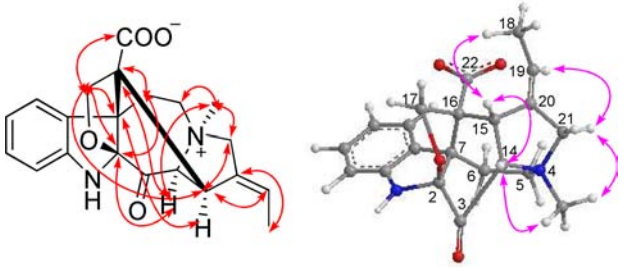


Figure 2. Key HMBC (left) and NOESY (right) correlations of **1**.

N-Methyl aspidodasycarpine (**2**) is a new natural product. Indeed, Joule et al. previously synthesized it as a *N*-methyl derivative of aspidodasycarpine.[7] The structure of **2** was unambiguously determined by HRESIMS as well as 1D and 2D NMR data. This is the first report of NMR data for **2** recorded in DMSO-d_6, which may assist in the identification of this compound.

By comparison with known monoterpenoid indole alkaloids, compound **1** was regarded as a rearranged monoterpenoid indole alkaloid with an unprecedented 7/5 ring system in the nonindole part of the skeleton. The unique biogenetic origin of **1** can plausibly be traced back to rhazimol, which could be formed by bond formation between C-7 and C-16 of 19-*E*-geissoschizine.[11] A plausible biosynthetic pathway of calophyline A (**1**) is proposed in Scheme 1. This pathway involved oxidation, Meisenheimer rearrangement, dehydrogenation, [1, 3] σ rearrangement, Mannich addition, and quaternization, starting from rhazimol (Scheme 1).

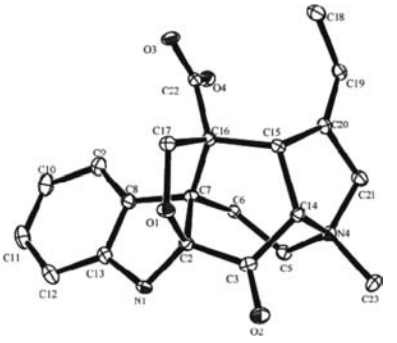


Figure 3. Single crystal X-ray structure of **1**.

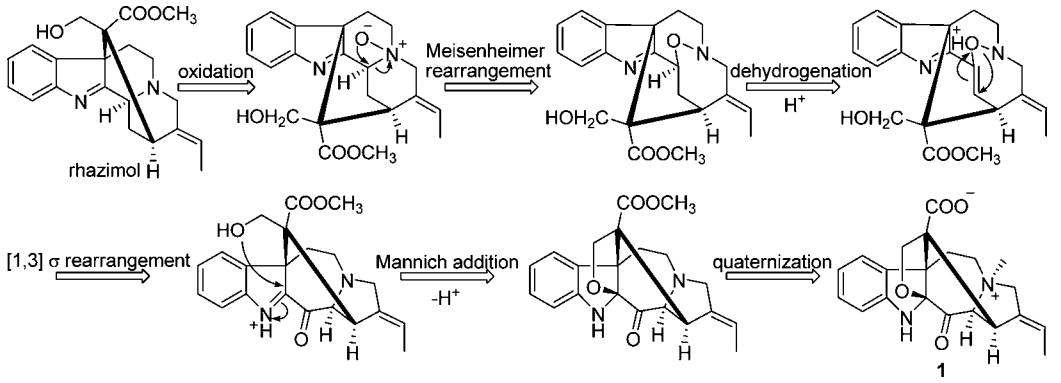
The cytotoxicity of compounds **1**–**8** was evaluated against seven human cancer cell lines (lung cancer: A549, breast cancer: MCF-7, prostate cancer: PC-3, glioma: U87MG, multiple myeloma: U266, MM1.S, and MM1.R) according to the MTT method.[12] Compound **2** selectively exhibited prostate cancer (PC-3) cell growth, with an IC_{50} value of 39.81 μM. Compound **7** showed weak cytotoxicity against MCF-7, U87MG, and PC-3 cell lines, with an IC_{50} value of 37.79, 39.0, and 44.42 μM, respectively. It is worth noting that all of these compounds have no toxicity on

(10) Crystallographic Data for **1**: $C_{21}H_{22}N_2O_4 \cdot 2CH_3OH$, M_r = 430.49; size 0.25 × 0.20 × 0.20 mm^3, orthorhombic, space group $P2_12_12_1$, a = 10.1186 (3) Å, b = 12.1557 (3) Å, c = 16.7470 (4) Å, $\alpha = \beta = \gamma = 90°$, V = 2059.85 (8) Å^3, Z = 4, D_{calc} = 1.388 g/cm^3, λ = 0.7107Å, μ(Mo Kα) = 0.100 mm^{-1}, $F(000)$ = 920, T = 150 K. Of the 6506 reflections in $h(-12/6)$, $k(-15/16)$, $l(-18/22)$ that were collected in range $2.95° \le \theta \le 29.19°$, completeness θ_{max} = 91.75%, 3860 were unique (R_{int} = 0.0247). 3509 reflections with $|F|^2 \ge 2\sigma|F|^2$, 286 parameters, 0 restraints, GOF = 1.044. Final R indices: R_1 = 0.0421, wR_2 = 0.1003. R indices (all data): R_1 = 0.0479, wR_2 = 0.1059. Flack parameter = − 1.4 (11). The maximum and minimum peaks on the final difference Fourier map corresponded to 0.314 and − 0.414 e Å^{-3}, respectively. Crystallographic data (including structure factors) of compound **1** (deposition number: CCDC 878941) have been deposited with the Cambridge Crystallographic Data Center. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

(11) Cai, X. H.; Du, Z. Z.; Luo, X. D. *Org. Lett.* **2007**, *9*, 1817–1820.

(12) Alley, M. C.; Scudiero, D. A; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

Scheme 1. Plausible Biosynthetic Pathway for **1**



peripheral blood mononuclear cells (PBMC) at a concentration of 50 μM. The anticancer mechanisms of compounds **2** and **7** will be reported in the near future.

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Supporting Information Available. Experimental details, HRESIMS, 1D and 2D NMR spectra of **1**, and X-ray crystallographic data of **1** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.