

# Melohenines A and B, Two Unprecedented Alkaloids from *Melodinus henryi*

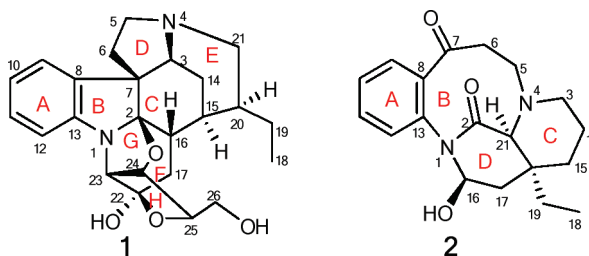
Tao Feng,<sup>†,‡</sup> Xiang-Hai Cai,<sup>†</sup> Yan Li,<sup>†</sup> Yuan-Yuan Wang,<sup>†</sup> Ya-Ping Liu,<sup>†,‡</sup> Ming-Jin Xie,<sup>‡</sup> and Xiao-Dong Luo<sup>\*,†</sup>

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China, Graduate School of Chinese Academy of Sciences, Beijing 100039, P. R. China, and Department of Chemistry, Yunnan University, Kunming 650091, P. R. China

xdluo@mail.kib.ac.cn

Received August 13, 2009

## ABSTRACT



A phytochemical study on *Melodinus henryi* has led to the isolation of two novel alkaloids, melohenines A (1), a monoterpenoid indole alkaloid with additional skeletal carbons arranged compactly in eight rings, and melohenine B (2), an alkaloid with an unprecedented 6/9/6/6 tetracyclic ring system regarded as a key intermediate from indole to quinoline alkaloids. Their structures were elucidated by means of spectroscopic methods and further confirmed by X-ray diffraction analysis.

Monoterpenoid indole alkaloids, which originate from the condensation of tryptophan with secologanin,<sup>1</sup> have long attracted the great interest of many chemists for their unusual carbon skeletons as well as potential bioactivities.<sup>2</sup> A series of novel monoterpenoid indole alkaloids, (19,20) *E/Z*-alstoscholarine, scholarisines A–G, were isolated previously

from different parts of *Alstonia scholaris* in our laboratory.<sup>3</sup> Plants of the genus *Melodinus* (Apocynaceae) have been proven to be good sources of monomeric and dimeric indole alkaloids as well as quinoline alkaloids.<sup>4</sup> The biosynthetic pathway among these kinds of alkaloids has stimulated considerable interest in many laboratories. Some alkaloids,

\* To whom correspondence should be addressed. Tel: +86-871-5223177. Fax: +86-871-5150227.

<sup>†</sup> Kunming Institute of Botany.

<sup>‡</sup> Graduate School of Chinese Academy of Sciences.

<sup>‡</sup> Yunnan University.

(1) Hutchinson, C. R. *Tetrahedron* **1981**, *37*, 1047–1065.

(2) (a) Saxton, J. E. *Nat. Prod. Rep.* **1995**, *12*, 385–411. (b) Bonjoch, J.; Sole, D. *Chem. Rev.* **2000**, *100*, 3455–3482. (c) Beckers, T.; Mahboobi, S. *Drugs Future* **2003**, *28*, 767–785. (d) Drewes, S. E.; George, J.; Khan, F. *Phytochemistry* **2003**, *62*, 1019–1025. (e) Islam, M. N.; Iskander, M. N. *Mini-Rev. Med. Chem.* **2004**, *4*, 1077–1104. (f) Lorence, A.; Nessler, C. L. *Phytochemistry* **2004**, *65*, 2735–2749. (g) O'Connor, S. E.; Maresh, J. J. *Nat. Prod. Rep.* **2006**, *23*, 532–547. (h) Lim, K. H.; Kam, T. S. *Org. Lett.* **2006**, *8*, 1733–1735.

(3) (a) Cai, X. H.; Du, Z. Z.; Luo, X. D. *Org. Lett.* **2007**, *9*, 1817–1820. (b) Cai, X. H.; Tan, Q. G.; Liu, Y. P.; Feng, T.; Du, Z. Z.; Li, W. Q.; Luo, X. D. *Org. Lett.* **2008**, *10*, 577–580. (c) Feng, T.; Cai, X. H.; Zhao, P. G.; Du, Z. Z.; Li, W. Q.; Luo, X. D. *Planta Med.* **2009**, in press DOI: 10.1055/s-0029-1185900.

(4) (a) Bernauer, K.; Englert, G.; Vetter, W.; Weiss, E. *Helv. Chim. Acta* **1969**, *52*, 1886–1905. (b) Mehri, M. H.; Rabaron, A.; Sevenet, T.; Plat, M. M. *Phytochemistry* **1978**, *17*, 1451–1452. (c) Rabaron, A.; Mehri, M. H.; Sevenet, T.; Plat, M. M. *Phytochemistry* **1978**, *17*, 1452–1453. (d) Baassou, S.; Mehri, H. M.; Rabaron, A.; Plat, M. *Tetrahedron Lett.* **1983**, *24*, 761–762. (e) Mehri, M.; Baassou, S.; Plat, M. *J. Nat. Prod.* **1991**, *54*, 372–379. (f) Mehri, H.; Plat, M. *J. Nat. Prod.* **1992**, *55*, 241–244. (g) He, Y. L.; Chen, W. M.; Feng, X. Z. *J. Nat. Prod.* **1994**, *57*, 411–414. (h) Zhang, Y. W.; Yang, R.; Cheng, Q.; Ofuji, K. *Helv. Chim. Acta* **2003**, *86*, 415–419.

such as meloscine,<sup>5</sup> epimeloscine,<sup>6</sup> scandine,<sup>7</sup> and deoxoapopine,<sup>6</sup> continue to be challenging targets for total synthesis. Simultaneously, pharmacological investigations on the crude and purified alkaloids of some *Melodinus* plants have demonstrated promising antitumor,<sup>8</sup> antimitotic,<sup>9</sup> and antibacterial activities.<sup>10</sup>

The interesting chemical and pharmacological significance of the *Melodinus* plants prompted us to phytochemically investigate the *Melodinus henryi*, a cane used for treating meningitis and fracture distributed in China, Thailand, and Burma.<sup>11</sup> As a result, two unique alkaloids, melohenine A (**1**), bearing 24 skeletal carbons arranged rigidly in eight rings, and melohenine B (**2**), a ketolactam derivative, regarded as a key intermediate from indole to quinoline alkaloids, have been isolated. In addition, both **1** and **2** were evaluated their cytotoxicity against five human cancer cell lines.

Melohenine A (**1**), colorless crystals (CH<sub>3</sub>OH–H<sub>2</sub>O, 8:2), possessed a molecular formula C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub> as established by the HRESIMS (*m/z* 411.2281 [M + H]<sup>+</sup>) in association with <sup>1</sup>H and <sup>13</sup>C NMR data, indicating 11 degrees of unsaturation. The IR absorption bands at 3406, 3380, 1485, and 1090 cm<sup>-1</sup> revealed the existence of hydroxyl groups and an aromatic ring. The <sup>1</sup>H, <sup>13</sup>C NMR and DEPT data (Table 1) displayed signals for a substituted indole ring [ $\delta_C$  105.7 (s, C-2), 55.4 (s, C-7), 112.6 (d, C-12), 124.4 (d, C-10), 124.8 (d, C-9), 130.6 (d, C-11), 136.7 (s, C-8), 148.6 (s, C-13);  $\delta_H$  6.88 (1H, d, *J* = 7.4 Hz, H-12), 7.01 (1H, t, *J* = 7.4 Hz, H-10), 7.23 (1H, t, *J* = 7.4 Hz, H-11), 7.36 (1H, d, *J* = 7.4 Hz, H-9)].<sup>12</sup> Besides the indole ring signals, the <sup>13</sup>C NMR and DEPT spectra displayed one methyl group, seven methylenes, seven methines, and one sp<sup>3</sup> quaternary carbon (Table 1).

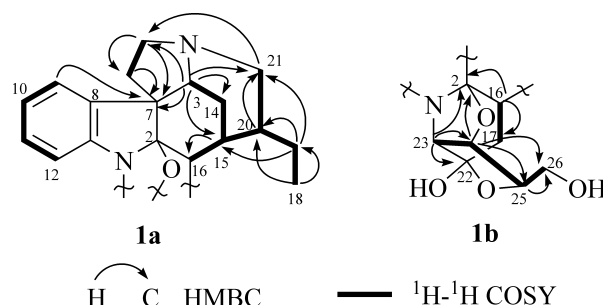
In the <sup>13</sup>C NMR spectrum, a characteristic sp<sup>3</sup> quaternary carbon at  $\delta_C$  55.4 was assigned to C-7, as supported by the HMBC correlations of H-9 and H-12 with it. Three downfield shifts at  $\delta_C$  55.3 (t), 49.9 (t), and 68.4 (d) were attributed to the carbons attached to N-4, corresponding to C-5, C-21, and C-3, respectively. In the HMBC spectrum, one triplet at  $\delta_H$  0.98 (3H, t, *J* = 7.5 Hz), assigned to the methyl group of CH<sub>3</sub>-18, showed correlations to C-19 and C-20, indicating the C-linkage of C-18/C-19/C-20. In addition, the HMBC spectrum also revealed the connections of C-5/C-6, C-3/C-

**Table 1.** <sup>1</sup>H (500 MHz) and <sup>13</sup>C (100 MHz) NMR Data of **1**<sup>a</sup> in CD<sub>3</sub>OD

entry	$\delta_H$ ( <i>J</i> in Hz)	$\delta_C$	HMBC ( <sup>1</sup> H– <sup>13</sup> C)
2		105.7 s	
3	3.84 (1H, br s)	68.4 d	2, 5, 7, 8, 14, 15
5a	3.52 (1H, overlap)	55.3 t	3, 6, 7, 21
5b	3.81 (1H, m)		
6a	2.28 (1H, dd, 15.5, 8.0)	32.7 t	2, 5, 7, 8
6b	3.52 (1H, overlap)		
7		55.4 s	
8		136.7 s	
9	7.36 (1H, d, 7.4)	124.8 d	7, 11, 13
10	7.01 (1H, t, 7.4)	124.4 d	8, 9, 11, 12
11	7.23 (1H, t, 7.4)	130.6 d	9, 13
12	6.88 (1H, d, 7.4)	112.6 d	8, 10
13		148.6 s	
14a	1.81 (1H, m)	24.0 t	7, 15, 16, 20
14b	2.01 (1H, m)		
15	1.79 (1H, m)	34.0 d	14, 16, 19, 20, 21
16	1.92 (1H, m)	36.2 d	2, 14, 15, 17, 22
17a	1.65 (1H, m)	42.9 t	2, 16, 22, 23
17b	1.96 (1H, m)		
18	0.98 (3H, t, 7.5)	11.4 q	19, 20
19	1.37 (2H, m)	24.2 t	15, 18, 20, 21
20	1.70 (1H, m)	40.9 d	15, 19, 21
21a	2.89 (1H, t, 13.5)	49.9 t	5, 15, 19, 20
21b	3.39 (1H, m)		
22		106.1 s	
23	4.60 (1H, d, 3.5)	74.8 d	2, 13, 22, 24
24	3.99 (1H, d, 2.0)	79.1 d	22, 25
25	4.18 (1H, m)	81.5 d	24, 26
26	3.73 (2H, m)	62.0 t	24, 25

<sup>a</sup> Data were assigned by the HSQC, HMBC, <sup>1</sup>H–<sup>1</sup>H COSY, and ROESY spectra.

14/C-15/C-16, and C-15/C-20/C-21. The above evidence, along with proton spin systems detected from the <sup>1</sup>H–<sup>1</sup>H COSY spectrum, H-9/H-10/H-11/H-12, H-3/H-14/H-15/H-16, H-5/H-6, H-18/H-19/H-20/H-21, and H-15/H-20, strongly suggested that compound **1** possessed rings A–E similar to those of dihydrodesoxyisostrychnidine,<sup>13</sup> which led to the establishment of a partial structure **1a** (Figure 1).



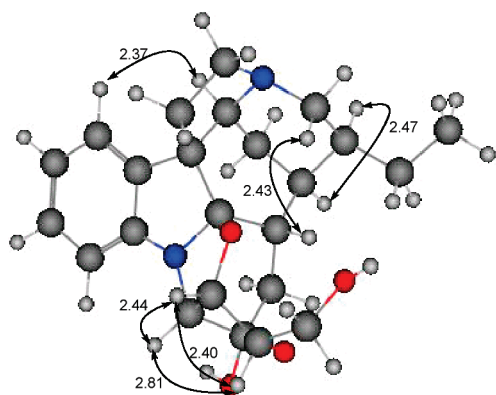
**Figure 1.** Key 2D NMR correlations of fragments of **1**.

The HMBC correlations of H-16 with C-17 and C-22, H-17 with C-22, and H-23 with C-2, C-13, and C-22, along

- (5) (a) Schultz, A. G.; Dai, M. S. *Tetrahedron Lett.* **1999**, 40, 645–648. (b) Selig, P.; Bach, T. *Angew. Chem., Int. Ed.* **2008**, 47, 5082–5084. (c) Selig, P.; Bach, T. *Chemtracts* **2008**, 21, 64–66. (d) Selig, P.; Herdtweck, E.; Bach, T. *Chem.–Eur. J.* **2009**, 15, 3509–3525.
- (6) Overman, L. E.; Robertson, G. M.; Robichaud, A. J. *J. Am. Chem. Soc.* **1991**, 113, 2598–2610.
- (7) Denmark, S. E.; Cottell, J. J. *Adv. Synth. Catal.* **2006**, 348, 2397–2402.
- (8) (a) Yan, K. X.; Hong, S. L.; Feng, X. Z. *Yaoxue Xuebao* **1998**, 33, 597–599. (b) He, X.; Zhou, Y. L.; Huang, Z. H. *Huaxue Xuebao* **1992**, 50, 96–101.
- (9) David, B.; Sevenet, T.; Morgat, M.; Guenard, D.; Moisand, A.; Tollon, Y.; Thoison, O.; Wright, M. *Cell Motil. Cytoskeleton* **1994**, 28, 317–326.
- (10) Au, K. S.; Gray, D. E. *Biochem. Pharmacol.* **1969**, 18, 2673.
- (11) Tsiang, Y.; Li, P. Y. *Flora of China*; Science Press: Beijing, 1977; Vol. 63, p 24.
- (12) (a) Varea, T.; Kan, C.; Remy, F.; Sevenet, T.; Quirion, J. C.; Husson, H. P.; Hadi, H. A. *J. Nat. Prod.* **1993**, 56, 2166–2169. (b) Salim, A. A.; Garson, M. J.; Craik, D. J. *J. Nat. Prod.* **2004**, 67, 1591–1594.

with the  $^1\text{H}$ – $^1\text{H}$  COSY cross peak between H-16 and H-17, suggested the linkage of C-16/C-17/C-22/C-23/N-1/C-2, which established the ring F as depicted. In addition, the HMBC correlations of H-23 with C-24, and H-24 with C-2, together with  $^1\text{H}$ – $^1\text{H}$  COSY correlation of H-23 with H-24, established a substituted tetrahydro-oxazole ring G. Meanwhile, the HMBC correlations of H-24 with C-25 and C-26, and H-25 with C-26, coupled with  $^1\text{H}$ – $^1\text{H}$  COSY correlations of H-24/H-25/H-26, established the C-linkage of C-24/C-25/C-26. Taking the degrees of unsaturation into consideration, a five-membered ring H was assumed. The partial structure **1b** was therefore established as shown in Figure 1. These data suggested that **1** was an unusual  $\text{C}_{24}$  indole alkaloid with an eight ring system.

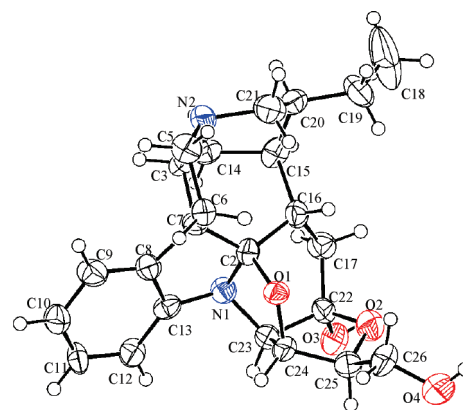
In the ROESY spectrum (Figure 2), the NOE correlations of H-9/H-3 and H-15/H-20 suggested that H-3, H-15 and



**Figure 2.** Key ROESY correlations of **1** with interatomic distances (Å) as calculated by the Molecular Operating Environment software.

H-20 were all  $\alpha$ -oriented, whereas the correlation of H-16 with H-21a suggested that H-16 was  $\beta$ -oriented. Furthermore, H-23, H-24, and H-25 were assigned on the same side on the basis of the NOE correlations among them. However, the ROESY spectrum could not identify the relative configurations at C-2, C-22, C-23, C-24, and C-25.

Since the structure of ring H and relative configuration at C-2, C-22, C-23, C-24, and C-25 were uncertain, further solid evidence was necessary. Fortunately, after many attempts to crystallize **1** with different solvents, a single crystal of **1** was finally obtained from MeOH– $\text{H}_2\text{O}$  (8:2) solvent, and an X-ray crystallographic analysis was realized (Figure 3), which clarified not only the planar structure but also the relative configuration of **1**. Biogenetically, **1** was considered to be a *Strychnos*-type alkaloid derivative. Since the spiro-center at C-7 of strychnine was previously identified as *R*,<sup>14</sup> the absolute configuration disclosed by X-ray diffraction analysis at chiral carbons of **1** could be elucidated as

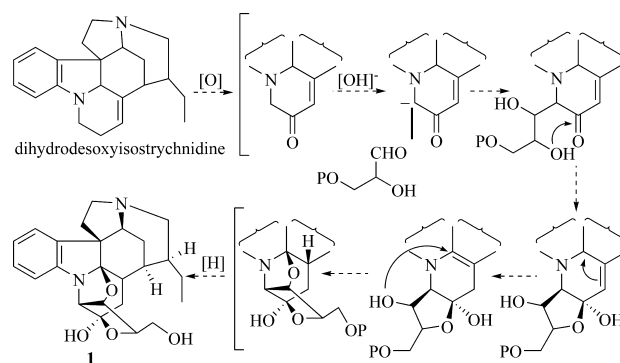


**Figure 3.** X-ray structure of **1** showing relative configuration.

*2R,3S,7R,15S,16S,20S,22R,23R,24S,25S* on the basis of the relative configuration.

The biosynthesis of **1** is shown in Scheme 1. Briefly, dihydrodesoxyisostrychnidine<sup>13</sup> might be oxidized to form

**Scheme 1.** Plausible Biogenetic Pathway of **1**



a ketone group at C-22<sup>15</sup> and underwent an aldol condensation with glyceraldehyde phosphate to form a new C–C bond of C-23–C-24. Then, a nucleophilic addition of the –OH at C-25 with the ketone group formed a hemiketal moiety at C-22 and built ring H. Subsequently, an enamine among N-1, C-2, and C-16 might be formed by double-bond migration from C-16=C-17 to C-2=C-16.<sup>3b</sup> Finally, another nucleophilic addition of the –OH at C-24 with the double bond of C-2=C-16 constructed ring G.

Melodinine B (**2**), colorless crystals (MeOH), possessed a molecular formula of  $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3$  as evidenced by the HRESIMS at  $m/z$  329.1858 [ $\text{M} + \text{H}$ ]<sup>+</sup>, indicating nine degrees of unsaturation. The  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and DEPT spectra displayed signals for an *ortho*-disubstituted phenyl ring [ $\delta_{\text{C}}$  124.2 (d, C-12), 126.8 (d, C-10), 128.4 (d, C-9), 131.8 (d, C-11), 138.6 (s, C-13), 140.0 (s, C-8);  $\delta_{\text{H}}$  7.24 (1H, d,  $J$  =

(13) Weissmann, Ch.; Heshmat, O.; Bernauer, K.; Schmid, H.; Karrer, P. *Helv. Chim. Acta* **1960**, *43*, 1165–1170.

(14) (a) Peerdeman, A. F. *Acta Crystallogr.* **1956**, *9*, 824. (b) Nagarajan, K.; Weissmann, Ch.; Schmid, H.; Karrer, P. *Helv. Chim. Acta* **1963**, *46*, 1212–1231.

(15) (a) Hagen, T. J.; Cook, J. M. *Tetrahedron Lett.* **1988**, *29*, 2421–2424. (b) Hagen, T. J.; Narayanan, K.; Names, J.; Cook, J. M. *J. Org. Chem.* **1989**, *54*, 2170–2178.

7.5 Hz, H-12), 7.28 (1H, t,  $J = 7.5$  Hz, H-10), 7.42 (1H, dd,  $J = 7.5, 1.5$  Hz, H-9), 7.51 (1H, td,  $J = 7.5, 1.5$  Hz, H-11)]. In addition to the phenyl ring, the alkaloid possessed one methyl, seven methylenes, two methines, one quaternary carbon, one amide group, and one carbonyl group (Table 2). These data suggested a tetracyclic ring system of **2**.

**Table 2.**  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  (100 MHz) NMR Data of **2**<sup>a</sup> in  $\text{CDCl}_3$

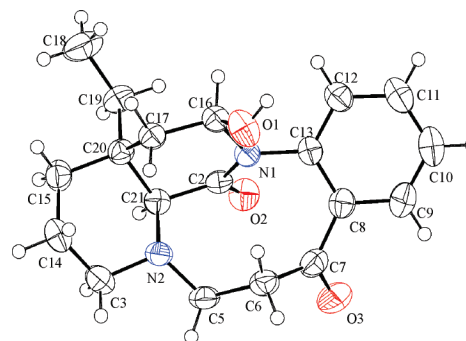
entry	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$	HMBC ( $^1\text{H}$ – $^{13}\text{C}$ )
2		172.6 s	
3a	2.40 (1H, dd, 12.5, 2.5)	55.0 t	5, 14, 15, 21
3b	3.01 (1H, overlap)		
5a	3.01 (1H, overlap)	54.5 t	3, 6, 7, 21
5b	3.07 (1H, m)		
6a	2.60 (1H, dd, 13.4, 11.0)	42.7 t	5, 7
6b	3.41 (1H, dd, 13.4, 8.0)		
7		203.6 s	
8		140.0 s	
9	7.42 (1H, dd, 7.5, 1.5)	128.4 d	8, 10, 11, 13
10	7.28 (1H, t, 7.5)	126.8 d	8, 9, 11, 12
11	7.51 (1H, td, 7.5, 1.5)	131.8 d	9, 10, 12, 13
12	7.24 (1H, d, 7.5)	124.2 d	8, 10, 11, 13
13		138.6 s	
14a	1.77 (1H, m)	22.3 t	3, 15, 20
14b	1.95 (1H, m)		
15a	1.20 (1H, dd, 13.5, 4.5)	32.8 t	3, 14, 17, 19, 20
15b	1.68 (1H, m)		
16	5.64 (1H, d, 5.3)	83.9 d	2, 13, 17, 20
17a	2.01 (1H, d, 15.0)	40.0 t	16, 19, 20, 21
17b	2.25 (1H, dd, 15.0, 6.0)		
18	0.89 (3H, t, 7.0)	7.3 q	19, 20
19a	1.34 (1H, q, 7.0)	35.7 t	17, 18, 20, 21
19b	1.41 (1H, q, 7.0)		
20		37.7 s	
21	2.73 (1H, s)	75.5 d	2, 3, 5, 17, 20
–OH	8.13 (1H, br s)		

<sup>a</sup> Data were assigned by the HSQC, HMBC,  $^1\text{H}$ – $^1\text{H}$  COSY, and ROESY spectra.

In the  $^{13}\text{C}$  NMR spectrum, a signal at  $\delta_{\text{C}}$  203.6 (s) was assigned to the carbon of a carbonyl group, which was further assigned to C-7 on the basis of the HMBC correlations of  $\delta_{\text{H}}$  2.60 (1H, dd,  $J = 13.4, 11.0$ , H-6a), 3.41 (1H, dd,  $J = 13.4, 8.0$ , H-6b), and H-9 with  $\delta_{\text{C}}$  203.6 (s, C-7). In addition, a quaternary signal at  $\delta_{\text{C}}$  172.6 (s) was ascribed to the carbon of an amide group at C-2, based on the key HMBC correlations of  $\delta_{\text{H}}$  5.64 (1H, d,  $J = 5.3$  Hz, H-16) and 2.73 (1H, s, H-21) with  $\delta_{\text{C}}$  172.6 (s, C-2). The above data indicated the existence of a 2,7-diketone moiety in **2**, which

should be derived from epivincanol by the cleavage of  $\text{C}_2$ – $\text{C}_7$  bond.<sup>16</sup> Detailed analysis of 1D and 2D NMR and MS data showed that the other patterns of **2** were identical to those of epivincanol. The NOE correlations of H-21 with  $\text{CH}_2$ -19 suggested that both C-19 and H-21 were on the same side. In addition, the coupling constant of H-16 (d,  $J = 5.3$  Hz) indicated a  $\beta$ -OH group at C-16, in accordance with that of epivincanol.<sup>16</sup>

It is noteworthy that **2** might be a key intermediate of indole to quinoline alkaloids, and an X-ray diffraction finally confirmed this unusual carbon skeleton (Figure 4), showing a 6/9/6/6 tetracyclic ring system.



**Figure 4.** X-ray structure of **2** showing relative configuration.

Compounds **1** and **2** were tested for cytotoxicity against SK-BR-3, SMMC7721, HL-60, PANC-1, and A549 cell lines using the MTT method as previously reported.<sup>17</sup> Cisplatin (Sigma) was used as the positive control. However, both were found to be inactive with  $\text{IC}_{50}$  values of  $>40 \mu\text{M}$ .

**Acknowledgment.** We are grateful to the National Natural Science Foundation of China (C30670214), the National Basic Research Program of China (973 Program 2009CB522300), and the Chinese Academy of Sciences (XiBuZhiGuang Project) for partial financial support.

**Supporting Information Available:** Detailed description of the experimental procedures, 1D and 2D NMR spectra, MS spectra, and X-ray crystallographic data (CIF) of **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL9018826

(16) Pfaffli, P.; Hauth, H. *Helv. Chim. Acta* **1978**, *61*, 1682–1695.

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