

# Melotenine A, a Cytotoxic Monoterpenoid Indole Alkaloid from *Melodinus tenuicaudatus*

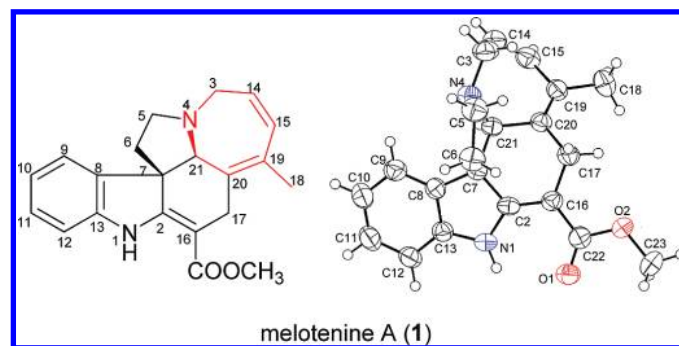
Tao Feng,<sup>†,‡</sup> Yan Li,<sup>†</sup> Ya-Ping Liu,<sup>†,‡</sup> Xiang-Hai Cai,<sup>†</sup> Yuan-Yuan Wang,<sup>†</sup> and  
Xiao-Dong Luo<sup>\*,†</sup>

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

xdluo@mail.kib.ac.cn

Received January 1, 2010

## ABSTRACT



Melotenine A (1), an unprecedented skeleton with a 6/5/5/6/7 pentacyclic rearranged ring system, was isolated from *Melodinus tenuicaudatus*. The structure was elucidated by means of spectroscopic methods and further confirmed by the single-crystal X-ray diffraction analysis. A possible biogenesis was also proposed. Melotenine A exhibited potential inhibition against five human cancer cell lines.

The genus *Melodinus* (Apocynaceae) comprises 53 species all over the world, and 11 of them are distributed in Guangxi and Yunnan provinces, People's Republic of China.<sup>1</sup> The phytochemical constituents of *Melodins* sp. have been investigated extensively. Up to now, more than 80 compounds have been isolated and characterized.<sup>2</sup> Most of the compounds are attributed to monomeric and dimeric monoterpenoid indole as well as quinoline alkaloids and are considered to originate from the condensation of tryptophan with secologanin.<sup>3</sup> Many characteristic *Melodinus* alkaloids, such as meloscine,<sup>4</sup> epimeloscine,<sup>5</sup> scandine,<sup>6</sup> and deoxoapo-

dine,<sup>5</sup> have for a long time attracted great interest of synthetic organic chemists as challenging targets due to their marked diversity and complicated architectures. Pharmacological investigations on the crude and purified alkaloids from some *Melodinus* plants have demonstrated promising antitumor,<sup>7</sup> antimetabolic,<sup>8</sup> and antibacterial activities.<sup>9</sup> Our previous study

(3) (a) Stoeckigt, J.; Zenk, M. H. *FEBS Lett.* **1977**, *79*, 233–237. (b) Stoeckigt, J.; Panjikar, S. *Nat. Prod. Rep.* **2007**, *24*, 1382–1400.

(4) (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.

(5) Overman, L. E.; Robertson, G. M.; Robichaud, A. J. *J. Am. Chem. Soc.* **1991**, *113*, 2598–2610.

(6) Denmark, S. E.; Cottell, J. J. *Adv. Synth. Catal.* **2006**, *348*, 2397–2402.

(7) (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.

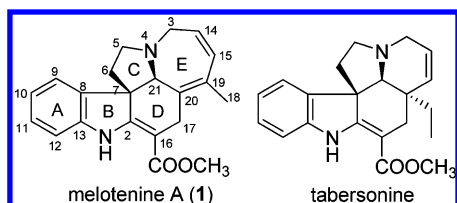
<sup>†</sup> Kunming Institute of Botany.

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

(1) Tsiang, Y.; Li, P. Y. *Flora of China*; Science Press: Beijing, 1977; Vol. 63, pp 17–30.

(2) Buckingham, J. *Dictionary of Natural Productson on CD-ROM*; CRC Press: Boca Raton, 2006.

on *M. henryi* reported two novel carbon skeletons, melodinine A, a complicated monoterpenoid alkaloid bearing 24 skeletal carbons arranged compactly in eight rings, and melodinine B, a key intermediate from indole to quinoline alkaloids.<sup>10</sup> As part of searching for novel and bioactive monoterpenoid indole alkaloids,<sup>11</sup> we now report an unprecedented alkaloid rearranged from the apidospermane skeleton, named melotenine A (**1**), together with tabersonine,<sup>12</sup> a known apidospermane alkaloid considered to be the precursor of melotenine A, from *M. tenuicaudatus*. Compound **1** displayed a stronger inhibitory effect against five human cancer cell lines than that of cisplatin.



*M. tenuicaudatus* cane was collected in Yunnan province, P. R. China. A voucher species (No. Cui 20081129) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences. An air-dried and powdered sample (14 kg) was extracted with 90% EtOH (24 h × 3). The extract was partitioned between EtOAc and 0.5% HCl solution. The acidic water-soluble materials, adjusted to pH 9–10 with 10% ammonia solution, were extracted with EtOAc to give an alkaloidal extract (17 g). The extract was subjected to silica gel column chromatography (petroleum ether–acetone, 1:0 to 0:1) to afford fractions I–VII. Fraction I (1.8 g) was separated repeatedly by silica gel column chromatography (petroleum ether–Me<sub>2</sub>CO, 12:1) to yield **1** (3 mg) and tabersonine (20 mg).

Compound **1**,<sup>13</sup> isolated as colorless crystals (Me<sub>2</sub>CO), possessed a molecular formula of C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub> as established by HRESIMS ([M + H]<sup>+</sup> at *m/z* 335.1772). The UV spectrum showed absorption maxima characteristic of a β-anilinoacrylate chromophore (328, 298, 265, and 221 nm), while the IR spectrum showed absorption bands due to NH (3440 cm<sup>−1</sup>) and conjugated ester (1680 cm<sup>−1</sup>) functions.<sup>14</sup>

The <sup>1</sup>H NMR spectrum revealed the existence of an *ortho*-disubstituted phenyl ring [ $\delta_{\text{H}}$  6.79 (1H, d, *J* = 7.4 Hz, H-12),

6.87 (1H, t, *J* = 7.4 Hz, H-10), 7.13 (1H, t, *J* = 7.4 Hz, H-11), 7.31 (1H, d, *J* = 7.4 Hz, H-9)], an indolic NH group [ $\delta_{\text{H}}$  9.06 (1H, br s)], and two methyls [ $\delta_{\text{H}}$  3.79 (3H, s, OMe), 1.87 (3H, s, H-18)] (Table 1). The <sup>13</sup>C NMR spectrum

**Table 1.** <sup>1</sup>H (500 MHz) and <sup>13</sup>C (100 MHz) NMR Data of **1**<sup>a</sup> in CDCl<sub>3</sub> ( $\delta$  in ppm, *J* in Hz)

entry	$\delta_{\text{H}}$	$\delta_{\text{C}}$
N <sub>1</sub> –H	9.06 (1H, br s)	
2		163.0 s
3a	3.62 (1H, br d, 13.7)	54.6 t
3b	3.30 (1H, br d, 13.7)	
5	3.02 (2H, m)	52.2 t
6a	2.42 (1H, m)	39.3 t
6b	1.90 (1H, m)	
7		55.1 s
8		137.4 s
9	7.31 (1H, d, 7.4)	123.1 d
10	6.87 (1H, t, 7.4)	120.7 d
11	7.13 (1H, t, 7.4)	127.9 d
12	6.79 (1H, d, 7.4)	108.9 d
13		143.4 s
14	5.98 (1H, overlap)	135.0 d
15	5.98 (1H, overlap)	131.0 d
16		91.3 s
17a	3.78 (1H, br d, 16.0)	25.4 t
17b	3.00 (1H, br d, 16.0)	
18	1.87 (3H, s)	18.7 q
19		128.1 s
20		134.7 s
21	3.74 (1H, s)	67.7 d
COOMe		168.4 s
COOMe	3.79 (3H, s)	51.0 q

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

displayed a total of 21 carbon resonances which were assigned to two methyls ( $\delta_{\text{C}}$  18.7, 51.0), four methylenes ( $\delta_{\text{C}}$  25.4, 39.3, 52.2, 54.6), seven methines ( $\delta_{\text{C}}$  67.7, 108.9, 120.7, 123.1, 127.9, 131.0, 135.0), and eight quaternary carbons ( $\delta_{\text{C}}$  55.1, 91.3, 128.1, 134.7, 137.4, 143.4, 163.0, 168.4) (Table 1). The signals at  $\delta_{\text{C}}$  168.4 and 91.3 were readily assigned to C-2 and C-16, respectively, corresponding to the acrylate double bond.

In the HMBC spectrum (Figure 1), the correlations of  $\delta_{\text{H}}$  3.78 (1H, d, *J* = 16.0 Hz, H-17a) and 3.00 (1H, d, *J* = 16.0 Hz, H-17b) with C-16 suggested the linkage between C-16 and CH<sub>2</sub>-17. The correlations of H-17 with  $\delta_{\text{C}}$  134.7 (s, C-20) and of  $\delta_{\text{H}}$  3.74 (1H, s, H-21) with  $\delta_{\text{C}}$  134.7 (s, C-20) established the linkage of C-17/C-20/C-21. The HMBC correlations of  $\delta_{\text{H}}$  2.42 (1H, m, H-6a) and 1.90 (1H, m, H-6b) with  $\delta_{\text{C}}$  55.1 (s, C-7) indicated the direct connection of CH<sub>2</sub>-6 to C-7. In addition, the HMBC correlation of H-6 with  $\delta_{\text{C}}$  52.2 (t, C-5), together with <sup>1</sup>H–<sup>1</sup>H COSY correlations of CH<sub>2</sub>-6 with CH<sub>2</sub>-5, suggested the direct connection between C-5 and C-6 (Figure 1). The downfield NMR data of CH<sub>2</sub>-5

(8) David, B.; Sevenet, T.; Morgat, M.; Guenard, D.; Moisand, A.; Tollon, Y.; Thoison, O.; Wright, M. *Cell Motil. Cytoskeleton* **1994**, *28*, 317–326.

(9) Au, K. S.; Gray, D. E. *Biochem. Pharmacol.* **1969**, *18*, 2673.

(10) Feng, T.; Cai, X. H.; Li, Y.; Wang, Y. Y.; Liu, Y. P.; Xie, M. J.; Luo, X. D. *Org. Lett.* **2009**, *11*, 4834–4837.

(11) (a) 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. (b) Feng, T.; Cai, X. H.; Zhao, P. G.; Du, Z. Z.; Li, W. Q.; Luo, X. D. *Planta Med.* **2009**, *75*, 1537–1541. (c) 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. (d) Cai, X. H.; Du, Z. Z.; Luo, X. D. *Org. Lett.* **2007**, *9*, 1817–1820.

(12) (a) Plat, M.; Men, J. L.; Janot, M. M.; Wilson, J. M.; Budzikiewicz, H.; Durham, L. J.; Nakagawa, Y.; Djerassi, C. *Tetrahedron Lett.* **1962**, *7*, 271–276. (b) Ziegler, F. E.; Bennett, G. B. *J. Am. Chem. Soc.* **1973**, *95*, 7458–7464.

(13) Melotenine A (**1**): colorless crystals (Me<sub>2</sub>CO); mp 174 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = −136.5 (c 0.20, CHCl<sub>3</sub>); UV (CHCl<sub>3</sub>)  $\nu_{\text{max}}$  (log  $\epsilon$ ) 328 (3.92), 298 (3.82), 265 (4.21), 221 (4.64) nm; IR (KBr)  $\nu_{\text{max}}$  3440, 2948, 1680, 1610, 1436, 1244 cm<sup>−1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* 334; HRESIMS *m/z* 335.1772 [M + H]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>, 335.1759).

(14) Lim, K. H.; Hiraku, O.; Komiyama, K.; Kam, T. S. *J. Nat. Prod.* **2008**, *71*, 1591–1594.

allowed the attachment of a nitrogen atom (N-4) to C-5. The HMBC correlation between  $\delta_{\text{H}}$  3.74 (1H, s, H-21) and C-5 revealed the connection of N-4 with C-21. The above information established rings A, B, C, and D as depicted in Figure 1, which showed the same patterns as those of tabersonine.

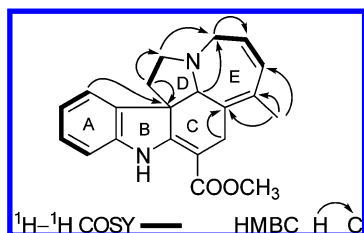


Figure 1. Key 2D NMR correlations of **1**.

A singlet at  $\delta_{\text{H}}$  1.87 (3H, s, H-18) in the  $^1\text{H}$  NMR spectrum, assigned to a methyl group, showed key HMBC correlations with  $\delta_{\text{C}}$  128.1 (s, C-19), 134.7 (s, C-20), and 131.0 (d, C-15), which established the fragments of C-18/C-19 and C-20/C-19/C-15/C-14. The downfield signal at  $\delta_{\text{C}}$  54.6 (t, C-3) suggested the attachment between C-3 and N-4. The HMBC correlations of  $\delta_{\text{H}}$  3.62 (1H, d,  $J = 13.7$  Hz, H-3a) and 3.30 (1H, d,  $J = 13.7$  Hz, H-3b) with  $\delta_{\text{C}}$  135.0 (d, C-14), as well as  $^1\text{H}$ – $^1\text{H}$  COSY correlations between H-3 and  $\delta_{\text{H}}$  5.98 (1H, overlap, H-14), suggested the connection between C-3 and C-14. These data established a seven-membered ring E (Figure 1). Since the overlapped signals of H-14 and H-15, the 2D NMR correlations were not clear enough to support the C-linkage of C-3/C-14/C-15/C-19. A sample of **1** was dissolved in  $\text{Me}_2\text{CO}-d_6$  to give clear  $^1\text{H}$  NMR signals of H-14 [ $\delta_{\text{H}}$  6.08 (1H, dd,  $J = 11.0, 6.0$  Hz)] and H-15 [ $\delta_{\text{H}}$  6.03 (1H, d,  $J = 11.0$  Hz)] (full spectrum: see Supporting Information), which clarified the uncertain details. A single-crystal X-ray diffraction confirmed the structure of **1** possessing an unusual skeleton (Figure 2).<sup>15</sup>

A plausible biogenetic pathway for **1** was proposed (Scheme 1). Compound **1** might be derived from tabersonine.<sup>12</sup> In brief, tabersonine was oxidated to produce 19-*R*-hydroxytabersonine.<sup>16</sup> The latter might undergo a Wagner–Meerwein rearrangement<sup>17</sup> involving the formation of carbocation intermediate and a 1,2-alkyl shift to produce the novel skeleton **1**. Since the tertiary carbocation is favored

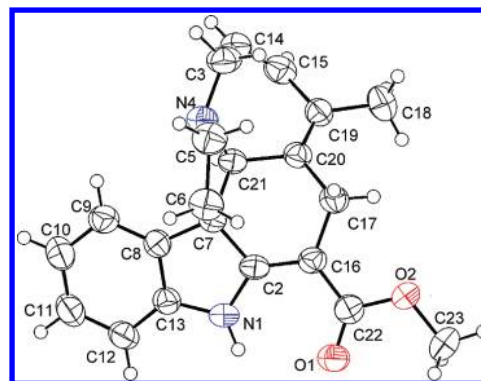


Figure 2. X-ray structure of **1**.

Scheme 1. Plausible Biogenetic Pathway to **1**

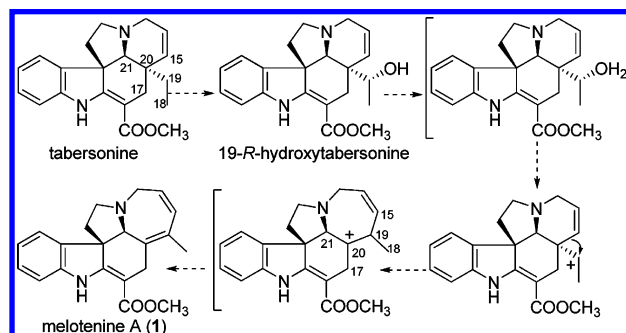


Table 2. Cytotoxicity of **1** and Tabersonine

cells	IC <sub>50</sub> (μM)		
	<b>1</b>	tabersonine	cisplatin
SK-BR-3	2.8	11.7	21.7
SMMC-7721	5.2	18.8	18.1
HL-60	0.9	5.4	2.6
PANC-1	3.6	30.5	24.8
A-549	10.7	25.9	15.8

over secondary carbocation and the usual objective in the rearrangement is to achieve a tertiary status at the positive center, the reaction is readily rationalized. The absolute configuration at C-7 and C-21 of tabersonine was determined as *R* and *S*,<sup>12</sup> respectively, so the absolute configuration at stereogenic centers of **1** might be identified as 7*R*, 21*S* according to the relative configuration.

Compound **1** and tabersonine were evaluated for their cytotoxicity against five human cancer cell lines, SK-BR-3

(15) Crystal data for melotonine A (**1**):  $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2$ , MW = 334.41; monoclinic, space group  $P2_1$ ;  $a = 6.5866$  (13) Å,  $b = 10.932$  (2) Å,  $c = 24.192$  (5) Å,  $\alpha = 90.00$ ,  $\beta = 90.00$  (10),  $\gamma = 90.00$ ,  $V = 1741.9$  (6) Å<sup>3</sup>,  $Z = 4$ ,  $d = 1.275$  g/cm<sup>3</sup>, crystal dimensions 0.26 × 0.14 × 0.10 mm was used for measurement on a SHELXL-97 with a graphite monochromator, Mo K $\alpha$  radiation. The total number of reflections measured was 4176, of which 1933 were observed,  $I > 2\sigma(I)$ . Final indices:  $R_1 = 0.1489$ ,  $wR_2 = 0.1376$ . The crystal structure of **1** was solved by direct method SHELXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHELXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 730414). Copies of these data can be obtained free of charge via [www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html) (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

(16) (a) Andriamialisoa, R. Z.; Diatta, L.; Rasoanaivo, P.; Langlois, N.; Potier, P. *Tetrahedron* **1975**, 31, 2347–2348. (b) Langlois, N.; Diatta, L.; Andriamialisoa, R. Z. *Phytochemistry* **1979**, 18, 467–471.

(17) (a) Guerard, K. C.; Chapelle, C.; Giroux, M.-A.; Sabot, C.; Beaulieu, M.-A.; Achache, N.; Canesi, S. *Org. Lett.* **2009**, 11, 4756–4759. (b) Lodeiro, S.; Wilson, W. K.; Shan, H.; Matsuda, S. P. T. *Org. Lett.* **2006**, 8, 439–442.

breast, SMMC-7721 hepatocellular carcinoma, HL-60 myeloid leukemia, PANC-1 pancreatic cancer, and A-549 lung cancer, using the MTT method reported previously<sup>18</sup> with minor revision.<sup>19</sup> The results showed that **1** exhibited stronger inhibitory activity with low IC<sub>50</sub> values than that of cisplatin

---

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

(19) 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% CO<sub>2</sub> at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100  $\mu$ L 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 initial density of  $1 \times 10^5$  cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40  $\mu$ M 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.

(Table 2). Unfortunately, intensive pharmacological investigation on **1** could not be carried out due to the limited amount available.

**Acknowledgment.** The authors are grateful to the National Basic Research Program of China (973 Program 2009CB522300), Chinese Academy of Sciences (KSCX2-YW-R-202), and the National Natural Science Foundation of China for partial financial support.

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

OL1000022