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

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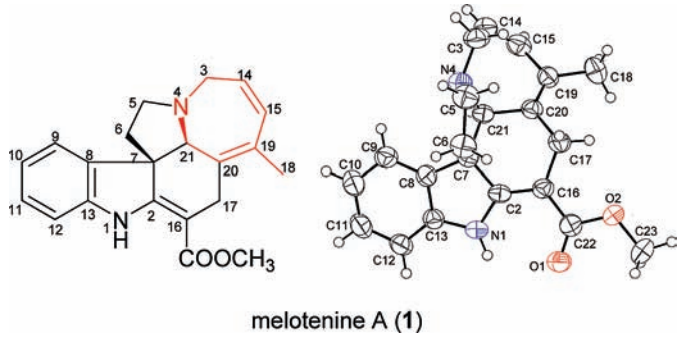
ABSTRACT



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.[1] The phytochemical constituents of *Melodins* sp. have been investigated extensively. Up to now, more than 80 compounds have been isolated and characterized.[2] 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.[3] Many characteristic *Melodinus* alkaloids, such as meloscine,[4] epimeloscine,[5] scandine,[6] and deoxoapodine,[5] 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,[7] antimitotic,[8] and antibacterial activities.[9] Our previous study

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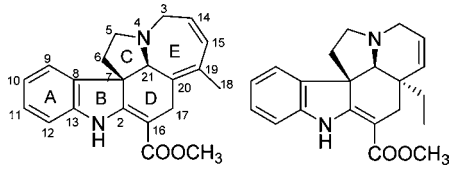
on *M. henryi* reported two novel carbon skeletons, melodinine A, a complicated monoterpenoid alkaloid bearing 24 skeletal carbons arranged compactly in eight rings, and melodinine B, a key intermediate from indole to quinoline alkaloids.[10] As part of searching for novel and bioactive monoterpenoid indole alkaloids,[11] we now report an unprecedented alkaloid rearranged from the apidospermane skeleton, named melotenine A (**1**), together with tabersonine,[12] 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.



melotenine A (**1**) tabersonine

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_2CO, 12:1) to yield **1** (3 mg) and tabersonine (20 mg).

Compound **1**,[13] isolated as colorless crystals (Me_2CO), possessed a molecular formula of $C_{21}H_{22}N_2O_2$ as established by HRESIMS ($[M + H]^+$ 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^{-1}) and conjugated ester (1680 cm^{-1}) functions.[14]

The ^1H NMR spectrum revealed the existence of an *ortho*-disubstituted phenyl ring [δ_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 [δ_H 9.06 (1H, br s)], and two methyls [δ_H 3.79 (3H, s, OMe), 1.87 (3H, s, H-18)] (Table 1). The ^{13}C NMR spectrum displayed a total of 21 carbon resonances which were assigned to two methyls (δ_C 18.7, 51.0), four methylenes (δ_C 25.4, 39.3, 52.2, 54.6), seven methines (δ_C 67.7, 108.9, 120.7, 123.1, 127.9, 131.0, 135.0), and eight quaternary carbons (δ_C 55.1, 91.3, 128.1, 134.7, 137.4, 143.4, 163.0, 168.4) (Table 1). The signals at δ_C 168.4 and 91.3 were readily assigned to C-2 and C-16, respectively, corresponding to the acrylate double bond.

Table 1. ^1H (500 MHz) and ^{13}C (100 MHz) NMR Data of **1**[a] in $CDCl_3$ (δ in ppm, J in Hz)

entry	δ_H	δ_C
N_1−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
*C*OOMe		168.4 s
COO*Me*	3.79 (3H, s)	51.0 q

[a] Data were assigned by HSQC, HMBC, ^1H−^1H COSY, and ROESY spectra.

In the HMBC spectrum (Figure 1), the correlations of δ_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_2-17. The correlations of H-17 with δ_C 134.7 (s, C-20) and of δ_H 3.74 (1H, s, H-21) with δ_C 134.7 (s, C-20) established the linkage of C-17/C-20/C-21. The HMBC correlations of δ_H 2.42 (1H, m, H-6a) and 1.90 (1H, m, H-6b) with δ_C 55.1 (s, C-7) indicated the direct connection of CH_2-6 to C-7. In addition, the HMBC correlation of H-6 with δ_C 52.2 (t, C-5), together with ^1H−^1H COSY correlations of CH_2-6 with CH_2-5, suggested the direct connection between C-5 and C-6 (Figure 1). The downfield NMR data of CH_2-5

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(13) Melotenine A (**1**): colorless crystals (Me_2CO); mp 174 °C; $[\alpha]^{25}_D$ − 136.5 (*c* 0.20, $CHCl_3$); UV ($CHCl_3$) ν_{max} (log ε) 328 (3.92), 298 (3.82), 265 (4.21), 221 (4.64) nm; IR (KBr) ν_{max} 3440, 2948, 1680, 1610, 1436, 1244 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 334; HRESIMS *m/z* 335.1772 $[M + H]^+$ (calcd for $C_{21}H_{22}N_2O_2$, 335.1759).

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allowed the attachment of a nitrogen atom (N-4) to C-5. The HMBC correlation between δ_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.

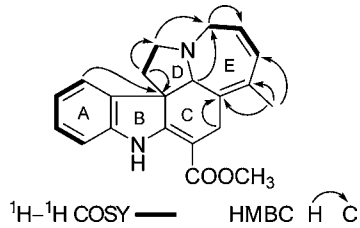

Figure 1. Key 2D NMR correlations of **1**.

A singlet at δ_H 1.87 (3H, s, H-18) in the ^{1}H NMR spectrum, assigned to a methyl group, showed key HMBC correlations with δ_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 δ_C 54.6 (t, C-3) suggested the attachment between C-3 and N-4. The HMBC correlations of δ_H 3.62 (1H, d, J = 13.7 Hz, H-3a) and 3.30 (1H, d, J = 13.7 Hz, H-3b) with δ_C 135.0 (d, C-14), as well as ^{1}H−^{1}H COSY correlations between H-3 and δ_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 Me_2CO-d_6 to give clear ^{1}H NMR signals of H-14 [δ_H 6.08 (1H, dd, J = 11.0, 6.0 Hz)] and H-15 [δ_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).[15]

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

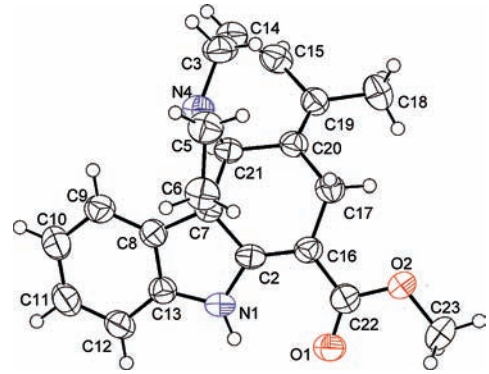

Figure 2. X-ray structure of **1**.

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

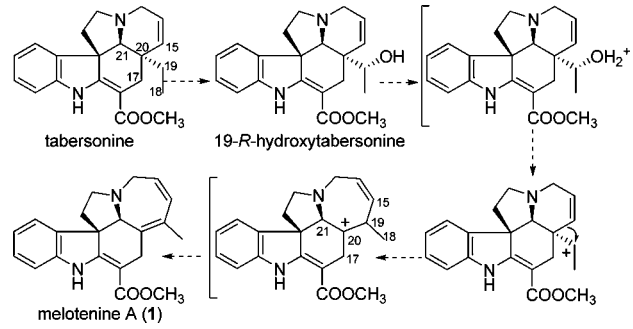

Table 2. Cytotoxicity of **1** and Tabersonine

	IC_{50} (μM)		
cells	**1**	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

(15) Crystal data for melodinine A (**1**): $C_{21}H_{22}N_2O_2$, MW = 334.41; monoclinic, space group $P2_1$; a = 6.5866 (13) Å, b = 10.932 (2) Å, c = 24.192 (5) Å, α = 90.00, β = 90.00(10), γ = 90.00, V = 1741.9 (6) Å^3, Z = 4, d = 1.275 g/cm^3, crystal dimensions 0.26 × 0.14 × 0.10 mm was used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα 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 SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-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 (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).

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breast, SMMC-7721 hepatocellular carcinoma, HL-60 myeloid leukemia, PANC-1 pancreatic cancer, and A-549 lung cancer, using the MTT method reported previously[18] with minor revision.[19] The results showed that **1** exhibited stronger inhibitory activity with low IC_{50} values than that of cisplatin (Table 2). Unfortunately, intensive pharmacological investigation on **1** could not be carried out due to the limited amount available.

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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.

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(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_2 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 μL 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 1×10^5 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.