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Alkaloids from *Menispermum dauricum*

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Abstract

The alkaloids, dechloroacutumidine and 1-epidechloroacutumine, together with three known alkaloids, acutumidine, acutumine, and dechloroacutumine, were isolated from the rhizomes of *Menispermum dauricum* and their structures established by spectral and chemical methods. The cytotoxicity of each compound against the growth of human cell lines was studied, and acutumine selectively inhibited T-cell growth.

Keywords: *Menispermum dauricum*; Menispermaceae; T-cell inhibition; Dechloroacutumidine; 1-Epidechloroacutumine

1. Introduction

Menispermum dauricum DC. (Menispermaceae) occurs widely in China and its rhizome is a traditional Chinese medicine officially listed in the Chinese Pharmacopoeia as an analgesic and antipyretic. Previous studies revealed the presence of three chlorine-containing alkaloids, acutumidine (**1**), acutumine (**2**), acutuminine and a dechlorine analogue, dechloroacutumine (**3**) in either its roots (Tomita et al., 1967), leaves (Okamoto et al., 1969), or roots cultured in chlorine-deficient medium (Sugimoto et al., 1998). Owing to its importance in traditional Chinese medicine, we re-investigated the alkaloid components of this plant. Two new dechlorinated compounds, dechloroacutumidine (**4**) and 1-epidechloroacutumine (**5**), together with the known alkaloids, acutumidine (**1**), acutumine (**2**), and dechloroacutumine (**3**), were isolated and characterized from the rhizomes.

The development of selective T-cell cytotoxic agents, which can be potentially used for the specific therapy of T-cell related leukemia and lymphoma remains an important task. Alkaloids **1–5** were evaluated for cytotoxity against human MOLT-4, HUT 78, and transformed human B-lymphocytes, and the results obtained are described below.

2. Results and discussion

Ethanol extracts of the rhizomes of *M. dauricum* were treated with 2% tartaric acid. The acidic solution was basified with aqueous ammonia to pH 9–10 and extracted with $CHCl_3$ and *n*-BuOH, successively. The $CHCl_3$ extract was subjected to column chromatographic separation on Sephadex LH-20 and silica gel repeatedly to afford alkaloids **1–5**.

Dechloroacutumidine (**4**), obtained as a white amorphous powder, showed a positive reaction with Dragendorff's reagent. EIMS spectral ions of **4** did not show characteristic isotopic patterns for the presence of chlorine. The molecular formula of **4** was assigned as $C_{18}H_{23}O_6N$ (*m*/*z* 349.1532 [M^+], requires 349.1525) by HREIMS. The EIMS spectral fragmentation pattern and UV absorption, which were very similar to those of acutumidine (**1**) (Tomita et al., 1971), as well as the molecular formula, suggested that **4** was a dechlorinated analogue of **1**. The ^1H NMR spectrum of **4** exhibited three methoxyl groups, two one-proton singlets at δ 5.03 and 5.42, and two, mutually-coupled, one-proton doublets at δ 2.54 and 2.92 (Table 1). Compared with the spectrum of **1**, the H-10 signal at δ 4.99 disappeared and two additional aliphatic proton signals were observed, constituting an additional $–CH_2–CH_2$-system by $^1H–^1H$ and $^1H–^{13}C$ COSY analysis. The ^{13}C NMR spectrum of **4** (Table 1) exhibited eighteen carbons, consisting of three methyls, five methylenes, two

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methines, and eight quaternary carbon atoms, which was very similar to **1**, except for one more methylene and one less methine than **1**. The signals of C-9 at δ 48.1 and C-10 at δ 58.4 in **1** were shifted to δ 38.6 and δ 40.4 in **4**, respectively, which strongly suggested that **4** differed from **1** only in the absence of a chlorine atom at C-10.

Table 2 shows the important HMBC correlations of **4**, supporting the proposed structure of **4** as dechloroacutumidine. The CD spectral patterns of **4** and **1** were very similar, with a negative Cotton effect near 240 nm, and a positive Cotton effect near 265 nm. Therefore, the absolute stereochemistry of **1** was elucidated as being the same as that of **1**. Dechlorination of acutumidine (**1**) with *n*-Bu_3SnH/AIBN afforded a product, which was identical to **4** by comparison of TLC, MS and NMR data. This further confirmed the structure of **4** unambiguously. The ^1H NMR and ^{13}C NMR data (Table 1) were assigned by various 2D NMR experiments.

1-Epidechloroacutumine (**5**) was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{19}H_{25}NO_6$ (*m*/*z* 363.1690 [M^+], requires 363.1682) by HREIMS, the same as that of dechloroacutumine (**3**). The mass spectrum exhibited a series of significant fragment ions at *m*/*z* 335, 320, 220, 209, 181, 166, 150, which were almost the same as those of **3**. The ^1H and ^{13}C NMR spectroscopic data (Table 1) showed the presence of one *N*-methyl group (δ_H 2.33; δ_C 35.5), three methoxyl groups (δ_H 3.74, 3.98, 4.17; δ_C 59.1, 60.6, 60.8), two singlet methines at C-1 and C-3 (δ_H 4.54, 5.20; δ_C 77.6, 102.5), one isolated methylene at C-5 (δ_H 2.10, 2.87, ABq, J = 15.1 Hz; δ_C 47.1), and two $-CH_2-CH_2-$units at $-C_9-C_{10}-$ and $-C_{14}-C_{15}-$, very similar with the data of **3**. However, the C-1 signal of **5** at δ 77.6 was downfield compared to the corresponding signal at δ 72.7 of **3**, suggesting strongly that **5** was different from **3** only in the stereochemistry of the hydroxyl group at C-1. Important HMBC correlations (Table 2) of **5** revealed similarities to those of **3**. The CD spectral patterns of **5** and **3** were also very similar, having a negative Cotton effect near 240 nm, and a positive Cotton effect near 270 nm. The above data suggested that **5** was the C-1 epimer of **3**, which was further confirmed by the NOESY experiments showing important correlation between H-1 and H-9a (Fig. 1). Comparatively, there was no correlation between H-1 and H-9a in the NOESY spectrum of **3**. Thus the structure of **5** was unambiguously elucidated as 1-epidechloroacutumine. The ^1H NMR and ^{13}C NMR data (Table 1) were assigned by various 2D NMR experiments.

Known alkaloids **1**, **2**, and **3** were characterized by analysis of their MS and NMR spectroscopic data to literature values (Sugimoto et al., 1998; Tomita et al., 1967). In addition, **3** was characterized by chemical transformation of **2** by treatment with *n*-Bu_3SnH/AIBN.

Alkaloids **1–5** were evaluated for cytotoxity against human MOLT-4, HUT 78, and transformed human B-lymphocytes. Acutumine (**2**) showed moderate selective cytotoxity to T-cells (IC_{50} = 13.2 μM), whereas **1**, **3**, **4** and **5** showed no activity to either T-cells or B-cells.

Table 1
NMR spectroscopic data of alkaloids **4** (in $CDCl_3$) and **5** (in C_5D_5N)

No.	**4**		**5**	
	δ_H	δ_C	δ_H	δ_C
1	5.03, *s*	72.2	4.54, *s*	77.6
2		188.2		188.3
3	5.42, *s*	103.5	5.20 *s*	102.5
4		204.4		199.4
5	2.54, *d*, 17.1 Hz 2.92, *d*, 17.1Hz	45.4	2.10, *d*, 15.1 Hz 2.87, *d*, 15.1Hz	47.1
6		192.2		193.9
7		136.6		138.9
8		163.3		158.9
9	2.32, *m* 2.79, *m*	38.6	1.23, *m* 2.29, *m*	31.4
10	1.63, *m* 2.70, *m*	40.4	1.80, *m* 2.57, *m*	34.4
11		66.6		67.4
12		53.4		56.4
13		73.8		53.8
14	1.93, *m* 2.94, *m*	28.5	1.97, *m* 2.18, *m*	31.8
15	2.94, *m* 3.03, *m*	44.6	2.41, *m* 2.93, *m*	53.8
2-OMe	3.74, *s*	58.7	3.85, *s*	60.6
7-OMe	3.98, *s*	60.3	3.63, *s*	59.1
8-OMe	4.17, *s*	60.9	4.02, *s*	60.8
N-CH_3			2.33, *s*	35.5
N-H	4.99, *br s*			
1-OH	7.93, *br s*			

3. Experimental

3.1. General

UV spectra were recorded on a Shimadzu 160A instrument. NMR spectra were recorded using a Bruker DRX 500 NMR spectrometer with chemical shifts being referenced to TMS as internal standard. EI and HREIMS data were obtained using Finnigan-450 and

Table 2
Important HMBC correlations of alkaloids **4** and **5**

H	C	
	4	**5**
H-1	3, 4, 10, 12	3, 4, 10, 12
H-3	1, 2, 4, 11	1, 2, 4, 11
H-5	11, 13, 14	11, 13, 14
H-9	11, 12	11, 12
H-10	1, 4, 12, 13	1, 4, 12, 13
N-Me	–	13, 15
2-OMe	2	2
7-OMe	7	7
8-OMe	8	8

MAT-711 mass spectrometers. CD: JASCO J-500 A spectropolarimeter. CC was performed with silica gel 60 (Qingdao Marine Chemical Co. Qingdao, People's Republic of China), 100–200 mesh. TLC was carried out on precoated silica gel GF-254 plates, 0.2 mm thick (Qingdao Marine Co. Qingdao), detected by UV light.

3.2. Plant material

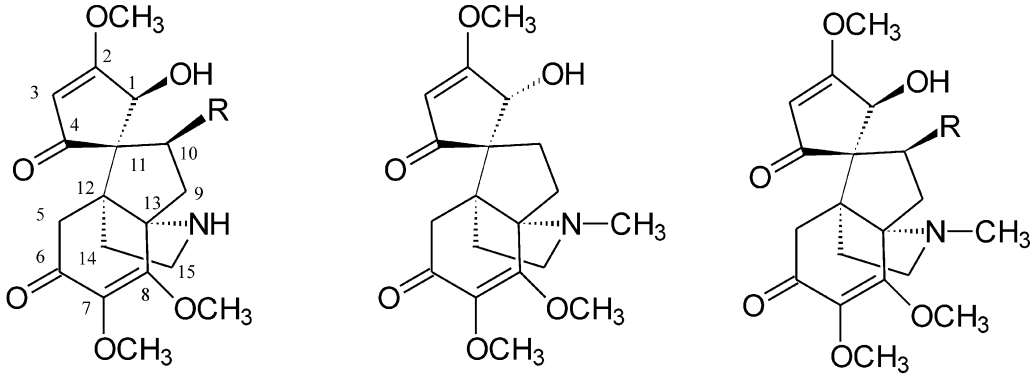
The rhizomes of *M. dauricum* were collected from Anshan, Liaoning Province, People's Republic of China, in May 1999. The materials were authenticated by Professor Ji-Xian Guo of the School of Pharmacy, Shanghai Medical University. A voucher specimen has been deposited in the Herbarium of Shanghai Institute of Material Medica (No. SIMM99051301).

3.3. Extraction and isolation

The dried powdered rhizome of *M. dauricum* (20 kg) was extracted with ethanol (95%, 50 l). The ethanol extract was concentrated in vacuo and residue was treated with 2% (w/w) tartaric acid solution. The acid solution was basified to pH 9–10 with aqueous ammonia (25%, w/w) and extracted with $CHCl_3$ (2 l) and *n*-BuOH (2 l), successively. The $CHCl_3$ residue (35 g) was subjected to silica gel chromatography eluting with $CHCl_3$–MeOH (20:1–1:1) to afford 20 fractions. Fr. 10 (560 mg) was further purified by silica gel and Sephedex LH-20 column chromatography eluting with $CHCl_3$–Me_2CO (10:1), and then 90% aq. MeOH to afford **1** (50 mg), **2** (150 mg), and **3** (10 mg), **4** (10 mg), **5** (8 mg), respectively.

3.5. Dechloroacutumidine (*4*)

$[\alpha]_d^{25}$ −68° (MeOH; *c* 0.2). CD $\Delta\varepsilon_{325.7}$ +4.8 $\Delta\varepsilon_{275.8}$ +15.8, $\Delta\varepsilon_{227.5}$ −17.5 (MeOH, 5.0×10^{-5} M). UV λ_{max}^{MeOH} (log ε): 251 (4.21), 275 (3.89) nm; HREIMS *m/z* 349.1532 [M^+] ($C_{18}H_{23}O_6N$ requires 349.1525); EIMS (60 eV) *m/z* 349 [M^+] (82), 321 [M–CO]$^+$ (100), 194 (71), 152 (31), 135 (13); for ^{1}H NMR and ^{13}C NMR data, see Table 1.



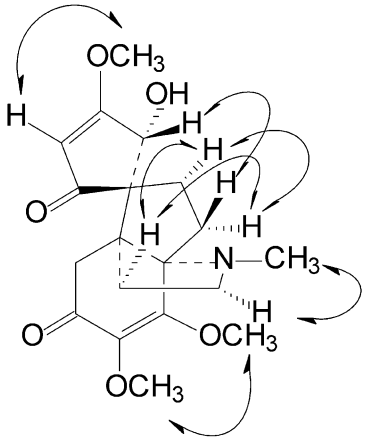


Fig. 1. Important NOESY correlations of **5**.

3.6. 1-Epidechloroacutumine (**5**)

$[\alpha]_D^{25}$ −45° (MeOH; *c* 0.2). CD $\Delta\varepsilon_{270.8}$ +15.8, $\Delta\varepsilon_{226.9}$ −17.5 (MeOH, 5.0×10^{-5} M). UV λ_{max}^{MeOH} (log ε) 245 (3.87), 270 (3.71) nm; HREIMS *m*/*z* 363.1690 [M^+] ($C_{19}H_{25}NO_6$ requires 363.1682); EIMS (60 eV) *m*/*z* 363 [M^+] (63), 335 (60), 320 (89), 220 (10), 208 (100), 181 (67), 166 (35), 150 (31); for ^{1}H NMR and ^{13}C NMR data, see Table 1.

3.7. Dechlorination of **1**

A solution of **1** (30 mg) in dry toluene (30 ml) containing *n*-Bu_3SnH (0.5 ml) and a catalytic amount of AIBN was heated under reflux and then maintained for 3 h. Aqueous KF (35%) was then added to the reaction solution after cooling to decompose the excess *n*-Bu_3SnH. The solution was basified with saturated aq. $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ residue was applied to a silica gel column eluted with $CHCl_3$–MeOH (30:1) to give (**4**) (15 mg), which was identified by comparing MS and NMR spectra.

3.8. Dechlorination of **2**

A sample of **2** (30 mg) was processed as above to **3** (10 mg), whose identity was established by MS and NMR spectra.

3.9. Bioassay

The proliferation of human cell lines was measured by the MTT colorimetric assay (Loveland et al., 1992). MOLT-4, HUT78, and transformed human B-lymphocytes were cultured at a concentration of 20,000 cells/well/100 μl in RPMI 1640 media supplemented with 10% fetal bovine serum, antibiotics, HEPES and L-glutamine in 96-round bottom plates in triplicate. Test alkaloids were added to the cells at final concentrations of 0.01–100 μM in 100 μl. Equivalent concentrations of solvent DMSO were used as control. After 72 h incubation at 37 °C in 5% CO_2, 2 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added. Plates were incubated for 4 h, supernatants were removed, and 100 μl of DMSO was added to each well. Color intensity was measured at 570 nm.

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