Cytotoxic Oxoisoaporphine Alkaloids from *Menispermum dauricum*

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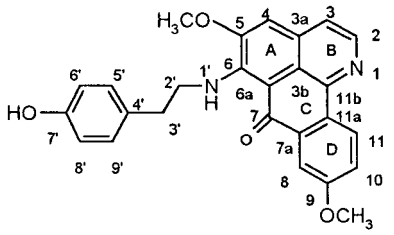
Four new oxoisoaporphine alkaloids, daurioxoisoporphines A−D (**1**−**4**), were isolated from the rhizomes of *Menispermum dauricum*. The structures of these alkaloids were established by spectroscopic methods. The cytotoxic evaluation of **1** and **2** is reported against four cancer cell lines.

Menispermum dauricum DC. (Menispermaceae) occurs widely in the People's Republic of China. The rhizomes of the plant are used in traditional Chinese medicine and are officially listed in the Chinese Pharmacopoeia as an analgesic and antipyretic. Previous studies have revealed that *M. dauricum* is the source of naturally occurring oxoisoaporphine alkaloids.[1] Nine oxoisoaporphine alkaloids have been isolated from the intact plant[2−7] and from the cultured plant roots treated with ketoconazole, a cytochrome P-450 inhibitor.[8] The ethanol extract of the plant has exhibited antitumor activity.[9] In the present work, four new oxoisoaporphine alkaloids, daurioxoisophines A−D (**1**−**4**), were isolated from the plant rhizomes and structurally characterized. In preliminary screening, **1** and **2** exhibited cytotoxic activities against a small panel of cancer cell lines.



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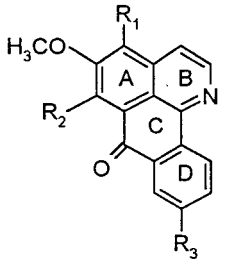

2 R_1=OCH_3, R_2=NH_2, R_3=OCH_3

3 R_1=H, R_2=$NHCH_3$, R_3=OCH_3

4 R_1=H, R_2=OCH_3, R_3=OH

Daurioxoisoporphine A (**1**) was obtained as yellow crystals, mp 234−235 °C. Its UV absorption (λ_{max} 215, 255, 447, 475 nm) indicated a highly conjugated system similar to those of oxoisoaporphine[10] or oxoaporphine alkaloids.[11]

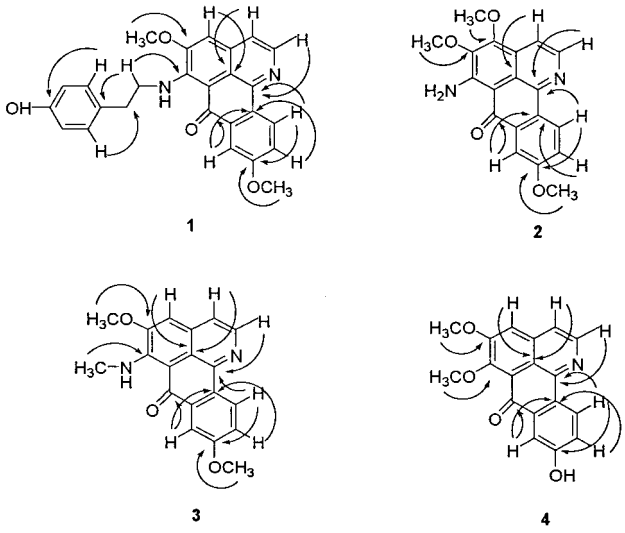

Figure 1. HMBC correlations of compounds **1**−**4**.

Compound **1** was assigned the molecular formula $C_{26}H_{22}N_2O_4$ by HREIMS (M^+ *m*/*z* 426.1570, calcd 426.1566). The MS and NMR spectral data obtained for **1** suggested that the alkaloid is composed of a tyramine unit and an oxoisoaporphine or oxoaporphine unit. A significant fragment at *m*/*z* 383 $[M - 43]^+$ in the EIMS of **1** was characteristic of an oxoaporphine or an oxoisoaporphine alkaloid,[12] and the base peak at *m*/*z* 319 $[M - 107]^+$ was characteristic of a tyramine moiety.[8] The presence of a tyramine moiety was also supported by the ^{1}H NMR (δ 4.25, 2H, dt, J = 12.7, 7.2 Hz, H-2′; 3.10, 2H, t, J = 7.2 Hz, H-3′; 7.42, 2H, d, J = 8.4 Hz, H-5′ and H-9′; 7.21, 2H, d, J = 8.4 Hz, H-6′ and 8′) and ^{13}C NMR (δ 49.3, C-2′; 37.4, C-3′; 129.8, C-4′; 130.6, C-5′ and C-9′; 116.5, C-6′ and C-8′; 159.8, C-7′) spectra. Besides the tyramine unit, the ^{1}H NMR spectrum displayed signals for two methoxyl groups at δ 3.82 and 3.84 and six aromatic protons. Among the aromatic protons, the two appearing as a typical AB quartet at δ 7.63 (1H, d, J = 5.1 Hz) and 8.86 (1H, d, J = 5.1 Hz) were assignable either to the protons at C-2 and C-3 of an oxoisoaporphine or to those at C-4 and C-5 of an oxoaporphine. In turn, the three mutually coupled aromatic protons at δ 7.56 (dd, J = 8.8, 2.7 Hz), 8.44 (d, J = 2.7 Hz), and 9.43 (d, J = 8.8 Hz) were assigned to a 1,2,4-trisubstituted D ring system, and the isolated signal at δ 7.33 (s) could be attributed to the B ring. Although oxoaporphine and oxoisoaporphine alkaloids have minor differences in NMR spectra,[5] their differentiation can be made unambiguously by HMBC correlations.[8] The HMBC correlations of **1** (Figure 1) suggested it is an oxoisoaporphine alkaloid. The strong deshielding of the

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Table 1. ^{1}H NMR Data of Compounds **1**−**4** (in $CDCl_3$)

position	1	2	3	4
2	8.86 d (5.1)	8.90 d (5.2)	8.11, d (5.1)	8.82 d (5.5)
3	7.63 d (5.1)	7.59 d (5.2)	7.39, d (5.1)	7.67 d (5.5)
4	7.33 s		7.02 s	7.57 s
8	8.44 d (2.7)	8.42 d (2.7)	7.32, d (2.7)	8.34 d (2.6)
10	7.56 dd (2.7, 8.8)	7.54 dd (2.7,8.8)	7.96, dd (2.7, 8.8)	7.64 dd (2.6, 8.8)
11	9.43 d (8.8)	9.39 d (8.8)	8.91, d (8.8)	9.21 d (8.8)
OMe-4		4.08 s		
OMe-5	3.82 s	3.90 s	3.95, s	3.90 s
OMe-6				4.20 s
OMe-9	3.84 s	3.83 s	3.95, s	
Me-N			3.52, s	
1′	13.30 br	6.35 br	12.50 br	
		10.55 br		
2′	4.25 dt (7.2, 12.7)			
3′	3.10 t (7.2)			
5′	7.42 d (8.4)			
6′	7.21 d (8.4)			
8′	7.21 d (8.4)			
9′	7.42 d (8.4)			

[a] Values in parentheses are *J* values in Hz.

imine proton by the carbonyl group at C-7, which resonated at δ 13.30, implied that the tyramine unit is attached to C-6 of the oxoisoaporphine through a C−N bond.[8]

The ^{13}C NMR spectrum of **1** showed 26 carbons consisting of two methyls, two methylenes, 10 methines, and 13 quaternary carbon atoms. ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC experiments revealed that eight carbons including two methylenes, four methines, and two quaternary carbon atoms were assignable to a tyramine skeleton. Important correlations from the HMBC NMR spectrum are shown in Figure 1, which are quite similar to those of tyraminoporphine, an oxoisoaporphine alkaloid isolated from the roots of *M. dauricum* cultured in a medium containing ketoconazole.[8] The HMBC data and biogenetic considerations supported the placement of the methoxyl group resonating at δ 3.82 at C-5 of the B ring and the attachment of a second methoxyl group at δ 3.84 to C-9 of the D ring. Thus, **1** was determined as 4-demethoxytyraminoporphine. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were assigned unambiguously by various 2D NMR experiments. In a previous study, a minor compound isolated from *M. dauricum* cultured roots was shown to have the same molecular weight as **1** by MS, but no other data were obtained to support its structural identification.[13]

Daurioxoisoporphine B (**2**) was obtained as a yellow amorphous powder. Its UV absorption (λ_{max} 219, 255, 350, 430, 455 nm) indicated a highly conjugated system similar to **1**. A significant fragment at *m/z* 293 $[M - 43]^+$ in the EIMS of **2** was characteristic of an oxoisoaporphine or an oxoaporphine alkaloid.[12] Compound **2** was assigned the molecular formula $C_{19}H_{16}N_2O_4$ by HREIMS (M^+ *m/z* 336.110, calcd 336.111). The ^{1}H NMR spectrum showed signals for three methoxyl groups at δ 3.83, 3.90, and 4.08, three aromatic protons at δ 7.54, 8.42, and 9.39, and a typical AB quartet at δ 8.90 (d, J = 5.2 Hz) and 7.59 (d, J = 5.2 Hz), assignable either to the protons at C-2 and C-3 of an oxoisoaporphine or to those at C-4 and C-5 of an oxoaporphine.[3−8] The aromatic ^{1}H NMR signals at δ 7.54 (dd, J = 2.7, 8.8 Hz), 8.42 (d, J = 2.7 Hz), and 9.39 (d, J = 8.8 Hz) were assignable to a 1,2,4-trisubstituted D ring system. Two signals at δ 6.35 and 10.55, exchangeable with D_2O, were assigned as amino protons. The strong deshielding of the amino proton that resonated at δ 10.55 by the carbonyl group at C-7 implied that an amino group was attached to C-6 of the oxoisoaporphine through a C−N bond.

Table 2. ^{13}C NMR Data of Compounds **1**−**4** (in $CDCl_3$)

position	1	2	3	4
2	142.3	142.0	141.9	144.0
3	119.5	115.1	119.0	119.3
3a	130.3	126.2	118.8	133.9
3b	119.1	120.4	119.0	118.6
4	111.5	151.0	110.7	112.4
5	153.3	141.1	153.4	156.8
6	150.9	153.4	151.7	155.7
6a	106.4	103.3	106.1	123.9
7	181.8	182.0	181.7	182.6
7a	135.1	135.1	134.5	135.0
8	108.3	108.5	107.1	112.8
9	161.0	161.3	160.6	161.2
10	121.5	120.9	121.3	122.6
11	123.1	127.8	126.6	128.0
11a	130.4	130.3	129.8	128.9
11b	142.8	143.9	142.5	148.0
OMe-4		60.6		
OMe-5	55.5	61.2	55.7	56.3
OMe-6				61.3
OMe-9	55.8	55.5	55.7	
Me-N			34.0	
2′	49.3			
3′	37.4			
4′	129.8			
5′	130.6			
6′	116.5			
7′	157.8			
8′	116.5			
9′	130.6			

The ^{13}C NMR spectrum of **2** showed 19 carbons consisting of three methyls, five methines, and 11 quaternary carbon atoms. Important HMBC correlations are shown in Figure 1. Besides one methoxyl group at C-9 of the D ring, the two other methoxyl groups were indicated to be at C-4 and C-5 of the B ring by the 2D NMR data. Thus, **2** was deduced as 6-amino-4,5,9-trimethoxyoxoisoaporphine.

Daurioxoisoporphine C (**3**) was obtained as an amorphous yellow powder. Its UV absorption and EIMS fragmentation characteristics were similar to those of **1** and **2**. Compound **3** was assigned the molecular formula $C_{19}H_{16}N_2O_3$ by HREIMS (M^+ *m/z* 320.114, calcd 320.116). The ^{1}H NMR spectrum showed signals for two methoxyl groups (δ 3.95) and one doublet signal for an *N*-methyl group (δ 3.52, J = 5.4 Hz), which were different from **1** and **2**. The signal at δ 12.50, exchangeable with D_2O, was assigned as an imine proton. The strong deshielding of the imine proton by the carbonyl group at C-7 implied that an $-NHCH_3$ functionally was attached to C-6 of the oxoisoaporphine alkaloid through a C−N bond. The ^{13}C NMR

Table 3. Cytotoxicity of Compounds **1** and **2** against Four Cancer Cell Lines

	IC $_{50}$ (μM)		
cell line	**1**	**2**	VP-16[b]
A549	8.8	>50	0.5
HL-60	>50	>50	5.4
MCF-7	3.0	6.2	12.3
P-388	30.5	9.6	0.1

[a] Key to cell lines used: A 549, human lung carcinoma; HL-60, human leukemia; MCF-7, human breast cancer; P388, mouse leukemia. [b] Positive control substance.

spectrum of **3** showed 19 carbons consisting of three methyls, six methines, and 10 quaternary carbon atoms. Important correlations revealed by HMBC are shown in Figure 1. Thus, **3** was deduced as 6-methylamino-5,9-dimethoxyoxoisoaporphine.

Daurioxoisoporphine D (**4**) was obtained as an amorphous yellow powder. Its UV absorption and EIMS data were similar to those of **1**−**3**. Compound **4** was assigned the molecular formula $C_{18}H_{13}NO_4$ by HREIMS (M^+ *m/z* 307.0849, calcd 307.0844). The ^{1}H NMR spectrum showed signals for two methoxyl groups at δ 3.90 and 4.20, which were confirmed as being attached to C-5 and C-6, respectively, from the HMBC spectrum. The ^{13}C NMR spectrum of **4** showed 18 carbons consisting of two methyls, six methines, and 10 quaternary carbon atoms. Thus, **4** was deduced as 5,6-dimethoxyl-9-hydroxyoxoisoaporphine.

It is noteworthy that compounds **1**−**3**, three new oxoisoaporphine alkaloids, having tyramine, amino, and methylamino functions, respectively, were produced by the plant without treatment with a P-450 inhibitor. Compounds **1** and **2** were evaluated for their cytotoxic activities against four cancer cell lines in a small tumor panel (Table 3) and were both significantly active against the MCF-7 human breast cancer cell line.

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained a Perkin-Elmer 599 B spectrometer. UV spectra were recorded on a Shimazu 160A instrument. NMR spectra were recorded using a Bruker DRX 500 NMR spectrometer in $CDCl_3$. Chemical shifts are presented on the δ (ppm) scale using TMS as internal standard. EIMS and HREIMS data were obtained by Finnigan-450 and MAT-711 mass spectrometers, respectively. Column chromatography was performed with Si gel 60 (Qingdao Marine Chemical Co. Qingdao, People's Republic of China), 100−200 mesh. VP-16 was purchased from Sigma Chemical Co.

Plant Material. The roots of *Menispermum dauricum* were collected from Anshan, Liaoning Province, People's Republic of China, in May 1999. The materials were authenticated by Prof. 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).

Extraction and Isolation. An ethanol extract of the air-dried roots of *M. dauricum* (20 kg) was treated with 2% tartaric acid solution. The tartaric acid soluble portion was basified with aqueous ammonia and extracted with chloroform and butanol, respectively, to give a chloroform residue (35 g) and butanol residue (40 g). The chloroform residue was subjected to Si gel chromatography by elution with $CHCl_3$−MeOH (20:1−1:1) and gave 20 fractions. Fraction 5 (200 mg) was further purified by Si gel column chromatography eluted with hexane−acetone (10:1) to afford 25 fractions. Fraction 5 was purified by Si gel column chromatography eluted with hexane−acetone (20:1) and yielded compound **1** (20 mg). Fraction 7 was purified by Si gel column chromatography eluted with hexane−acetone (17:1) and afforded compound **2** (9 mg). Fraction 10 was purified by Si gel column chromatography eluted with hexane−acetone (13:1) and yielded compound **3** (10 mg). Fraction 12 was purified by Si gel column chromatography eluted with hexane−acetone (8:1) and afforded compound **4** (8 mg).

Daurioxoisoporphine A (1): yellow crystals ($CHCl_3$), mp 234−235 °C; UV (MeOH) λ_{max} (log ϵ) 215 (4.2), 255 (2.5), 448 (3.8), 476 (4.3) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 426 [M^+] (8.5%), 383 [M − 43]$^+$ (0.9), 319 (100), 303 (12.8), 276 (5.1), 206 (4.3), 131 (0.9), 85 (0.9); HREIMS *m/z* found [M^+] 426.1570, $C_{26}H_{22}N_2O_4$ requires 426.1566.

Daurioxoisoporphine B (2): yellow amorphous powder, UV (MeOH) λ_{max} (log ϵ) 219 (2.8), 255 (3.2), 350 (4.5), 430 (2.6), 455 (4.1) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 336 [M^+] (4.9), 321 (15.7), 293 [M − 43]$^+$ (6.1), 278 (7.8), 250 (3.5), 221 (9.6), 178 (17.4), 155 (28.7), 149 (23.5), 118 (20), 99 (100), 86 (47.8); HREIMS *m/z* found [M^+] 336.110, $C_{19}H_{16}N_2O_4$ requires 336.111.

Daurioxoisoporphine C (3): yellow amorphous powder UV (MeOH) λ_{max} (log ϵ) 215 (4.32), 250 (3.42), 310 (2.43), 360 (2.42), 406 (3.34), 420 (4.33), 458 (3.43) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 320 [M^+] (100), 277 [M − 43]$^+$ (23.6), 234 (14.7), 205 (6.7), 139 (4.7), 133 (2.9); HREIMS *m/z* found [M^+] 320.1140, $C_{19}H_{16}N_2O_3$ requires 320.1138.

Daurioxoisoporphine D (4): yellow amorphous powder, UV (MeOH) λ_{max} (log ϵ) 210 (4.32), 245 (3.54), 356 (4.23), 420 (3.45), 460 (4.63) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 307 [M^+] (100), 264 [M − 43]$^+$ (22.2), 235 (21.4), 219 (14.5), 170 (7.3), 127 (4.2), 119 (4.7); HREIMS found [M^+] 307.0849, $C_{18}H_{13}NO_4$ requires 307.0844.

Cytotoxicity Assays. P-388, MCF-7, HL-20, and A-549 cells were obtained from Shanghai Institute of Materia Medica and maintained as suspension cultures in 90% (v/v) Dulbecco's modified Eagle's medium containing 4500 mg glucose/L, 10% (v/v) horse serum, and 100 IU penicillin, 0.1 mg streptomycin, and 0.25 μg amphotericin B/mL at 37 °C in a 5% CO_2 in air atmosphere. Experiments were performed in 96-well microtiter plates (2×10^5 cell/mL). Growth inhibitory effects of compounds on tumor cells were measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma Chemical Co. St. Louis, MO) assay with minor modification.[14,15] Briefly, adherent tumor cells were seeded into 96-well microculture plates and allowed to adhere for 24 h before drug addition. The cell densities were selected on the basis of the results of preliminary tests to maintain the control cells in an exponential phase of growth during the period of the experiment and to obtain a liner relationship between the optical density (OD) and the number of viable cells. The cytotoxicity results obtained are listed in Table 3; IC_{50} values ≤ 10 μM are considered significantly active.

References and Notes

(1) Sugimoto, Y. In *Medicinal and Aromatic Plants* XI; Bajaj, Y. P. S., Ed.; Springer-Verlag: Berlin, 1999.
(2) Kunitomo, J.; Satoh, M. *Tetrahedron* **1983**, *39*, 3261−3265.
(3) Kunitomo, J.; Kaede, S.; Satoh, M. *Chem. Pharm. Bull.* **1985**, *33*, 2778−2782.
(4) Hu, S.; Xu, S.; Yao, X.; Cui, C.; Tezuka, Y.; Kikuchi, T. *Chem. Pharm. Bull.* **1993**, *41*, 1866−1868.
(5) Hou, C.; Xue, H. *Acta Pharm. Sin.* **1985,** *20*, 112−117.
(6) Takani, M.; Takasu, Y.; Takahashi, K. *Chem. Pharm. Bull.* **1983**, *31*, 3091−3093.
(7) Zhao, S.; Ye, W.; Tan, N.; Zhao, H.; Xia, Z. *J. China Pharm. Univ.* **1989**, *20*, 312.
(8) Sugimoto, Y.; Babiker, H. A. A.; Inanaga, S.; Kato, M.; Isogai, A. *Phytochemistry* **1999**, *52*, 1431−1435.
(9) Chen, C.-F. *Zhonghua Yaoxue Zazhi* **1992**, *44*, 381−388.
(10) Kunitomo, J.; Satoh, M. *Chem. Pharm. Bull.* **1982**, *30*, 2659−2660
(11) Manske, R. H. F. *The Alkaloids*; Academic Press: New York, 1973.
(12) Cong, P.-Z. *Mass Spectrometry*EnDash−*Applications in Natural Organic Chemistry*; Academic Press: Beijing, 1987.
(13) Sugimoto, Y.; Uchida, S.; Inanaga S.; Isogai, A. *J. Plant Physiol.* **1997**, *150*, 376−380.
(14) 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.
(15) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.

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