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Oxoisoaporphines from *Menispermum dauricum*

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Abstract

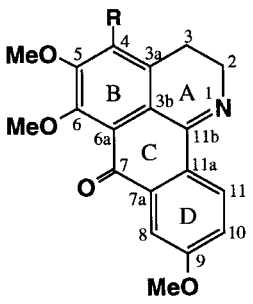
Two oxoisoaporphine alkaloids, 2,3-dihydrodauriporphine and tyraminoporphine, together with the known alkaloid dauriporphine, were isolated from *Menispermum dauricum* roots cultured in a medium containing ketoconazole, a cytochrome P-450 inhibitor. Structures of the alkaloids were established by spectroscopic, crystallographic and chemical methods.

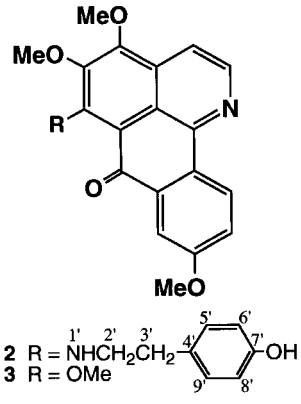
Keywords: *Menispermum dauricum*; Menispermaceae; Root culture; Oxoisoaporphine alkaloids; Dauriporphine; 2,3-Dihydrodauriporphine; Tyraminoporphine

1. Introduction

Menispermum dauricum (Menispermaceae) has been reported to be the only source of naturally occurring oxoisoaporphine alkaloids (Sugimoto, 1999). Seven oxoisoaporphines have been identified and isolated from intact *M. dauricum* plants: menisporphine (Kunitomo & Satoh, 1983), 2,3-dihydromenisporphine (Kunitomo, Kaede & Satoh, 1985), 6-*O*-demethylmenisporphine (Hu et al., 1993), bianfugecine, bianfugedine (Hou & Xue, 1985), dauriporphine (bianfugenine) (Takani, Takasu & Takahashi, 1983) and dauriporphinoline (Zhao, Ye, Tan, Zhao & Xia, 1989).

In a previous paper (Sugimoto, Uchida, Inanaga & Isogai, 1997) we reported that cultured *M. dauricum* roots treated with ketoconazole, a cytochrome P-450 inhibitor, produced tyramine and two unidentified alkaloids with molecular weights of 353 and 426. Precursor administration experiments showed that both alkaloids are derived from tyrosine. This paper reports on their isolation and structural elucidation.





2. Results and discussion

Chromatographic separation of the basic fraction from cultured *M. dauricum* roots yielded two alkaloids, **1** and **2**, as yellow needles and orange prisms, respectively.

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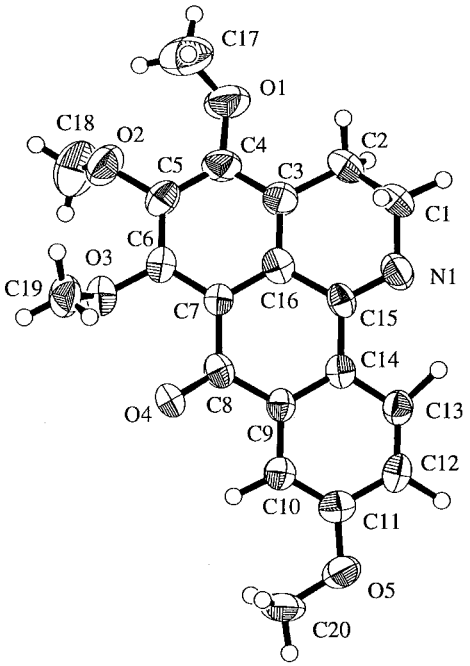

Fig. 1. Molecular structure of 2,3-dihydrodauriporphine (**1**).

1 was assigned the molecular formula $C_{20}H_{19}NO_5$ by HRESIMS (354.1341 for $(M+H)^+$). The ^1H NMR spectrum, analyzed with the aid of ^1H-^1H COSY, ^{13}C-^1H COSY and HMQC, displayed signals for four methoxy groups at δ 3.84, 3.89, 3.91 and 3.92, three aromatic protons at δ 7.30 (*dd, J* = 8.8, 2.7 Hz), 7.52 (*d, J* = 2.7 Hz), and 8.19 (*d, J* = 8.8 Hz), and mutually coupled aliphatic protons at δ 2.77 (*t, J* = 7.9 Hz) and 3.96 (*t, J* = 7.9 Hz). The aromatic proton signals are assignable to a 1,2,4-trisubstituted benzene ring system. The aliphatic proton signals can be attributed 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; the above spectral data are quite similar to those of 2,3-dihydromenisporphine (**4**) (Kunitomo et al., 1985). Comparing the spectral data of the two compounds, an aromatic proton appears at δ 7.00 in **4**, which was replaced by an aromatic methoxy group in **1**. The ^{13}C NMR spectrum of **1** showed twenty carbons, including four methyls, two methylenes, three methines and eleven quaternary carbon atoms as determined by a DEPT experiment.

On the basis of spectral data, **1** was deduced to be an oxoisoaporphine-type alkaloid with four methoxy groups, three of which are located in the B ring at C-4, 5, 6, and one in the D ring at C-9. This structure was also supported by data from HMBC and NOESY experiments, as well as EIMS. For further confirmation of the deduced structure, **1** was treated with chromium trioxide in acetic acid as described by Kunitomo et al. (1985). The dehydrogenated product was isolated by prep. HPLC. MS and ^1H NMR spectral data of the product matched those of dauriporphine (**3**) (Takani et al., 1983). Moreover, single crystal X-ray diffraction data of **1** supported the deduced structure as shown in Fig. 1, **1** named 2,3-dihydrodauriporphine. Dauriporphine (**3**), which is known to be produced by *M. dauricum*, was also isolated during the course of this study.

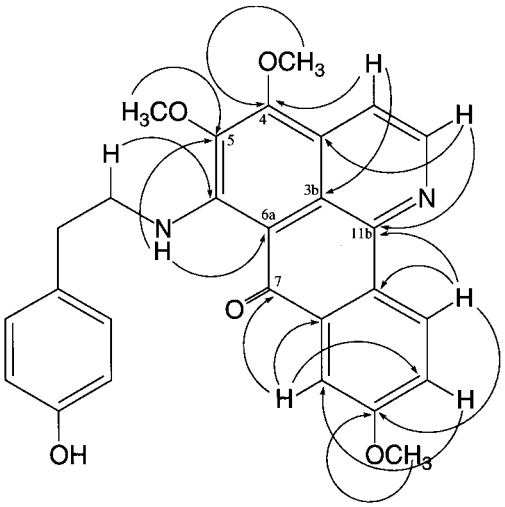

Fig. 2. Important HMBC correlations of tyraminoporphine (**2**).

2 was assigned the molecular formula $C_{27}H_{24}N_2O_5$ by HRESIMS (457.1770 for $(M+H)^+$). Mass and NMR spectral data suggested that the alkaloid is composed of tyramine and an oxoisoaporphine or an aporphine unit. The ^1H NMR spectrum displayed, besides the tyramine unit, signals for three methoxy groups at δ 3.87, 3.96 and 4.12, three aromatic protons at δ 7.44, 7.89 and 8.88, and a typical AB quartet at δ 7.88 (*d, J* = 4.9 Hz) and 8.66 (*d, J* = 4.9 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. The aromatic protons at δ 7.44 (*dd, J* = 8.8, 2.7 Hz), 7.89 (*d, J* = 2.7 Hz), and 8.88 (*d, J* = 8.8 Hz) are assignable to a 1,2,4-trisubstituted D ring system. The strong deshielding of the imine proton, which resonates at δ 12.51, by the carbonyl group at C-7, implies that the tyramine unit is attached to C-6 of the oxoisoaporphine through a C—N bond.

The ^{13}C NMR spectrum showed twenty-seven carbons consisting of three methyls, two methylenes, nine methines and thirteen quaternary carbon atoms. ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC experiments revealed that eight carbons, two methylenes, four methines and two quaternary carbon atoms are assignable to a tyramine skeleton. Important correlations revealed by HMBC are shown in Fig. 2. A connection between C-6a and C-7 is consistent with the deshielding effect of the carbonyl group at C-7 on the imine proton. In spite of several efforts including an INADEQUATE experiment, data supporting the con-

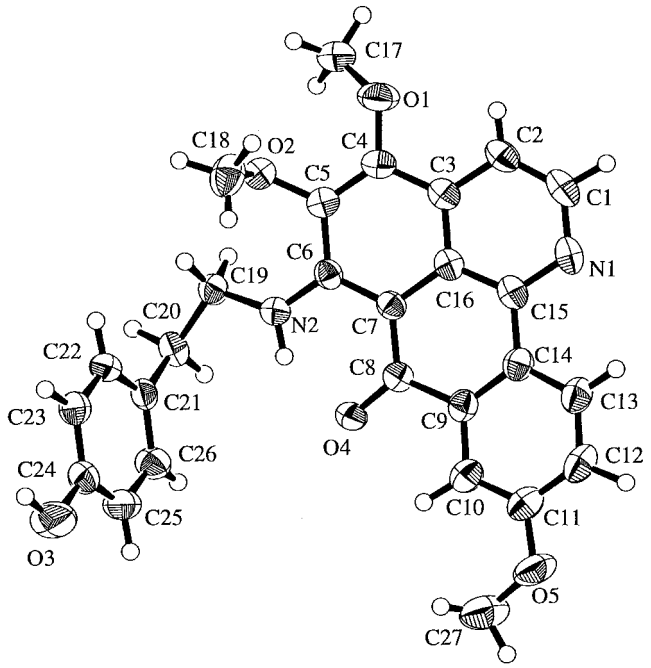

Fig. 3. Molecular structure of tyraminoporphine (**2**).

nections between C-4 and C-5, C-6a and C-3b, and C-11b and C-3b were not obtained. However, other possible connections of the carbons in question afford extraordinary skeletons composed of four, six, six and eight-membered rings or three, six, seven and nine-membered rings. Single-crystal X-ray diffraction analysis unambiguously established the molecular structure of **2** (Fig. 3). The new oxoisoaporphine alkaloid was named tyraminoporphine.

Previously, seven oxoisoaporphine alkaloids have been isolated from *M. dauricum*. Our results showed 2,3-dihydrodauriporphine (**1**) and tyraminoporphine (**2**) as two additional oxoisoaporphine-type alkaloids produced by *M. dauricum* root cultures treated with ketoconazole. However, without the P-450 inhibitor, production of alkaloids was not detected. Small modifications of the A and/or the B rings are expected to give a variety of oxoisoaporphines. It is noteworthy that a tyramine unit is attached to C-6 of the B ring in tyraminoporphine. In a separate experiment, aromoline, a bisbenzylisoquinoline alkaloid produced by *Stephania cepharantha* roots (Sugimoto, Sugimura & Yamada, 1988, 1989), also induced production of 2,3-dihydrodauriporphine (**1**) and dauriporphine (**3**) in *M. dauricum* roots. However, accumulation of tyraminoporphine (**2**) or tyramine was not observed. These findings suggest that induction of **2** is linked with tyramine accumulation. As reported previously (Sugimoto et al., 1997), an effective inhibition of oxidative coupling, by ketoconazole, in the early steps of benzylisoquinoline biosynthesis, leads to accumulation of tyramine in the roots. Excess tyramine seems to induce a biosynthetic pathway leading to production of tyraminoporphine in *M. dauricum* roots.

3. Experimental

3.1. General

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 for **1** and **2**, and in $CDCl_3$ for **3**, on a JEOL Lambda 400 spectrometer. MS spectra were obtained on a JEOL JMS-700 mass spectrometer in the EI or ESI mode. IR spectra were obtained as KBr disks on a JASCO FT/IR-8900m spectrometer.

3.2. Plant materials and culture conditions

Excised *Menispermum dauricum* roots, obtained from established cultures as described previously (Sugimoto et al., 1997), were grown in a B5 medium containing 3% sucrose and 7.5 μM NAA. The medium was further supplemented with 5 μM ketoconazole or 0.1 mM aromoline. The cultures were maintained in the dark at 27° on a rotary shaker at 70 rpm.

3.3. Extraction and isolation

Roots, from cultures supplemented with ketoconazole, were harvested, freeze-dried and powdered. The powder (20 g) was soaked overnight in MeOH and filtered. Treatment with MeOH was repeated three times and the combined filtrates were evaporated to dryness at 40°. The residue (5.75 g) was dissolved in 3% citric acid, made alkaline with NH_4OH and treated four times with $CHCl_3$. The combined $CHCl_3$ extracts were evaporated. The residue (357 mg) was chromatographed over silica gel with hexane–EtOAc. The proportion of EtOAc in the solvent system was increased stepwise. Fractions eluted with hexane–EtOAc (4:1) yielded 12 mg of a yellow powder, which gave compound **3** as yellow fibers on recrystallization from hexane–EtOAc. Fractions eluted with hexane–EtOAc (3:1) gave 20 mg of a yellow powder. Recrystallization of the powder from hexane–EtOAc gave compound **1** as yellow needles. Fractions eluted with hexane–EtOAc (2:1) yielded 21 mg of an orange powder which gave compound **2** as orange prisms on recrystallization from hexane–EtOAc.

Roots, treated with aromoline, were harvested and alkaloids were extracted and purified as mentioned previously. A 100 g dry root sample yielded 11 mg of compound **3** and 22 mg of compound **1**.

3.3.1. 2,3-Dihydrodauriporphine (*1*)

Mp 141.0–142.0°; UV λ_{max} nm (logϵ): 219.0 (4.35), 271.5 (4.44), 329.5 (3.78); HRESIMS: m/z $[MH]^+$

Table 1
^{1}H NMR (400 MHz) spectral data of 2,3-dihydrodauriporphine (**1**), dauriporphine (**3**) and tyraminoporphine (**2**)

Position	**1** (DMSO-d_6)			**3** ($CDCl_3$)			**2** (DMSO-d_6})		
	δ (ppm)	m	J (Hz)	δ (ppm)	m	J (Hz)	δ (ppm)	m	J (Hz)
2	3.96	*t*	7.9	8.68	*d*	5.6	8.66	*d*	4.9
3	2.77	*t*	7.9	7.94	*d*	5.6	7.88	*d*	4.9
8	7.52	*d*	2.7	7.87	*d*	2.7	7.89	*d*	2.7
10	7.30	*dd*	2.7, 8.8	7.32	*dd*	2.7, 8.8	7.44	*dd*	2.7, 8.8
11	8.19	*d*	8.8	8.80	*d*	8.8	8.88	*d*	8.8
4-OMe	3.92	*s*		4.27	*s*		4.12	*s*	
5-OMe	3.91[a]	*s*		4.05[a]	*s*		3.87	*s*	
6-OMe	3.84[a]	*s*		4.17[a]	*s*				
9-OMe	3.89	*s*		3.98	*s*		3.96	*s*	
1′							12.51	*br s*	
2′							4.08	*m*	
3′							2.94	*t*	7.3
5′							7.14	*d*	8.7
6′							6.69	*d*	8.7
8′							6.69	*d*	8.7
9′							7.14	*d*	8.7

354.1341 [$C_{20}H_{20}NO_5$ requires 354.1341]; EIMS: *m*/*z* (rel. int.) 353 (100), 338 (75.0), 322 (22.9), 294 (16.6); IR ν_{max} cm^{-1}: 1663, 1605, 1559, 1464, 1347, 1280, 1124, 1027; ^{1}H and ^{13}C NMR spectral data are shown in Tables 1 and 2, respectively.

3.3.2. Tyraminoporphine (*2*)

Mp 229° (dec); UV λ_{max} nm (logϵ): 216.5 (4.28), 263.0 (4.33) 361.5 (3.58), 450.5 (3.86), 479.0 (3.99); HRESIMS: *m*/*z* $[MH]^+$ 457.1770 [$C_{27}H_{25}N_2O_5$ requires 457.1763]; EIMS: *m*/*z* (rel. int.) 456 (22.7), 349 (100), 319 (8.5), 256 (11.8); IR ν_{max} cm^{-1}: 1610, 1569, 1516, 1451, 1374, 1291, 1267, 1147; ^{1}H and ^{13}C NMR spectral data are shown in Tables 1 and 2, respectively.

3.3.3. Dauriporphine (*3*)

Mp 167.0–167.5°. HRESIMS: *m*/*z* $[MH]^+$ 352.1171 [$C_{20}H_{18}NO_5$ requires 352.1185]; EIMS, UV and IR spectra matched literature values (Takani et al., 1983). ^{1}H and ^{13}C NMR spectral data are shown in Tables 1 and 2, respectively.

3.4. Identification of the dehydrogenated product of compound *1* as dauriporphine

Compound **1** was treated with an equivalent of CrO_3 in acetic acid at room temperature for 30 min. The reaction mixture was made alkaline with NH_4OH and the alkaloids were extracted with EtOAc. The extract was analyzed for alkaloids by HPLC. The stationary phase was Develosil ODS-3 (4.6 × 150 mm) and the solvent was 90% MeOH with 0.2% NH_4OH. The flow rate was 0.3 ml/min. A short pre-column (4.6 × 30 mm) was placed between the injector and the separation column. Rts of compound **1** and dauriporphine were 13.4 and 17.6 min, respectively. Further stirring did not increase the dehydrogenated product. Additional CrO_3 decomposed both the starting material and the product.

Table 2
^{13}C NMR (100 MHz) spectral data of 2,3-dihydrodauriporphine (**1**), dauriporphine (**3**) and tyraminoporphine (**2**)

Position	**1** (DMSO-d_6)		**3** ($CDCl_3$)		**2** (DMSO-d_6)	
	δ (ppm)	m[a]	δ (ppm)	m	δ (ppm)	m
2	46.4	*t*	143.1	*d*	141.4	*d*
3	18.5	*t*	114.5	*d*	114.4	*d*
3a	125.6	*s*	128.5	*s*	125.5	*s*
3b	122.3	*s*	120.1	*s*	119.2	*s*
4	154.4	*s*	153.1	*s*	153.1	*s*
5	149.1[b]	*s*	146.3[b]	*s*	142.3	*s*
6	153.1[b]	*s*	160.9[b]	*s*	153.4	*s*
6a	119.4	*s*	116.5	*s*	103.0	*s*
7	181.3	*s*	181.5	*s*	179.3	*s*
7a	134.1	*s*	135.0	*s*	128.3	*s*
8	108.5	*d*	108.8	*d*	107.3	*d*
9	161.3	*s*	161.4	*s*	160.3	*s*
10	120.6	*d*	121.6	*d*	120.4	*d*
11	126.2	*d*	126.9	*d*	126.3	*d*
11a	127.9	*s*	129.5	*s*	134.0	*s*
11b	153.4	*s*	147.4	*s*	141.8	*s*
4-OMe	60.9	*q*	61.8	*q*	61.3	*q*
5-OMe	61.2[c]	*q*	61.8	*q*	61.0	*q*
6-OMe	61.4[c]	*q*	61.8	*q*		
9-OMe	55.5	*q*	55.7	*q*	55.4	*q*
2′					47.4	*t*
3′					39.0	*t*
4′					128.7	*s*
5′					129.7	*d*
6′					115.1	*d*
7′					155.8	*s*
8′					115.1	*d*
9′					129.7	*d*

[a] Peak multiplicities were confirmed by DEPT spectra.
[b,c] Assignments may be interchanged within the same column.

The dehydrogenated product was purified by prep. HPLC. The stationary phase was Capcell Pak C_{18} (20 × 250 mm) and the solvent was 90% MeOH with 0.2% NH_4OH. The flow rate was 8 ml/min. A short pre-column (4.6 × 10 mm) was placed between the injector and the separation column. Rts of compound **1** and dauriporphine were 10.1 and 12.7 min, respectively. ^{1}H-NMR and MS spectra of the dehydrogenated product matched those of authentic dauriporphine (Takani et al., 1983).

3.5. X-ray crystallographic analyses of 2,3-dihydrodauriporphine (*1*) and tyraminoporphine (*2*)

(**1**) A yellow needle crystal of $C_{20}H_{19}NO_5$ having

appropriate dimensions of $0.62 \times 0.10 \times 0.10$ mm, Mr 353.37, orthorhombic, $a = 18.377$ (9), $b = 19.066$ (7), $c = 4.879$ (4) Å, $V = 1709$ (1) Å^3, space group $P2_12_12_1$ (No. 19), $Z = 4$, $D_c = 1.373$ g cm^{-3}, μ(Mo Kα) = 0.99 cm^{-1}, $F_{000} = 744$. (**2**) An orange prismatic crystal of $C_{27}H_{24}N_2O_5$ having appropriate dimensions of $0.28 \times 0.20 \times 0.04$ mm, Mr 456.50, monoclinic, $a = 10.970$ (5), $b = 10.565$ (5), $c = 19.047$ (4) Å, $V = 2210$ (1) Å^3, space group $P2_1/c$ (No. 14), $Z = 4$, $D_c = 1.372$ g cm^{-3}, μ(Mo Kα) = 0.95 cm^{-1}, $F_{000} = 960$. The data were collected on a Rigaku AFC-7R four-circle diffractometer with graphite monochromated MoKα radiation and a 18 kW rotating anode generator. The structures were solved by direct methods (SIR92) (Altomare et al., 1994) and expanded using Fourier techniques (Beurskens et al., 1994). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1019 observed reflections ($I > 2.00\ (\sigma)$) and 236 variable parameters for **1** and on 1891 observed reflections ($I > 2.00\ (\sigma)$) and 308 variable parameters for **2**, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation. All data have been deposited at the Cambridge Crystallographic Data Center.

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