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Isoquinoline and isoindole alkaloids from *Menispermum dauricum*

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Abstract

Three isoquinoline alkaloids and an isoindole alkaloid, along with eight known compounds, were isolated from the roots of *Menispermum dauricum* (Menispermacese). The alkaloids were characterized as 7-hydroxy-6-methoxy-1(2*H*)-isoquinolinone, 6,7-dimethoxy-*N*-methyl-3,4-dioxo-1(2*H*)-isoquinolinone, 1-(4-hydroxybenzoyl)-7-hydroxy-6-methoxy-isoquinoline and 6-hydroxy-5-methoxy-*N*-methylphthalimide, on the basis of spectral evidence including 1D- and 2D-NMR and MS analyses.

Keywords: *Menispermum dauricum*; Menispermaceae; Isoquinoline; Isoindole; Alkaloids

1. Introduction

The roots of *Menispermum dauricum* DC. (Menispermaceae), known in Chinese medicine as Bei-Dou-Gen, are used for treating sore throats, colitis, dysentery, and rheumatic arthralgia (Anon., 1977). Previous studies on this plant have shown the presence of alkaloids belonging to various classes such as bisbenzylisoquinoline, aporphine, proporphine, protoberberine and oxoisoaporphine (Tomita and Okamoto, 1965; Tomita et al., 1971; Okamoto et al., 1971; Kunitomo et al., 1983; Hou and Xue, 1985; Kunitomo and Miyata, 1986; Zhao et al., 1989; Pang et al., 1991; Hu et al., 1993; Pan et al., 1999; Sugimoto et al., 1999; Yu et al., 2002). In particular, the natural occurrence of oxoisoaporphine alkaloids is apparently restricted to this plant species (Sugimoto et al., 1999; Yu et al., 2001), with the exception of lakshminine found in *Sciadoternia toxifera* (Killmer et al., 2003). We now report the isolation of twelve compounds from this plant species including seven simple isoquinolines, one benzylisoquinoline, two isoindoles, and two phenolic derivatives.

2. Results and discussion

The alkaloidal fraction of the roots was subjected to silica gel and Sephadex LH-20 chromatographic steps to afford 12 compounds. Preliminary results on the identification of five quinolones, namely, northalifoline (Chou et al., 1994), thalifoline, corydaldine, *N*-methylcorydaldine, and doryphornine (Krane and Shamma, 1982), were reported by Zhang et al. (2001). In addition, 5,6-dimethoxy-*N*-methylphthalimide (Chen et al., 1996), salicylic acid, and protocatechualdehyde (Chen et al. 1981) were obtained during the course of isolation. The structures of these isolates were determined by comparison of the spectral and physical data with those reported in literature.

N-demethyldoryphornine (**1**), obtained as colorless rods from methanol, mp 257–259 °C, displayed in its CI–MS a quasimolecular ion peak $[M+H]^+$ at *m*/*z* 192, consistent with a molecular formula $C_{10}H_9NO_3$ ($[M]^+ = 191$). The IR spectrum showed absorption bands for an hydroxyl group (3139 cm^{-1}) and an δ-lactam ring (1634 and 1507 cm^{-1}), whereas the UV spectrum exhibited an absorption maximum at 243 nm. These data suggested an isoquinolone structure (Zhang et al., 2001). The ^1H NMR spectrum revealed the presence of methoxyl (δ 3.85, s) and hydroxyl (δ 10.91, br s) groups, as well as two aromatic singlets (δ 7.08 and 7.47, s) and a pair of doublets (δ 6.95 and 6.42, d, $J = 6.8$ Hz).

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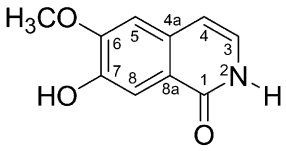

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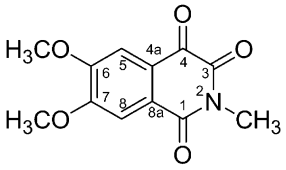

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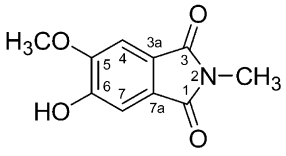

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Chart 1.

A combination of ^{13}C DEPT, ^{13}C–^{1}H COSY and HMBC spectroscopic data led to the assignment of the ^{1}H and ^{13}C NMR signals of **1** (Table 1). The locations of the hydroxyl and methoxyl groups were determined by an NOE experiment. Thus, when the methoxyl signal at δ 3.85 was irradiated, an enhancement (5.3%) of the H-5 signal (δ 7.08) was observed. The methoxyl group was therefore assigned to C-6 and hence the hydroxyl group to C-7. All available evidence suggested that **1** is a new alkaloid possessing the structure 7-hydroxy-6-methoxy-1(2*H*)-isoquinolinone (Chart 1).

The EI-MS of **2** ([M^+] at *m*/*z* 249) was consistent with the molecular formula $C_{12}H_{11}NO_5$. The UV absorption maxima at 211 and 256 nm, and a broad IR absorption band centered at 3468 cm^{-1} (OH stretching), as well as two strong and sharp bands at 1693 and 1669 cm^{-1}, were characteristic for a dioxo structure (Achenbach et al., 1991). The ^{1}H NMR spectrum of **2** displayed resonance of an *N*-methyl group, two methoxyl groups, and two *para*-coupled aromatic protons (Table 1). From the NMR spectra, it was evident that the alkaloid contained two additional carbonyls and one less double bond than in *N*-methylcorydaldine. Comparison of the ^{13}C NMR spectral data of **2** with those of 1-desmethoxy-4,5-dioxodehydroasimilobine (Achenbach et al., 1991), particularly the carbon signals of the dioxo part of ring B, led to the assignment of two carbonyls on C-3 and C-4. The HMBC results (Table 1) supported such a structural assignment. Compound **2** was therefore elucidated as 6,7-dimethoxy-*N*-methyl-3,4-dioxo-1(2*H*)-isoquinolinone (Chart 1).

Table 1
Spectroscopic NMR data and HMBC correlations of **1–4** (ppm, *J* in Hz)[a]

	1[b]			**2**[c]			**3**[d]			**4**[c]		
Position	^{1}H	^{13}C	HMBC	^{1}H	^{13}C	HMBC	^{1}H	^{13}C	NMBC	^{1}H	^{13}C	HMBC
1	–	165.4	–	–	162.3	–	–	154.1	–	–	169.0	–
3	6.95 (*d*, 7)	161.2	–	–	157.8	–	8.26 (*d*, 5.6)	138.9	C-1, 4, 4a	–	169.0	–
3a	–	–	–	–	–	–	–	–	–	–	125.8	–
4	6.42 (*d*, 7)	104.8	C-8a	–	173.2	–	7.77 (d, 5.6)	122.1	C-5, 8a	7.30 (*s*)	105.7	C-3, 3a, 6
4a	–	132.2	–	–	124.8	–	–	134.6	–	–	–	–
5	7.08 (*s*)	107.0	C-4, 6, 7, 8a	7.59 (*s*)	108.3	C-4, 4a, 7	7.36 (*s*)	105.8	C-4, 6, 8a	–	152.0	–
6	–	152.5	–	–	153.8	–	–	149.8	–	–	151.6	–
7	–	146.3	–	–	155.6	–	–	155.1	–	7.25 (*s*)	109.7	C-1, 5, 7a
7a	–	–	–	–	–	–	–	–	–	–	124.2	–
8	7.47 (*s*)	110.6	C-4a, 6, 7	7.73 (*s*)	110.5	C-1, 6, 8a	7.22 (*s*)	107.9	C-4a, 7	–	–	–
8a	–	120.0	–	–	125.0	–	–	123.3	–	–	–	–
9	–	–	–	–	–	–	–	195.0	–	–	–	–
10	–	–	–	–	–	–	–	128.9	–	–	–	–
11/15	–	–	–	–	–	–	7.73 (*d*, 9)	133.9	C-9, 13	–	–	–
12/14	–	–	–	–	–	–	6.86 (*d*, 9)	116.1	C-10	–	–	–
13	–	–	–	–	–	–	–	164.2	–	–	–	–
NH	9.96 (*br s*)	–	–	–	–	–	–	–	–	–	–	–
NCH_3	–	–	–	3.46 (*s*)	27.4	–	–	–	–	3.12 (*s*)	23.9	C-1, 3
5-OCH_3	–	–	–	–	–	–	–	–	–	4.00 (*s*)	56.4	C-5
6-OCH_3	3.85 (*s*)	55.8	C-6	4.05 (*s*)	56.7	C-6	4.06 (*s*)	56.3	C-6	–	–	–
7-OH	10.91 (*br s*)	–	–	–	–	–	–	–	–	–	–	–
7-OCH_3	–	–	–	4.09 (*s*)	56.8	C-7	–	–	–	–	–	–

[a] Assignments were made with the aid of DEPT, ^{13}C-^{1}H COSY, HMBC, and NOE spectra.
[b] Recorded in DMSO-d_6.
[c] Recorded in $CDCl_3$–CD_3OD (1:1).
[d] Recorded in CD_3OD.

Alkaloid **3**, obtained as red needles from MeOH, gave a positive $FeCl_3$ test. The EI–MS result ([M^+] at *m/z* 295) and ^{13}C NMR spectral data were consistent with the molecular formula $C_{17}H_{13}NO_4$, and its IR and UV spectra suggested an oxobenzylisoquinoline structure (Baser, 1982). From the DEPT spectrum, it was apparent that the molecule contained a methoxyl group, eight aromatic methines, seven aromatic quaternary carbons, and a carbonyl group. The ^1H NMR spectrum of **3** further revealed the presence of a methoxyl (δ 4.06, 3H, s), two *para*-coupled aromatic protons [δ 7.36 (s) and 7.22 (s)], a pair of *ortho*-coupled protons of an isoquinoline ring [δ 8.26 and 7.77 (each 1H, d, $J = 5.6$ Hz)], as well as an AA′BB′ system of a *para*-substituted benzoyl moiety [δ 7.73 and 6.86 (each 2H, d, $J = 8.8$ Hz)]. All ^1H and ^{13}C NMR spectral signals could be assigned with the aid of DEPT, ^{13}C-^1H COSY and HMBC analyses as shown in Table 1. The location of the methoxyl group was determined by an NOE experiment (Fig. 1), in which irradiation of the methoxyl signal (δ 4.06) resulted in an enhancement (7.6%) of H-5. Hence, alkaloid **3** was established to be 1-(4-hydroxybenzoyl)-7-hydroxy-6-methoxy-isoquinoline.

Alkaloid **4** was isolated as pale yellow needles from $CHCl_3$. The EI–MS exhibited a [M^+] at *m/z* 207, consistent with the molecular formula $C_{10}H_9NO_4$. The UV spectrum of **4** revealed absorptions at 216, 240, and 298 nm, characteristic of an isoindole 1,3-dione skeleton (Chen et al., 1996). The IR spectrum exhibited absorption bands of hydroxyl (3290 cm^{-1}) and amidocarbonyl groups (1710 and 1685 cm^{-1}). A comparison of the NMR spectroscopic data of **4** (Table 1) with those of 5,6-dimethoxy-*N*-methylphthalimide (Chen et al., 1996) suggested that the former contained only one methoxyl group. The location of the methoxyl group was then deduced from the NOE data. Thus, on irradiation of the methoxyl signal at δ 4.00, an enhancement of H-4 (δ 7.30) was observed. Taking all evidence together with the HMBC data, the methoxyl group was assigned to C-5 and the hydroxyl group to C-6. Hence, compound **4** was determined to be 6-hydroxy-5-methoxy-*N*-methylphthalimide (Chart 1).

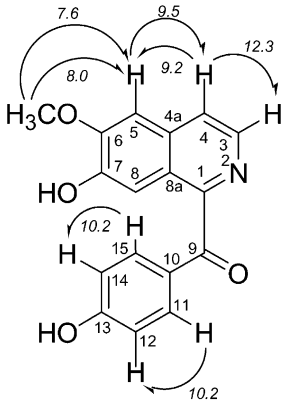

Fig. 1. The structure and NOE results (%) of **3**.

3. Experimental

3.1. General

Mps are uncorrected. UV spectra were recorded on a Milton Roy Spectrophotometer Array 3000 spectrometer. IR spectra were measured on a Perkin-Elmer 16 PC FT-IR spectrometer. MS (CI or EI mode) were recorded on a Finnigan MATTSQ 7000 spectrometer. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz), and 2D NMR spectra were determined on a Jeol JNM-EX 400 spectrometer. Column chromatography (CC) was carried out with silica gel (200–400 mesh, Merck) and Sephadex LH-20 (Merck). TLC was conducted on silica gel 60 F_{254} and RP-18 F_{254} S (Merck).

3.2. Plant materials

The roots of *M. dauricum* were collected in Nanlin County, Anhui Province, China in April, 1995. The plant material was identified by Dr. Min-Jian Qin, and a voucher specimen (no. 950414) has been deposited in the Herbarium of China Pharmaceutical University.

3.3. Extraction and isolation

The roots of *M. dauricum* (5.0 kg) were extracted with 3% aqueous HCl (10.0 l×3) at room temperature. The combined solution was adjusted to pH 9 with 20% aqueous NH_3, filtered, and extracted with hexane (800 ml×5). The combined organic phase was concentrated at reduced pressure to afford a residue (162.5 g). The residue was redissolved in 1% aqueous HCl solution (1400 ml), filtered, adjusted to pH 9 with 20% aqueous NH_3, and extracted with Et_2O (600 ml×5). Evaporation of the Et_2O yielded a dark brown residue (72.0 g), which was submitted to column chromatography on silica gel (ca. 1500 g) eluted with 0–30% MeOH in $CHCl_3$ gradient mixtures. Fractions were combined on the basis of TLC behavior. Fractions 16–30 were combined (4.3 g) and applied to silica gel (ca. 300 g) using $CHCl_3$–CH_3COCH_3–CH_3OH (98:1:1–90:5:5, gradient) as eluting solvent to afford thalifoline (60 mg), corydaldine (60 mg), *N*-methylcorydaldine (105 mg), and **4** (32 mg). Fractions 90–131 (7.9 g) were combined and separated using the same conditions as above to afford northalifoline (37 mg), doryphorine (30 mg), 5,6-dimethoxy-*N*-methylphthalimide (23 mg), salicylic acid (12 mg), and protocatechualdehyde (4 mg), and **1** (23 mg). Fractions 132–140 and 154–160 were separated by Sephadex LH-20 using MeOH as solvent to afford **2** (28 mg) and **3** (59 mg), respectively.

3.3.1. 7-Hydroxy-6-methoxy-1(2H)-isoquinolinone (1)

Colorless rods (MeOH), mp 257–259 °C; UV λ_{max} nm (log ϵ): 243 (3.24), 279 (1.14), 290 (1.17), 325 (0.83); IR

(KBr) ν_{max} cm^{-1}: 3139, 2963, 2156, 1634, 1605, 1507, 1470, 1280, 1198, 1065, 843; CIMS: *m/z* 192 $[M+1]^+$, 154; Anal. calcd for $C_{10}H_9NO_3$: C, 62.81; H, 4.75; N, 7.33. Found: C, 62.90; H, 4.72; N, 7.26. For ^1H and ^{13}C NMR spectral data, see Table 1.

3.3.2. 3,4-Dioxo-6,7-dimethoxy-N-methyl-1(2H)-isoquinolinone (2)

Yellow powder, mp 185–189 °C; UV λ_{max} nm (log ϵ): 211 (1.85), 228 (1.38), 256 (1.30), 363 (0.26); IR (KBr) ν_{max} cm^{-1}: 3468, 1724, 1693, 1669, 1585, 1517, 1412, 1429, 1325, 1231, 1012, 887; EIMS: *m/z* 249 $[M]^+$, 221 $[M^+–CO]$, 177, 164, 136, 121, 93; Anal. calcd for $C_{12}H_{11}NO_5$: C, 57.82; H, 4.45; N, 5.62. Found: C, 57.79; H, 4.51; N, 5.54. For ^1H and ^{13}C NMR data, see Table 1.

3.3.3. 1-(4-Hydroxybenzoyl)-7-hydroxy-6-methoxy-isoquinoline (3)

Red needles, mp 230–232 °C; UV λ_{max} nm (log ϵ): 233, 252, 276, 297, 342; IR (KBr) ν_{max} cm^{-1}: 3268, 3066, 1660, 1643, 1598, 1505, 1482, 1284, 1158, 858, 761; EIMS: *m/z* 295 $[M]^+$, 278 $[M^+–OH]$, 266 $[M^+–OH–H_2O]$, 251, 121; Anal. calcd for $C_{17}H_{13}NO_4$: C, 69.13; H, 4.44; N, 4.75. Found: C, 69.18; H, 4.48; N, 4.68. For ^1H and ^{13}C NMR spectral data, see Table 1.

3.3.4. 6-Hydroxy-5-methoxy-N-methylphthalimide (4)

Light yellow needles ($CHCl_3$); mp 213–215 °C UV λ_{max} nm (log ϵ): 216 (3.04), 240 (3.49), 298 (0.62), 349 (0.54); IR (KBr) ν_{max} cm^{-1}: 3293, 3088, 2946, 1758, 1710, 1685, 1602, 1432, 1379, 1017, 744; EIMS: *m/z* 207 $[M]^+$, 192 $[M^+–Me]$, 179 $[M^+–CO]$, 163, 150, 136, 122, 107; Anal. calcd for $C_{10}H_9NO_4$: C, 57.96; H, 4.38; N, 6.76. Found: C, 57.90; H, 4.46; N, 6.69; for ^1H and ^{13}C NMR spectral data, see Table 1.

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