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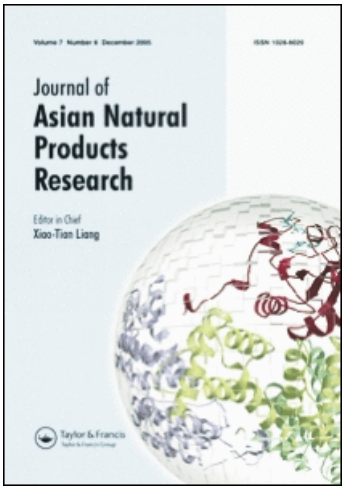

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Chemical constituents of the stems of *Ephedra sinica*

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Chemical constituents of the stems of *Ephedra sinica*

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A new naphthalene derivative, 1-methyl-2,3-methylenedioxy-6-naphthalenecarboxylic acid methyl ester (**1**), and a new alkaloid, (±)-1-phenyl-2-imido-1-propanol (**2**), together with the four known compounds, ephedrine, pseudoephedrine, *N*-methylephedrine, and 6-methoxykynurenic acid, have been isolated from the stems of *Ephedra sinica*, a famous traditional Chinese herbal medicine. The structures of **1** and **2** were determined by spectroscopic methods, including 1D- and 2D-NMR experiments as well as HR-EI-MS analysis.

Keywords: *Ephedra sinica*; Ephedraceae; naphthalene derivative; phenylalkylamine alkaloid

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1. Introduction

Many species of the genus *Ephedra* are characterized by the presence of (−)-ephedrine and related alkaloids [1]. *Ephedra sinica* Stapf., distributed mainly in the northeast and northwest of China, has been used as a Chinese folk medicine for a long time, and its stems have been used to treat rheum, asthma, edema, and cough with dyspnea [2]. It also provides extracts used in folk medicine as depurative, hypotensive, antiasthmatic, sympathomimetic, and astringent agents [3]. As part of an ongoing study on the chemical constituents from famous Chinese herbal medicine, we report herein a new naphthalene derivative and a new phenylalkylamine alkaloid from the stems of the titled plant, along with the four compounds of the previously known structures.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder ($CHCl_3$). The HR-EI-MS of **1** showed a molecular ion peak at *m/z* 244.0730 $[M]^+$, corresponding to the molecular formula $C_{14}H_{12}O_4$. The characteristic UV absorptions at λ_{max} 341, 302, 291, 255, 248, and 210 nm, indicating the presence of the naphthalene moiety. The IR absorption bands at 2955, 2918, 2851, 1717, 1617, and 1465 cm^{-1} suggested the presence of methyl, carboxyl, and aromatic ring in the molecule. The EI-MS data of the base peak at *m/z* 244 $[M]^+$ and another strong peak at *m/z* 213 $[M-31]^+$ indicated that **1** contained one methoxyl. The ^{13}C and DEPT NMR spectra showed that the structure of **1** consisted of one methyl, one methoxyl, one methylenedioxyl, four sp^2-hybridized methines, one carbonyl carbon, and six sp^2-hybridized quaternary carbons. These findings were consistent with the ^1H NMR information of suggesting the presence of three aromatic protons from the signals of an ABX coupling system at δ_H 7.83 (1H, d, $J = 9.0$ Hz), 7.96 (1H, d, $J = 9.0$ Hz), and 8.41 (1H, s) as well as one aromatic proton singlet at δ_H 7.10 (1H, s). In addition, the ^1H NMR spectrum also exhibited one methyl signal

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at δ_H 2.57 (3H, s) and one methoxy signal at δ_H 3.97 (3H, s), as well as a methylenedioxyl signal at δ_H 6.08 (2H, s). With the aid of HSQC experiment, the HMBC spectrum was used to establish the connections of the above partial structures, which showed cross-peaks of the methylenedioxyl protons with C-2 and C-3, the methoxyl protons with 6-C═O, as well as the methyl protons with C-1, C-2, and C-1a. This information further revealed a methylenedioxyl attaching to a naphthalene and a carboxylic acid methyl ester located at C-6, as well as the methyl group was assigned to C-1 in the molecule (Figure 1). The above inferences were also supported by the NOE difference spectra, which gave NOE enhancements to H-8 (δ_H 7.83) as irradiating CH_3-1 (δ_H 2.57). Thus, compound **1** was determined as 1-methyl-2,3-methylenedioxy-6-naphthalenecarboxylic acid methyl ester.

Compound **2**, colorless needles, was assigned the molecular formula $C_9H_{11}NO$ by HR-EI-MS $[M]^+$ at *m/z* 149.0229, along with the ^1H and ^{13}C NMR spectra. The IR absorption bands at 3281, 3033, 2927, 1662, 1602, and 1478 cm^{-1} indicated the presence of hydroxyl, imidogen, methyl, and benzene ring in the molecule. Except for a single-substituted benzene ring moiety exhibited by the typical signals in the ^1H and ^{13}C NMR spectra of **2** (Table 2), the signals of one tertiary methyl, one methine bearing an oxygen atom, and one sp^2-hybridized quaternary carbon as well as two other proton signals were observed in the ^1H and ^{13}C NMR spectra. The disappearance of the signals at δ_H 5.80 (1H, d, $J = 4.0$ Hz) and 10.59 (1H, s) in the D_2O exchange experiment of ^1H NMR, combined with the molecular formula, suggested that **2** possessed a hydroxyl and an imido group. The HMBC spectra showed long-range correlations between H-1 and C-2, C-3, C-1′, C-2′, and C-6′; H-3 and C-1, C-2, and 1-OH; and C-1, C-2 and C-1′, 2-NH, and C-2 allowed the assignments of the whole structure of **2**. Optical rotation of **2** was obtained as zero and no signal appeared on ORD spectra. Therefore, the structure of **2** was a racemate and deduced to be (±)-1-phenyl-2-imido-1-propanol.

Four known compounds, ephedrine [4,5], pseudoephedrine [5], *N*-methylephedrine [5], and 6-methoxykynurenic acid [6], were isolated. Their structures were identified by the comparison of the spectroscopic data (^1H NMR, ^{13}C NMR, and MS) with those described in the literature.

3. Experimental

3.1 General experimental procedures

Melting point was detected on a Boetius apparatus and is uncorrected. Optical rotation was obtained in MeOH at 20°C, using a Perkin-Elmer polarimeter. ORD was taken on a JASCO-815 polarimeter. The UV spectra were determined on a JASCO V-650 spectrophotometer and IR spectra were measured on a Nicolet 5700 spectrophotometer. The 1D- and 2D-NMR were recorded on Mercury 300 and 400 spectrometer, respectively, using DMSO-d_6 and $CDCl_3$

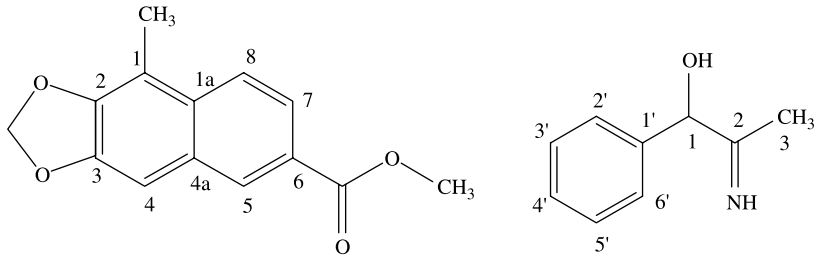

Figure 1. Structures of compounds **1** and **2**.

as solvent. MS measurements were carried out on a VGZAB-2F spectrometer. Column chromatography was carried out on silica gel (200–300 mesh), while preparative TLC was accomplished on silica gel GF_{254}. Silica gel for TLC and column chromatography was obtained from Qingdao Marine Chemical Inc., Qingdao, China.

3.2 Plant material

The stems of *E. sinica* Stapf. were collected in March 2007 at Bo-Zhou market, An-Hui Province, China, and identified by Prof. Lin Ma, Institute of Materia Medica, Chinese Academy of Medical Sciences. A voucher specimen (No. 337-12) is deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

The dried stems of *E. sinica* (30 kg) were exhaustively extracted with 95% EtOH (3×) to give a crude extract (3.5 kg). The whole extract was suspended in 60% EtOH and extracted several times with petroleum. The EtOH was then removed under reduced pressure, and the resultant residue (1700 g) was dissolved in water. The aqueous layer was then extracted with EtOAc and *n*-BuOH, successively. The EtOAc residue (85 g) was fractionated over a silica-gel column, eluted with $CHCl_3$—MeOH of increasing polarity (from $CHCl_3$ to $CHCl_3$—MeOH 1:1), to give 143 fractions (each 500 ml). Fractions 1–26 were then combined to give fraction A. Fraction A was further separated by column chromatography over silica gel, eluting with petroleum–EtOAc (from 50:1 to 1:1). Fraction 4 of A gave compound **1** (3 mg). The *n*-BuOH residue (67 g) was subjected to chromatography over silica gel and eluted with $CHCl_3$—MeOH of increasing polarity (from $CHCl_3$ to $CHCl_3$—MeOH 1:1) to afford 61 fractions (each 500 ml). Fractions 1–4, 5–10, and 11–19 were then combined to obtain fractions B, C, and D, respectively. Fractions B, C, and D were subsequently subjected to column chromatography on silica gel, eluting with petroleum–EtOAc (from 7:3 to 1:1) to B and C, with $CHCl_3$—MeOH (from 100:1 to 1:1) to D. Fraction 15 of B gave pseudoephedrine (7 mg). Fractions 4 and 17 of C yield compound **2** (20 mg) and *N*-methylephedrine (7 mg), respectively. Fractions 6–18 and 30–31 of D gave ephedrine (3 g) and 6-methoxykynurenic acid (10 mg), respectively.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for compound **1** (in $CDCl_3$).

	1		
Position	δ_C (DEPT)	δ_H	HMBC correlations
1	111.5 s		
2	147.4 s		
3	147.4 s		
4	103.0 d	7.10 s	C-2, C-5, C-1a, C-4a
5	130.2 d	8.41 s	C-4, C-7, C-1a, 6-C═O
6	125.6 s		
7	123.9 d	7.96 d (9.0)	C-5, C-1a, 6-C═O
8	123.2 d	7.83 d (9.0)	C-1, C-6, C-4a
1a	133.0 s		
4a	129.9 s		
1-CH_3	10.9 q	2.57 s	C-1, C-2, C-1a
OCH_2O	101.1 t	6.08 s	C-2, C-3
6-C═O	167.5 s		
OCH_3	52.1 q	3.97 s	6-C═O

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Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for compound **2** (in DMSO-d_6).

	2		
Position	δ_C (DEPT)	δ_H	HMBC correlations
1	73.5 d	5.15 d (4.0)	C-2, C-3, C-1′, C-2′, C-6′
2	157.5 s		
3	8.9 q	1.54 s	C-1, C-2
1′	141.9 s		
2′, 6′	125.6 d	7.23–7.33 m	C-1, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′
3′, 5′	128.0 d		
4′	127.0 d		
1-OH		5.80 d (4.0)	C-1, C-2, C-1′
2-NH		10.59 s	C-2

*3.3.1 1-Methyl-2,3-methylenedioxy-6-naphthalenecarboxylic acid methyl ester (**1**)*

White amorphous powder ($CHCl_3$); IR (KBr) ν_{max} (cm^{-1}): 3293, 2955, 2918, 2851, 1717, 1617, 1465, 1439, 1293, 1259, 1244, 1094, 1024, 951, 821, and 771; ^{1}H and ^{13}C NMR spectral data, see Table 1; EI-MS *m/z*: 244 $[M]^+$ (100), 213 (82), 127 (27), and 83 (22); HR-EI-MS *m/z*: 244.0730 $[M]^+$ (calcd for $C_{14}H_{12}O_4$, 244.0736).

*3.3.2 1-Phenyl-2-imido-1-propanol (**2**)*

Colorless needles (MeOH); mp 111–112°C; $[\alpha]_D^{25}$ 0 ($c = 0.4$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3281, 3033, 2927, 1662, 1602, 1478, 1011, 959, 845, and 698; ^{1}H and ^{13}C NMR spectral data, see Table 2; EI-MS *m/z*: 149 $[M]^+$ (15), 122 (25), 105 (100), 91 (25), and 77 (63); HR-EI-MS *m/z*: 149.0229 $[M]^+$ (calcd for $C_9H_{11}NO$, 149.0841).

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References

[1] S.W. Pelletier, *Chemistry of the Alkaloids* (Van Nostrand, New York, 1970), p. 24.
[2] P.G. Xiao, *Modern Chinese Material Media* (Chemical Industry Press, Beijing, 2001), Vol. 3, p. 297.
[3] M.A.M. Nawwar, H.I. El-Sissi, and H.H. Barakat, *Phytochemistry* **23**, 2937 (1984).
[4] H. Oshio, M. Tsukui, and T. Matsuoka, *Chem. Pharm. Bull.* **26**, 2096 (1978).
[5] H.K. Kim, Y.H. Choi, W.T. Chang, and R. Verpoorte, *Chem. Pharm. Bull.* **51**, 1382 (2003).
[6] A.N. Starratt and S. Caveney, *Phytochemistry* **42**, 1477 (1996).