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EXPERIMENTAL RESEARCH

Ephedrate A, a new phenol compound from the root of *Ephedra sinica*

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[Abstract] **Objective** To study the chemical constituents of the roots of *Ephedra sinica* Stapf. **Methods** The roots of *E. sinica* were extracted with 90% ethanol and isolated and purified by column chromatography on silica gel, Sephadex LH 20, and semipreparative RP-HPLC. All the compounds were identified based on spectral analysis (including MS, ^{1}H-NMR and ^{13}C-NMR, and 2D NMR) and compared with the reported literature. Their cytotoxicities were evaluated against three human cancer cell lines. **Results** Two compounds were isolated from the roots of *E. sinica* and identified as (*E*)-eicosyl 3,4-dihydroxy-5-methoxycinnamate (**1**) and (*E*)-hexadecyl-ferulate (**2**). **Conclusion** Compound **1** was a new phenol named ephedrate A, and compound **2** was newly isolated from this plant. These two compounds showed no obvious cytotoxic activity, and their medicinal activities need to be studied further.

[Key words] *Ephedra sinica* Stapf; Ephedraceae; Chemical constituents

1 Introduction

Ephedra sinica Stapf (Ephedraceae) known as "Ma Huang" has been used as an important medicinal herb in China for thousands of years[1]. The aerial parts of *Ephedra* plants are known for containing ephedrine alkaloids[2-5], and the underground parts of Ephedra plants are said to have therapeutic effects opposite to those of aerial parts and have been used as an antiperspirant in Traditional Chinese Medicine. Compared with the ephedrine alkaloids, few non-ephedrine-constituents have been reported[6-7]. In our previous study, six proanthocyanidins, nine flavonoids, three terpenoids, and one lignan were isolated from the ethanolic extract of the roots of *E. sinica*[8-10]. As part of an ongoing study on the constituents from the roots of *E. sinica*, we reported herein a new phenol derivative, ephedrate A (**1**), and a known compound, (*E*)-hexadecyl-ferulate (**2**) [11] (Fig. 1).

2 Materials and methods

2.1 General Experimental Procedures

The NMR spectra were recorded on a Bruker AC 500NMR spectrometer (BrukerBioSpin,

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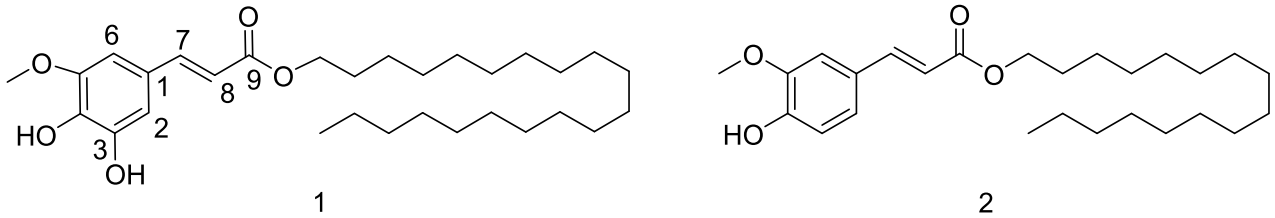

Fig. 1 Structures of compounds 1 and 2.

Fällanden, Switzerland) with TMS as an internal standard. The ESI-MS data were measured on a Bruker amaZon SL spectrometer (Bruker, Fällanden, Switzerland). The HR-ESI-MS data were measured on a Bruker micro TOF-QII mass spectrometer (Bruker, Fällanden, Switzerland). YMC gel (ODS-A, 12 nm, S-50 mm, YMC, Kyoto, Japan) was used for column chromatography (CC). The SiO_2 GF_{254} used for TLC was supplied by the Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 gel (GE Healthcare, Uppsala, Sweden) was used. HPLC was conducted on a Hitachi L-2400 with a YMC ODS column (Hitachi, Tokyo, Japan). Spots were detected on TLC under ultraviolet light or by heating after spraying with 5% H_2SO_4 in EtOH (v/v).

2.2 Plant Materials

The roots of *Ephedra sinica* were purchased from a local herb-drug store and identified by Professor M. L. Deng of the Department of Pharmacy, Changchun University of Chinese Medicine, China. The voucher specimen (No. 200407005) was deposited there.

2.3 Extraction and Isolation

The air-dried roots of *E. sinica* (10 kg) were extracted twice with 90% EtOH (each 40 L) and concentrated under vacuum. The extract was fractionated by successive partitioning with petroleum ether and EtOAc. The EtOAc fraction (100 g) was directly chromatographed over a silica gel column by gradient elution with $CHCl_3$/MeOH (100 : 0, 100 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1, and 1 : 1, each 3 L) to produce seven fractions (Frs. A–G). Fr. B (15.0 g) was separated by silica gel CC eluted with petroleum ether–EtOAc (10 : 1, each 300 mL) to yield 12 portions (Frs. B-1–B-12). Fr. B-6 (3.4 g) was purified by CC over ODS using a middle-pressure liquid chromatograph, using gradient elution from 60% to 100% MeOH at a flow rate of 20 mL/min to yield 25 portions (Frs. B6-1–B6-25). Fr. B6-18 was further purified with semipreparative HPLC, eluting with MeOH/H_2O = 85 : 15 at a flow rate of 3 mL/min to yield compounds **1** (61.0 mg) and **2** (3.8 mg).

Ephedrate A: White amorphous powder; ^1H and ^{13}C NMR data: see Table 1; HR-ESIMS *m/z* 490.365 4 (calcd for $C_{30}H_{50}O_5$, 490.365 8).

Table 1 ^1H and ^{13}C NMR spectroscopic data for compound 1 (500/125 MHz, in $CDCl_3$, δ in ppm, *J* in Hz)

Pos.	δ_C	δ_H	Pos.	δ_C	δ_H
1	126.8		16	29.7	1.26, m
2	109.3	6.82, d (2.0)	17	29.7	1.26, m
3	144.1		18	29.7	1.26, m
4	134.6		19	29.7	1.26, m
5	147.0		20	29.7	1.26, m
6	103.1	6.66, d (2.0)	21	29.7	1.26, m
7	144.7	7.55, d (16.0)	22	29.7	1.26, m
8	116.5	6.28, d (16.0)	23	29.7	1.26, m
9	167.3		24	29.7	1.26, m
10	65.4	4.18, t (6.5)	25	29.6	1.26, m
11	28.8	1.69, m	26	29.4	1.26, m
12	26.0	1.39, m	27	31.9	1.26, m
13	29.3	1.26, m	28	22.7	1.26, m
14	29.6	1.26, m	29	14.1	0.88, t (7.1)
15	29.7	1.26, m	OCH_3	56.3	3.91, s

2.4 Bioassays

The human cancer cell lines, HeLa, HCT-116, and A549, were purchased from ATCC. The HCT-116 cells were grown and maintained in an RPMI-

1640 medium with 10% FBS, whereas the other cells were grown in a DMEM medium with 10% FBS. The cell viability was determined using an MTT assay with 50 μM of compounds **1** and **2**, as previously described[12].

3 Results and discussion

3.1 Structure Elucidation

Compound **1** was assigned a molecular formula of $C_{30}H_{50}O_5$ based on its positive HR-ESI-MS at *m/z* 490.365 4 $[M]^+$, indicating six degrees of unsaturation. The ^1H NMR spectrum of compound **1** exhibits signals characteristic of the 3-hydroxyferuloyl moiety, a phenolic methyl at 3.91, two *meta* phenyl protons [δ_H 6.66 and 6.82 (each 1H, d, J = 2.0 Hz)], and two *trans* olefinic protons [δ_H 7.55 and 6.28 (each 1H, d, J = 16.0 Hz)]. The presence of an aliphatic alcohol moiety is indicated by the triplet signal at δ_H 0.88 (J = 7.1 Hz) (terminal methyl), the broad singlet at δ 1.29 for CH_2, and the downfield triplet at δ_H 4.18 (J = 6.7 Hz), corresponding to a methylene adjacent to an oxycarbonyl function (Table 1). The ^{13}C NMR spectrum, together with HSQC, reveals 30 signals for two methyls, nineteen methylene, two methines, six aromatic carbons, and one carbonyl carbon peak (Table 1). The HMBC correlations from H-2 to C-4, C-6, and C-7; H-6 to C-2, C-4, C-5, and C-7; and H-7 to C-2, C-6, C-8, and C-9 (Fig. 2 and Table 1). These data suggest that compound **1** was almost the same as docosyl *trans*-3-hydroxyferulate[13]. The distinction is attributed to the absence of two methylenes, as is evident from the molecular weight, which is smaller by 28 amu than that of the known analog. Thus, the structure of compound **1** was identified to be eicosyl *trans*-3-hydroxyferulate.

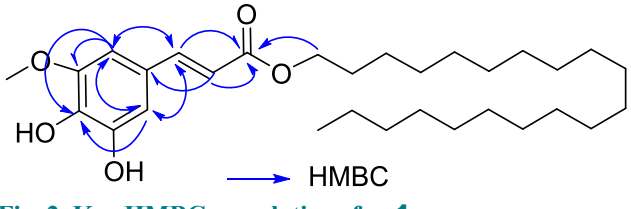

Fig. 2 Key HMBC correlations for 1.

The known compound **2** was identified as (*E*)-hexadecyl-ferulate (**2**)[11] by comparing the NMR and MS data with the reference data.

As a conventional activity screening, compounds **1** and **2** were tested for their cytotoxic activities against human cancer cell lines HeLa, HCT-116, and A549. However, none of them displayed prominent cytotoxicity activities with 50 μM.

3.2 Conclusion

In our ongoing search for new nonephedrine constituents from underground parts of *Ephedra species*, a new phenol derivative ephedrate A (**1**), together with a known compound (*E*)-hexadecyl-ferulate (**2**), was isolated from the roots of *E. sinica*. All compounds were novelly isolated and identified from the genus *Ephedra*. These two compounds showed no obvious cytotoxic activity, and their medicinal activities need to be further studied.

4 Conflicts of interest

These authors have no conflict of interest to declare.

5 Acknowledgments

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