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Two new guaiane-type sesquiterpenoids from the fruits of *Daucus carota* L.

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ABSTRACT

Two new guaiane-type sesquiterpenoids containing an interesting epoxy unit, daucuside (**1**) and daucusol (**2**) were isolated from the fruits of *Daucus carota* L. Their chemical structures were elucidated on the basis of MS, NMR spectroscopic analyses coupled with chemical degradation and they were also evaluated for the cytotoxic effects against two human gastric cancer cell lines BGC-823 and AGS.

1. Introduction

Daucus carota L. (Umbelliferae) is a biennial herb widely distributed throughout the world. Its fruits, commonly known as "nanheshi" in China, have been used as a traditional Chinese medicine for the treatment of ancylostomiasis, dropsy, chronic kidney diseases and bladder afflictions [1]. In addition, very interesting pharmacological effects such as antibacterial [2], antifungal [3], anthelmintic, hepatoprotective [4] and cytotoxic [5] activities have been reported for the plant of *D. carota*. Previous phytochemical investigations on the genus *Daucus* indicated the presence of sesquiterpenes [6–8], chromones [9], flavonoids [10,11], coumarins [6,12] and anthocyanins [13,14]. As a part of an ongoing research project on the chemistry of bioactive natural products from the fruits of *D. carota* L. [15–18], we have isolated two new guaiane-type sesquiterpenoids, daucuside (**1**) and daucusol (**2**). Herein, we describe the isolation and structural elucidation of them.

2. Experimental

2.1. General

Optical rotations were measured using a Rudolph Autopol IV digital polarimeter with a 0.5 dm length cell. HR-ESI-MS spectra were taken on a Bruker Daltonics Apex III mass spectrometer. All NMR spectra were recorded on a Bruker ARX-500 and ARX-125 MHz NMR spectrometer equipped with a CH dual 5ϕ probe. Samples were dissolved in 0.6 ml CD_3OD or C_5D_5N and transferred into a 5 mm NMR tube. All chemical shifts are expressed as δ (ppm) relative to the internal standard TMS ($\delta = 0$ ppm), and scalar coupling constants are reported in Hz. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Lcd., China), Sephadex LH-20 (Amersham Pharmacia Biotech) and ODS (35–50 μm, Alltech) were used for column chromatography. Preparative HPLC was performed using ODS column (Waters Sunfire ODS-C_{18}, 10 mm i.d.×250 mm).

2.2. Plant material

The fruits of *Daucus carota* L. were purchased in September 2007 from Hangzhou, Zhejiang Province, P. R. China, and

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identified by one of the authors (Lin Zhang). A voucher specimen was deposited in the Herbarium of the College of Biomedical Engineering and Instrument Sciences, Zhejiang University, P. R. China.

2.3. Extraction and isolation

The air-dried fruits of *D. carota* L. (2 kg) were refluxed two times with 95% aqueous EtOH. The combined EtOH extracts were concentrated, suspended in H_2O, and then partitioned, successively, with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH to give four different polar parts. The *n*-BuOH layer (4.2 g) was subjected to silica gel CC with a gradient of $CHCl_3$/MeOH (15:1–8:1) to afford eight fractions (1–8). Fraction 5 was loaded onto a silica gel CC with $CHCl_3$/MeOH (9:1) to provide five fractions (A1–A5). Fraction A2 was separated by repeated HPLC purification with 20% aqueous MeOH to afford **1** (7.5 mg). The $CHCl_3$ layer (40.5 g) was fractionated by silica gel CC with a gradient of petroleum ether/EtOAc (7:1–1:7) to obtain ten fractions (1–10). Fraction 6 (4.6 g) was chromatographed on silica gel CC eluted with petroleum ether/EtOAc (3:1–1:1) to give five fractions (B1–B5). Fraction B3 was separated by Sephadex LH-20 CC with MeOH followed by repeated silica gel CC with $CHCl_3/Et_2O$ (8:1) to yield **2** (8.6 mg).

Daucuside (=(1β,4αH,5α,10βH)-2β,7α,8α-trihydroxy-10,11-epoxy-guaiane 2-*O*-β-D-glucopyranoside; **1**): amorphous powder. $[\alpha]_D^{22}$ −16.4° (*c* 1.0, MeOH). ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Table 1. HR-ESI-MS *m/z* 455.2252 (calcd for $C_{21}H_{36}O_9Na$, $[M+Na]^+$, 455.2257).

Daucusol (=(1β,4αH,5α,10βH)-2β,7α-dihydroxy-10,11-epoxy-guaiane; **2**): amorphous solid. $[\alpha]_D^{22}$ +9.2° (*c* 1.0, MeOH): ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD and C_5D_5N, respectively, 125 MHz), see Table 1. HR-ESI-MS *m/z* 253.1809 (calcd for $C_{15}H_{25}O_3$, $[M-H]^-$, 253.1804).

2.4. Enzymatic hydrolysis and determination of the absolute configuration of the monosaccharide

A solution of **1** in 0.1 M acetate buffer (pH 4.0, 1.0 ml) was treated with naringinase (Sigma Chemical Co., 3.0 mg) and then the reaction mixture was stirred at 40 °C for 36 h. The reaction mixture was passed through a Sep-Pak C_{18} cartridge using H_2O and MeOH. The H_2O layer was concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 ml), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 ml) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 ml each) and then the *n*-hexane extract was analyzed by GC–MS under the following conditions: capillary column, EQUITY™-1 (30 m × 0.25 mm × 0.25 μm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas; detection in EI mode, ionization potential, 70 eV; ion-source temperature, 280 °C [19]. D-glucose in **1** was confirmed by comparison of the retention times of its derivatives with those of standard D-glucose and L-glucose derivatives prepared in a similar way.

Table 1
^{1}H and ^{13}C NMR Data of **1** and **2** (δ in ppm, *J* in Hz).

Position		**1** [a]		**2** [a]		**2** [b]
		δ_H [c]	δ_C [d]	δ_C [d]	δ_C [d]	δ_C [d]
1	β	2.00 (m)	55.5	1.79 (dd, 13.2, 9.0)	57.3	57.2
2	α	3.88 (t, 8.7)	84.4	3.96 (td, 9.0, 5.9)	74.3	73.5
3	α	2.43 (dt, 14.9, 8.7)	42.9	2.43 (dt, 13.9, 8.9)	44.4	44.5
	β	1.49 (ddd, 14.9, 4.5, 2.6)		1.14 (ddd, 13.9, 5.8, 3.0)		
4	α	1.98 (m)	33.5	1.98 (m)	32.9	32.3
5	α	2.11 (ddd, 13.1, 13.0, 6.5)	39.4	2.15 (ddd, 13.0, 13.0, 6.5)	40.2	39.6
6	α	1.94 (m)	40.6	1.87 (dd, 13.0, 6.9)	43.0	43.2
	β	1.54 (t, 13.0)		1.66 (t, 13.0)		
7			72.7		73.0	72.2
8	α			1.96 (m)	33.6	33.8
	β	3.90 (dd, 14.6, 8.7)	73.0	1.89 (dd, 13.2, 5.1)		
9	α	1.98 (m)	48.0	2.01 (m)	37.8	39.6
	β	2.35 (dd, 14.0, 9.4)		1.69 (dd, 13.4, 9.9)		
10			74.8		75.8	74.7
11			79.9		80.1	79.2
12		1.19 (s)	25.7	1.19 (s)	24.7	25.2
13		1.29 (s)	28.3	1.25 (s)	27.8	28.2
14		1.31 (s)	28.6	1.29 (s)	28.1	28.5
15	β	0.90 (d, 7.2)	18.5	0.94 (d, 7.2)	18.9	19.1
Glc-1		4.21 (d, 7.8)	105.5			
2		3.12 (t, 8.4	75.7			
3		3.34 (t, 8.7)	78.5			
4		3.27 (t, 8.7)	72.0			
5		3.24 (ddd, 8.7, 5.3, 1.9)	78.0			
6		3.82 (dd, 11.8, 1.9)	63.1			
		3.65 (dd, 11.7, 5.3)				

[a] In CD_3OD.
[b] In C_5D_5N.
[c] At 500 MHz.
[d] At 125 MHz.