Two New Guaiane Sesquiterpenes from the Fruits of *Daucus carota*

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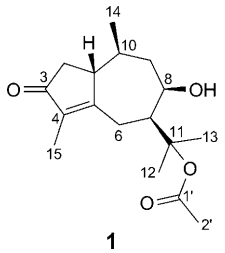
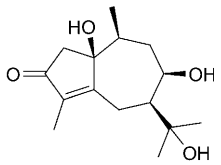
Two new sesquiterpenes, 11-(acetyloxy)torilolone (**1**) and 1β-hydroxytorilolone (**2**) were obtained from the fruits of *Daucus carota*. Their structures were elucidated on the basis of various spectroscopic analyses and chemical evidence.

Introduction. – *Daucus carota* L. is a biennial herb, belonging to the family Umbelliferae, which is commonly distributed throughout the world. The fruits of the plant are widely used as traditional Chinese medicines for the treatment of a variety of human diseases, such as ancylostomiasis, dropsy, chronic kidney disease, and bladder afflictions, *etc.* [1]. In modern pharmacological research, it has exhibited a wide range of pharmacological effects, including antibacterial [2], antifungal [3], anthelmintic, hepatoprotective [4], and cytotoxic [5] activities. Previous chemical investigations on the plant of *D. carota* have demonstrated the occurrence of sesquiterpenes [6], chromones [7], flavonoids [8], coumarins [6a][9], and anthocyanins [10]. As a part of our continuous investigation on bioactive constituents [11], we initiated a chemical investigation of this plant. In this article, we report the isolation and structure elucidation of two new guaiane sesquiterpenes from the fruits of *D. carota*.

Results and Discussion. – The fruits of *D. carota* were extracted with 95% EtOH to give a residue which was chromatographed on silica gel, *ODS*, and *Sephadex LH-20* columns to afford two novel sesquiterpenes, **1** and **2**.

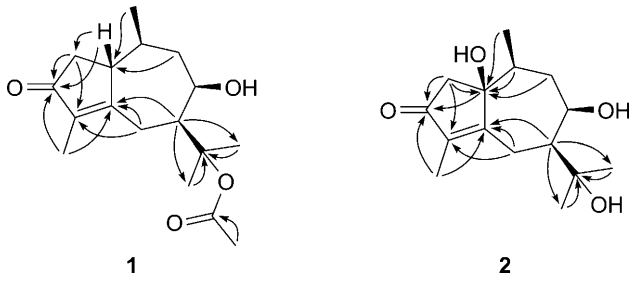


1



2

11-(Acetyloxy)torilolone (**1**) was obtained as a colorless oil, $[\alpha]_D^{22} = -28.5$ ($c = 0.7$, $CHCl_3$/MeOH 1:2). Compound **1** was considered a novel sesquiterpene, since the *SciFinder Scholar* search gave only the registry structure with no reference and predicted properties. The molecular formula was established as $C_{17}H_{26}O_4$ from the data of the positive-ion mode HR-FAB-MS (*m/z* 295.1901, $[M+H]^+$). The UV spectrum showed a characteristic band of an α,β-unsaturated ketone with an absorption maximum at 240 nm. An analysis of the NMR spectra revealed that the two compounds **1** and **2** were closely related guaiane-type sesquiterpenes [12]. The ^{1}H- and ^{13}C-NMR spectra of **1** displayed the presence of an AcO group. In addition, the ^{13}C-NMR and DEPT spectra showed the remaining 15 C-atom signals: four Me, three CH_2, and four CH groups, as well as four quaternary C-atoms, including one ketone CO group. The ^{1}H,^{1}H-COSY and HMQC data led to the partial structure of C(6)–C(7)–C(8)–C(9)–C(10)(–C(14))–C(1)–C(2) (*Fig. 1*). The presence of an α,β-unsaturated cyclopentenone unit was deduced from the HMBCs from H–C(1) (δ(H) 2.45–2.50) to C(2) (δ(C) 42.5) and C(3) (δ(C) 211.7), CH_2(2) (δ(H) 2.57, 2.07) to C(3) and C(4) (δ(C) 135.8), and Me(15) (δ(H) 1.70) to C(3), C(4), and C(5) (δ(C) 179.4). The presence and location of the ^{i}Pr group at C(7) was elucidated by the HMBC correlations of Me(12)/C(7) and C(11), and of Me(13)/C(7) and C(11). The connectivity of the two rings was determined by the HMBC correlations of Me(14) (δ(H) 1.03) with C(1) (δ(C) 52.7), and of CH_2(6) (δ(H) 3.04, 2.48) with C(4) and C(5). The correlations between H–C(8) (δ(H) 5.30) and C(1′) (δ(C) 172.3) though ^3J(C,H) was not observed, indicating that the AcO group and C(11) were connected through an O-atom. From the above data, the constitution of **1** was deduced as 11-(acetyloxy)torilolone. In the NOESY spectrum, **1** displayed NOESY correlations between H–C(7)/H–C(8), H–C(8)/H_α–C(9), and Me(14)/H_β–C(9), suggesting that H–C(7), H–C(8), and H–C(10) all have α-orientation (*Fig. 2*). The β-orientation of H–C(1) was suggested by the NOESY correlations between H–C(1) and Me(14). Based on the above results, the structure of **1** was established as (1β,7β,8β,10β)-8,11-dihydroxy-4-guaien-3-one 11-acetate, namely 11-(acetyloxy)torilolone.



1 **2**

Fig. 1. *HMBCs* (H → C) *of* **1** *and* **2**

1β-Hydroxytorilolone (**2**) was isolated as a colorless oil, $[\alpha]_D^{22} = -2.2$ ($c = 0.7$, MeOH). The molecular formula was determined to be $C_{15}H_{24}O_4$ by HR-FAB-MS (*m/z* 269.1750, $[M+H]^+$). The UV spectrum showed an absorption maximum of an α,β-unsaturated ketone at 240 nm. Compounds **1** and **2** have very similar patterns in their spectra (^{1}H-, ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMQC, and HMBC), while the ^{1}H-NMR

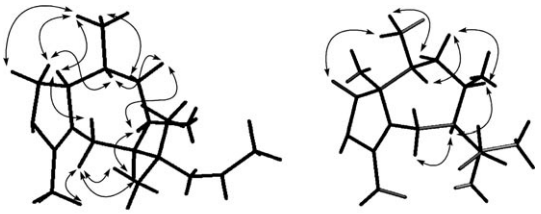


Fig. 2. *Key NOESY correlations* (H ↔ H) *of* **1** *and* **2**

spectrum of **2** did not display signals for H–C(1) and an AcO group. Therefore, compound **2** has a similar structure, except for the difference in the presence of an extra OH group at C(1) and the absence of an AcO group at C(11). The configuration of H–C(7), H–C(8), and H–C(10) was deduced to be the same as that of **1**, on the basis of the NOESY spectrum (*Fig. 2*). The β-configuration of HO–C(1) was suggested by the ^{1}H-NMR data of Me(14) (δ(H) 1.06), since the value of the chemical shift of Me(14) is δ(H) 1.07 for β-configuration and δ(H) 0.77 for α-orientation [12b]. Thus, the structure of **2** was established as 1β-hydroxytorilolone, (1β,7β,8β,10β)-1,8,11-trihydroxy-4-guaien-3-one.

Experimental Part

General. TLC: silica gel *GF254* pre-coated plates (*Qingdao Haiyang*). Column chromatography (CC): D_{101} (Chemical Plant of Nankai University, Tientsin, P. R. China), silica gel (SiO_2; 200–300 mesh, *Qingdao Haiyang Chemical Co. Ltd.*, P. R. China), *Sephadex LH-20* (*Amersham Pharmacia Biotech*), and *ODS* (35–50 μm, *Alltech*). Optical rotations: *JASCO DIP-370* digital polarimeter in a 0.5 dm length cell. IR Spectra: *JASCO FT/IR-300E* (KBr disk method) spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: *Bruker ARX-400* MHz NMR spectrometer, with TMS as the internal reference, and chemical shifts are expressed in δ [ppm]. HR-FAB-MS: *JEOL JMS-700 MStation.*

Plant Material. The fruits of *D. carota* were purchased in September 2007 from Hangzhou, Zhejiang Province, P. R. China, and identified by one of the authors (*L. Z.*). A voucher specimen was deposited in the Herbarium of the College of Biomedical Engineering and Instrument Sciences, Zhejiang University, P. R. China.

Extraction and Isolation. The air-dried fruits of *D. carota* (10 kg) were refluxed two times with 95% aq. EtOH. The combined EtOH extracts were concentrated, suspended in H_2O, and then partitioned with petroleum ether (PE), $CHCl_3$, AcOEt, and BuOH successfully to give four different polar parts. The $CHCl_3$ layer (167.0 g) was subjected to SiO_2 CC with a gradient of PE/AcOEt to give ten fractions (*Frs. C1–C10*). *Fr. C6* (16.5 g) was subjected to SiO_2 CC with a gradient of PE/acetone 1:0–1:1 to give five subfractions (*Frs. C6.1–C6.5*). *Fr. C6.2* (0.89 g) was fractionated by *Sephadex LH-20* and SiO_2 ($CHCl_3$/Et_2O 8:1) CC to yield **1** (25.5 mg). The AcOEt layer (8.7 g) was fractionated by SiO_2 CC with a gradient of PE/AcOEt 6:4–0:1 to give eight fractions (*Frs. A1–A8*). *Fr. A3* (2.3 g) was purified by SiO_2 and *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) CC to give **2** (176.5 mg).

11-(Acetyloxy)torilolone (=*(1β,7β,8β,10β)-8,11-Dihydroxyguai-4-en-3-one 11-Acetate*; *2-[(5*S*,6*R*,8*S*,8a*R*)-1,2,4,5,6,7,8,8a-Octahydro-6-hydroxy-3,8-dimethyl-2-oxoazulen-5-yl]propan-2-yl Acetate*; **1**). Colorless oil. $[\alpha]_D^{22} = -28.5$ (c = 0.7, $CHCl_3$/MeOH 1:2). UV (MeOH): 240. ^{1}H- and ^{13}C-NMR: *Table.* HR-FAB-MS: 295.1901, ($[M+H]^+$, $C_{17}H_{27}O_4^+$; calc. 295.1909).

1β-Hydroxytorilolone (=*(1β,7β,8β,10β)-1,8,11-Trihydroxyguai-4-en-3-one*; *(5*S*,6*R*,8*S*,8a*S*)-4,5,6,7,8,8a-Hexahydroazulen-6,8a-dihydroxy-5-(2-hydroxypropan-2-yl)-3,8-dimethyl-2(1*H*)-one*; **2**). Colorless

Table. *[1]H- and [13]C-NMR Data* (in CD_3OD) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)[a])	δ(C)[b])	δ(H)[c])	δ(C)[d])
H_β–C(1) or C(1)	2.45–2.50 (*m*)	52.7		80.4
CH_2(2)	2.57 (*dd*, J = 18.4, 6.2, H_α), 2.07 (*dd*, J = 18.4, 3.0, H_β)	42.5	2.49 (*d*, J = 18.2, H_α), 2.36 (*d*, J = 18.2, H_β)	50.7
C(3)		211.7		209.9
C(4)		135.8		135.5
C(5)		179.4		178.2
CH_2(6)	3.04 (*d*, J = 13.6, H_α), 2.48 (*t*, J = 13.6, H_β)	26.8	2.73 (*d*, J = 13.4, H_α), 2.56 (*dd*, J = 13.4, 10.4, H_β)	23.0
H_α–C(7)	1.66[e])	51.3	1.46–1.52 (*m*)	51.3
H_α–C(8)	5.30 (*td*, J = 8.0, 4.0)	73.9	4.23–4.28 (*m*)	71.3
CH_2(9)	1.62–1.66 (*m*, H_α), 2.18 (*ddd*, J = 14.4, 7.8, 0.8, H_β)	41.4	1.69 (H_α)[e]), 2.15–2.21 (*m*, H_β)	39.7
H_α–C(10)	1.42–1.47 (*m*)	35.0	1.60–1.70 (*m*)	39.1
C(11)		73.4		74.8
Me(12)	1.22 (*s*)	28.7	1.31 (*s*)	28.9
Me(13)	1.24 (*s*)	27.2	1.34 (*s*)	28.5
Me_β(14)	1.03 (*d*, J = 6.7)	23.4	1.06 (*d*, J = 6.9)	19.1
Me(15)	1.70 (*d*, J = 1.2)	8.2	1.70 (*s*)	8.1
C(1′)		172.3		
Me(2′)	2.05 (*s*)	21.8		

[a]) At 500 MHz. [b]) At 125 MHz. [c]) At 400 MHz. [d]) At 100 MHz. [e]) Overlapped signals.

oil. $[\alpha]_D^{22} = -2.2$ (c = 0.7, MeOH). UV (MeOH): 240. ^{1}H- and ^{13}C-NMR: *Table*. HR-FAB-MS: 269.1750, ($[M+H]^+$, $C_{15}H_{25}O_4^+$; calc. 269.1753).

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