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One new guaiane-type sesquiterpene glycoside from the fruits of *Daucus carota* L.

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A new guaiane-type sesquiterpene glycoside, torilolone 8-*O*- β -D-glucopyranoside (**1**), together with a known analogue compound, torilolone 11-*O*- β -D-glucopyranoside (**2**), was 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. The cytotoxic activities of two isolated compounds were evaluated against two human gastric cancer cell lines BGC-823 and AGS using MTT assay.

1. Introduction

Daucus carota L. (wild carrot) is one of many umbelliferous plants distributed throughout the world. Its fruits are widely used as a traditional Chinese medicine for the treatment of ancylostomiasis, dropsy, chronic kidney diseases and bladder afflictions (Pant and Manandhar 2007) due to a wide range of pharmacological effects, including antibacterial (Rossi et al. 2007), antifungal (Tavares et al. 2008), anthelmintic, hepatoprotective (Bishayee et al. 1995) and cytotoxic (Yang et al. 2008) activities. Previous phytochemical investigations on *D. carota* indicated the presence of sesquiterpenes (Ahmed et al. 2005; Dhillon et al. 1989; Cool 2001), chromones (Czepa and Hofmann 2003), flavonoids (Gebhardt et al. 2005; Gupta and Niranjana 1982), coumarins (Ahmed et al. 2005; Ivie et al. 1982) and anthocyanins (Kurilich et al. 2005; Hemingson and Collins 1982). Our interest in identification of bioactive agents from native plants (Fu et al. 2005; Li et al. 2007; Wang et al. 2008) prompted us to conduct a chemical investigation on *D. carota* L. The present paper reports the isolation and structure elucidation of one new and one known guaiane-type sesquiterpene glycoside (**1** and **2**) for the readers' convenience since only one paper reported the NMR assignments of **2** in C₅D₅N (Park et al. 2006), largely different from those in CD₃OD showed in this paper. Their cytotoxic activities against two human gastric cancer cell lines BGC-823 and AGS were also reported.

2. Investigations, results and discussion

Compound **1** was obtained as an amorphous powder, [α]_D²² –35.8° (*c*=0.3, MeOH). The molecular formula was established as C₂₁H₃₄O₈ from the data of positive-ion HRESIMS (*m/z* 437.2164 [M+Na]⁺). Its UV spectrum was characteristic as an α,β -unsaturated ketone with an absorption maximum at 245 nm. A careful analysis of their NMR spectra revealed that the two compounds **1** and **2** were closely related guaiane-type sesquiterpenoids (Chikamatsu et al. 1969; Park et al. 2006; Aguilar-Guadarrama and Rios 2004). The ¹³C NMR data (Table)

combined with analysis of the HMQC spectrum revealed 21 carbon signals due to four quaternary carbons, nine methines, four methylenes and four methyls, of which 15 were assigned to the aglycone part including one ketonic carbonyl carbon at δ_C 211.9 along with two olefinic carbons at δ_C 180.3, 135.1 and the remaining 6 were ascribed to a glucopyranoside unit. On acidic hydrolysis of **1**, in addition to the aglycone, D-glucose was received, which was confirmed by GC-MS analysis following conversion to the trimethylsilyl thiazolidine derivatives (Hara et al. 1987). The ¹H NMR spectrum of **1** exhibited a set of signals assignable to a β -glucopyranosyl moiety with an anomeric proton resonanced at δ_H 4.47 (d, *J* = 7.7 Hz). The ¹H NMR spectrum of the aglycone moiety showed four methyl proton signals [δ_H 1.69 (3H, s), 1.35 (3H, s), 1.34 (3H, s) and 1.05 (3H, d, *J* = 6.5 Hz)] and one carbinyl proton signal [4.54 (td, *J* = 7.8 and 4.2 Hz)], coupled with information from the ¹³C NMR spectrum (two oxygenated carbons at δ_C 76.1 and 74.6). The partial structure of C6–C7–C8–C9–C10(–C14)–C1–C2 (Fig. 1) was deduced from the ¹H–¹H COSY and HMQC spectral data. An α,β -unsaturated cyclopentenone unit bearing one methyl group was confirmed by the HMBC correlations of H-2 (δ_H 2.57, 2.07)/C-1 (δ_C 53.1), C-3 (δ_C 211.9), C-4 (δ_C 135.1) and C-5 (δ_C 180.3) as well as Me-15 (δ_H 1.69)/C-3 and C-5. The presence and location of the isopropyl group at C-7 was also elucidated by the HMBC correlations between Me-12 (δ_H 1.35)/C-7 (δ_C 51.0), C-11 (δ_C 74.6) and Me-13 (δ_H 1.34)/C-7, C-11. Further, the HMBC correlations from Me-14 (δ_H 1.05) to C-1, along with H-6 (δ_H 2.88, 2.55) to C-1, C-4 and C-5 indicated the connectivity of the two rings. From the above data, the gross planar structure of the aglycone of **1** was confirmed. The β -D-glucopyranosyl moiety located at C-8 was determined by the HMBC correlation between Glc-H-1 (δ_H 4.47) and C-8, which was supported by the glycosylation shift observed at C-8 (+4.9) as compared to its aglycone which was isolated from the same extract of the fruits of *D. carota* L. The stereochemistry of **1** was confirmed by careful assignments of NOESY data (Fig. 2). The configurations of H-7 and H-8 should be α and α , respectively, which was

Table: ^1H - and ^{13}C -NMR Data (in CD_3OD) of **1** and **2** (δ in ppm, J in Hz)

Position		1		2	
		$\delta_{\text{H}}^{\text{a)}$	$\delta_{\text{C}}^{\text{b)}$	$\delta_{\text{H}}^{\text{a)}$	$\delta_{\text{C}}^{\text{b)}$
1	β	2.45 (m)	53.1	2.38 (m)	53.5
2	α	2.07 (dd, 17.9, 2.8)	42.5	2.01 (dd, 18.4, 2.2)	42.6
	β	2.57 (dd, 17.9, 6.6)		2.51 (dd, 18.4, 6.1)	
3			211.9		212.1
4			135.1		135.4
5			180.3		180.7
6	α	2.88 (d, 13.6)	26.7	2.97 (d, 13.5)	25.6
	β	2.55 (t, 13.6)		2.39 (t, 13.5)	
7	α	1.58 (dd, 9.8, 4.2)	51.0	1.64 (m)	51.4
8	α	4.54 (td, 7.8, 4.2)	76.1	4.22 (dt, 11.9, 4.2)	70.3
9	α	2.20 (dd, 13.7, 5.5)	40.8	2.00 (dd, 13.8, 8.0)	45.0
	β	1.80 (ddd, 13.7, 10.2, 4.3)		1.64 (ddd, 13.8, 9.6, 4.5)	
10	α	1.30 (m)	35.0	1.35 (m)	34.7
11			74.6		82.0
12		1.35 (s)	28.5	1.39 (s)	25.1
13		1.34 (s)	28.6	1.39 (s)	26.4
14	β	1.05 (d, 6.5)	23.6	0.98 (d, 6.6)	23.6
15		1.69 (s)	8.2	1.67 (s)	8.5
Glc-1		4.47 (d, 7.7)	101.3	4.50 (d, 7.7)	98.5
2		3.15 (t, 8.5)	75.6	3.10 (t, 8.4)	75.5
3		3.30 (t, 9.4)	78.5	3.33 (t, 9.2)	78.7
4		3.25 (t, 9.2)	72.2	3.18 (t, 9.5)	72.2
5		3.34 (m)	78.5	3.20 (m)	78.0
6		3.87 (dd, 11.5, 1.9)	63.3	3.76 (d, 11.5)	63.2
		3.65 (d, 11.2, 5.0)		3.50 (dd, 11.5, 5.1)	

^{a)} At 400 MHz, ^{b)} At 100 MHz

suggested by the obvious NOE correlations between Glc-H-1/H-7, H-8 and H-7/H-8. The NOESY correlations of H-1/H β -9, H β -6 and Me-14 indicated that the orientations of H-1 and H-10 were β and α , respectively. Thus, the structure of **1** was established as torilolone 8-*O*- β -D-glucopyranoside,

namely (1 β ,7 β ,8 β ,10 β)-8,11-dihydroxy-4-guaien-3-one 8-*O*- β -D-glucopyranoside.

Compound **2** was isolated as an amorphous powder, $[\alpha]_{\text{D}}^{22} -37.2^\circ$ ($c=0.2$, MeOH). The elemental formula was determined to be $\text{C}_{21}\text{H}_{34}\text{O}_8$ and its UV spectrum showed an absorption

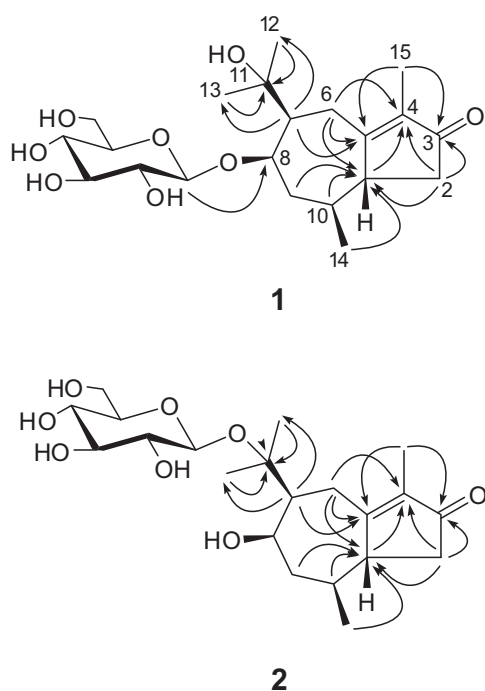


Fig. 1: The Key HMBC (H \rightarrow C) Correlations of **1** and **2**

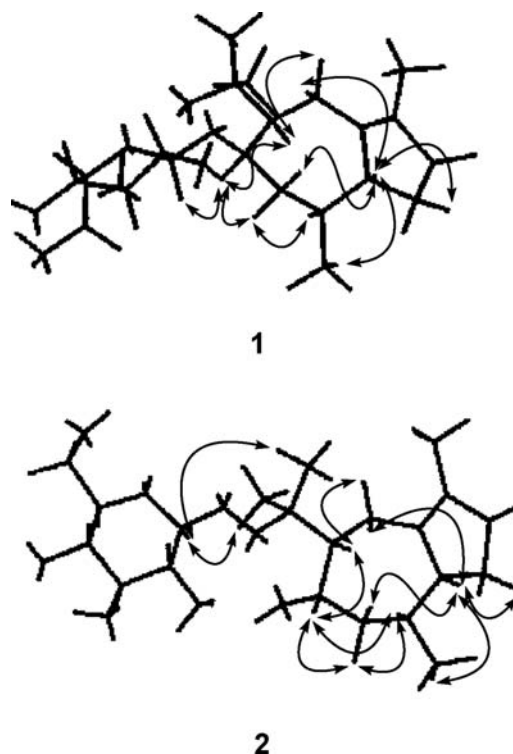


Fig. 2: The Key NOESY Correlations (H \rightarrow H) of **1** and **2**

maximum of an α,β -unsaturated ketone at 244 nm similar to **1**. The ^1H and ^{13}C NMR data of **2** (Table) were almost superimposable with **1**, except in the ^{13}C NMR spectrum, C-8 and C-11 of **2** were shifted by -5.8 and $+7.4$ ppm than those of **1**, suggesting that **2** had a β -D-glucopyranosyl moiety located at C-11 in **2** not at C-8 in **1** (Fig. 1). Furthermore, the β -D-glucopyranosyl moiety linked to C-11 in **2** was deduced from the HMBC correlation between Glc-H-1 (δ_{H} 4.50) and C-11 (δ_{C} 82.0). The stereochemistry of H-1, H-7, H-8 and H-10 was deduced to be the same as that of **1**, on the basis of the NOESY spectrum (Fig. 2). Based on the above results, the structure of **2** was determined as (1 β ,7 β ,8 β ,10 β)-8,11-dihydroxy-4-guaiane-3-one 11-O- β -D-glucopyranoside, named as torilolone 11-O- β -D-glucopyranoside, which was a known compound (Kitajima et al. 1998).

The two guaiane-type sesquiterpene glycosides **1** and **2** were screened for cytotoxicity against two human gastric cancer cell lines BGC-823 and AGS at a concentration of 100 $\mu\text{g/mL}$. The results indicated that compound **1** and **2** displayed little cytotoxicity against the two selected cancer cell lines.

3. Experimental

3.1. Equipment

Optical rotations were measured using a Rudolph Autopol IV digital polarimeter in a 0.5 dm length cell. ^1H - and ^{13}C -NMR were recorded using a Bruker NMR spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). HRESIMS was taken on a Bruker Daltonics Apex III mass spectrometer. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., 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-C18, 10 mm i.d. \times 250 mm).

3.2. Plant material

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

3.3. Extraction and isolation

The air-dried fruits of *D. carota* L. (10 kg) were refluxed two times with 95% aqueous EtOH. The combined EtOH extracts were concentrated, suspended in H_2O , and then partitioned with petroleum ether, CHCl_3 , EtOAc and *n*-BuOH successfully to give four different polar parts. The *n*-BuOH layer (32.6 g) was subjected to silica gel column chromatography (CC) with a gradient of $\text{CHCl}_3/\text{MeOH}$ (15:1–8:1) to give eight fractions (1–8). Fraction 4 (9.8 g) was loaded on a silica gel CC eluted with $\text{CHCl}_3/\text{MeOH}$ (95:1–9:1) to obtain six fractions (A1–A6). Fraction A3 was chromatographed on silica gel CC [$\text{CHCl}_3/\text{MeOH}$ (9:1)] and then further separated by HPLC purification with 20% aqueous MeOH to afford **1** (5 mg) and **2** (19 mg).

3.4. Torilolone 8-O- β -D-glucopyranoside (**1**)

Amorphous powder; $[\alpha]_{\text{D}}^{22} -35.8^\circ$ ($c=0.3$, MeOH); UV (MeOH): 245 nm; ^1H NMR (CD_3OD , 400 MHz) and ^{13}C NMR (CD_3OD , 100 MHz), see Table; positive-ion HRESIMS m/z 437.2164 (calcd for $\text{C}_{21}\text{H}_{34}\text{O}_8\text{Na}$, 437.2151).

3.5. Acid hydrolysis and determination of the absolute configuration of the monosaccharides

A solution of **1** (2 mg) in 1 M HCl (dioxane- H_2O , 1:1, 2 ml) was heated at 80 $^\circ\text{C}$ for 3 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (2 ml \times 3) to remove the aglycone. The H_2O layer was concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. In the acid hydrolysate of **1**, D-glucose was confirmed by comparison of the retention times of their derivatives with those of standard D-glucose and L-glucose derivatives prepared in a similar way using the method previously described (Li et al. 2007a; Hara et al. 1987). The sugar in **2** was also identified by the same method.

3.6. Cytotoxicity assay

The two human gastric cancer cell lines BGC-823 and AGS were maintained in RPMI 1640 medium at 37 $^\circ\text{C}$ under a humidified atmosphere of 95% air and 5% CO_2 . The cell growth inhibition was assessed by colorimetric MTT Assay *in vitro* as previously reported (Li et al. 2007b).

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