Phytochemistry Letters 14 (2015) 35–38

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Two new eudesmane sesquiterpenoids from *Daucus carota* L.

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ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form 19 August 2015
Accepted 24 August 2015
Available online xxx

Keywords:
Daucus carota L.
Eudesmane sesquiterpenoids
X-ray diffraction
Antioxidant activity

ABSTRACT

Two new eudesmane sesquiterpenoids were isolated from the fruits of *Daucus carota* L. The structures were established based on the interpretation of high-resolution MS and 1D- and 2D-NMR data, the absolute configuration of compound **1** was determined by single-crystal X-ray diffraction. The new compounds were elucidated as (1βH, 3βH, 5βH, 7αH)-4α, 10α-dimethyl-7β-isopropyl-1α, 3α, 4β, 11-tetrahydroxy decahydronaphthalene (1) and (1βH, 3βH, 5βH, 7αH)-4α, 10α-dimethyl-7β-isopropyl-1α, 3α, 4β-trihydroxy decahydronaphthalene-11-*O*-β-D-glucopyranoside (2). The compound **1** showed significant antioxidant activity.

1. Introduction

The root of *Daucus carota* L. is one of the most commonly used vegetables all over the world, the fruits of the plant also have been used in Traditional Chinese Medicine for the treatment of a variety of human diseases, such as ancylostomiasis, dropsy, chronic kidney disease and bladder afflictions, etc. (Pant and Manandhar, 2007). The chemical constituents of the fruits of *D. carota* L. have been studied by many medicinal plant and food chemistry groups, which resulted in the isolation of sesquiterpenoids (Ahmed et al., 2005; Dhillon et al., 1989; Cool, 2001), flavonoids (Gebhardt et al., 2005; Gupta and Niranjan, 1982), anthocyanins (Kurilich et al., 2005; Hemingson and Collins, 1982) and coumarins mainly (Ahmed et al., 2005; Ivie et al., 1982). Our previous phytochemical research led to the isolation of many guaiane-type sesquiterpenes and eudesmane type sesquiterpenes (Fu et al., 2009; Fu et al., 2010a; Fu et al., 2010b; Fu et al., 2010c; Fu et al., 2010d; Yi et al., 2009; Xu et al., 2015). In this paper, we report the isolation and characterization of a rare eudesmane sesquiterpenoid (**1**) and its glycoside (**2**) (Fig. 1). The absolute configuration of compound **1** was also confirmed by single-crystal X-ray diffraction analysis. The antioxidant activity of compound **1** was described in this paper.

2. Results and discussion

Compound **1** was obtained as colorless crystals. The molecular formula was established as $C_{15}H_{28}O_4$ by HR–ESI–MS analysis (295.1879 $[M+Na]^+$), with two degrees of unsaturation. The ^{13}C NMR data (Table 1) showed the presence of 15C-atoms which were attributed by DEPT and HSQC analyses to four methyls (δ_C 13.6, 16.1, 28.9 and 29.9), four methylenes (δ_C 20.1, 20.5, 37.2 and 37.9), four methines (δ_C 41.5, 44.1, 76.2 and 76.5), and three quaternary carbons (δ_C 38.4, 72.7 and 74.5). Further assignments of all hydrogen and carbon signals were achieved by its HSQC, ^1H–^1H COSY, DEPT, HMBC and NOESY experiments. The structure must be bicyclic to account for two degrees of unsaturated sesquiterpenoid required by the molecular formula which was constructed further by interpretation of the 2D-NMR analysis. The fragments (1) CH-(2) CH2-(3) CH and (5) CH-(6) CH2-(7) CH-(8) CH2-(9) CH2 were deduced from COSY cross-peaks (Fig. 2a). The long range correlations from H-5 to C-1, C-3, C-7, C-9, C-15 and from Me-15 to C-1, C-5, C-9, C-10 established two six-membered ring A and B. It was confirmed that the A/B ring fusion was decahydronaphthalene ring (Fig. 2a) and Me-15 was assigned in the site of decahydronaphthalene ring. Four oxygenated carbons were assigned to C-1 (δ_C 76.5), C-3 (δ_C 76.2), C-4 (δ_C 74.5) and C-11 (δ_C 72.7) by integrated 2D-NMR analysis. Two methyls (C-12 and C-13) showed HMBCs with the oxygenated quaternary C-11, which assembled a substituted *i*-Pr group, HMBCs from these two methyls to C-7 showed that this fragment was located at C-7. Further interpretation of the HMBC spectra showed that

Abbreviations: HUVEC, human umbilical vein endothelial cell; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.phytol.2015.08.016
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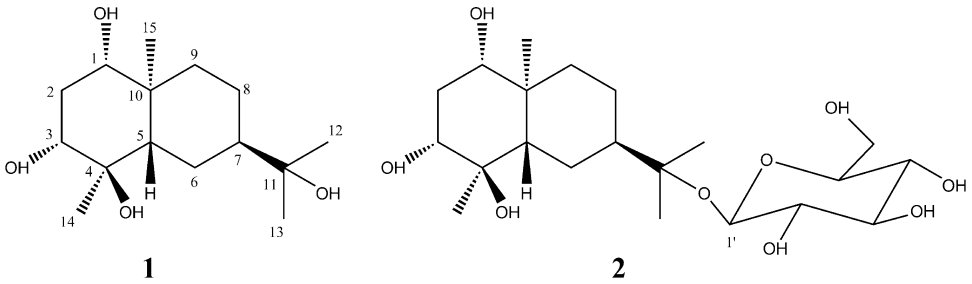

Fig. 1. The structures of constituents isolated from *D. carota* L.

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

Position		1[a]		2[b]	
		δ_H[c]	δ_C[d]	δ_H[c]	δ_C[d]
1	β	2.98 (dd, 7.5, 7.0)	76.5	2.95 (dd, 7.5, 7.0)	76.3
2	α	1.51 (m)	37.2	1.48 (m)	37.0
	β	1.35 (m)		1.33 (m)	
3	β	3.11 (dd, 7.5, 7.0)	76.2	3.10 (dd, 7.5, 7.0)	76.1
4			74.5		74.2
5	β	1.25 (dd, 7.5, 7.0)	44.1	1.24 (dd, 7.5, 7.0)	43.8
6	β	1.84 (m)	20.1	1.85 (m)	19.9
	α	1.24 (m)		1.23 (m)	
7	α	1.41 (m)	41.5	1.51 (m)	39.2
8	β	1.55 (m)	20.5	1.53 (m)	20.3
	α	1.30 (m)		1.28 (m)	
9	α	1.29 (m)	37.9	1.26 (m)	37.6
	β	1.23 (m)		1.21 (m)	
10			38.4		38.2
11			72.7		81.6
12		1.00 (s)	28.9	0.99 (s)	26.1
13		0.99 (s)	29.9	0.98 (s)	27.5
14	α	0.73 (s)	16.1	0.73 (s)	15.8
15	α	0.63 (s)	13.6	0.65 (s)	13.6
Glc-1′				5.05 (d, 7.5)	99.1
2′				4.23 (1H, t, 7.5)	74.3
3′				3.95 (1H, t, 7.5)	77.5
4′				4.06 (1H, t, 7.5)	71.7
5′				4.15 (1H, q, 7.5)	77.9
6′				4.62 (1H, dd, 12.0, 7.5)	62.8
				4.33 (1H, dd, 12.0, 7.5)	

[a] In DMSO-d_6.
[b] In pyridine-d_5.
[c] Recorded at 600 MHz.
[d] Recorded at 150 MHz.

Me-14 was attached to C-4. Thus, the structure could be proposed as 4,10-dimethyl-7-isopropyl-decalin structure.

In order to confirm the structure and determine the absolute configuration of compound **1**, it was crystallized from MeOH to afford needle crystals, with one of these analyzed by X-ray crystallography (Fig. 3) (CCDC contains the Supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html). On the basis of the resonant scattering of light atoms in Cu Kα radiation, it provided the ring fusion of decahydronaphthalene structure and the absolute configuration. The configurations of the asymmetric centers at C-1, C-3, C-4, C-5, C-7 and C-10 could be determined as S, R, R, S, R and S, respectively. The *trans*-decahydronaphthalene ring was also proved by chemical shift of C-15, the angular methyl group of *trans*-decahydronaphthalenes appearing around δ_C 18.5, and that of the *cis*-isomers around δ_C 28–31 (Ando et al., 1994). The relative configuration was also determined by analysis of the NOESY data (Fig. 2b), The NOESY correlations Me-14/Me-15/H-7 and H-1/H-3/H-5 indicated that 1-OH, 3-OH, Me-14, Me-15 were in α-positions and 4-OH, 7-(*i*-Pr) were in β-positions. Thus, compound **1** was identified as (1βH, 3βH, 5βH, 7αH)-4α, 10α-dimethyl-7β- isopropyl-1α, 3α, 4β, 11-tetrahydroxy decahydronaphthalene.

Compound **2** was obtained as amorphous powder. Its molecular formula was determined as $C_{21}H_{38}O_9$ by HR–ESI–MS analysis (457.2425 $[M+Na]^+$), the positive ESI–MS gave a fragment at m/z 273.5 ($[M+H-162]^+$), besides a quasi-molecular ion at m/z 435.5 ($[M+H]^+$), indicating the potential presence of one hexose unit.

The ^{1}H, ^{13}C NMR, HSQC and DEPT data of **2** showed the presence of four methyls (two geminal methyls, two angular methyls), five methylenes (one oxygenated), nine methines (seven oxygenated), and three quaternary carbons (two oxygenated) (Table 1). The hexose was suggested to be a D-glucose by comparison of data with those reported in the literature (Zhang et al., 2007), and further confirmed by GC analysis after the enzymatic hydrolysis and preparation of its thiazolidine derivative (Hara et al., 1987). The coupling constant of H-1′ (δ_H 5.05, 1H, d, J = 7.5 Hz) indicated the D-glucose was a β-linkage. The aglycone spectral features were closely related to compound **1**, except that the chemical shift of C-11 shifted downfield for 8.9 ppm, and the chemical shifts of C-7, C-12 and C-13 had shifted up field for 2.3, 2.8 and 2.4 ppm, respectively, which suggested that C-11 of **1** should be

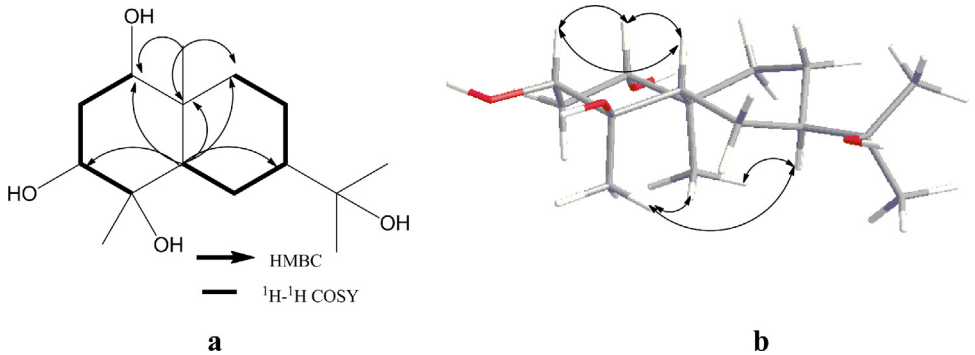

Fig. 2. Key ^{1}H–^{1}H COSY, HMBC (a) and NOESY (b) correlations.

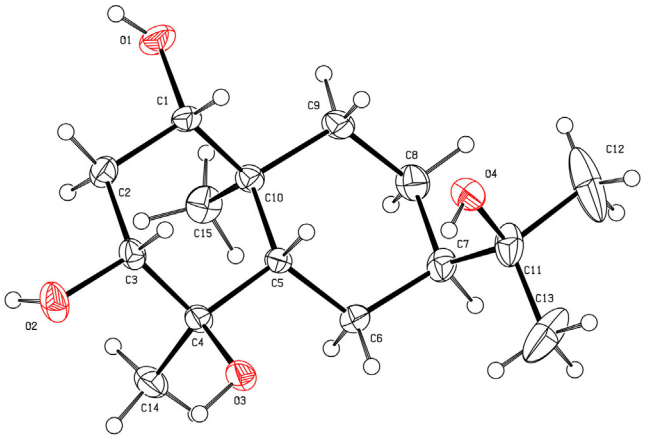

Fig. 3. X-ray crystal structure of compound **1**.

glycosylated. The HMBC correlation achieved between the anomeric proton at δ_H 5.05 (H-1′) and C-11 (δ_C 81.6) also supported a C-11 location for the sugar unit. Thus, the structure of compound **2** was established as (1βH, 3βH, 5βH, 7αH)-4α, 10α-dimethyl-7β-isopropyl-1α, 3α, 4β-trihydroxy decahydronaphthalene-11-*O*-β-D-glucopyranoside.

Compound **1** showed no significant cytotoxicity on HUVEC (Fig. 4 left). However, it showed significant protection on H_2O_2-induced cytotoxicity in HUVEC (Fig. 4 right). Reactive oxygen species (ROS) are generated under various physiological and pathological conditions such as aging, carcinogenesis, and inflammation. An increase in intracellular ROS level has been shown to damage tissues and cells. Therefore, the results suggest that compound **1** could possess the ability to protect oxidative damages for further applications.

3. Experimental

3.1. General experimental procedures

The melting points (uncorrected) were determined on a Fisher–Johns melting point apparatus (Fisher Scientific, U.S.A.). HR–ESI–MS and ESI–MS spectra were taken on a Bruker Daltonics Apex III mass spectrometer. All NMR spectra were recorded on a Bruker ARX-600 and ARX-150 MHz NMR spectrometer equipped with a CH dual 5 φ probe. Samples were dissolved in 0.6 ml DMSO-d_6 or pyridine-d_5, and transferred into a 5 mm NMR tubes. 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. DEPT, ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra were recorded using conventional pulse sequences. Thin-layer chromatography was performed with silica gel GF254 pre-coated plates (Qingdao Haiyang). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China) and Sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. The water used in the experiment was doubly distilled in the laboratory. Other chemicals and solvents were of analytical grade.

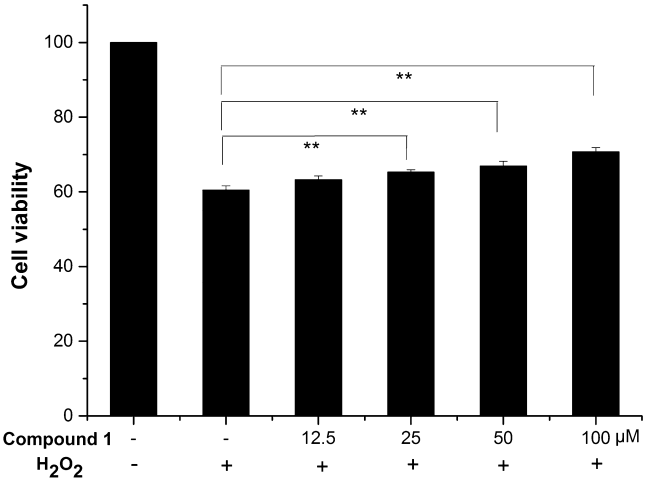

Fig. 4. Examination of cytotoxic effect of compound **1** on HUVEC (left) and protection of HUVEC cells from H_2O_2-induced cell death (right), $^{**}p < 0.01$ indicates a significant difference compared with control group.

3.2. Plant material

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

3.3. 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 with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH successively to give four different polar parts. 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 8 (2.5 g) was chromatographed on silica gel CC eluted with petroleum ether/EtOAc (3:1–1:2) to give six fractions (A1–A6). Fraction A5 was separated by Sephadex LH-20CC with MeOH followed by repeated silica gel CC with $CHCl_3$/EtOAc (7:3) to yield the compound **1** (78 mg). *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 B7 was loaded onto a silica gel CC with $CHCl_3$/MeOH (88:12) to provide four fractions (B1–B4). Fraction B3 was separated by repeated Sephadex LH-20CC with MeOH to afford compound **2** (43.5 mg).

Compound **1** colorless needle crystals (MeOH); mp 112–114 °C; ^{1}H NMR and ^{13}C NMR (DMSO-d_6) see Table 1; Positive HR–ESI–MS *m/z* 295.1879 (calcd. for $C_{15}H_{28}O_4Na$, $[M+Na]^+$, 295.1885).

Compound **2** amorphous powder (MeOH); mp 155–157 °C; ^{1}H NMR and ^{13}C NMR (pyridine-d_5) see Table 1; Positive HR–ESI–MS *m/z* 457.2425 (calcd. for $C_{21}H_{38}O_9Na$, $[M+Na]^+$, 457.2413), Positive ESI–MS *m/z* 435.5 ($[M+H]^+$), 273.5 ($[M+H-162]^+$).

3.4. X-ray crystallographic data of compound **1**

Compound **1** was crystallized in MeOH. A monocrystal was selected and mounted on a Gemini A Ultra CCD diffractometer using Cu Kα radiation (λ = 1.541 84 Å). The structure was solved using the SHELXS-97 program and refined with SHELXL-97. Refinement resulted in $R = 0.0351$ for **1**. Resonant scattering, principally from the O atoms, resulted in a Flack (Flack, 1983) parameter $X = 0.07$ (19) and a Hooft 50 parameter $Y = 0.06$ (7) based on 1125 Friedel pairs for **1**.

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

A solution of **2** 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 lcysteine 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, EQUITYTM-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 (Hara et al., 1987). D-glucose in **2** 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.

3.6. Antioxidant activity assay

The human umbilical vein endothelial cell (HUVEC) were purchased from the American type culture collection (ATCC, USA). Cells were cultured in ECM medium (Sciencell, USA). The complete medium contained 5% FBS (Sciencell, USA), 1% penicillin–streptomycin and 1% ECGS. Cells were cultured at 37 °C with 5% CO_2. Hydrogen peroxide (H_2O_2, Yongda, Tianjin, China) was diluted in phosphate-buffered saline.

The cytotoxicity of the compound **1** was evaluated by MTT (Solarbio, Beijing, China) (Chow et al., 2005). Cells were seeded in 96-well plates at 2.0×10^4 cells/well in a final volume of 100 μl. After 16 h, fresh free medium with different concentrations (12.5, 25, 50, 100 μM) of compound **1** was added to each well. After treatment for 24 h, cells were treated with 10 μL MTT (10 mg/ml) each well for another 2 h. The crystals were dissolved in dimethyl sulfoxide (DMSO). The optical density was measured at 570 nm with a microplate reader. The cytotoxicity was calculated using untreated cells at 100%.

The antioxidant activity of the compound **1** was investigated through establishing an oxidation stress model with H_2O_2 (Ham et al., 2012). HUVEC at 2.0×10^4 per well were seeded in 96-well plates. After 16 h incubation, cells were treated with H_2O_2 (1400 μM) together with or without compound **1** (12.5, 25, 50 and 100 μM) for 3 h. Then replace the supernatant with fresh serum free medium following 10 μL MTT (10 mg/ml) each well for another 2 h. The resulting crystals were dissolved in DMSO. The optical density was measured at 570 nm. Then the cell viability was calculated. The experiments were repeated three times, and the data are expressed as the means ± SD of three independent experiments.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by the National Science Foundation of China (Grant No. U1303122), Jiangsu Provincial Science & Technology Program (BE2014654), and the Foundation for Outstanding Young Scientist in Shandong Province (BS2009SW046).

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