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Rare trisubstituted sesquiterpenes daucanes from the wild *Daucus carota*

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Abstract

Phytochemical and biological investigation of the roots of the wild *Daucus carota* ssp. *carota* afforded three new and four known compounds, including four sesquiterpenes daucane esters (**1**–**3** [new], and **4**), one polyacetylene (**5**), one sesquiterpene coumarin (**6**), and sitosterol glucoside. The structures of the new compounds were determined by comprehensive NMR studies, including DEPT, COSY, NOESY, HMQC and HMBC analyses. Based on an agar diffusion assay, **1**, **2** and **4**–**6** were screened and found to contain a range of low antibacterial activities against four gram positive (*Staphylococcus aureus*, *Streptomyces scabies*, *Bacillus subtilus*, *Bacillus cereus*) and two gram negative species (*Pseudomonas aeruginosa*, *Escherichia coli*) as well as antifungal against *Fusarium oxysporum* and *Aspergillus niger* using cup agar diffusion assay.

Keywords: *Daucus carota* ssp. *carota*; Apiaceae; Daucane sesquiterpenes; Sesquiterpene coumarin; Polyacetylene; Antibacterial

1. Introduction

The genus *Daucus* (Apiaceae) comprises weedy plants of about 60 species, widely distributed and commonly cultivated for their fleshy edible roots. *Daucus carota* L. ssp. *carota* (wild carrot) is indigenous to Europe and is used as antibacterial, stimulant (Emilio, 1994), antiseptic, carminative, diuretic, hepatoprotective (Bishayee et al., 1995), antisteroidogenic (Majumder et al., 1997), anti-inflammatory (Porchezhian et al., 2000) and in treatment of jaundice and stomach disorders. The seeds of *D. carota* are used for the treatment of swelling and tumors, while the roots are used as poultice in mammary and uterine carcinoma as well as skin cancer. Seed oil of this species afforded daucane-type sesquiterpenes (Dhillon et al., 1989; Mazzoni et al., 1999), such as *trans*-dauc-8-ene-4β-ol, *trans*-dauca-8,11-diene, dauca-5,8-diene, acora-4,9-diene, acora-4,10-diene, carotol and daucol, furocoumarins (Ceska et al., 1986). Additional constituents include flavonoids (Gupta and Niranjan, 1982), polyacetylenes (Lund, 1992), fatty oil (Vesna et al., 1989) and β-carotene (Bicudo-De-Almeida et al., 1998). This investigation of the roots of the wild *D. carota* L. ssp. *carota* in search for new bioactive constituents afforded three new sesquiterpene daucane derivatives, **1**–**3**, and four known compounds, with all of tested constituents exhibiting selective inhibition of particular bacterial strains.

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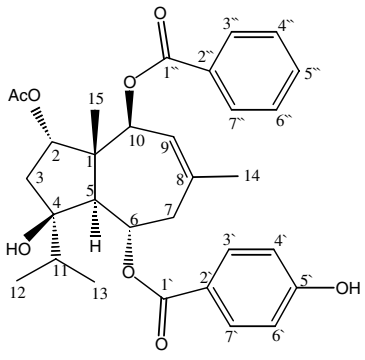

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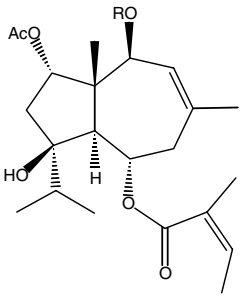


2 R = benzoate
3 R = cinnamate

2. Results and discussion

Compound **1** was isolated as white powder, $[\alpha]_D^{50} + 22.8$ ($c = 0.025, CHCl_3$), its IR spectrum showed absorption bands at 3421 cm^{-1} (OH) and 1714 cm^{-1} (C=O). The molecular formula of **1**, C_{31} $H_{36}O_8$, was determined from HRCIMS (*m/z* 535.2326), and ^{13}C NMR spectral data. The ^1H and ^{13}C NMR (Tables 1 and 2) exhibited signals for a daucane sesquiterpene nucleus, including a doublet at δ_H 5.11 (1H, d, $J = 6$ Hz, H-2) that correlated in the ^1H–^1H COSY spectrum with a signal at δ_H 2.12 (1H, dd, $J = 6$, 16 Hz, H-3β). In the same experiment, a signal at δ_H 5.36 (1H, ddd, $J = 10.5$, 10.5, 2.5 Hz, H-6) exhibited correlations with three signals at δ_H 2.71 (1H, d, $J = 10.5$ Hz, H-5), 2.72 (1H,d, $J = 14.5$, 2.5 Hz, H-7β) and 2.30 (1H, dd, $J = 14.5$, 10.5 Hz, H-7α). The two broad singlets at δ_H 5.35 and 5.77 were assigned to H-9 and H-10, respectively. The two methyl doublet signals at δ_H 0.95 (3H, d, $J = 7$ Hz, H-12) and 0.80 (3H, d, $J = 7$ Hz, H-13) coupled in the ^1H–^1H COSY spectrum with a signal at δ_H 1.85 (1H, qq, $J = 7$ Hz, H-11), which assigned for an isopropyl moiety. Four aromatic protons appeared as two doublets at δ_H 7.95 (2H, d, $J = 8.5$ Hz, H-3′,7′) and 6.90 (2H, d, $J = 8.5$ Hz, H-4′,6′), were typical for a *p*-hydroxybenzoyloxy group. Other protons at δ_H 7.45 (2H, t, $J = 7.5$ Hz H-4″,6″), 7.55 (1H, t, $J = 7.5$ Hz, H-5″) and 8.10 (2H, d, $J = 7.5$ Hz, H-3″,7″) were assigned for a benzoyloxy moiety. The ^{13}C NMR spectral data of **1** (Table 2) exhibited 31 carbon signals that were resolved by DEPT experiment into: 15 methines, 2 methylenes, 5 methyls and 9 quaternary carbons. The three signals at δ_C 170.1, 166.4 and 165.9 could be assigned by HMBC to the three carbonyl carbons of the acetyloxy, *p*-hydroxybenzoyloxy and benzoyloxy groups, respectively. Four oxygen bearing carbons were assigned at

Table 1
^1H NMR of compounds **1**–**3** (500 MHz,$CDCl_3$, δ-values)

Protons	1	2	3[a]
H-2β	5.11 d (6)	5.07	5.06
H-3β	2.12 dd (6,16)	1.87	1.85
H-3α	1.93 d (16)	2.10	2.09
H-5α	2.71 d (10.5)	2.61	2.58
H-6β	5.36 ddd (10.5, 10.5, 2.5)	5.23	5.21
H-7β	2.72 dd (14.5, 2.5)	2.69	2.67
H-7α	2.30 dd (14.5, 10.5)	2.23	2.21
H-9	5.35 br s	5.33	5.29
H-10α	5.77 br s	5.74	5.61
H-11	1.85 qq (7)	1.82	1.80
H-12	0.95 d (7)	0.89	0.89
H-13	0.80 d (7)	0.87	0.88
H-14	1.85 br s	1.83	1.84
H-15	1.36 s	1.34	1.28
H-3′,7′	7.95 d (8.5)		
H-4′,6′	6.90 d (8.5)		
H-3″,7″	8.10 d (7.5)	7.95	7.60
H-4″,6″	7.45 t (7.5)	7.45	7.45
H-5″	7.55 t (7.5)	7.55	7.60
H-3′		6.20 qq (7.3, 1.5)	6.18
H-4′		2.03 dq (7.3, 1.5)	2.02
H-5′		1.90 dq (1.5, 1.5)	1.89
AcO	1.86 s	1.84	1.93

[a] H-α at δ_H 6.39 ($J = 16$ Hz), H-β at 7.64 ($J = 16$ Hz).

Table 2
^{13}C NMR of compounds **1**–**3** (125 MHz, $CDCl_3$, δ-values)

Carbons	1	2	3[a]
C-1	51.2 (s)	51.1	51.0
C-2	79.8 (d)	79.7	79.5
C-3	39.4 (t)	39.3	39.3
C-4	85.7 (s)	85.6	85.5
C-5	54.7 (d)	54.6	54.5
C-6	69.5 (d)	68.9	68.8
C-7	41.1 (t)	41.2	41.1
C-8	131.6 (s)	127.3	127.4
C-9	128.4 (d)	128.5	128.6
C-10	75. 9 (d)	75.8	75.3
C-11	37.1 (d)	36. 9	36.9
C-12	18.1 (q)	17.4	17.4
C-13	17.6 (q)	18.2	18.1
C-14	26.0 (q)	26.1	26.0
C-15	15.5 (q)	15.5	15.3
C-1′	166.4 (s)		
C-2′	122.3 (s)		
C-3′,7′	132.1 (d)		
C-4′,6′	115.5 (d)		
C-5′	160.5 (s)		
C-1″	165.9 (s)	165.7	166.2
C-2″	131.5 (s)	131.5	134.2
C-3″,7″	129.5 (d)	129.5	128.8
C-4″,6″	128.5 (d)	128.5	128.2
C-5″	133.2 (d)	133.2	130.4
C-1′		168.2 (s)	168.2
C-2′		129.5 (s)	127.4
C-3′		140.1 (d)	140.1
C-4′		20.5 (q)	20.4
C-5′		16.0 (q)	15.9
AcO	170.1 (s), 20.9 (q)	170.0, 20.9	170.1,20.8

[a] C-α at δ_C 117.8, C-β at δ_C 145.1.

δ_C 79.8 (C-2), 85.7 (C-4), 69.5 (C-6) and 75.9 (C-10). Other proton and carbon signals were determined by HMQC and HMBC. The position of the acyl groups was determined by combination of HMBC and NOESY (Fig. 1). In a HMBC experiment, H-2 (δ_H 5.11) showed a cross peak with the carbon signal at δ_C 170.1, locating the acetyl moiety at C-2; H-6 (δ_H 5.36) exhibited a cross peak with the carbon signal at δ_C 166.4, placing the *p*-hydroxy-benzoyloxy moiety at C-6. The relative stereochemistry of the chiral centers could be deduced from a NOESY experiment, where H-2, H-6 and H-15 correlated with each other, indicating the β-configuration of these protons, while H-10 correlated with H-5, suggesting the α-configuration of H-10. Therefore, compound **1** was assigned as 2α-acetyloxy-4β-hydroxy-6α-*p*-hydroxy-benzoyloxy-10β-benzoyloxy-dauc-8-ene, a new natural products.

Compound **2** exhibited similar ^{1}H NMR spectra with those of compound **1**. However, a few differences were observed, including the presence of an angeloyloxy moiety in **2**, instead of *p*-hydroxybenzoyloxy in **1**. The ^{1}H NMR spectrum showed a typical angeloyeloxy signals at δ_H 6.20 (1H, qq, J = 7.3, 1.5 Hz, H-3′), 2.03 (3H, dq, J = 7.3, 1.5 Hz, H-4′) and 1.90 (3H, dq, J = 1.5, 1.5 Hz, H-5′). The ^{13}C NMR spectral data of **2** (Table 2) revealed 29 carbon signals that were classified by DEPT experiment into: 12 methines, 2 methylenes, 7 methyls and 8 quaternary carbons. The three signals at δ_C 170.0, 168.2 and 165.7 could be assigned by the HMBC spectrum to the three carbonyl carbons of the acetyloxy, angeloyoxyl and benzoyloxy groups, respectively. Other signals were determined by HMQC and HMBC. Furthermore, the placement of angeloyloxy moiety at C-6 was suggested by the isopropyl methyl doublets collapsed to two close doublets (ca. Δ 0.13 ppm)

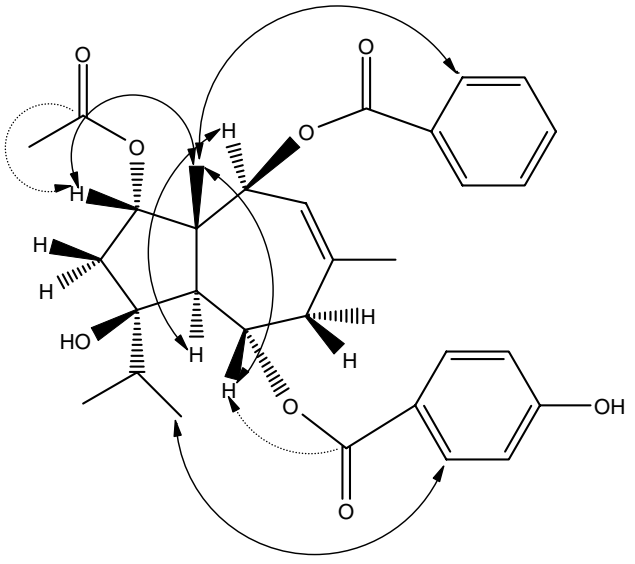


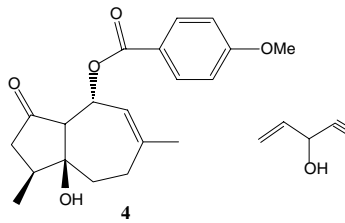
Fig. 1. Selected HNBC →, and NOESY ↔ of compound **1**.

(Miski and Mabry, 1985; Garg et al., 1987). This finding was supported by a HMBC experiment, the angeloyloxy carbonyl carbon (δ_C 168.2) exhibited correlation with H-6 (δ_H 5.23) and the acetyloxy carbonyl carbon (δ_C 170.0) showed correlation with H-2 (δ_H 5.07). The relative stereochemistry of **2** was same as **1**, a NOESY experiment showed correlation between H-6β, H-15β, H-3β, between H-5α, H-10α and between H-6β, H-2β. Therefore, compound **2** was determined as 2α-acetyeloxy-4β-hydroxy-6α-angeloyloxy- 10β-benzyoloxy-dauc-8-ene, a new natural product.

Compound **3** was isolated as a mixture with **2** (3:2). However, its structure could be easily determined from comparison of the 1D and 2D NMR with those of **2**. The ^{1}H NMR spectrum exhibited two doublets at δ_H 7.64 (δ_C 145.1) and 6.39 (δ_C 117.8), that coupled with each other in the ^{1}H–^{1}H COSY spectrum. The large coupling (J = 16 Hz) of the signals was consistence with *trans*-configuration. Both signals correlated in a HMBC spectrum with the carbonyl carbon at $_C$ 166.2 (C-1″). Furthermore, the HMBC spectrum showed correlation between the quaternary carbon at δ_C 134.2 (C-2″) and the two proton signals at δ_H 6.39 and 7.45. This accumulated data established the presence of a cinnamoyloxy moiety in **3**, instead of a benzoyloxy moiety in **2**. The placement of the cinnamoyloxy moiety at C-10 was supported by a HMBC experiment, the carbonyl carbon at δ_C 166.2 (C-1″) correlated with H-10 at δ_H 5.61. The other protons and carbons were similar to those of **2** (Tables 1 and 2). The molecular formula of **3** was established as $C_{31}H_{40}O_7$ on the basis of HRCIMS, which gave an ion peak $[M + H - H_2O]^+$ at *m*/*z* 507.2769. Therefore, compound **3** was identified as 2α-acetyloxy-4β-hydroxy-6α-angeloyloxy- 10β-cinnamoyloxydauc-8-ene, a new natural product.

The structure of the known compounds fercomin (**4**) (Miski and Mabry, 1989), falcarindiol (**5**) (Driss et al., 1987) and ferulenol (**6**) (Lund, 1992) was determined from NMR spectral comparison with those reported in the literature.

It appears to us that this is the first chemical investigation of the roots of the wild *D. carota*. Ferulenol (**5**) is



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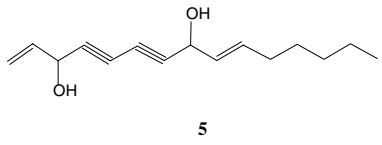

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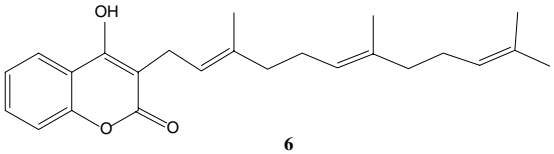

6

the major constituents of the wild *D. carota*. However, accumulation of sesquiterpene-daucans type in the roots of the wild species showed a chemical relation to the genus *Ferula* (family Apiaceae) (Ahmed, 1991; Galal et al., 2001). This is the first report of trisubstituted daucans containing two phenyl moieties from the genus *Daucus*, which are rare in the genus *Ferula* (Miski and Mabry, 1985).

Antibacterial activity of the compounds **1**, **2** and **4**–**6** was evaluated against the gram positive species *Staphylococcus aureus*, *Streptomyces scabies*, *Bacillus subtilis* and *Bacillus cereus* as well as the gram negative species *Pseudomonas aeroginosa*, *Escherichia coli*. Also, were tested against two fungal strains *Fusarium oxysporum* and *Aspergillus niger*. From preliminary studies, the minimum inhibitory concentration (MIC) of **1** and **2** was less than 2 mg/mL against all microorganism except *E. coli* in which the tested compounds exhibited no antibacterial activity at the doses tested. Compound **6** had an estimated MIC less than 2.5 mg/mL against *S. aureus*, *S. scabies*, *B. subtilis*, *B. cereus* and *P. aeroginosa* and between 5 and 4.5 mg/mL against *E. coli*, *F. oxysporum* and *A. niger*. Compounds **4** and **5** had MIC less than 2.5 mg/mL against all selected microorganisms but show no antibacterial effect against *F. oxysporum* and *E. coli*. As a point of biological-activity comparisons, the antifungal terpenoid ascaridole and the commercially available fungicide vinclozolin have a MIC at ca. 4 mg/mL under the same bioassay system (Paré et al., 1993).

3. Experimental

3.1. General

^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$) and the 2D spectra were recorded on Varian 500 MHz, with TMS as an internal standard. Mass spectra were determined by VG-ZAB2E (70 eV) and optical rotations were determined with a JASCO-20 C automatic recording spectropolarimeter. TLC: precoated silica gel type 60 (Merck); CC: silica gel type 60 (Merck). HPLC was performed in the reversed phase on Knauer pump 64 and different refractometer (column: RP-8, 250 × 25 mm, flow = 17 mL/min, elution with MeOH–H_2O mixtures, refractive index).

3.2. Plant material

Roots of *D. carota*, Linn. ssp. *carota* were collected in the flowering stage May 2002, from the Wadi Shuib, Jordan, by one of the authors (A.A. Ahmed). A voucher specimen was deposited in the Center for Jordanian Studies, Jordan Natural History Museum.

3.3. Biological activitiy

3.3.1. Antimicrobial assay

The antibacterial activity of the compounds **1**, **2** and **4**–**6** was determined against *S. aureus*, *S. scabies*, *B. subtilis*, *B. cereus*, *P. aeroginosa* and *E. coli* and the antifungal activity of those compounds was determined against *F. oxysporum* and *A. niger* using cup agar diffusion assay (United States Pharmacopia, XXII, 1990) (see Table 3). The media used were nutrient agar (Oxoid). The over night culture of each organism was diluted with saline to contain 1×10^6 cell/mL for bacteria and 1×10^5 spores/mL for fungi (Padual-Desai et al., 1976). An aliquot of 3 mL of diluted culture was spread onto the surface of nutrient agar plate (40 mL each) and the excess bacterial suspension was withdrawn. The assay plates were incubated at 35 °C and left for 24 h for bacteria and 7 days for fungi to observe the inhibition zones around the wells and then were measured.

3.4. Extraction and isolation

Air-dried roots (250 g) were ground and extracted with MeOH–CH_2Cl_2 (1:1) at room temperature. The extract was concentrated in vacuo to obtain a residue of 20 g. The residue was prefractionated by column chromatography (6 × 120 cm) on a silica gel column eluting with *n*-hexane (3 L) followed by a gradient elution with *n*-hexane–CH_2Cl_2 up to 100% CH_2Cl_2 and finally with CH_2Cl_2–MeOH (85:15). The *n*-hexane–CH_2Cl_2 (1:4) gave two fractions, the first one was purified by TLC (*n*-hexane–ether, 1:4) to give compound **1** (4 mg), the second fraction was purified by HPLC (MeOH–H_2O, 73:27) to afford **2** (2 mg) and a mixture of **2** and **3** (1.5 mg). The CH_2Cl_2(100%) fraction was subjected to a Sephadex LH-20 column (2x60) eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5) to afford compound **4** (30 mg). The CH_2Cl_2–MeOH (85:15) fraction was subjected to Sephadex LH-20 column (2 × 60) eluted with *n*-hexane–CH_2Cl_2–MeOH to give four fractions. The *n*-hexane–CH_2Cl_2–MeOH (7:4:1) afforded compound **6**

Table 3
Antimicrobial activity of compounds **1**, **2** and **4**–**6**

Microorganism	MIC-minimum inhibitory concenteration (mg/mL)				
	1	**2**	**4**	**5**	**6**
S. aureus	1.8	1.6	2.3	2.2	2.4
S. scabies	1.2	1.4	2.1	1.5	2.2
B. subtilis	1.0	1.2	1.7	1.4	2.0
B. cereus	1.5	1.3	1.4	1.6	2.1
P. aeroginosa	1.3	1.5	1.0	1.4	2.3
E. coli					4.8
F. oxysporum	0.5	0.4			4.6
A. niger	0.3	0.25	1.0	0.5	4.7

These data are means of four replicates.

(10 mg), *n*-hexane–CH_2Cl_2–MeOH (7:4:2) was further purified by TLC to afford compound **5** (1.5 mg).

3.4.1. 2α-Acetyloxy-4β-hydroxy-6α-p-hydroxybenzoyloxy- 10β-benzoyloxy-dauc-8-ene (**1**)

White powder; $[\alpha]_D^{25} + 22.8^\circ$ ($c = 0.30$, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}): 3421, 2963, 2925, 1714, 1272, 1164, 770 cm^{-1}; CIMS $[M - H]^+$ *m/z* 535, $[M + H - H_2O]^+$ *m/z* 519, $[519 - benzoate]^+$ *m/z* 415; HRCIMS: *m/z* 535.2326 (calc. for $C_{31}H_{34}O_8$, 535.2332); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

3.4.2. 2α-Acetyloxy-4β-hydroxy-6α-angeloyloxy-10β-benzoyloxydauc-8-ene (**2**)

Amorphous white powder; $[\alpha]_D^{25} + 8.8^\circ$ ($c = 0.10$, $CHCl_3$); IR (ν_{max}^{KBr} cm^{-1}): 3500, 2980, 2960, 1730, 1260, 1164, 1032, 770 cm^{-1}; CIMS $[M + H - H_2O]^+$ *m/z* 481, $[M - benzoate]^+$ *m/z* 277; HRCIMS: *m/z* 481.2585 $[M + H - H_2O]^+$ (calc. for $C_{29}H_{37}O_6$, 481.2590). ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

3.4.3. 2α-Acetyloxy-4β-hydroxy-6α-angeloyloxy- 10β-cinnamoyloxydauc-8-ene (**3**)

Amorphous yellowish powder; IR (ν_{max}^{KBr} cm^{-1}): 3500, 2980, 2960, 1730, 1260, 1164, 1032, 770 cm^{-1}; CIMS $[M + H - H_2O]^+$ *m/z* 507, $[M - cinnamate]^+$ *m/z* 377; HRCIMS: *m/z* 507.2769 $[M + H - H_2O]^+$ (calc. for $C_{31}H_{39}O_6$, 507.2747). ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Acknowledgments

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