Chemistry of Natural Compounds, Vol. 45, No. 6, 2009

COMPOUNDS FROM FLOWERS OF *Daucus carota* L. SSP. *carota* AND THEIR ANTIOXIDANT ACTIVITY

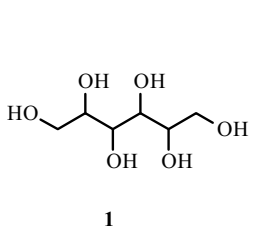
Yurdanur Akgul,[1*] Lale Yildiz Aktas,[2] and Huseyin Anil[1]

UDC 547.972

Daucus carota L. ssp. *carota*, wild carrot, belongs to the family Apiaceae (Umbelliferae). It is a tall robust biennial spiny-fruited herb that grows in dried-out fields [1]. It is indigenous to Europe and has antibacterial, stumulant [2], antisteroidogenic [3], anti-flammatory [4], antiseptic, carminative, diuretic, and hepatoprotective properties [5]. Chemical studies on the seed oil and the root of *Daucus carota* L. have revealed a variety of chemical counstituents such as daucane-type sesquiterpenes [6–9], flavonoids [10], polyacetylenes [11], fatty oils [12], β-carotene [13], and essential oil [14].

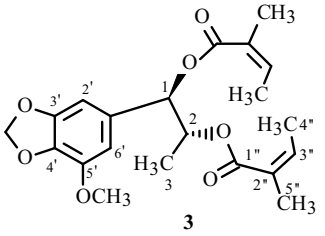
The dichloromethane–methanol (1:1) extract of the flowers of *Daucus carota* L. was partitioned between water and *n*-butanol. The *n*-butanol soluble portion was subjected to successive column chromatographic separations using silica gel to afford compounds **2–5**. The water soluble fraction give compound **1**. Identification of compounds **1–5** was performed using 1D (^{1}H, ^{13}C)-2D NMR (HMBC, HMQC, COSY), LC/MS-APCI, and literature data.

Compound **1** was obtained as a white amorphous solid from the water soluble fraction. The positive LC/MS-APCI spectrum of **1** showed a molecular ion peak at *m/z* 183.10 $[M+H]^+$ corresponding to the molecular formula $C_6H_{14}O_6$. The main fragment peaks were recorded at *m/z* $[M–OH]^+$ 165.10 and *m/z* $[M–CH=OH]^+$ 152.10. The ^{1}H NMR and ^{13}C NMR spectra revealed signals for eight aliphatic hydrogens at δ_H 3.62 (2H), 3.71 (4H), and 3.77 (2H) and three carbons at δ_C 63.51, 69.65, and 71.28. The melting point of compound **1** was not identified (decomposed above 149°C). The melting point of the acetylated derivative **1a** was found to be 121°C. The ^{1}H NMR spectrum of **1a** showed signals at δ_H 5.42 (2H, d, J = 8.0 Hz), 5.06 (2H, m), 4.21 (2H, dd, J = 2.8, 2.8 Hz), 4.06 (2H, dd, J = 5.2, 5.2 Hz), and 2.06, 2.04, and 2.02 (18H, s). The ^{1}H–^{1}H COSY spectrum showed a correlation between signals at δ_H 5.42 and 5.06, δ_H 5.06 and 4.21, δ_H 5.06 and 4.06, and δ_H 4.21 and 4.06. The ^{13}C NMR spectrum showed the presence of three carbonyl signals at δ_C 170.71, 170.05, and 169.85, three methyl signals at δ_C 21.01, 20.83, and 20.76, and three carbon signals at δ_C 62.06, 67.69, and 68.14. Furthermore, the HMQC spectrum showed a correlation between the carbon at δ_C 68.14 and the proton signals at δ_H 5.06; between δ_C 67.79 and the proton signals at δ_H 5.42; between δ_C 62.02 and the proton signals at δ_H 4.21 and 4.06. Compound **1** was identified as D,L-iditol based on NMR and LC/MS-APCI data. Also compound **1** was confirmed to be D,L-iditol by comparison with the melting point of **1a** in the literature [18]. This compound was isolated and reported for the first time from *Daucus carota* L. The plant is a new source of D- and L-iditol.



1

(D, L-Iditol)



3

1) Ege University, Faculty of Science, Chemistry dept., 35100 Bornova-Izmir, Turkey, fax: + 90 232 388 82 64, e-mail: yurdanur.akgul@ege.edu.tr; 2) Ege University, Faculty of Science, Biology dept., 35100 Bornova-Izmir, Turkey. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 742–744, November–December, 2009. Original article submitted April 7, 2008.

0009-3130/09/4506-0889

TABLE 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compound **1** (400 MHz and 125 MHz in D_2O) and Compound **1a** ($CDCl_3$)

C atom	**1**		**1a**		
	δ_C	δ_H (J/Hz)	δ_C	δ_H (J/Hz)	H–H COSY
1	63.57	3.73–3.66(2H, m)	62.06	4.21 (2H, dd, J = 2.8, H-1a, 6a)	H1a, H1b, H2
2	69.65	3.62–3.57 (1H)	67.69	5.06 (1H, m)	H1a, H6a, H3
3	71.27	3.81 (1H, d, J = 2.8)	68.14	5.42 (1H,d, J = 8.0)	H4
4	71.27	3.78 (1H, d, J = 2.4)	68.14	5.42 (1H, d, J = 8.0)	H5
5	69.65	3.62–3.57 (1H)	67.69	5.06 (1H, m)	H6b,H1b, H4
6	63.57	3.73–3.66 (2H, m)	62.06	4.06 (2H, dd, J = 5.2, H-1b, 6b)	H6a, H6b, H5
CH_3			21.01	2.06 (6H, s)	
			20.83	2.03 (6H, s)	
			20.75	2.01 (6H, s)	
C=O			170.71		
			170.05		
			169.85		

TABLE 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Compound **3** (400 MHz and 125 MHz in $CDCl_3$)

C atom	δ_C	δ_H (J/Hz)	H–H COSY	HMBC
1	77.43	5.78 (1H, d, J = 6.4)	H2	H2′, H2, H3
2	71.44	5.36 (1H, m)	H1, H3	H1, H3
3	17.01	1.11 (3H, d, J = 6.8)	H2	H1
1′	131.93			H1
2′	107.51	6.56 (1H, d, J = 1.2)		H1, H6′
3′	143.76			OMe, H2′, H6′
4′	135.56			H2′, H6′
5′	149.18			H2′, H6′, OCH_2O
6′	101.80	6.58 (1H, d, J = 1.6)		H2′
OMe	56.81	3.89 (3H, s)		OMe
1″	167.35			H2, H5″
	166.86			H1, H5″
2″	128.04			H4″, H5″
	127.70			
3″	139.13	6.11 (2H, m)	H4″	H4″, H5″
	138.31			
4″	15.99	1.97 (6H, m)	H3″	
	15.88			
5″	20.73	1.88 (6H, m)		H3″
OCH_2O	101.87	5.96 (2H, s)		OCH_2O

Compound **2** was isolated as a yellow powder from the *n*-butanol portion. It was identified by comparison with the reported spectroscopic data [15] as kaempferol-3-*O*-*β*-glucoside [15], which was isolated and reported for the first time from the *Daucus carota* L.

Compound **3** was isolated as an oil from the *n*-butanol soluble fraction after repeated silica gel column chromatography. The ^{1}H NMR spectrum of the compound showed signals due to two aromatic protons (δ_H 6.57, d and 6.56, d), two olefinic protons (δ_H 6.11, m), two aliphatic protons (δ_H 5.77, d and 5.36, m), one methoxy group (δ_H 3.89, s), one methylenedioxy group (δ_H 5.96, s), and three signals from five methyl groups (δ_H 1.97, 1.88, 1.11). The ^{13}C NMR spectrum showed the presence of six aromatic carbons, four olefinic carbons, one methyleneoxy carbon, one methoxy carbone, two aliphatic oxygen-substituted carbons, and methyl carbons (Table 2). The ^{1}H–^{1}H COSY spectrum showed a correlation between H1 (δ_H 5.77) and H2 (δ_H 5.36); between H2 (δ_H 5.36) and H3 (δ_H 1.11); between H3″ (δ_H 6.11) and H4″, H5″ (δ_H 1.97, 1.88). Compound **3** had a molecular ion peak in the LC/MS-APCI spectrum at *m/z* $[M+H]^+$ 391.15 corresponding to the molecular formula $C_{21}H_{26}O_7$.

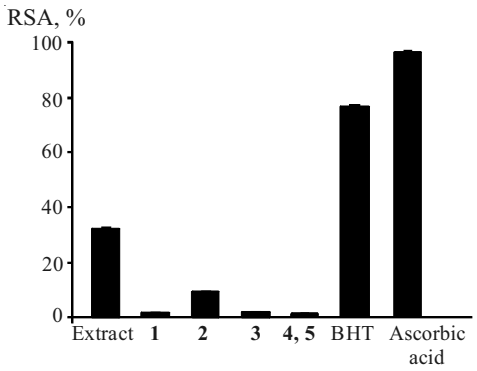


Fig. 1. Antioxidant Activity of Isolated Compounds.

Also the mass spectrum gave a base peak at *m/z* 291.15 corresponding to the loss of the one angelate. The NOESY spectrum of compound **3** indicated that there was no cross peak between H1 and H2 (J = 6.4Hz). The data indicated that **3** was laserine [16]. The structure was confirmed by HMBC and HMQC experiments. Compound **3** was isolated and reported for the first time from *Daucus carota* L.

Compounds **4** and **5** were isolated as a mixture (1:1, stigmasterol and sitosterol). Their structures were identified easily from ^{1}H, ^{13}C NMR spectra, and correlated literature data [17].

The antioxidant capacity of isolated compounds was evaluated using the DPPH method [19]. Although the dichloromethane–methanol (1:1) extract of the flowers of *Daucus carota* L. has significant antioxidant activity (32.57%), isolated compounds showed slight activity. Compound **2** has higher free radical-scavenging activity (9.12%) than the other compounds (Fig. 1).

General Experimental Procedures. Optical rotation: Perkin–Elmer 341 Polarimeter; NMR: Varian AS-400 on Mercury plus console; LC/MS-APCI: ALIGENT 1100 MSD. All solvents used were of analytical grade (Carlo Erba).

Silicagel Merck 7734 and Silicagel Merck 13895 were used for column cromatograpy and PTLC. TLC was carried out using Kieselgel 60 F_{254} (Merck 5554); spots were visualized by spraying with 20% $H_2SO_{4\ aq}$.

Plant Material. Flowers of *Daucus carota* L. ssp. *carota* were collected in June 2006 from Sasali-Izmir (Turkey) and were identified by Dr. Serdar Gokhan Senol, Department of Botany, Faculty of Science, Ege university. A voucher specimen (EGE.HERB:40759) is preserved in the herbarium of Ege university.

Extraction and Isolation. The shade-dried flowers (898 g) were extracted exhaustively with dichloromethane–methanol (1:1, v/v) and concentrated under reduced pressure to afford 185.10 g of extract. Twenty grams of extract was partioned between *n*-butanol and water. Compound **1** was isolated from the water fraction. After removal of the water, it was dissolved in MeOH–CH_2Cl_2 (4:1 v/v). Precipitate 1 was obtained (900 mg). The *n*-butanol soluble fraction was chromatographed using a silica gel column (400 g, hexane–CH_2Cl_2 increasing CH_2Cl_2 and 90:10:1 CH_2Cl_2–MeOH–water, CH_2Cl_2–MeOH–water 80:20:2, CH_2Cl_2–MeOH–water 70:30:3). Twenty fractions were collected (A-U). Fraction K gave compound **3** (eluting solvent: hexane–CH_2Cl_2 40:60). After purification with PTLC (hexane–CH_2Cl_2–ethyl acetate 10:10:3), compound **3** was obtained (20 mg). Compounds **4** and **5** were isolated from fraction R. After purification with PTLC (hexane–CH_2Cl_2–ethyl acetate 10:10:5), 6 mg mixture of compound was obtained. Compound **2** was obtained from fraction S- (elueting solvent: CH_2Cl_2–MeOH–water 80:20:2, CH_2Cl_2–MeOH–water 70:30:3). After purification with PTLC (CH_2Cl_2–MeOH–water 70:30:3), 20 mg of compound **2** was obtained.

D,L-Iditol (1): white amorphous solid; $[\alpha]_D^{25}$ 0° (*c* 0.1, MeOH); mp decomposed; LCMS/APCI *m/z* (rel. ints.): 183.10 (100), 165.10, 152.10; ^{1}H and ^{13}C NMR (Table 1).

Acetylation of Compound 1 (1a): 100 mg compound **1** was acetylated in pyridine with Ac_2O according to the usual precedure. White powder, mp 121°C [18]; ^{1}H and ^{13}C NMR (Table 1).

Kaempferol-3-*O*-*β*-glucoside (2): yellow amophous solid; ^{1}H and ^{13}C NMR [15].

Laserine (3): colorless oil; $[\alpha]_D^{25}$ +48° (*c* 0.1, CH_2Cl_2); LCMS/APCI *m/z* (rel. ints.): 391.15 (0.3), 291.15 (100), 282.30 (20.5), 83.10 (45.7); 60.10 (31.4); ^{1}H and ^{13}C NMR (Table 2).

Antioxidant Activity. The free radical scavenging activity was assayed spectrophotometrically [19]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical has a deep violet color due to its unpaired electron, and radical scavenging activity can be followed spectrophotometrically by a loss of absorbance at 515 nm. An appropriate dilution series at six different

concentrations (0.05–1 mg sample/mL methanol or 70% ethanol–water) was prepared, and 0.1 mL of each dilution was added to 1.9 mL of a 6.0×10^{-5} M methanol solution of DPPH, shaken well by vortex, and allowed to react at room temperature. The free radical scavenging activity of samples was calculated according to the formula:

DPPH radical scavenging activity (%) = [(negative control A_{515} – sample A_{515})/negative control A_{515})] × 100

As a blank, 70% ethanol–water or methanol solvent (0.1 mL) was used. DPPH solution (1.9 mL, 6.0×10^{-5} M) and 70% ethanol–water or methanol solvent (0.1 mL) was used as a negative control. BHT and ascorbic acid were used as a positive control. All determinations were performed in triplicate.

ACKNOWLEDGMENT

The authors thank Assoc. Prof. Stephan Thomas Astley for proofing the manuscript. Thanks are also due to Dr. Serdar Gokhan Senol for plant identification.

REFERENCES

1. L. W. Mitich, *Weed Techol.,* **10**, 455 (1996).
2. L. G. Emilio, *Phytochemistry*, **37**, 597 (1994).
3. P. K. Majumder, S. Dasgupta, R. K. Mukhopadhaya, U. K. Majumdar, and M. Gupta, *Ethnopharmacology*, **57**, 209 (1997).
4. E. Porchezhian, S. H. Ansari, and M. Ali, *Biochem. Syst. Ecol.,* **21**, 649 (2000).
5. A. Bishayee, A. Sarkar, and M. Chatterjee, *Ethnopharmacology*, **47**, 69 (1995).
6. R. S. Dhillon, V. K. Gautam, P. S. Kalsi, and B. R. Chabra, *Phytochemistry*, **28**, 639 (1989).
7. V. Mazzoni, F. Tomi, and J. Casanova, *Ethnopharmacology*, **24**, 1735 (1985).
8. O. Ceska, S. K. Chaudhary, P. J. Warrington, and M. Ashwood-Smitt, *Phytochemistry,* **25**, 81 (1986).
9. A. A. Ahmed, M. M. Bishr, M. A. El-Shanawany, E. Z. Attia, and S. A. Ross, *Phytochemistry*, **66**, 1680 (2005).
10. K. R. Grupta and G. S. Niranjan, *Planta Med.,* **46**, 240 (1982).
11. E. D. Lund, *Phytochemistry*, **31**, 3621 (1992).
12. K. Wesna, I. Rada, S. Katiea, and M. Mitan, *Acrang. Pharmazie*, **44**, 166 (1989).
13. M. L. Bicuda-de-Almeida, G. R. Dias, and R. Sasaki, *Boll. Chim. Farm.*, **137**, 290 (1998).
14. E. Porchezhian, S. H. Ansari, and M. Ali, *Indian J. Nat. Prod.,* **61**, 24 (2000).
15. Y. Lu and L. Y. Foo, *Food Chem.*, **80**, 71 (2003).
16. A. F. Baerrero, M. M. Herrador, and P. Arteaga, *Phytochemistry*, **31** (1), 203 (1992).
17. M. P. Dupont, G. Labres, C. Delaude, L. Tchissambou, and J. P. Gastmans, *Planta Med.*, **63** (3), 282 (1997).
18. W. W. Pigman and M. L. Wolfrom, *Advances in Carbohydrate Chemistry*, Academic Press Inc., 1949, 4, pp. 219, 239–240.
19. W. Brand-Williams, M. E. Cuvelier, and C. Berset, *Lebensmittel-Wissenschaft Technol.,* **28**, 25 (1995).