Sesquiterpene Lactones from *Daucus glaber*

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Six new sesquiterpene lactone esters, daucoguaianolactones A–F (**1**–**6**), and one new sesquiterpene lactone, daucoeudesmanolactone A (**7**) were isolated from the leaves and stems of *Daucus glaber* (FORSSK.) THELL., along with the known sesquiterpene lactones talasins A and B and badkhysin. The structures of **1**–**6** were established on the basis of extensive 1D- and 2D-NMR spectroscopic studies and that of **7** by X-ray crystallography. Compounds **4**, **7**, and talasins A and B showed moderate cytotoxicity against P-388 leukemia cells.

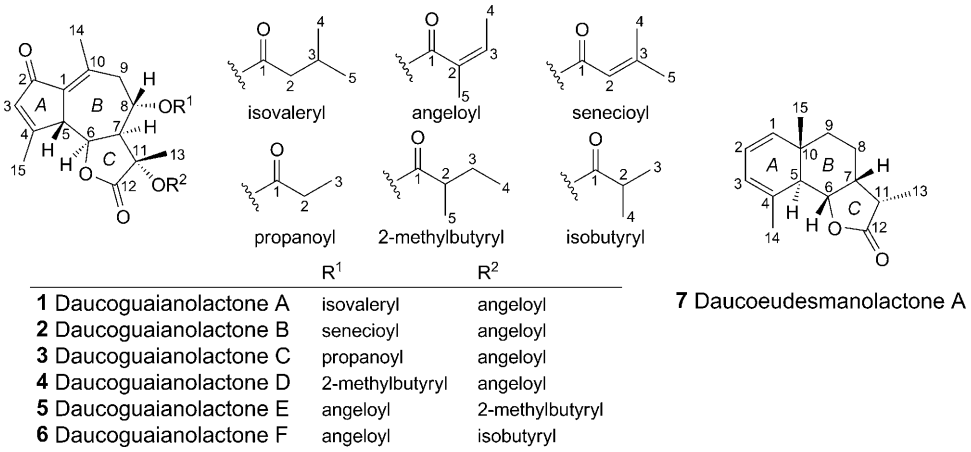
Introduction. – The genus *Daucus* (Apiaceae or Umbelliferae) comprises about 60 annual and biennial species distributed mainly in Europe, Africa, and West Asia [1]. *Daucus glaber* (FORSSK.) THELL. is an annual wild herb growing widely in the Mediterranean coast region and East Asia. In Egypt, it grows wild in the sandy dunes, plains, and sea coast in the northern Nile Delta, along the Mediterranean, and in Sinai peninsula [1][2]. The compositions of the essential oils from the fruits, leaves, and stems of *D. glaber* have been reported [3].

In the present study, from the aerial parts of *D. glaber*, we isolated seven new sesquiterpene lactones, daucoguaianolactones A–F[1]) (**1**–**6**) and daucoeudesmanolactone A[1]) (**7**) (*Fig. 1*), along with the three known sesquiterpene lactones talasins A and B and badkhysin, and elucidated the structures of the new compounds.

Results and Discussion. – By sequential silica gel and reversed-phase silica gel (*RP-18*) column chromatography, *Sephadex LH-20* column chromatography, and prep. HPLC, the hexane-soluble portion of a MeOH extract of the leaves and stems of *D. glaber* yielded daucoguaianolactones A–F (**1**–**6**) and daucoeudesmanolactone A (**7**), along with three known sesquiterpene lactones, talasins A and B, and badkhysin.

Daucoguaianolactone A (**1**) was obtained as a white powder. Its molecular formula was determined to be $C_{25}H_{32}O_7$ from the $[M+H]^+$ peak at m/z 445.2234 in the HR-ESI-MS. The UV spectrum of **1** showed absorption bands at 230 (sh, log ε 4.12) and 251 (log ε 4.18) nm. The IR spectrum indicated the presence of CO groups (1791, 1729, and 1716 cm^{-1}). The ^{13}C-NMR and DEPT spectra revealed a total of 25 C-atoms, *i.e.*, seven Me, two CH_2, and seven CH groups, of which two were O-bearing CH groups and two

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



	R^1	R^2
1 Daucoguaianolactone A	isovaleryl	angeloyl
2 Daucoguaianolactone B	senecioyl	angeloyl
3 Daucoguaianolactone C	propanoyl	angeloyl
4 Daucoguaianolactone D	2-methylbutyryl	angeloyl
5 Daucoguaianolactone E	angeloyl	2-methylbutyryl
6 Daucoguaianolactone F	angeloyl	isobutyryl

Fig. 1. *Sesquiterpene lactones from* Daucus glaber[1])

olefinic, as well as nine quaternary C-atoms of which four were olefinic, one an aliphatic O-bearing, and four CO groups. Analysis of the ^{1}H,^{1}H-COSY and HMQC plots revealed the presence of a C_5-chain fragment consisting of two O-bearing CH, two CH, and one CH_2 group (C(5)–C(6)–C(7)–C(8)–C(9)). Analysis of the ^{1}H,^{1}H-COSY, HMQC, and HMBC data of **1** also indicated the presence of an isovaleryloxy (= 3-methyl-1-oxobutoxy) group (δ(H)/δ(C) 0.98/22.4, 0.98/22.5, 2.08–2.18/25.6, and 2.20/43.5; δ(C) 172.0) and an angeloyloxy (= (2-methyl-1-oxobut-2-en-1-yl)oxy) group (δ(H)/δ(C) 1.90–1.93/20.2, 2.02/16.0, and 6.24/141.5; δ(C) 126.4 and 166.5) (*Fig. 2*, *Tables 1* and *2*). In the HMBC spectrum, H–C(3) was correlated to C(1), C(2), C(4), and C(5) and H–C(5) to C(1), C(3), and C(4), indicating that C(5) of the C_5-chain fragment formed a cyclopentenone ring together with C(1), C(2), C(3), and C(4). Me(15) correlated to C(3) and C(5), and H–C(3) and H–C(5) to C(15), suggesting that Me(15) was connected to C(4). Me(14) correlated to C(1), C(9), and C(10) and CH_2(9) to C(1), C(10), and C(14), which indicated that the olefinic quaternary C(10) atom was connected to C(1) of the cyclopentenone ring, to C(9) of the C_5-chain fragment, and also to Me(14). The HMBCs from H–C(6) to C(11) and C(12) and from H–C(8) to C(11) and the IR absorption band at 1791 cm^{-1} suggested the presence of a γ-lactone moiety involving C(6) of the C_5-chain fragment and the C(12)=O group. The

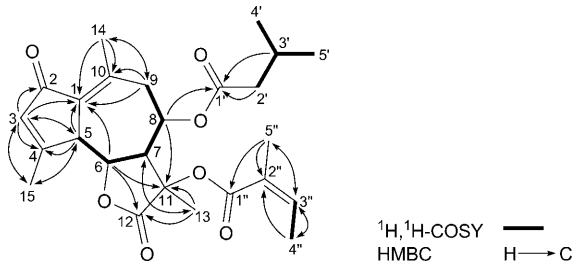


Fig. 2. *^{1}H,^{1}H-COSY and selected HMBCs of* **1**

Table 1. *^{1}H-NMR Data* ($CDCl_3$) *of Compounds* **1**–**7**[1]). δ in ppm[a]), J in Hz.

	1[b])	**2**[b])	**3**[b])	**4**[b])	**5**[c])	**6**[c])	**7**[b])
H–C(1)							5.40–5.44 (*m*)
H–C(2)							5.71–5.73[d]) (*m*)
H–C(3)	6.20–6.22 (*m*)	6.20–6.22 (*m*)	6.20 (*s*-like)	6.20–6.22 (*m*)	6.20 (*s*-like)	6.20–6.22 (*m*)	5.73–5.75[d]) (*m*)
H–C(5)	3.57 (br. *d*, *J* = 11.3)	3.59 (br. *d*, *J* = 11.2)	3.57 (br. *d*, *J* = 11.3)	3.58 (br. *d*, *J* = 11.3)	3.58 (*d*, *J* = 11.4)	3.58 (*d*, *J* = 11.3)	2.46 (*d*-like, *J* = 9.8)
H–C(6)	4.69 (*dd*, *J* = 11.3, 10.0)	4.69 (*dd*, *J* = 11.2, 9.9)	4.69 (*dd*, *J* = 11.3, 9.9)	4.70 (*dd*, *J* = 11.3, 9.9)	4.67 (*dd*, *J* = 11.4, 9.9)	4.66 (*dd*, *J* = 11.3, 9.9)	4.75 (*dd*, *J* = 9.8, 6.7)
H–C(7)	3.55 (br. *dd*, *J* = 11.1, 10.0)	3.55 (*dd*-like, *J* = 11.1, 9.9)	3.55 (*dd*, *J* = 11.2, 9.9)	3.57 (*dd*, *J* = 11.2, 9.9)	3.50 (*dd*, *J* = 11.2, 9.9)	3.52 (*dd*, *J* = 11.1, 9.9)	2.65–2.73 (*m*)
H–C(8) or CH_2(8)	5.56 (*ddd*, *J* = 11.1, 11.1, 3.4)	5.57 (*ddd*, *J* = 11.1, 11.0, 3.3)	5.55 (*ddd*, *J* = 11.2, 11.1, 3.3)	5.55 (*ddd*, *J* = 11.2, 11.1, 3.4)	5.62 (*ddd*, *J* = 11.2, 11.0, 3.3)	5.61 (*ddd*, *J* = 11.1, 11.0, 3.3)	1.62–1.70, 1.45–1.55 (2*m*)
H_a–C(9)	2.83 (*dd*, *J* = 19.0, 3.4)	2.87 (*dd*, *J* = 19.1, 3.3)	2.84 (*dd*, *J* = 19.1, 3.3)	2.80 (*dd*, *J* = 19.1, 3.4)	2.90 (*dd*, *J* = 19.2, 3.3)	2.90 (*dd*, *J* = 19.0, 3.3)	1.83–1.91 (*m*)
H_b–C(9)	2.46 (br. *dd*, *J* = 19.0, 11.1)	2.47 (br. *dd*, *J* = 19.1, 11.0)	2.47 (br. *dd*, *J* = 19.1, 11.1)	2.45 (br. *dd*, *J* = 19.1, 11.1)	2.49 (br. *dd*, *J* = 19.2, 11.0)	2.50 (br. *dd*, *J* = 19.0, 11.0)	1.45–1.55 (*m*)
H–C(11)							2.83 (*dq*, *J* = 8.8, 7.4)
Me(13)	1.63 (*s*)	1.63 (*s*)	1.62 (*s*)	1.62 (*s*)	1.59 (*s*)	1.60 (*s*)	1.22 (*d*, *J* = 7.4)
Me(14)	2.26 (*s*)	2.26 (*s*)	2.25 (*s*)	2.25 (*s*)	2.26 (*s*)	2.27 (*s*)	1.98 (*d*, *J* = 1.3)
Me(15)	2.27 (*s*)	2.27 (*s*)	2.27 (*s*)	2.27 (*s*)	2.27 (*s*)	2.27 (*s*)	0.89 (*s*)
Acyloxy group at C(8):							
CH_2(2′) or H–C(2′)	2.20 (*d*, *J* = 8.0), 2.20 (*d*, *J* = 5.7)	5.62–5.64 (*m*)	2.36 (*q*, *J* = 7.5)	2.32–2.41 (*m*)			
H–C(3′), Me(3′), or CH_2(3′)	2.08–2.18 (*m*)		1.18 (*t*, *J* = 7.5)	1.70–1.81, 1.41–1.52 (2*m*)	6.18 (*qq*, *J* = 7.3, 1.5)	6.19 (*qq*, *J* = 7.3, 1.5)	
Me(4′)	0.98 (*d*, *J* = 6.6)	2.20 (*s*-like)		0.93 (*t*, *J* = 7.4)	2.03 (*dq*, *J* = 7.3, 1.5)	2.03 (*dq*, *J* = 7.3, 1.5)	
Me(5′)	0.98 (*d*, *J* = 6.6)	1.93 (*s*-like)		1.18 (*d*, *J* = 7.0)	1.90–1.93 (*m*)	1.90–1.92 (*m*)	
Acyloxy group at C(11):							
H–C(2″)					2.41–2.34 (*m*)	2.56 (*sept.*, *J* = 7.0)	
H–C(3″), CH_2(3″), or Me(3″)	6.24 (*qq*, *J* = 7.3, 1.5)	6.23 (*qq*, *J* = 7.3, 1.5)	6.22 (*qq*, *J* = 7.3, 1.5)	6.23 (*qq*, *J* = 7.3, 1.5)	1.67–1.76, 1.42–1.51 (2*m*)	1.20 (*d*, *J* = 7.0)	
Me(4″)	2.02 (*dq*, *J* = 7.3, 1.5)	2.02 (*dd*-like, *J* = 7.3, 1.5)	2.02 (*dq*, *J* = 7.3, 1.5)	2.01 (*dq*, *J* = 7.3, 1.5)	0.92 (*t*, *J* = 7.4)	1.17 (*d*, *J* = 7.0)	
Me(5″)	1.90–1.93 (*m*)	1.90–1.93 (*m*)	1.90–1.93 (*m*)	1.90–1.93 (*m*)	1.18 (*d*, 7.0)		

[a]) Chemical shifts δ referenced to residual $CHCl_3$ (δ 7.26). [b]) Recorded at 400 MHz. [c]) Recorded at 500 MHz. [d]) Assignments may be reversed.

Table 2. *^{13}C-NMR Data* ($CDCl_3$) *of Compounds* **1**–**7**[1]). δ in ppm[a])

	1[b])	**2**[b])	**3**[b])	**4**[b])	**5**[c])	**6**[c])	**7**[b])
C(1)	129.2	129.0	129.1	129.1	129.1	129.1	137.3
C(2)	195.0	195.1	195.0	195.0	195.0	195.0	120.5[d])
C(3)	136.2	136.1	136.1	136.1	136.2	136.2	121.3[d])
C(4)	169.3	169.4	169.3	169.3	169.4	169.3	135.9
C(5)	48.1	48.1	48.1	48.1	48.1	48.1	46.5
C(6)	78.5	78.6	78.5	78.5	78.6	78.6	80.0
C(7)	47.4	47.5	47.4	47.4	47.9	47.6	37.1
C(8)	67.4	66.4	67.5	67.2	67.2	67.1	18.3
C(9)	44.0	44.3	44.1	44.0	44.4	44.2	34.0
C(10)	145.0	145.3	144.9	145.0	145.0	145.1	33.3
C(11)	77.6	77.7	77.6	77.7	77.9	77.8	38.1
C(12)	173.5	173.7	173.5	173.5	173.4	173.4	179.5
C(13)	20.6	20.6	20.6	20.5	20.5	20.5	11.0
C(14)	20.0	20.0	20.0	20.0	20.4	20.0	20.8
C(15)	20.3	20.3	20.3	20.3	20.4	20.0	20.4
Acyloxy group at C(8):							
C(1′)	172.0	165.2	173.3	175.6	166.6	166.5	
C(2′)	43.5	114.9	27.8	41.3	126.9	126.8	
C(3′)	25.6	159.9	9.0	26.3	140.5	140.7	
C(4′)	22.4	20.5		11.8	15.9	15.9	
C(5′)	22.5	27.7		16.5	20.4	20.4	
Acyloxy group at C(11):							
C(1″)	166.5	166.6	166.5	166.6	176.0	176.3	
C(2″)	126.4	126.5	126.4	126.3	40.7	33.7	
C(3″)	141.5	141.4	141.6	141.6	26.3	18.8[d])	
C(4″)	16.0	16.0	16.0	16.0	11.6	18.4[d])	
C(5″)	20.2	20.2	20.2	20.2	16.4		

[a]) Chemical shifts δ referenced to $CDCl_3$ (δ 77.03). [b]) Recorded at 100 MHz. [c]) Recorded at 125 MHz. [d]) C-Atoms bearing the same superscript in the same column are exchangeable.

correlations from H–C(7) to C(13) and from Me(13) to C(7), C(11), and C(12) indicated that Me(13) was connected to the quaternary C(11) atom. The HMBCs from H–C(8) (δ(H) 5.56) to C(1′) (δ(C) 172.0) of the isovaleryloxy group indicated that the isovaleryloxy group was connected to C(8), and accordingly, the angeloyloxy group to C(11). From these observations, the gross structure of **1** was elucidated as shown in *Fig. 2*. Its relative configuration was deduced from the NOESY data. The NOE correlation H–C(6)/H–C(7) indicated that the cycloheptene ring (*B* ring) and the γ-lactone ring (*C* ring) were *cis*-fused. The NOE correlations H–C(5)/H–C(8), H–C(5)/Me(13), and H–C(8)/Me(13) indicated that H–C(5), H–C(8), and Me(13) were β-oriented, whereas H–C(6) and H–C(7) were α-oriented (*Fig. 3*). Accordingly, the structure of **1** was elucidated to be (5β,6β,7β,8α,11α)-11-(angeloyloxy)-8-(isovaleryloxy)-2-oxoguaia-1(10),3-dieno-6,12-lactone[1]).

Daucoguaianolactone B (**2**) was obtained as colorless needles. The $[M+H]^+$ ion peak at *m*/*z* 443.2058 in the HR-ESI-MS determined its molecular formula to be $C_{25}H_{30}O_7$. The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1** and suggested that **2** was a congener of **1** having an angeloyloxy moiety as in **1** but a different ester side chain. Analysis of the ^{1}H- and ^{13}C-NMR, HMQC, and HMBC

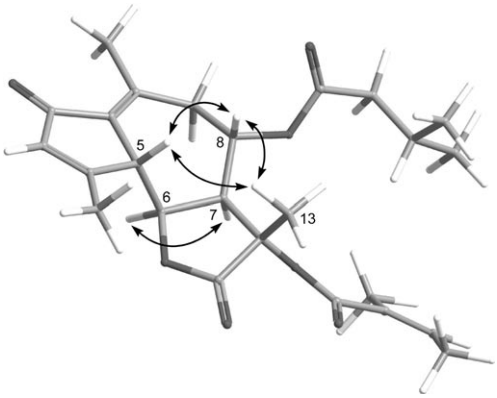


Fig. 3. *Key NOE correlations of* **1**

spectra revealed that the different ester side chain was a senecioyloxy group (δ(H)/δ(C) 1.93/27.7, 2.20/20.5, and 5.62–5.64/114.9; δ(C) 159.9 and 165.2). An HMBC from H–C(8) (δ(H) 5.57) to the CO group of the senecioyloxy group (δ(C) 165.2) confirmed that the senecioyloxy (=(3-methyl-1-oxobut-2-en-1-yl)oxy) group was connected to C(8) and accordingly the angeloyloxy group to C(11). From these observations and the NOESY data, the structure of **2** was elucidated to be (5β,6β,7β,8α,11α)-11-(angeloyloxy)-2-oxo-8-(senecioyloxy)guaia-1(10),3-dieno-6,12-lactone[1]).

Daucoguaianolactone C (**3**) was obtained as a white powder. Its molecular formula $C_{23}H_{28}O_7$ was determined from the $[M+H]^+$ peak at *m*/*z* 417.1900 in the HR-ESI-MS. Comparison of its ^{1}H- and ^{13}C-NMR spectra with those of **1** indicated that **3** was an analogue of **1** in which the isovaleryloxy group in **1** was replaced by a propanoyloxy group (δ(H)/δ(C) 1.18/9.0 and 2.36/27.8; δ(C) 173.3). Thus, the structure of **3** was identified as (5β,6β,7β,8α,11α)-11-(angeloyloxy)-2-oxo-8-(propanoyloxy)guaia-1(10),3-dieno-6,12-lactone[1]). This structure was also confirmed by the ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY data.

Daucoguaianolactone D (**4**) was obtained as colorless needles. Its molecular formula $C_{25}H_{32}O_7$ was established from the $[M+H]^+$ peak at *m*/*z* 445.2256 in the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra of **4** were very similar to those of **1**, except for the signals caused by one of the two ester side-chain moieties. The data suggested that **4** was also a congener of **1** having an angeloyloxy moiety and a different ester side chain. Analysis of ^{1}H- and ^{13}C-NMR, HMQC, and HMBC spectra revealed that the different ester side chain was a 2-methylbutyryloxy group (δ(H)/δ(C) 0.93/11.8, 1.18/16.5, 1.41–1.52 and 1.70–1.81/26.3, and 2.32–2.41/41.3; δ(C) 175.6). The HMBC spectrum of **4** showed a correlation from H–C(8) (δ(H) 5.55) to the CO group of the 2-methylbutyryloxy group (δ(C) 175.6), confirming that the 2-methylbutyryloxy group was at C(8) and accordingly the angeloyloxy group at C(11). From these observations, the structure of **4** was elucidated to be (5β,6β,7β,8α,11α)-11-(angeloyloxy)-8-[(2-methylbutyryl)oxy]-2-oxoguaia-1(10),3-dieno-6,12-lactone[1]), and this structure was confirmed by the ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY data.

Daucoguaianolactone E (**5**) was isolated as a white powder. Its molecular formula was determined to be $C_{25}H_{32}O_7$ from the $[M+H]^+$ peak at *m*/*z* 445.2231 in the HR-

ESI-MS. Its ^{1}H- and ^{13}C-NMR spectra were quite similar to those of **4** with characteristic signals ascribable to an angeloyloxy group and a 2-methylbutyryloxy group. The HMBC correlation from H–C(8) (δ(H) 5.62) to the CO group of the angeloyloxy group (δ(C) 166.6), however, indicated that the angeloyloxy group was at C(8) and the 2-methylbutyryloxy group at C(11). The structure of **5** was thus elucidated as (5β,6β,7β,8α,11α)-8-(angeloyloxy)-11-[(2-methylbutyryl)oxy]-2-oxoguaia-1(10),3-dieno-6,12-lactone[1]). This structure was confirmed by the ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY data.

Daucoguaianolactone F (**6**) was obtained as a white powder. Its molecular formula was determined to be $C_{24}H_{30}O_7$ from the $[M+H]^+$ peak at *m*/*z* 431.2066 in the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR, COSY, and HMBC data revealed that **6** was also a sesquiterpene lactone of the guaiane type with two ester side chains at C(8) and C(11). Comparison of the chemical shifts of the protons and C-atoms of **6** with those of **5** revealed that **6** had the same sesquiterpene lactone structure with an angeloyloxy group as in **5** and another ester side chain, which was shown to be an isobutyryloxy group (δ(H)/δ(C) 1.17/18.4, 1.20/18.8, and 2.56/33.7; δ(C) 176.3). A cross-peak between H–C(8) (δ(H) 5.61) and the CO group (δ(C) 166.5) of the angeloyloxy group in the HMBC spectrum revealed that the angeloyloxy group was at C(8) and, accordingly, the isobutyryloxy group at C(11) of the sesquiterpene lactone structure. From these observations and the NOESY data, the structure of **6** was established as (5β,6β,7β,8α,11α)-8-(angeloyloxy)-11-(isobutyryloxy)-2-oxoguaia-1(10),3-dieno-6,12-lactone[1]).

Daucoeudesmanolactone A (**7**) was obtained as colorless prisms. Its molecular formula was established as $C_{15}H_{20}O_2$ from the $[M+H]^+$ peak at *m*/*z* 233.1536 in the HR-ESI-MS. The IR spectrum showed an absorption band at 1772 cm^{-1}, a characteristic band of a γ-lactone moiety. The ^{13}C-NMR and DEPT spectra showed the presence of 15 C-atoms, *i.e.*, three Me, two CH_2, and seven CH groups of which one was an O-bearing CH, and three were olefinic CH groups, and three quaternary C-atoms, of which one was an aliphatic, one an olefinic, and one a CO group. The ^{1}H,^{1}H-COSY and HMQC data indicated the presence of three chain fragments, *i.e.*, fragment *A*, a C_3 chain (C(1)–C(2)–C(3)) of three olefinic CH groups, fragment *B*, a C_5 chain (C(5)–C(6)–C(7)–C(8)–C(9)) in which C(6) was an O-bearing CH group, and fragment *C*, a C_2 chain (C(11)–C(13)) in which C(13) was a secondary Me group (*Fig. 4*). Further analysis of the ^{1}H,^{1}H-COSY spectrum indicated that C(7) of fragment *B* was connected to C(11) of fragment *C*. Connection between these molecular fragments and other skeletal C-atoms were determined on the basis of HMBC data. HMBC correlations from H–C(5) and Me(14) to C(3), from H–C(3), H–C(5), H–C(6), and Me(14) to C(4), and from H–C(3) and Me(14) to C(5) indicated that C(3) of fragment *A*, C(5) of fragment *B*, and Me(14) were connected to the olefinic quaternary C(4) atom. The correlations from Me(15) to C(1), C(5), C(9), and C(10), from H–C(1) to C(5), C(9), and C(10), from H_a–C(9) to C(1), from H_b–C(9) to C(5), and from CH_2(9) to C(10) and C(15) revealed that C(1) of fragment *A*, C(9) of fragment *B*, and Me(15) were connected to the quaternary C(10) atom. The correlations from H–C(6), H–C(11), and Me(13) to the C(12)=O group (δ(C) 179.5) indicated the presence of a γ-lactone linkage involving C(6) and C(12). From these observations, compound **7** was determined to be a sesquiterpene lactone having

an eudesmanolactone structure. The relative configuration of **7** was deduced from the NOESY plot. The NOE correlations H–C(5)/Me(13), H–C(6)/Me(15), and H–C(7)/Me(15) indicated that the *A*/*B* and *B*/*C* rings were *trans*- and *cis*-fused, respectively, and that H–C(6), H–C(7), and Me(15) were β-oriented, and that H–C(5) and Me(13) were α-oriented, which was further confirmed by the X-ray crystal structure (*Fig. 5*). The structure of **7** was thus elucidated as (5α,6α,7α,10β,11α)-eudesma-1,3-dieno-6,12-lactone[1]). This structure is the C(7) epimer of feropodin isolated from *Ferula oopoda* (Apiaceae) roots [4][5][6].

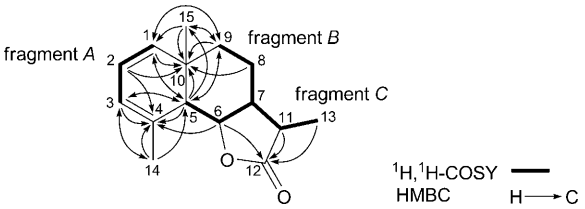


Fig. 4. *^{1}H,^{1}H-COSY and selected HMBCs of* **7**

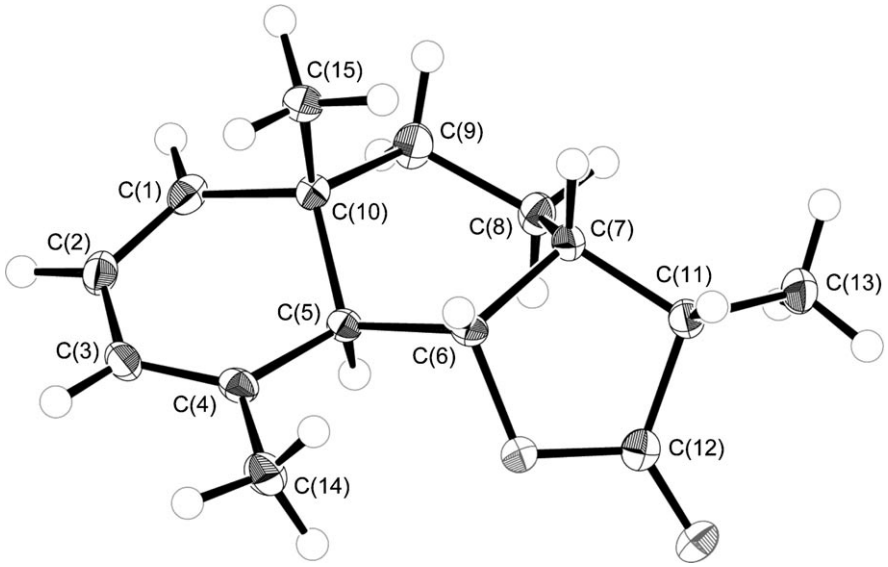


Fig. 5. *ORTEP Representation of daucoeudesmanolactone A* (**7**)

The three known compounds obtained were identified as talasins A and B [7][8], and badkhysin [9] by the analysis of their spectroscopic data.

Daucoguaianolactone D (**4**), daucoeudesmanolactone A (**7**), and talasins A and B were evaluated for their cytotoxicity against P-388 leukemia cells. The amount of the other isolated compounds was too small for the assay. The IC_{50} values of **4**, **7**, and talasins A and B were 12, 8.5, 14, and 12 μg/ml, respectively.

In this study, sesquiterpene lactones of the guaiane and eudesmane type were isolated from the leaves and stems of *Daucus glaber* (FORSSK.) THELL. Although many

sesquiterpene lactones have been obtained from plants belonging to the tribes Laserpitieae and Peucedaneae of the family Apiaceae [10], this is the first isolation of sesquiterpene lactones from a plant of the genus *Daucus*.

Experimental Part

General. CC = Column chromatography. Prep. reversed-phase HPLC: *Shimadzu-LC-6AD* pump unit equipped with a *SPD-10A* UV detector (220 or 254 nm); flow rate 10 ml/min; column 1, *Inertsil Prep-ODS* (10 μm, 20 × 250 mm; *GL Sciences Inc.*); column 2, *Mightysil RP-18 GP* (5 μm, 20 × 250 mm; *Kanto Chemical Co., Inc.*); solvent 1, MeOH/H_2O 65:35; solvent 2, MeOH/H_2O 62:38; solvent 3, MeOH/H_2O 60:40; solvent 4, MeOH/H_2O 55:45; solvent 5, MeCN/H_2O 45:55; solvent 6, MeCN/H_2O 30:70. M.p.: *Yanaco-MP-3* apparatus; uncorrected. Optical rotations: *Jasco-P-1030* digital polarimeter. UV Spectra: *Jasco-V-530* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco-FT/IR-620* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-DPX-400* spectrometer (400 and 100 MHz for ^1H and ^{13}C, resp.) or *Bruker-DRX-500* spectrometer (500 and 125 MHz for ^1H and ^{13}C, resp.). MS: *Micromass-LCT* spectrometer; in *m/z*.

Plant Material. Daucus glaber (FORSSK.) THELL. herb was collected from the northern regions of the Nile Delta, Egypt, in May 2005. A voucher specimen was kept at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University.

Extraction and Isolation. The leaves and stems of *D. glaber*, separated from the fruits and roots, were shade-dried and finely powdered (6.0 kg). The material was extracted with MeOH (60 l) three times at r.t., for 7 d each time. The combined MeOH extracts were concentrated to give a residue (1300 g), which was further suspended in a small amount of H_2O and sequentially treated with solvents of increasing polarities, *i.e.*, hexane (35 l), $CHCl_3$ (13 l), AcOEt (18 l), and BuOH (10 l). The hexane fraction gave, on concentration, a white precipitate (*Hex-ppt*, 1.5 g), which was separated by filtration. The filtrate was further concentrated to give 215 g of a hexane fraction (*Hex-sol*). A part (600 mg) of the *Hex-ppt* was subjected to HPLC (column 1, solvent 1) to give seven fractions: *Hex-ppt-frs. 1–7.* By HPLC (column 2, solvent 5), *Hex-ppt-fr. 3* (5.0 mg) and *Hex-ppt-fr. 7* (15.0 mg) afforded compounds **3** (2.7 mg) and **5** (1.4 mg), resp. By analogous HPLC (column 2, solvent 5, then column 2, solvent 3), *Hex-ppt-fr. 5* (50.0 mg) gave talasin B (15.3 mg), and by HPLC (column 2, solvent 5, then column 2, solvent 2), *Hex-ppt-fr. 6* (a 150 mg portion of *Hex-ppt-fr. 6* (460 mg)), gave compounds **1** (2.3 mg), **2** (4.3 mg), **4** (13.2 mg), **6** (1.7 mg), and talasins A (80 mg) and B (3.6 mg).

The hexane fraction of the MeOH extract, *Hex-sol* (215 g), was subjected to CC (SiO_2, $CHCl_3$/hexane 10:90 and 20:80, then AcOEt/hexane 5:95, 10:90, 20:80, and 50:50, and AcOEt). A fraction (9.2 g) eluted with AcOEt/hexane 5:95 was treated with MeOH, and the insoluble matter and the mother liquor were separated by filtration. The mother liquor was concentrated and subjected to CC (*Sephadex LH-20*, MeOH) to give 8 fractions. The fifth fraction (1.38 g) was purified by HPLC (column 1, solvent 1) to yield **7** (15.0 mg). Another fraction from the CC of *Hex-sol* (215 g), eluted with AcOEt/hexane 20:80, gave, on concentration, 9.2 g of residue. A part (5.00 g) of this residue was subjected to reversed-phase CC (*ODS*, MeOH/H_2O 75:25, 80:20, and 90:10, and MeOH) to yield four fractions. The first fraction (3.3 g), obtained by elution with 75% MeOH/H_2O, was treated with MeOH, and the insoluble matter was separated by filtration to give mother liquor. A part (400 mg) of the mother liquor (1.4 g) was further purified by HPLC (column 2, solvent 4) to give 12 fractions. The fifth fraction (12.6 mg) gave badkhysin (2.1 mg) when further subjected to HPLC (column 2 and solvent 6).

Daucoguaianolactone A (= *(5β,6β,7β,8α,11α)-11-(Angeloyloxy)-8-(isovaleryloxy)-2-oxoguaia-1(10),3-dieno-6,12-lactone* = rel-*(3*R*,3a*S*,4*R*,9a*S*,9b*R*)-2,3,3a,4,5,7,9a,9b-Octahydro-3,6,9-trimethyl-4-(3-methyl-1-oxobutoxy)-2,7-dioxoazuleno[4,5-*b*]furan-3-yl (2*Z*)-2-Methylbut-2-enoate*; **1**): White powder. $[\alpha]_D = -20$ (*c* = 0.07, MeOH). UV (MeOH): 230 (sh, 4.12), 251 (4.18). IR (film): 2956, 2919, 2851, 1791, 1729, 1716, 1688, 1563. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 445.2234 ($[M+H]^+$, $C_{25}H_{33}O_7^+$; calc. 445.2226).

Daucoguaianolactone B (= *(5β,6β,7β,8α,11α)-11-(Angeloyloxy)-2-oxo-8-(senecioyloxy)guaia-1(10),3-dieno-6,12-lactone* = rel-*(3*R*,3a*S*,4*R*,9a*S*,9b*R*)-2,3,3a,4,5,7,9a,9b-Octahydro-3,6,9-trimethyl-4-*

*[(3-methyl-1-oxobut-2-en-1-yl)oxy]-2,7-dioxoazuleno[4,5-*b*]furan-3-yl (2*Z*)-2-Methylbut-2-enoate*; **2**): Colorless needles (MeOH). M.p. 155–158°. $[\alpha]_D = -59$ ($c = 0.1$, MeOH). UV (MeOH): 251 (sh, 4.55). IR (film): 2921, 2852, 1790, 1713, 1691, 1643. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 443.2058 ($[M+H]^+$, $C_{25}H_{31}O_7^+$; calc. 443.2070).

Daucoguaianolactone C (= *(5β,6β,7β,8α,11α)-11-(Angeloyloxy)-2-oxo-8-(propanoyloxy)guaia-1(10),3-dieno-6,12-lactone* = rel-*(3*R*,3a*S*,4*R*,9a*S*,9b*R*)-2,3,3a,4,5,7,9a,9b-Octahydro-3,6,9-trimethyl-2,7-dioxo-4-(1-oxopropoxy)azuleno[4,5-*b*]furan-3-yl (2*Z*)-2-Methylbut-2-enoate*; **3**): White powder. $[\alpha]_D = -28$ ($c = 0.08$, MeOH). UV (MeOH): 229 (sh, 4.14), 251 (4.20). IR (film): 2921, 2852, 1793, 1716, 1689. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 417.1900 ($[M+H]^+$, $C_{23}H_{29}O_7^+$; calc. 417.1913).

Daucoguaianolactone D (= *(5β,6β,7β,8α,11α)-11-(Angeloyloxy)-8-[(2-methylbutyryl)oxy]-2-oxo-guaia-1(10),3-dieno-6,12-lactone* = rel-*(3*R*,3a*S*,4*R*,9a*S*,9b*R*)-2,3,3a,4,5,7,9a,9b-Octahydro-3,6,9-trimethyl-4-(2-methyl-1-oxobutoxy)-2,7-dioxoazuleno[4,5-*b*]furan-3-yl (2*Z*)-2-Methylbut-2-enoate*; **4**): Colorless needles (MeOH). M.p. 170–173°. $[\alpha]_D = -38$ ($c = 0.17$, MeOH). UV (MeOH): 230 (sh, 4.15), 251 (4.20). IR (film): 2920, 2851, 1794, 1714, 1687, 1564. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 445.2256 ($[M+H]^+$, $C_{25}H_{33}O_7^+$; calc. 445.2226).

Daucoguaianolactone E (= *(5β,6β,7β,8α,11α)-8-(Angeloyloxy)-11-[(2-methylbutyryl)oxy]-2-oxo-guaia-1(10),3-dieno-6,12-lactone* = rel-*(3*R*,3a*S*,4*R*,9a*S*,9b*R*)-2,3,3a,4,5,7,9a,9b-Octahydro-3,6,9-trimethyl-3-(2-methyl-1-oxobutoxy)-2,7-dioxoazuleno[4,5-*b*]furan-4-yl (2*Z*)-2-Methylbut-2-enoate*; **5**): White powder. $[\alpha]_D = -18$ ($c = 0.05$, MeOH). UV (MeOH): 222 (sh, 4.02), 252 (4.15). IR (film): 2917, 2851, 1794, 1734, 1691, 1563. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 445.2231 ($[M+H]^+$, $C_{25}H_{33}O_7^+$; calc. 445.2226).

Daucoguaianolactone F (= *(5α,6α,7α,10β,11α)-8-(Angeloyloxy)-11-(isobutyryloxy)-2-oxoguaia-1(10),3-dieno-6,12-lactone* = rel-*(3*R*,3a*S*,4*R*,9a*S*,9b*R*)-2,3,3a,4,5,7,9a,9b-Octahydro-3,6,9-trimethyl-3-(2-methyl-1-oxopropoxy)-2,7-dioxoazuleno[4,5-*b*]furan-4-yl (2*Z*)-2-Methylbut-2-enoate*; **6**): White powder. $[\alpha]_D = -24$ ($c = 0.08$, MeOH). UV (MeOH): 227 (sh, 4.06), 252 (4.16). IR (film): 2922, 2852, 1795, 1734, 1706, 1689, 1638, 1563. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 431.2066 ($[M+H]^+$, $C_{24}H_{31}O_7^+$; 431.2070).

Daucoeudesmanolactone A (= *(5α,6α,7α,10β,11α)-Eudesma-1,3-dieno-6,12-lactone* = rel-*(3*R*,3a*S*,5a*R*,9a*R*,9b*R*)-3a,4,5,5a,9a,9b-Hexahydro-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2(3*H*)-one*; **7**): Colorless prisms (MeOH). M.p. 137–138°. $[\alpha]_D = +60$ ($c = 0.1$, MeOH). UV (MeOH): 263 (3.64). IR (film): 3027, 2921, 2853, 1772. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 233.1536 ($[M+H]^+$, $C_{15}H_{21}O_2^+$; 233.1542).

Single-Crystal X-Ray Analysis of ***7****. Bruker-AXS-APEX-II-ULTRA* CCD area detector diffractometer with a rotating anode source (MoK_α radiation, λ 0.71073 Å). **7**: $C_{15}H_{20}O_2$, M_r 232.31, $0.22 \times 0.19 \times 0.17$ mm, orthorhombic, $P2_12_12_1$ (No. 19), $a = 6.9944(5)$ Å, $b = 12.4618(9)$ Å, $c = 14.4554(10)$ Å, $V = 1259.97(15)$ Å^3, $Z = 4$, $D_x = 1.225$ Mg m^{-3}, μ(MoK_α) = 0.079 mm^{-1}, T 100 K, 7160 reflections collected, 2822 unique ($R_{int} = 0.0238$). R indices for 152 parameters, 2672 reflections with $I > 2\sigma(I)$ in the θ range of 2.2–27.5 °, $R^1 = 0.0346$, $wR^2 = 0.0839$ ($I > 2\sigma(I)$), S (goodness-of-fit) = 1.031; $R^1 = 0.0369$, $wR^2 = 0.0859$ (all data), residual electron density (min/max) = −0.17/0.21 eÅ^{-3}. The substantial redundancy in data allows empirical absorption correction to be applied using multiple measurements of equivalent reflections with the SADABS *Bruker* program [11]. The structure was solved by direct methods with SHELXS-97 [12] and refined by full-matrix least-squares on F^2 with SHELXL-97 [13]. CCDC-708927 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif.

Assay for Cytotoxic Activity. Compounds **4**, **7**, talasin A, and talasin B were tested for the cytotoxic activity by the MTT (= 2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay. Murine P-388 leukemia cells ($3 \cdot 10^3$ cells) were suspended in RPMI-1640 medium (*Nissui Pharmaceutical Co., Ltd.*, Tokyo, Japan) supplemented with 5% fetal calf serum (*Mitsubishi Chemical Industry Co., Ltd.*, Tokyo, Japan) and kanamycin (100 µg/ml; 100 µl). The cell suspension (100 µl) was placed in each well of a 96-well plate and incubated at 37° in a humidified atmosphere of 7% CO_2. After 24 h incubation, test-compound solns. in dimethyl sulfoxide (10 µl) of various concentrations were added to the wells, and the plate was incubated for further 48 h at 37°. Then, MTT soln. (5 mg/ml; 20 µl) was added to each well. After incubation for 4 h, 10% sodium dodecyl sulfate soln. in 0.01M HCl (100 µl) was

added to each well. The formazan crystals formed in each well were dissolved by stirring with a pipette tip, and the optical density was recorded on a microplate reader (*Tosoh MPR-A4i*) at 550 nm. The cytotoxic activities given are the average of three replicate measurements.

REFERENCES

[1] V. Tackholm, 'Students' Flora of Egypt', 2nd ed., Cooperative Printing Company, Beirut, 1974, p. 394.
[2] L. Boulos, 'Flora of Egypt', Al Hadara Publishing, Cairo, Egypt, 2000, Vol. 2, p. 181.
[3] E.-S. S. Mansour, G. T. Maatooq, A. T. Khalil, E.-S. M. Marwan, A. A. Sallam, *Z. Naturforsch., C* **2004**, *59*, 373.
[4] S. V. Serkerov, *Khim. Prir. Soedin.* **1969**, *5*, 245.
[5] S. V. Serkerov, *Khim. Prir. Soedin.* **1971**, *7*, 667.
[6] S. V. Serkerov, *Khim. Prir. Soedin.* **1980**, *16*, 510.
[7] O. A. Konovalova, K. S. Rybalko, M. G. Pimenov, *Khim. Prir. Soedin.* **1973**, *9*, 122.
[8] O. A. Konovalova, K. S. Rybalko, V. I. Sheichenko, *Khim. Prir. Soedin.* **1975**, *11*, 590.
[9] U. Rychlewska, S. V. Serkerov, *Acta Crystallogr., Sect. C* **1991**, *47*, 1872.
[10] M. Holub, M. Buděšinský, *Phytochemistry* **1986**, *25*, 2015.
[11] G. M. Sheldrick, SADABS, Bruker Area Detector Absorption Correction Program, University of Göttingen, Göttingen, Germany, 1996.
[12] G. M. Sheldrick, SHELXS-97, Program for the Solution of Crystal Structures, University of Göttingen, Göttingen, Germany, 1997.
[13] G. M. Sheldrick, SHELXL-97, Program for the Refinement of Crystal Structures, University of Göttingen, Göttingen, Germany, 1997.

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