New Eudesmane Derivatives and Other Sesquiterpenes from the Epigeal Parts of *Dittrichia graveolens*

Ahmad Muhamad Abou-Douh

Natural Products Chemistry of Medicinal Plants Lab, Department of Chemistry, Aswan Faculty of Science, South Valley University; Aswan P. O. Box 205, Aswan 81511, Egypt.

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In bioassay-guided searches for novel bioactive natural products from higher plants of the Egyptian flora, two new eudesmane sesquiterpene derivatives, 3α-hydroxyilicic acid methyl ester (1) and 2α-hydroxy-4-*epi*-ilicic acid (2), together with 11 known sesquiterpenes were isolated from bioactive fractions of the active epigeal parts extracts of *Dittrichia graveolens* (L.) Greuter (Asteraceae) growing in the coastal regions of northwestern Egypt. Four other known sesquiterpene lactones with different carbon skeletons, named 2α-hydroxy-2*R*-xanthalongin (8), 4-*epi*-isoinuviscolide (9), 8-*epi*-helenalin (10), and bigelovin (11), were also isolated for the first time from the same source. The stereochemical structures of the isolated compounds were elucidated on the basis of physical and spectroscopic methods including UV, IR, ^{1}H-, ^{13}C-NMR, distortionless enhancement by polarization transfer, 2D NMR, ^{1}H–^{1}H correlation spectroscopy, ^{1}H–^{13}C heteronuclear single-quantum coherence, ^{1}H–^{13}C heteronuclear multiple-bond connectivity, ^{1}H–^{1}H nuclear Overhauser effect spectroscopy experiments, and high-resolution mass spectrometry, as well as some chemical transformations. The antimicrobial, antiinflammatory, and antipyretic activities of *D. graveolens* extracts and chromatographic fractions were carried out and the various bioactivities of our findings are discussed.

Key words *Dittrichia graveolens*; Asteraceae; new eudesmane derivative; antimicrobial; antiinflammatory; antipyretic

The genus *Inula* L. (Asteraceae, tribe Inuleae, subtribe Inulinae) comprises *ca.* 100 species and are distributed throughout Europe and Asia, although there are also several African species.[1)] Many species of *Inula* are traditionally used as folk medicine in the treatment of ischemic heart disease,[2)] angina pectoris,[3)] hypoglycemia,[4)] liver disorders,[5,6)] gastroduodenal diseases and ulcers,[7)] bronchia, rheumatic complaints, migraine and skin infection,[8)] as a lung tonic and analgesic,[8)] and as an antiproliferative.[6,9)] The characteristic constituents of the genus *Inula* are sesquiterpene lactones[10—13)] and essential oil groups.[14)] Some phenolic acids and flavonoids were detected as additional constituents of this genus.[15,16)]

Sesquiterpene lactones (STLs) occur in many plant families but are most widely distributed within the Asteraceae (Compositae, sunflower family) in which the majority of almost 4000 naturally occurring STLs have been found.[1)] These lactones have been reported to possess a diversity of conspicuous biological activities, such as cytotoxic, antitumorigenic, and highly bactericidal and fungicidal properties.[13,17—23)] Many of these compounds or plants containing them are a cause of allergic contact dermatitis in humans.[18)] On the other hand, some of these lactones have been reported to possess a wide spectrum of pharmacological activities, such as hypotensive, hyperglycemic (high doses) and hypoglycemic (lower doses),[24)] anthelminthic,[21)] diuretic,[25)] antiinflammatory, and antipyretic.[26—28)] They have also been used therapeutically as expectorants, cholagouge, and to lower blood pressure.[18)]

Dittrichia graveolens (L.) Greuter (Syn. *Inula graveolens*) is an annual herbaceous plant that grows about 80 cm high and is widespread in the Mediterranean area. It grows along roadsides, on waste ground, in humid and degraded soils, and near subsalt lands. Its presence in the coastal regions of northwestern Egypt has recently been recorded.[29)] This species is well known for its essential oil content.[30)] The essential oil is known as the most effective medication for loosening mucous obstructions and is useful against acute chronic respiratory conditions, such as coughs, colds, sinusitis laryngitis, and bronchitis and it also reduces acneic skin inflammation.[31)] Previous investigations reported the isolation of sesquiterpenes, flavonoids, and benzoic acid derivatives[22,32—35)] from *D. graveolens*. To the best of our knowledge, no chemical and bioactivity investigations have been carried out on *D. graveolens*, an endemic plant in Egypt. Moreover, this species has found no medicinal uses so far.

As part of our search for new biologically active natural products, the active epigeal parts extracts of *D. graveolens* were chemically investigated. The present paper describes the isolation and structural elucidation of two new eudesmane sesquiterpene derivatives, designated as 3α-hydroxyilicic acid methyl ester (**1**) and 2α-hydroxy-4-*epi*-ilicic acid (**2**), together with 15 known sesquiterpenes, which were isolated and characterized from bioactive fractions of the active epigeal parts extracts of *D. graveolens*. In addition, our findings on the various bioactivities of crude extracts and chromatographic fractions are reported. Antibacterial and antifungal properties were assayed *in vitro* using laboratory microbe cultures grown on an agar medium. Antiinflammatory and antipyretic activities were assayed *in vivo* using laboratory rats. The potential utility in pharmaceutical developments is discussed.

Results and Discussion

The crude CH_2Cl_2/MeOH extract of the air-dried epigeal parts of *D. graveolens* showed significant bioactivities (*vide infra*). This extract was then subjected to flash column chromatography over a Silica gel, eluted with hexane, Et_2O in hexane, Me_2CO in Et_2O, and MeOH to give 11 initial frac-

This paper is dedicated to Prof. Dr. Tom J. Mabry on the occasion of his 76th birthday.

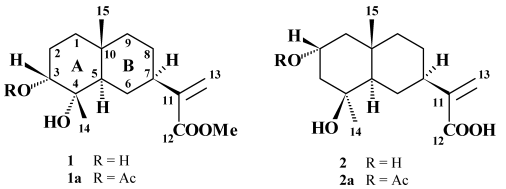
e-mail: ahmad_aboudouh@hotmail.com

tions. Individual fractions were refractionated using vacuum-liquid chromatography (VLC) on a Silica gel employing gradients of CH_2Cl_2 in hexane, EtOAc in CH_2Cl_2, and EtOAc. The fractions and/or subfractions were subjected to further separation and purification by size exclusion chromatography (SEC) on a Sephadex LH-20, preparative TLC, and high-pressure liquid chromatography (HPLC). These procedures and bioassays of the chromatographic fractions yielded two new eudesmane sesquiterpene derivatives (**1**) and (**2**), together with 11 known sesquiterpenes, as shown in Chart 1. Four other known sesquiterpene lactones with different carbon skeletons, named 2α-hydroxy-2*R*-xanthalongin (**8**), 4-*epi*-isoinuviscolide (**9**), 8-*epi*-helenalin (**10**), and bigelovin (**11**), were also isolated for the first time from the same source, as shown in Chart 2. The stereochemical structures of the isolated compounds were elucidated on the basis of physical (mp, $[\alpha]_D$) and spectroscopic methods including UV, IR, ^{1}H-, ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT), 2D NMR ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C heteronuclear-single quantum coherence (HSQC), ^{1}H–^{13}C heteronuclear multiple-bond connectivity (HMBC), and ^{1}H–^{1}H nuclear Overhauser effect spectroscopy (NOESY) experiments, and high-resolution mass spectrometry (HR-MS), as well as some chemical transformations.

3α-Hydroxyilicic acid methyl ester (**1**) was obtained as colorless needles, mp 123—125 °C, $[\alpha]_D^{25}$ −48.9° (MeOH). The molecular formula was determined to be $C_{16}H_{26}O_4$ by HR-MS and showed the exact mass at *m*/*z* 282.1829. This was then confirmed by electron impact (EI)-MS, which showed a molecular ion at *m*/*z* 282. The most prominent fragments are due to the gradual loss of up to two molecules of H_2O *m*/*z* 264 and 246, confirming the presence of two hydroxyl groups. The base peak at *m*/*z* 235 was attributed to a $[C_{14}H_{19}O_3]^+$ fragment. The UV spectrum showed absorption at λ_{max} 206 (log ε 3.96), indicating the presence of an exocyclic methylene group conjugated with a methyl ester group –CO_2Me. The IR spectrum exhibited a main absorption band at ν_{max} 1720 cm^{-1} corresponding to a conjugated ester –CO_2Me, and bands at 3540 and 3458 cm^{-1} due to two hydroxyl groups. In addition, the bands at 1625 and 912 cm^{-1} are characteristic for an exocyclic methylene group.

The ^{1}H-NMR spectrum of **1** (Table 1) integrated for 26 protons. The two upfield singlets at δ_H 1.13 and δ_H 1.03 were assigned to two tertiary methyl groups Me-14 and Me-15, respectively. The angular methyl singlet centered at δ_H 1.03 suggested an eudesmane framework, whereas a further three-proton singlet at δ_H 1.13 suggested the presence of a methyl group geminal to a hydroxyl function. A three-proton singlet centered at δ_H 3.77 indicated the presence of a methyl ester group –CO_2Me. The broadened singlets centered at δ_H 6.16 (H-13) and δ_H 5.60 (H-13′) are indicative for a conjugated exocyclic methylene group. The ^{1}H-NMR spectrum of **1** was very similar to that of the methyl ester of ilicic acid (vachanic acid) **12**.[36] The only remarkable difference was the lack of the 3*eq*-H signal at δ_H 1.70—1.60 (1H, m) in (**12**)[36] and the appearance of a new signal at δ_H 4.17 (1H, t, *J*= 2.7 Hz, H-3) attributed to a secondary hydroxyl group at C-3. The configuration at C-3 was deduced from measuring the small vicinal couplings of H-3 ($J_{3ax,2ax}$=$J_{3ax,2eq}$=2.7 Hz),[37,38] indicating the presence of an C-3 axial proton and an equatorial orientation of the C-3 hydroxyl group.

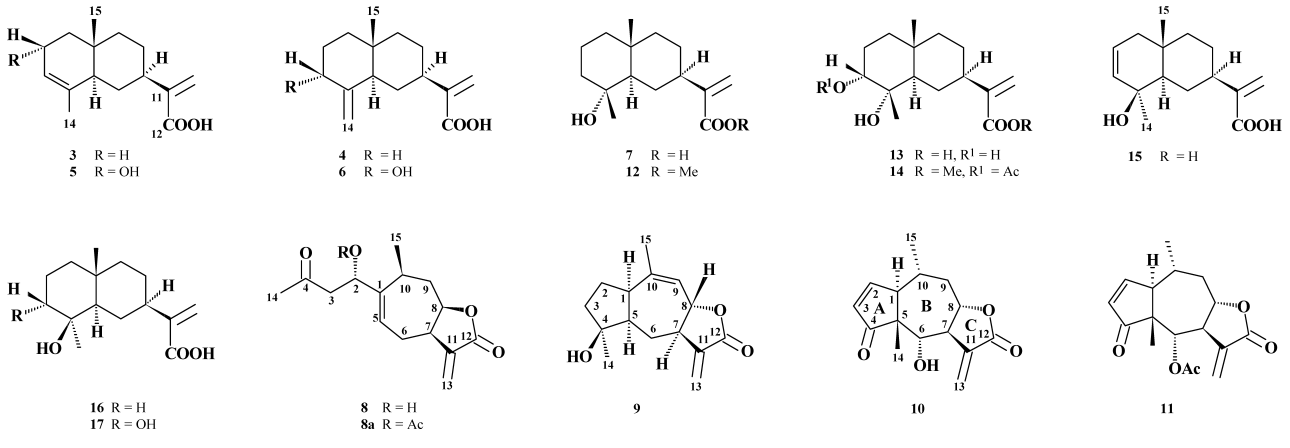
The ^{13}C-NMR spectrum of **1** (Table 1) displayed 16 carbon resonances. The two tertiary methyl groups at C-14 and C-15 were located at δ_C 22.5 (q) and δ_C 18.7 (q), respectively. Signals for carbon-bearing oxygen functions were observed at δ_C 167.9 (s, C-12), 76.4 (d, C-3), 75.8 (s, C-4), and 51.7 (q, C-16). An exocyclic methylene resonance was observed at δ_C 122.5 (t, C-13). These assignments were confirmed by 2D NMR, HSQC and HMBC spectra. The chemical shifts and multiplicities of the ^{13}C-singles that originated from the carbocyclic methyl ester moiety of **1** were found to be in agreement with those of vachanic acid (**12**)[36] except for the C-3 methylene carbon signal, which appeared at δ_C 43.4 (t) in vachanic acid, was replaced by an C-3 oxymethine carbon at δ_C 76.4 (d), indicating the presence of a secondary hydroxyl group at C-3 in compound **1**. On the other hand, the ^{13}C-NMR spectral data of **1** were similar to those of the eudesmane derivative 3α-hydroxyilicic acid (**13**)[39] except that the carboxyl group –COOH, which appeared at δ_C 170.5 (s, C-12) in **13**, was shifted upfield at δ_C 167.9 (s, C-12), and the



1 R = H
1a R = Ac

2 R = H
2a R = Ac

Chart 1.



3 R = H
5 R = OH

4 R = H
6 R = OH

7 R = H
12 R = Me

13 R = H, R¹ = H
14 R = Me, R¹ = Ac

15 R = H

16 R = H
17 R = OH

8 R = H
8a R = Ac

9

10

11

Chart 2.

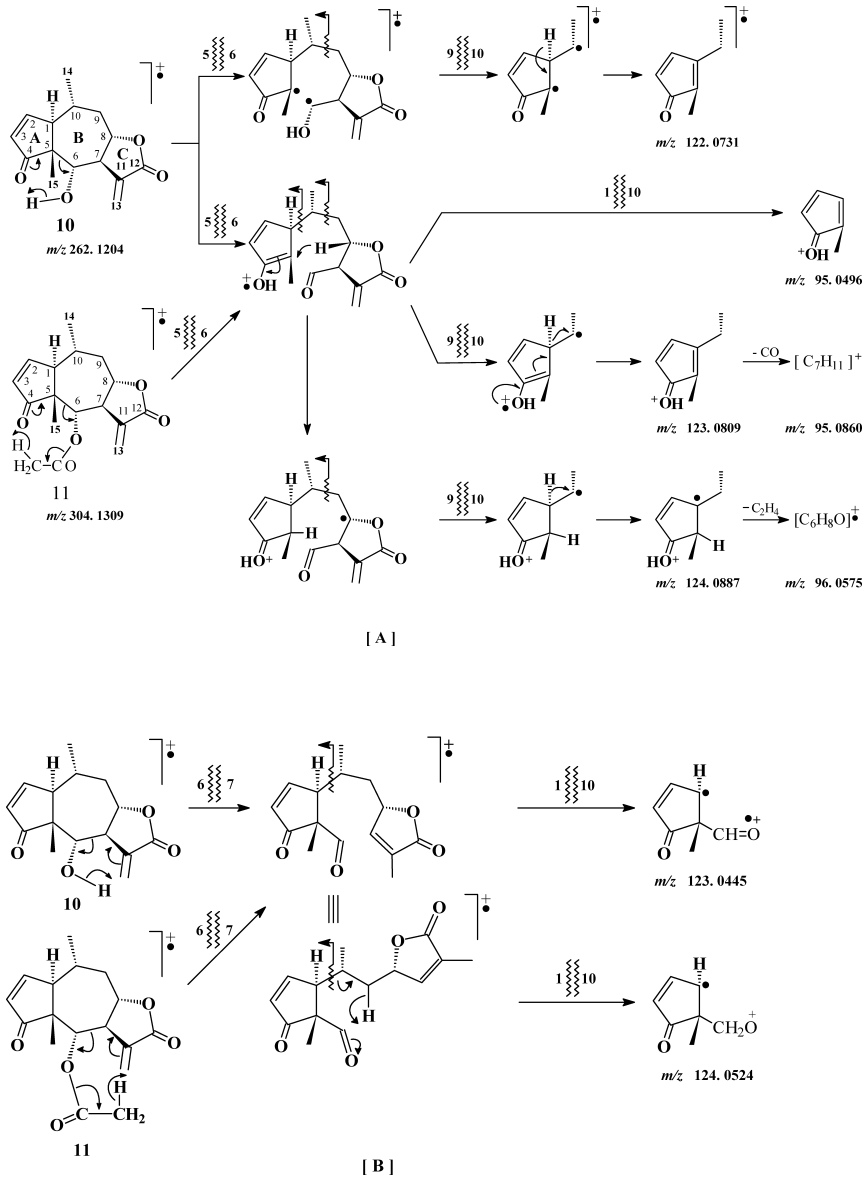


Chart 3. Mass Spectral Fragmentation Pattern of Compounds **10** and **11**

appearance of a new signal at δ_C 51.7 (q, C-16) indicating the presence of a methyl ester group $-CO_2Me$ compared with the latter compound. DEPT experiments (performed at 90° and 135°) of **1** were also carried out to ascertain the nature of the carbon atoms, as shown in Table 1.

Further support for the structure of **1** was obtained by acetylation using Ac_2O/pyridine for 24 h at room temperature to afford the monoacetate derivative **1a** (Chart 1) as a colorless gel, $[\alpha]_D^{25}$ −35.5° ($CHCl_3$), $C_{18}H_{28}O_5$ (anal. 324.1935). The IR and NMR spectral data of **1a** indicated the presence of an acetoxyl group (ν_{max} 1745, 1250 cm^{-1}; δ_H 2.12, 3H, s; δ_C 170.7, $-\underline{CO}CH_3$, s; 21.4, $-CO\underline{CH_3}$, q), a conjugated ester (ν_{max} 1720 cm^{-1}, δ_H 3.77, $-CO_2\underline{CH_3}$, s; $\delta_{C\text{-}12}$ 167.9, $-\underline{CO}OCH_3$, s; $\delta_{C\text{-}16}$ 51.7, $-CO_2\underline{CH_3}$, q), C-3 oxymethine signals (δ_H 4.78, t; $\delta_{C\text{-}3}$ 77.7, d), and a tertiary hydroxyl group (ν_{max} 3458 cm^{-1}; $\delta_{C\text{-}4}$ 75.8, s). The axial proton geminal to the acetoxyl group was shifted downfield at δ_H 4.78 (t, J=2.7 Hz, H-3*ax*),[38,40] further confirming the presence of a secondary hydroxyl group in the equatorial orientation at C-3 (Table 1). It was noteworthy that the ^{1}H- and ^{13}C-NMR spectral data of **1a** were identical to those of the methyl ester of 3α-acetylilicic acid (**14**),[38] prepared by methylation of the corresponding acid.

The HMBC spectrum (Fig. 1) of **1** established the placement of the hydroxyl and methyl groups at the C-3, C-4, C-14, and C-15 positions, respectively, *via* the correlations between H-3 and C-1, C-2, C-4, and C-14, cross-peaks between H_3-15 and C-1, C-5, C-9, and C-10, as well as cross-peaks between H_3-14 and C-3, C-4, and C-5. The relative stereochemistry of **1** was confirmed based on a nuclear Overhauser effect (NOE) experiment (Fig. 2), which gave diagnostic correlations. Thus the cross-peaks between H_3-14/H_3-15, H-3*ax*, and H-6*ax*, as well as between H-3*ax* and H-2*ax*, established the equatorial configuration of the two hydroxyl groups at C-3 and C-4. Furthermore, the observed NOE interactions between H_3-15, H-1*ax*, H-2*ax*, H-6*ax*, H-8*ax*, and H_3-14 confirmed the axial orientation of the tertiary methyl group H_3-14 and supported the equatorial orientation of the tertiary hydroxyl group at C-4. On the basis of the foregoing spectral data, compound **1** was designated as 3α-hydroxyilicic acid

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **1** and **2** (500/125 MHz, CD_3OD)[a]

Position	**1**				**2**			
	δ ^{1}H ppm	mult. (*J*, Hz)	δ ^{13}C ppm	DEPT	δ ^{1}H ppm	mult. (*J*, Hz)	δ ^{13}C ppm	DEPT
1*ax*	1.18 (1H)	m	49.2	CH_2	1.25 (1H)	m	50.5	CH_2
1*eq*	1.38 (1H)	m			1.92 (1H)	br d (4.5)		
2*ax*	1.45 (1H)	m	28.2	CH_2	4.19 (1H)[c]	dddd (12.0, 12.0, 4.5, 4.5)[c]	67.9[c]	CH
2*eq*	1.85 (1H)	ddt (12.5, 12.5, 2.7)			—	—		
3*ax*	4.17 (1H)[b]	t (2.7)[b]	76.4[b]	CH	2.67 (1H)	br t (12.0)	52.6	CH_2
3*eq*	—	—			2.00 (1H)	dd (12.0, 4.5)		
4	—	—	75.8	C	—	—	70.7	C
5*eq*	1.63 (1H)	dd (12.5, 2.7)	55.0	CH	1.65 (1H)	dd (12.0, 2.5)	50.0	CH
6*ax*	1.85 (1H)	ddt (12.5, 12.5, 2.7)	27.3	CH_2	1.80 (1H)	ddd (12.0, 12.0, 2.5)	27.8	CH_2
6*eq*	1.28 (1H)	m			1.29 (1H)	m		
7*eq*	2.50 (1H)	dddd (12.5, 12.5, 4.0, 4.0)	40.5	CH	2.53 (1H)	dddd (12.0, 12.0, 4.0, 4.0)	40.0	CH
8*ax*	1.46 (1H)	m	26.5	CH_2	1.59 (1H)	m	27.0	CH_2
8*eq*	1.60 (1H)	m			1.68 (1H)	m		
9*ax*	1.40 (1H)	m	44.6	CH_2	1.35 (1H)	m	43.9	CH_2
9*eq*					1.40 (1H)	m		
10	—	—	34.6	C	—	—	33.3	C
11	—	—	145.7	C	—	—	147.6	C
12	—	—	167.9	C	10.5 (1H)	br s	172.5	C
13	6.16 (1H)	br s	122.5	CH_2	6.31 (1H)	br s	122.6	CH_2
13′	5.60 (1H)	br s			5.67 (1H)	br s		
14	1.13 (3H)	s	22.5	CH_3	1.16 (3H)	s	26.7	CH_3
15	1.03 (3H)	s	18.7	CH_3	1.05 (3H)	s	18.8	CH_3
–COOMe	3.77 (3H)	s	51.7	CH_3	—		—	

a) δ (ppm) relative to TMS. Assignments were confirmed by 2D ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY. *b*) Acetate deriv. **1a** ($CDCl_3$): δ_H 4.78 (1H, t, *J*=2.7 Hz, H-3*ax*), 2.12 (s, –OAc); δ_C 77.7 (d, C-3), 170.7 (s, –<u>CO</u>CH_3), 21.4 (q, –CO<u>CH_3</u>). *c*) Acetate deriv. **2a** ($CDCl_3$): δ_H 4.91 (1H, dddd, *J*=12.0, 12.0, 4.5, 4.5 Hz, H-2*ax*), 2.05 (s, –OAc); δ_C 77.9 (d, C-2), 171.0 (s, –<u>CO</u>CH_3), 21.1 (q, –CO<u>CH_3</u>).

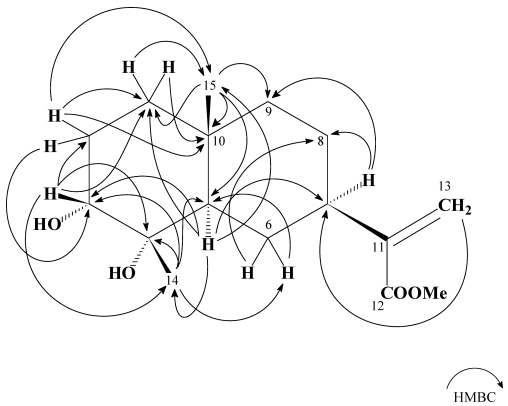


Fig. 1. Major ^{1}H–^{13}C Long-Range Correlations for **1**, Observed by HMBC Spectrum

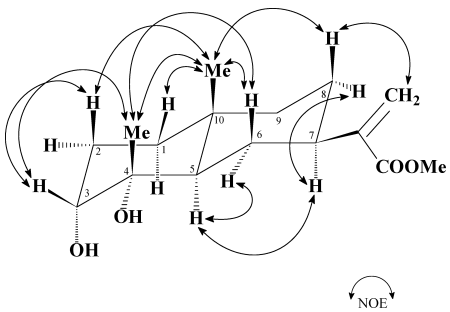


Fig. 2. Significant NOESY Spectrum Observed for **1**

methyl ester with the elucidated structure methyl-3α,4α-dihydroxyeudesm-11(13)-en-5α, 7αH-12-oate.

2α-Hydroxy-4-*epi*-ilicic acid (**2**) was obtained as colorless needles mp 162—164 °C, $[\alpha]_D^{25}$ +22.5° (MeOH). The molecular formula was established as $C_{15}H_{24}O_4$ by HR-MS and showed the exact mass at *m*/*z* 268.1673. This was then confirmed by EI-MS, which showed a molecular ion at *m*/*z* 268. The most prominent fragments are due to the stepwise elimination of two molecules of H_2O at *m*/*z* 250 and 232, confirming the presence of two hydroxyl groups. The base peak at *m*/*z* 87 was attributed to a $[C_7H_3]^+$ fragment. The UV spectrum showed absorption at λ_{max} 207.4 (log ε 3.89), indicating the presence of an exocyclic methylene group conjugated with a carboxyl group –COOH. The IR spectrum exhibited acid adsorption at ν_{max} 3300—2500 br, 1696 cm^{-1}, and showed adsorption bands at 3600 and 3455 cm^{-1} due to two hydroxyl groups. In addition, the bands at 1650 and 914 cm^{-1} are characteristic for an exocyclic methylene group.

The integrated values in the ^{1}H-NMR spectrum of **2** (Table 1) indicated the presence of 24 protons. The upfield singlet at δ_H 1.05 suggested an eudesmane framework and was assigned to the tertiary angular methyl group Me-15, whereas a further three-proton singlet centered at δ_H 1.16 suggested the presence of the tertiary methyl group Me-14, geminal to a hydroxyl function. The broadened singlets centered at δ_H 6.31 (H-13) and 5.67 (H-13′) are characteristic for a conjugated exocyclic methylene group. A broad singlet centered at δ_H 10.5 indicated the presence of a –COOH signal.[41] The ^{1}H-NMR spectrum of **2** was very similar to that of Δ^2-4-*epi*-ilicic acid (**15**)[42] and 4-*epi*-ilicic acid (**16**).[43] The noticeable differences were the lack of the two olefinic protons, which appeared as a multiplet at δ_H 5.65 (H-2, H-3) in **15**; and the lack of methylene protons, which appeared as a multiplet at δ_H 1.70—1.30 (H-2*ax*, H-2*eq*) in **16**; and the appearance of a new signal at δ_H 4.19 (1H, dddd, *J*=12.0, 12.0, 4.5, 4.5 Hz,

H-2) attributed to a secondary hydroxyl group at C-2.

The ^{13}C-NMR spectrum of **2** (Table 1) displayed 15 carbon resonances. The two tertiary methyl groups at C-14 and C-15 were located at δ_C 26.7 (q) and δ_C 18.8 (q), respectively. Resonances of carbon-bearing oxygen functions were observed at δ_C 172.5 (s, C-12), 70.7 (s, C-4), and 67.9 (d, C-2). An exocyclic methylene resonance was observed at δ_C 122.6 (t, C-13). These assignments were judged from 2D NMR, HSQC and HMBC spectra. The chemical shifts and multiplicities of the ^{13}C-signals originating from the carbocyclic moiety of **2** were found to be in agreement with those of Δ^2-4-*epi*-ilicic acid (**15**)[42] and 3α-hydroxy-4-*epi*-ilicic acid (**17**)[44] except for the two methine carbon signals, which appeared at δ_C 126.2 (d, C-2) and 133.9 (d, C-3) in **15** and the oxymethine carbon signals, which appeared at δ_C 74.0 (d, C-3) in **17**, were replaced with C-2 oxymethine carbon signals at δ_C 67.9 (d), confirming the presence of a secondary hydroxyl group at C-2 in compound **2**. The multiplicities of the individual ^{13}C peaks of **2** were also determined using DEPT experiments (at 90° and 135°), as shown in Table 1.

Further support for the structure of **2** was obtained by acetylation using Ac_2O/pyridine for 24 h at room temperature to afford the monoacetate derivative **2a** (Chart 1) as colorless crystals, mp 153—155 °C, $[\alpha]_D^{25}$ +38.7° ($CHCl_3$), $C_{17}H_{26}O_5$ (anal. 310.1778). The IR and NMR spectral data of **2a** indicated the presence of an acetoxyl group (ν_{max} 1740, 1250 cm^{-1}; δ_H 2.05, 3H, s; δ_C 171.0, –<u>CO</u>CH$_3$, s; 21.1, –CO<u>CH$_3$</u>, q), a carboxyl group (ν_{max} 3300—2500 br, 1696 cm^{-1}; δ_H 10.5, br s; $\delta_{C\text{-}12}$ 172.5, s), C-2 oxymethine signals (δ_H 4.91, dddd; $\delta_{C\text{-}2}$ 77.9, d), and a tertiary hydroxyl group (ν_{max} 3455 cm^{-1}; $\delta_{C\text{-}4}$ 70.7, s). The proton geminal to the acetoxyl group was shifted downfield at δ_H 4.91 (dddd, J=12.0, 12.0, 4.5, 4.5 Hz, H-2), providing further confirmation for the presence of a secondary hydroxyl group at C-2 (Table 1).

The HMBC experiment (Fig. 3) of **2** confirmed the placement of the hydroxyl and methyl groups at the C-2, C-4, C-14, and C-15 positions, respectively, through the correlations between H-2 and C-1, C-3, C-10, and C-15, cross-peaks between H$_3$-15 and C-1, C-5, C-9, and C-10, as well as cross-peaks between H$_3$-14 and C-3, C-4, and C-5. The relative stereochemistry of **2** was established based on a NOESY experiment (Fig. 4), which gave diagnostic correlations. The interactions observed between the methyl group H$_3$-14 and both H-3*eq*, and H-5*eq*, established the equatorial configuration of the tertiary methyl group H$_3$-14 and axial orientation of the tertiary hydroxyl group at C-4. Furthermore, the observed NOE's between H$_3$-15, H-1*ax*, H-2*ax*, H-6*ax*, and H-8*ax* supported the equatorial orientation of H$_3$-14. The observed cross-peaks between the signals of H-2*ax* and H-1*ax*, H-3*ax*, and H$_3$-15 confirmed the equatorial configuration of the hydroxyl group at C-2. In the light of the above spectral data, compound **2** was designated as 2α- hydroxy-4-*epi*-ilicic acid with the established structure 2α,4β-dihydroxyeudesm-11(13)-en-5α,7αH-12-oic acid.

2α-Hydroxy-2*R*-xanthalongin (**8**) was isolated as a colorless gum, $[\alpha]_D^{25}$ +25.7° ($CHCl_3$), $C_{15}H_{20}O_4$ (anal. 264.1360). The IR and ^{13}C-NMR spectral data of **8** demonstrated the presence of a secondary hydroxyl group (ν_{max} 3600 cm^{-1}; $\delta_{C\text{-}2}$ 75.4, d). The ^{1}H-NMR spectrum of **8** exhibited oxymethine signals at δ_H 4.49 (1H, dd, J=9.0, 3.5 Hz, H-2).[45] On acetylation to yield **8a** (Chart 2), the proton geminal to the acetoxyl group was shifted downfield at δ_H 5.40 (1H, dd, J=9.0, 4.0 Hz, H-2), further confirmation of the presence of a secondary hydroxyl group at C-2.[46] The ^{13}C-NMR spectral assignments of **8** and its acetate derivative **8a** (Table 2) are not available in the literature.

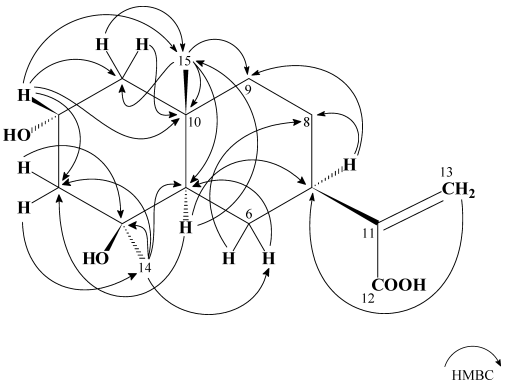


Fig. 3. Major ^{1}H–^{13}C Long-Range Correlations for **2**, Observed by HMBC Spectrum

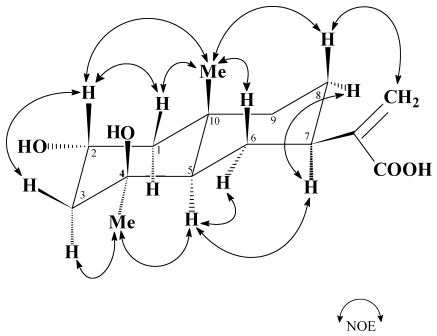


Fig. 4. Significant NOESY Spectrum Observed for **2**

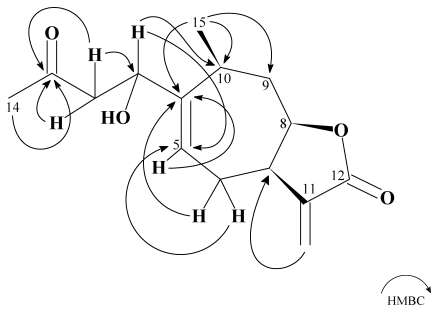


Fig. 5. Major ^{1}H–^{13}C Long-Range Correlations for **8**, Observed by HMBC Spectrum

The position of the OH group at C-2 was determined from the COSY correlation of H-2 (δ_H 4.49) with both H-3α and H-3β protons. Additional evidence was given by the HMBC long-range correlation of H-2 with C-5 (δ_C 121.5) and C-10 (δ_C 31.9), as shown by arrows in Fig. 5. The relative stereochemical assignments of the C-2 hydroxyl group were confirmed based on a NOESY experiment (Fig. 6), which gave diagnostic cross-peak correlations between H-2β and each of H-3β, H-3α, H-5, and H$_3$-15, indicated the α-orientation of the hydroxyl group at C-2. The absolute configuration of the stereogenic center at C-2 in **8** was established using Mosher ester methodology.[47] Compound **8** was treated with (*R*)-(−)-

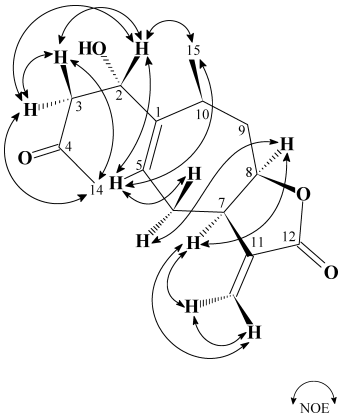


Fig. 6. Significant NOESY Spectrum Observed for **8**

and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride and work-up in the usual manner to obtain the (*S*)- and (*R*)-Mosher esters. Analysis of the $\Delta\delta_{H(S-R)}$ data between the (*S*)- and (*R*)-esters of **8** showed a positive difference in chemical shift (+0.12 ppm) for the protons at C-3. This effect indicated that the absolute configuration at C-2 is *R*. From the above spectral data, the structure of **8** was amended and fully characterized as 2α-hydroxy-2*R*-xanthalongin. Compound **8** represents a new addition to the list, as a sesquiterpene derivative has not been previously described in *Inula* spp.[45,46]

4-*epi*-Isoinuviscolide (**9**) was isolated as colorless crystals, mp 144—146 °C $[\alpha]_D^{25}$ −59.5° ($CHCl_3$), $C_{15}H_{20}O_3$ (anal. 248.1411). The ^{13}C-NMR spectral assignments of pure **9** (Table 2) have not been previously reported. Compound **9** was isolated for the first time from *D. graveolens* and characterized as 4-*epi*-isoinuviscolide by comparison of the spectroscopic (IR, ^{1}H-NMR, and EI-MS) data with those described in the literature.[10]

8-*epi*-Helenalin (**10**) was isolated as colorless needles, mp 183—185 °C $[\alpha]_D^{25}$ −14.5° ($CHCl_3$), $C_{15}H_{18}O_4$ (anal. 262.1204). The mass spectral fragmentation pattern of **10** supported the structural assignment, and showed significant peaks at *m*/*z* 124 (base peak), 95, 96, 122, and 123 which was rationalized as shown in Chart 3. Compound **10** has not been recorded before for this species and was identified as 8-*epi*-helenalin by comparison of the spectroscopic (IR, ^{1}H-, and ^{13}C -NMR) data with those published in the literature.[48]

Bigelovin (**11**) was isolated as colorless needles, mp 180—182 °C $[\alpha]_D^{25}$ +53.8° ($CHCl_3$), $C_{17}H_{20}O_5$ (anal. 304.1309). The mass spectral fragmentation pattern of **11** supported the structural assignment, and showed significant fragments at *m*/*z* 96 (base peak), 95, 122, 123, and 124 which was rationalized as shown in Chart 3. Compound **11** was isolated for the first time from *D. graveolens* and characterized as bigelovin by comparison of the spectroscopic (IR, ^{1}H-, and ^{13}C-NMR) data with those described in the literature.[48]

Other sesquiterpene acids isolated from the same plant material were fully characterized as isocostic acid (**3**),[32] costic acid (**4**),[32] 2α-hydroxyisocostic acid (**5**),[32] 3α-hydroxycostic acid (viscic acid) (**6**),[32] and ilicic acid (**7**).[32] In addition, six sesquiterpene lactones, namely 4,5-dioxo-11βH-xanth-1(10)-en-12,8β-oilde (**18**),[34] 8-*epi*-xanthatin-1β,5β-epoxide (**19**),[33] xanthalongin (tomentosin) (**20**),[32,33] 4H-xanthalongin (**21**),[32] graveolide (**22**),[35] and 11αH,13-dihydroconfertin (**23**)[34] were identified by analysis of physical (mp, $[\alpha]_D$) and spectroscopic evidence and confirmed by comparison of their spectral data with those published in the literature.

Table 2. ^{13}C-NMR Spectral Data for Compounds **8**, **8a** and **9** (125 MHz, $CDCl_3$)[a]

Carbon No.	**8**	DEPT	**8a**	**9**	DEPT
1	148.9	C	143.6	47.2	CH
2	75.4	CH	76.0	26.3	CH_2
3	43.3	CH_2	47.7	42.0	CH_2
4	213.8	C	204.4	80.5	C
5	121.5	CH	126.0	53.4	CH
6	26.2	CH_2	26.5	31.7	CH_2
7	42.9	CH	42.5	47.9	CH
8	80.2	CH	79.7	83.7	CH
9	36.7	CH_2	36.8	126.7	CH
10	31.9	CH	31.7	143.1	C
11	139.3	C	139.1	141.7	C
12	170.6	C	170.2[b]	172.2	C
13	122.4	CH_2	122.6	119.6	CH_2
14	24.6	CH_3	30.7	25.6	CH_3
15	22.2	CH_3	22.2	21.5	CH_3
–$\underline{CO}CH_3$		C	170.1[b]		
–$CO\underline{CH_3}$		CH_3	21.2		

a) δ (ppm) relative to TMS. Assignments were confirmed by 2D-hetero COSY HSQC and HMBC. *b*) Signals may be interchangeable.

Antimicrobial Activity The crude plant extracts and chromatographic fractions of the epigeal parts of *D. graveolens* were tested using a disc diffusion assay[49] against nine species of microorganisms (four bacteria and five fungi). Bacterial species comprised two gram-positive strains, *Bacillus cereus* AUMC 52 and *Staphylococcus aureus* AUMC 54, and two gram-negative strains, *Escherichia coli* AUMC 53 and *Serratia marcescens* AUMC 55. Activities were compared against the antibiotic chloramphenicol, which was used as a positive control. Fungal test species were selected based on human pathogenic and phytopathogenic fungal species and included *Aspergillus flavus* AUMC 4028 (aflatoxigenic species) and *Aspergillus niger* AUMC 4059 (obtained from deteriorated food materials). In addition, the yeast *Candida albicans* AUMC 3880, *Geotrichum candidum* AUMC 2570 and *Scopulariopsis brevicaulis* AUMC 4069 (pathogenic fungi, obtained from cases of human skin diseases) were selected. Activities were compared against the antifungal agent tioconazole (Trosyd®), which was used as a positive control.

In antibacterial testing, the crude CH_2Cl_2/MeOH (1 : 1) extract and chromatographic fractions **C**, **D**, **E**, **F**, and **I** exhibited high inhibitory activity against a gram-positive bacterium, *Bacillus cereus*, compared with the standard reference antibiotic chloramphenicol.

In antifungal testing, the crude CH_2Cl_2/MeOH (1 : 1) extract and fractions **C** and **H** displayed the same inhibitory activity as the positive control tioconazole against the human pathogenic fungi *S. brevicaulis*. It was also noteworthy that fractions **F** and **G** exhibited higher antifungal activity than tioconazole against the same human pathogenic fungal species. The results of the antimicrobial activity of *D. graveolens* extracts and chromatographic fractions are recorded in Table 3. The observed antimicrobial activity could be attributed to the presence of sesquiterpene lactones[17—22] and sesquiterpene acids.[23]

Table 3. Antimicrobial Activity of *D. graveolens* Extracts and Chromatographic Fractions[a)]

Microorganism	Inhibition zone, mm[c)]												Reference drugs	
	CH_2Cl_2/MeOH (1 : 1) extract	MeOH 80% extract	Fractions **A**, **B**	Fraction **C**	Fraction **D**	Fraction **E**	Fraction **F**	Fraction **G**	Fraction **H**	Fraction **I**	Fraction **J**	Fraction **K**	Chloram-phenicol	Tioconaz-ole
Bacterial species[b)]														
Bacillus cereus AUMC 52	20.0	10.0	00.0	16.0	20.0	16.0	20.0	14.0	13.0	15.0	12.0	10.0	30.0	Nt[d)]
Escherichia coli AUMC 53	10.0	08.8	08.0	07.0	06.0	00.0	00.0	14.0	00.0	00.0	00.0	10.0	30.0	Nt[d)]
Serratia marcescens AUMC 55	14.0	12.0	08.0	00.0	08.0	12.0	10.0	07.0	08.0	00.0	08.0	16.0	40.0	Nt[d)]
Staphylococcus aureus AUMC 54	06.0	08.0	10.0	12.0	12.0	10.0	08.0	14.0	14.0	14.0	08.0	08.0	38.0	Nt[d)]
Fungal species[b)]														
Aspergillus flavus AUMC 4028	08.0	00.0	00.0	00.0	10.0	10.0	07.0	00.0	00.0	07.0	00.0	00.0	Nt[d)]	16.0
Aspergillus niger AUMC 4059	06.0	00.0	00.0	06.0	10.0	12.0	08.0	08.0	00.0	00.0	00.0	00.0	Nt[d)]	14.0
Candida albicans AUMC 3880	07.0	00.0	07.0	10.0	07.0	16.0	06.0	14.0	10.0	14.0	00.0	00.0	Nt[d)]	40.0
Geotrichum candidum AUMC 2570	09.0	00.0	00.0	00.0	12.0	06.0	10.0	00.0	14.0	00.0	00.0	00.0	Nt[d)]	22.0
Scopulariopsis brevicaulis AUMC 4069	10.0	00.0	06.0	10.0	00.0	06.0	14.0	12.0	10.0	08.0	00.0	00.0	Nt[d)]	10.0

a) The crude extracts, chromatographic fractions and reference drugs=200 μg/ml. *b*) AUMC represents the strain number in Assiut University Mycological Center. *c*) 50%—100% mm=high inhibition activity; 25%—<50% mm=medium inhibition activity; <25% mm=low inhibition activity; 00.0=no inhibition compared with the reference drugs. *d*) Nt=Not tested.

Antiinflammatory Activity The antiinflammatory activity of *D. graveolens* extracts and chromatographic fractions was tested using a reduction in yeast-induced edema bioassay. Results were compared with the reference drug indomethacin (8 mg/kg body weight), with maximum effects being obtained after 2 h.

From the obtained data, we can conclude that fractions **D**, **E**, **G**, and **I**—**K** exhibited more potent activity than the other fractions, exerting their maximum antiinflammatory activity after 3 h and continuing for 4 h. MeOH 80% and CH_2Cl_2/MeOH (1 : 1) extracts and fraction **H** showed similarly potent antiinflammatory activity, respectively, as the positive control indomethacin with maximum effects being observed after 4 h. Fraction **F** had the least potent fraction compared with the plant extracts and other tested fractions, because it exhibited low potency after 3—4 h. Fraction **K** was the most active fraction because it had nearly the same antiinflammatory activity as indomethacin. Therefore it can be used as an antiinflammatory agent at the dose of 300 mg/kg body weight for inhibiting the edema induced by yeast injection after carrying out other pharmacological studies on its toxicity. The results of antiinflammatory activity and inhibitory effects are shown in Table 4. The observed antiinflammatory activity could be attributed to the presence of sesquiterpene lactones,[26,27] sesquiterpene acids,[26,50] triterpens and sterols,[51] and flavonoids.[50]

Antipyretic Activity The antipyretic activity of *D. graveolens* extracts and chromatographic fractions was tested using a reduction in yeast-induced fever bioassay. Results were compared with the reference drug indomethacin (8 mg/kg body weight), with maximum effects being obtained after 1 h.

From the observed data, we can conclude that fraction **D** showed a marked antipyretic activity at the dose of 300 mg/kg body weight and controlled hyperthermia after 1 h without any decrease in its activity. MeOH 80% and CH_2Cl_2/MeOH (1 : 1) extracts, as well as fractions **K**, **E**, **J**, and **G** showed marked antipyretic activity and controlled hyperthermia after 2 h without any decrease in activity. Fraction **I** showed maximum antipyretic activity after 3 h. Fraction **H** had less activity compared with the plant extracts and other tested fractions at the same dose level, whereas fraction **F** did not exhibit any antipyretic activity. Fractions **D** and **K** were very potent as antipyretic agents because they showed nearly the same antipyretic activity as the positive control indomethacin. Therefore they can be used as antipyretic agents

Table 4. Antiinflammatory Activity and Inhibitory Effects of *D. graveolens* Extracts and Chromatographic Fractions on Yeast-Induced Rat Paw Edema

Group	Thickness of the right paw (mm) after injection,[a] and % inhibition									
	0.5 h	Inhibition %	1 h	Inhibition %	2 h	Inhibition %	3 h	Inhibition %	4 h	Inhibition %
Control	7.5±0.23	—	7.5±0.29	—	8.2±0.25	—	8.4±0.24	—	8.5±0.20	—
Indomethacin[b]	6.9±0.13	08.0	6.4±0.24*	14.7	6.2±0.14**	24.4	5.9±0.31**	29.8	4.6±0.24**	45.9
CH_2Cl_2/MeOH (1 : 1) extract[c]	7.0±0.13	06.7	6.9±0.20	08.0	6.9±0.13*	15.9	6.8±0.20*	16.7	6.4±0.24**	24.7
MeOH 80% extract[c]	7.1±0.13	05.3	7.2±0.14	04.0	6.9±0.13*	15.9	6.8±0.13*	15.5	6.1±0.13**	28.2
Fraction **D**[c]	7.1±0.13	05.3	7.4±0.24	01.3	7.1±0.13*	13.4	6.5±0.35**	22.6	6.1±0.13**	28.2
Fraction **E**[c]	7.3±0.14	02.7	7.1±0.13	05.3	7.1±0.13*	13.4	6.4±0.34**	23.8	6.1±0.13**	28.2
Fraction **F**[c]	7.1±0.13	05.3	7.1±0.13	05.3	7.1±0.14*	13.4	7.0±0.20*	16.7	6.8±0.25*	20.0
Fraction **G**[c]	7.1±0.13	05.3	7.0±0.20	06.7	6.8±0.14*	17.1	6.4±0.24**	23.8	6.4±0.24**	24.7
Fraction **H**[c]	7.0±0.10	06.7	7.1±0.13	05.3	6.9±0.13*	15.9	7.0±0.25*	16.7	6.9±0.24**	18.8
Fraction **I**[c]	7.1±0.13	05.3	7.1±0.13	05.3	6.9±0.20*	15.9	6.5±0.20**	22.6	6.4±0.24**	24.7
Fraction **J**[c]	6.9±0.16	08.0	6.8±0.24	09.3	6.6±0.24*	19.5	6.4±0.13**	23.8	6.1±0.13**	28.2
Fraction **K**[c]	6.9±0.13	08.0	6.9±0.13	08.0	6.5±0.20*	20.7	5.9±0.13**	29.8	5.6±0.24**	34.1

a) Values are expressed as the means standard error±S.E.; $n=5$ animals per group. *b*) Dose=8 mg/kg. *c*) Dose=300 mg/kg. * $p \leq 0.05$; ** $p \leq 0.01$ compared with control group.

Table 5. Antipyretic Activity of *D. graveolens* Extracts and Chromatographic Fractions on Yeast-Induced Rat Fever

Group	Average rectal temperature (°C)[a]				
	0.5 h	1 h	2 h	3 h	4 h
Control	38.4±0.17	38.2±0.05	38.6±0.14	38.5±0.13	38.7±0.09
Indomethacin[b]	38.2±0.12	37.5±0.22**	37.2±0.11**	37.2±0.11**	37.4±0.08**
CH_2Cl_2/MeOH (1 : 1) extract[c]	38.6±0.12	37.9±0.09	37.5±0.23**	37.6±0.04**	37.6±0.10**
MeOH 80% extract[c]	38.4±0.15	38.1±0.08	37.5±0.16**	37.5±0.12**	37.5±0.19**
Fraction **D**[c]	38.2±0.06	37.5±0.20**	37.3±0.09**	37.3±0.21**	37.3±0.10**
Fraction **E**[c]	38.1±0.06	38.0±0.08	37.9±0.08**	37.8±0.11**	37.5±0.19**
Fraction **F**[c]	38.3±0.16	38.3±0.15	38.3±0.13	38.3±0.13	38.3±0.16
Fraction **G**[c]	38.2±0.11	38.1±0.03	38.0±0.03**	38.0±0.03**	38.0±0.06**
Fraction **H**[c]	38.2±0.09	38.2±0.14	38.5±0.13	38.4±0.17	38.1±0.03*
Fraction **I**[c]	38.3±0.14	38.6±0.18	38.6±0.18	37.9±0.09*	37.9±0.06**
Fraction **J**[c]	38.2±0.05	38.3±0.15	37.9±0.06**	37.3±0.10**	37.3±0.15**
Fraction **K**[c]	38.2±0.08	38.1±0.07	37.6±0.10**	37.3±0.09**	37.4±0.11**

a) Values are expressed as the means standard error±S.E.; $n=5$ animals per group. *b*) Dose=8 mg/kg. *c*) Dose=300 mg/kg. * $p \leq 0.05$; ** $p \leq 0.01$ compared with control group.

at the dose of 300 mg/kg body weight. Data on the antipyretic activity are listed in Table 5. The marked antipyretic activity could be attributed to the presence of sesquiterpene lactones[28] and flavonoids.[52]

The *in vitro* antimicrobial activity as well as the *in vivo* antiinflammatory and antipyretic activities of the new eudesmane sesquiterpene derivatives (**1**) and (**2**) are still under investigation. To our knowledge, sesquiterpene acids have been previously reported to have only antifungal and antiinflammatory activity.[23,26,50] The antidermatophytic activity of these compounds is due to the presence of a free –COOH group and to the olefinic bonds in their structures.[23]

Experimental

General Experimental Procedures All melting points were measured on a Kofler hot-stage microscope and are uncorrected. UV spectra were recorded on a Shimadzu UV-160A spectrometer in MeOH. IR spectra were recorded on a Shimadzu IR-450 spectrometer. ^{1}H- and ^{13}C-NMR, DEPT, 2D NMR ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC, and ^{1}H–^{1}H NOESY experiments were measured on a JEOL GSX-500 and a Bruker DRX-500 (Karlsruhe) spectrometers in CD_3OD, unless otherwise stated. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference and the coupling constants J in Hz. All MS and HR-MS were recorded under EI conditions using a Hitachi M-80 or a JEOL JMS-HX-110 mass spectrometer with a direct inlet system. Optical rotations ($[\alpha]_D$, values) were measured on a Perkin Elmer 192 Polarimeter, with a 10-cm microcell in MeOH and/or $CHCl_3$ at 25 °C. Silica gel (Merck, 70—230 mesh) was used for column chromatography. SEC was carried out on a Sephadex LH-20 (Pharmacia Biotech AB). VLC separation was carried out on a Silica gel using gradients of CH_2Cl_2 in hexane, EtOAc in CH_2Cl_2, and EtOAc. TLC and preparative TLC were carried out on Kieselgel 60 GF_{254} (Merck). TLC zones were visualized under UV light (254 and 366 nm) and/or by spraying with anisaldehyde/H_2SO_4 reagent. Preparative HPLC was performed using an RP-18 column (Nucleosil, C-18, 120 μm, 4 mm×250 mm, 5 μm, Bischoff, Leonberg) with MeOH/H_2O as a mobile phase; a variable wavelength monitor (Knauer, 254 and/or 264 nm); a differential refractometer for detection (RI-8110, Bischoff, Leonberg); R 401, Water associates, and an HPLC-pump (Knauer 64, Berlin) with a flow rate of 1.5 ml/min. All evaporations were done *in vacuo* on a rotary evaporator. Ether refers to diethyl ether throughout.

Plant Material The epigeal parts of *D. graveolens* (L.) Greuter were collected in September 2001 at the flowering stage from the coastal regions of northwestern Egypt. Identification was kindly verified by Professor Loutfy Boulos, Prof. of Flora, Department of Botany, Faculty of Science, Alexandria University, Alexandria, Egypt. A voucher specimen has been deposited at the Botany Department Herbarium, Aswan Faculty of Science, South Valley University, Aswan, Egypt, with the identification number 11099.

Extraction and Isolation The air-dried and finely powdered epigeal parts of *D. graveolens* (1.5 kg) were exhaustively extracted successively with CH_2Cl_2/MeOH (1 : 1, v/v), followed by 80% MeOH at ambient temperature, which furnished 35 and 22 g, respectively.

The crude CH_2Cl_2/MeOH (1 : 1) extract (35 g) was subjected to column chromatography on a Silica gel (1050 g, 4×120 cm) to give 11 fractions: fraction **A** (hexane, 11), fraction **B** (Et_2O/hexane 1 : 9, 11), fraction **C** (Et_2O/hexane 1 : 3, 11), fraction **D** (Et_2O/hexane 1 : 1, 11), fraction **E** (Et_2O/hexane 3 : 1, 11), fraction **F** (Et_2O, 11), fraction **G** (Me_2CO/Et_2O 1.5 : 8.5, 11), fraction **H** (Me_2CO/Et_2O 1 : 3, 11), fraction **I** (Me_2CO/Et_2O 1 : 1, 11), fraction **J** (Me_2CO, 11), and fraction **K** (MeOH, 11). Bioassay-guided analyses of the crude CH_2Cl_2/MeOH (1 : 1), MeOH 80% extracts, and chromatographic fractions were performed (*vide infra*).

Fractions **A** and **B** corresponding to elution in the range hexane–10% Et_2O in hexane were similar in TLC and NMR, and were thus combined. Fractions **A** and **B** (*ca.* 2.0 g overall) contained mainly waxes and volatile terpenes and were discarded. Fraction **G** corresponding to elution with 15% Me_2CO in Et_2O (*ca.* 2.0 g) was purified in preparatory HPLC using an RP-18 column with MeOH/H_2O (3 : 1, v/v) to afford **1** (12 mg, 0.0008% on dry plant material, t_R 3.5 min) and **2** (10 mg, 0.0007%, t_R 5.5 min). Fraction **D** (*ca.* 6.5 g) was subjected to gelfiltration on a Sephadex LH-20 eluted with C_6H_{14}/CH_2Cl_2/MeOH (7 : 4 : 1) and the resulting subfractions were further purified in preparatory HPLC using an RP-18 column with MeOH/H_2O (4 : 1, v/v) and a flow rate of 1.5 ml/min to afford **3** (8 mg, 0.0005%), **4** (15 mg, 0.001%), **5** (3 mg, 0.0002%), **6** (3.5 mg, 0.0002%), **7** (5000 mg, 0.33%), and **23** (40 mg, 0.003%). Fraction **E** (*ca.* 8.3 g) was absorbed on 5.0 g of a Silica gel and placed onto a VLC column (2.5 cm in diameter and 30 cm long) packed with 120 g of a Silica gel. Fraction **E** was separated into nine subfractions (**EA**—**EI**, 100 ml each), using gradients of CH_2Cl_2 in hexane and EtOAc in CH_2Cl_2, followed by EtOAc. The subfractions **EE**—**EI** were further purified by gelfiltration on a Sephadex LH-20 eluted with C_6H_{14}/CH_2Cl_2/MeOH (7 : 4 : 1) and preparatory HPLC using an RP-18 column with MeOH/H_2O (8.5 : 1.5, v/v) and a flow rate of 1.5 ml/min to afford **5** (7 mg, 0.0005%), **6** (5 mg, 0.0003%), **8** (11 mg, 0.0007%), **9** (8.5 mg, 0.0006%), **10** (7 mg, 0.0005%), and **11** (6.2 mg, 0.0004%). The other subfractions **EA**—**ED** were further purified by PTLC: hexane/Et_2O (1 : 1, 1 : 2, 1 : 4) and benzene/CH_2Cl_2 (1 : 1, with different amounts of Et_2O, several developments) to yield **18** (9 mg, 0.0006%), **19** (100 mg, 0.007%), **20** (19.5 mg, 0.001%), and **21** (18 mg, 0.001%). Fraction **C** (*ca.* 2.5 g) was subjected to gelfiltration on a Sephadex LH-20 eluted with C_6H_{14}/CH_2Cl_2/MeOH (7 : 4 : 0.5) and further purified by PTLC: hexane/Et_2O (4 : 1, 1 : 1, two developments) and preparatory HPLC using an RP-18 column with MeOH/H_2O (9 : 1, v/v) and a flow rate of 1.5 ml/min to afford **22** (500 mg, 0.033%), **23** (10 mg, 0.0007%), **4** (5 mg, 0.0003%), and **7** (100 mg, 0.007%).

3α-Hydroxyilicic Acid Methyl Ester; Methyl-3α,4α-dihydroxyeudesm-11(13)-en-5α,7αH-12-oate (**1**): Colorless needles from EtOH, mp 123—125 °C, $[\alpha]_D^{25}$ −48.9° (c=0.25, MeOH). HR-EI-MS m/z: 282.1829 $[M]^+$ ($C_{16}H_{26}O_4$, calcd. 282.1832). EI-MS (direct inlet) 70 eV, m/z (rel. int.): 282 $[C_{16}H_{26}O_4, M]^+$ (2), 267 $[C_{15}H_{23}O_4, M-Me]^+$ (10), 264 $[C_{16}H_{24}O_3, M-H_2O]^+$ (8), 250 $[C_{15}H_{22}O_3, M-MeOH]^+$ (60), 249 $[C_{15}H_{21}O_3, M-Me-H_2O]^+$ (23), 246 $[C_{16}H_{21}O_2, M-2\times H_2O]^+$ (12), 235 $[C_{14}H_{19}O_3, M-Me-MeOH]^+$ (base peak, 100), 231 $[C_{15}H_{19}O_2, M-Me-2\times H_2O]^+$ (47), 207 $[C_{13}H_{19}O_2, M-Me-MeOH-CO]^+$ (30), 192 (45), 149 (90), 121 (80), 71 (65), 55 (60). UV λ_{max} (MeOH) nm (log ε): 206 (3.96). IR (KBr) ν_{max} cm^{-1}: 3540, 3458 (–OH), 1720 (conjugated ester, –COOMe), 1625 and 912 (exocyclic methylene). ^{1}H-NMR (500 MHz in CD_3OD, solvent peak at δ_H 4.78 ppm, J in Hz): The ^{1}H assignments were achieved using 2D ^{1}H–^{1}H COSY, HSQC, and NOESY experiments: see Table 1. ^{13}C-NMR (125 MHz in CD_3OD, solvent peak at δ_C 49.0 ppm): The ^{13}C attributions were achieved in HSQC and HMBC experiments: see Table 1.

3α-Acetoxyilicic Acid Methyl Ester (**1a**): Compound **1** (6.5 mg) was acetylated with Ac_2O/pyridine (1 : 1, 5 ml) at room temperature for 24 h and worked up in the usual manner to yield the corresponding monoacetate **1a** (3.7 mg). Colorless gel, $[\alpha]_D^{25}$ −53.5° (c=0.27, $CHCl_3$). HR-EI-MS m/z: 324.1935 $[M]^+$ ($C_{18}H_{28}O_5$, calcd. 324.1937). UV λ_{max} (MeOH) nm (log ε): 211 (3.98). The spectroscopic data involving IR, ^{1}H- and ^{13}C-NMR, 2D NMR ^{1}H–^{1}H COSY, and HSQC experiments, and EI-MS are in accordance with those described in the literature.[38]

2α-Hydroxy-4-*epi*-ilicic Acid; 2α,4β-Dihydroxyeudesm-11(13)-en-5α,7αH- 12-oic Acid (**2**): Colorless needles from Me_2CO, mp 162—164 °C, $[\alpha]_D^{25}$ +22.5° (c=0.25, MeOH). HR-EI-MS m/z: 268.1673 $[M]^+$ ($C_{15}H_{24}O_4$, calcd. 268.1675). EI-MS (direct inlet) 70 eV, m/z (rel. int.): 268 $[C_{15}H_{24}O_4, M]^+$ (3), 253 $[C_{14}H_{21}O_4, M-Me]^+$ (14), 250 $[C_{15}H_{22}O_3, M-H_2O]^+$ (12), 235 $[C_{14}H_{19}O_3, M-Me-H_2O]^+$ (39), 232 $[C_{15}H_{20}O_2, M-2\times H_2O]^+$ (10), 217 $[C_{14}H_{17}O_2, M-Me-2\times H_2O]^+$ (52), 209 $[C_{13}H_{21}O_2, M-H_2O-ketene]^+$ (5), 204 $[C_{14}H_{20}O, M-2\times H_2O-CO]^+$ (68), 192 (5), 121 (23), 87 (base peak, 100), 84 (29), 55 (34). UV λ_{max} (MeOH) nm (log ε): 207.4 (3.89). IR (KBr) ν_{max} cm^{-1}: 3600, 3455 (–OH), 3300—2500 br, 1696 (–COOH), 1650, 914 (exocyclic methylene). ^{1}H-NMR (500 MHz in CD_3OD, solvent peak at δ_H 4.78 ppm, J in Hz): The ^{1}H assignments were achieved using 2D ^{1}H–^{1}H COSY, HSQC, and NOESY experiments: see Table 1. ^{13}C-NMR (125 MHz in CD_3OD, solvent peak at δ_C 49.0 ppm): The ^{13}C attributions were achieved in HSQC and HMBC experiments: see Table 1.

2α-Acetoxy-4-*epi*-ilicic Acid (**2a**): Compound **2** (7.0 mg) was acetylated with Ac_2O/pyridine (1 : 1, 5 ml) at room temperature for 24 h and worked up in the usual manner to yield the corresponding monoacetate **2a** (4.2 mg). Colorless crystals from MeOH, mp 153—155 °C, $[\alpha]_D^{25}$ +38.7° (c=0.25, $CHCl_3$). HR-EI-MS m/z: 310.1781 $[M]^+$ ($C_{17}H_{26}O_5$, calcd. 310.1778). EI-MS (direct inlet) 70 eV, m/z (rel. int.): 310 $[C_{17}H_{26}O_5, M]^+$ (3), 293 $[C_{17}H_{25}O_4, M-OH]^+$ (11), 250 $[C_{15}H_{22}O_3, M-HOAc]^+$ (16), 233 $[C_{15}H_{21}O_2, M-OH-HOAc]^+$ (33), 205 $[C_{14}H_{21}O, M-OH-HOAc-CO]^+$ (24), 191 (36), 148 (60), 92 (78), 71 (94), 55 (base peak, 100). UV λ_{max} (MeOH) nm (log ε): 210 (3.95). IR ($CHCl_3$) ν_{max} cm^{-1}: 3455 (–OH), 1740, 1250 (–OAc), 3300—2500 br, 1696 (–COOH), 1650, 914 (exocyclic methylene). ^{1}H-NMR (500 MHz in $CDCl_3$, solvent peak at δ_H 7.26 ppm, J in Hz): The ^{1}H assignments were achieved using 2D ^{1}H–^{1}H COSY and in HSQC experiments: see Table 1. ^{13}C-NMR (125 MHz in $CDCl_3$, solvent peak at δ_C 77.0 ppm): The ^{13}C attributions were achieved in HSQC and HMBC experi-

ments: see Table 1.

2α-Hydroxy-2*R*-xanthalongin (**8**): Colorless gum, $[\alpha]_D^{25}$ +25.7° (c=0.35, $CHCl_3$). HR-EI-MS *m/z*: 264.1360 $[M]^+$ ($C_{15}H_{20}O_4$, calcd. 264.1362). UV λ_{max} (MeOH) nm (log ε): 217.2 (3.75). The spectroscopic data including IR and EI-MS are in accordance with those described in the literature.[45] ^{1}H-NMR (500 MHz in $CDCl_3$, solvent peak at δ_H 7.26 ppm, *J* in Hz): The ^{1}H assignments were confirmed using 2D ^{1}H–^{1}H COSY and in HSQC experiments.[45] ^{13}C-NMR (125 MHz in $CDCl_3$, solvent peak at δ_C 77.0 ppm): The ^{13}C attributions were achieved in HSQC and HMBC experiments: see Table 2.

2α-Acetoxy-2*R*-xanthalongin (**8a**): Compound **8** (6.0 mg) was acetylated with Ac_2O/pyridine (1 : 1, 5 ml) at room temperature for 24 h and worked up in the usual manner to yield the corresponding monoacetate **8a** (3.2 mg). Plates from EtOH–hexane, mp 125—127 °C, $[\alpha]_D^{25}$ −46.2° (c=0.25, $CHCl_3$). HR-EI-MS *m/z*: 306.1465 $[M]^+$ ($C_{17}H_{22}O_5$, calcd. 306.1468). IR ($CHCl_3$) ν_{max} cm^{-1}: 1772, 1672, 1162 (α,β-unsaturated γ-lactone), 1715 (>C=O), 1666 (>C=C<), 1740, 1244 (–OAc). The spectroscopic data including ^{1}H-NMR and EI-MS are in agreement with those published in the literature.[46] ^{13}C-NMR (125 MHz in $CDCl_3$, solvent peak at δ_C 77.0 ppm): The ^{13}C attributions were achieved in HSQC and HMBC experiments: see Table 2.

4-*epi*-Isoinuviscolide (**9**): Colorless crystals from EtOAc, mp 144—146 °C, $[\alpha]_D^{25}$ −59.5° (c=0.35, $CHCl_3$). HR-EI-MS *m/z*: 248.1411 $[M]^+$ ($C_{15}H_{20}O_3$, calcd. 248.1413). The spectroscopic data including IR, ^{1}H-NMR, and EI-MS are in accordance with those described in the literature.[10] ^{13}C-NMR (125 MHz in $CDCl_3$, solvent peak at δ_C 77.0 ppm): The ^{13}C attributions were achieved in HSQC and HMBC experiments: see Table 2.

8-*epi*-Helenalin (**10**): Colorless needles from Me_2CO–Et_2O, mp 183—185 °C, $[\alpha]_D^{25}$ −14.5° (c=0.35, $CHCl_3$). HR-EI-MS *m/z*: 262.1204 $[M]^+$ ($C_{15}H_{18}O_4$, calcd. 262.1205). EI-MS (direct inlet) 70 eV, *m/z* (rel. int.): see Chart 3. The spectroscopic data including IR, ^{1}H-, and ^{13}C-NMR are in agreement with those reported in the literature.[48]

Bigelovin; 6-Acetoxy-8-*epi*-helenalin (**11**): Colorless needles from Me_2CO–CH_2Cl_2, mp 180—182 °C, $[\alpha]_D^{25}$ +53.8° (c=0.32, $CHCl_3$). HR-EI-MS *m/z*: 304.1309 $[M]^+$ ($C_{17}H_{20}O_5$, calcd. 304.1311). EI-MS (direct inlet) 70 eV, *m/z* (rel. int.): see Chart 3. The spectroscopic data including IR, ^{1}H-, and ^{13}C-NMR are in accordance with those published in the literature.[48]

Antimicrobial Assays Evaluation of the antimicrobial activity was carried out in the Mycological Center, Assuit University, Assuit, Egypt, according to the disc diffusion assay protocol[49] with some modifications. Bacterial strain inocula were prepared as cell suspensions at a concentration of approximately 10^5 CFU/ml for 24 h cultures grown in nutrient broth at 37±1 °C. Yeasts and filamentous fungal inocula were prepared as cell or spore suspensions at a concentration of approximately 10^4 CFU/ml for 48 h yeast cultures or 7-d mold cultures grown at 28±1 °C on Sabouraud's dextrose broth. Every inoculum was spread on a plate containing nutrient agar in the case of bacteria or Sabouraud's agar in the case of fungi. The crude plant extracts and chromatographic fractions, as well as the reference drugs chloramphenicol (Misr Co. for Pharm. Ind.) and tioconazole (Trosyd®, Pfizer Egypt) used as positive controls were dissolved in dimethyl sulphoxide (DMSO) to prepare 200 μg/ml solutions. The filter paper discs (5 mm diameter, Whatman 3 mm) were impregnated with 10 μl of the above-mentioned solutions. At the time of inoculation, discs were placed on the surface of agar medium in Petri dishes (3 discs per plate) under aseptic conditions. The cultures were incubated at 37±1 °C for 48 h for the antibacterial assay and at 28±1 °C for 3—7 d for the antifungal assay. The diameters of the inhibition zones around the discs were measured in millimeters to evaluate the antimicrobial activity.

The antimicrobial activity of the CH_2Cl_2/MeOH (1 : 1) and MeOH 80% extracts, and chromatographic fractions **A**—**K** of the epigeal parts of *D. graveolens* as well as the reference drugs chloramphenicol and tioconazole are shown in Table 3. Every experiment in the antibacterial and antifungal assays was performed in duplicate.

Pharmacological Studies. Animals Male albino rats (100—120 g) were housed under standardized environmental conditions of temperature and humidity at the Pre-Clinical Animal House, Pharmacology Department, Faculty of Medicine, Assiut University, Assiut, Egypt. They were fed a standard diet *ad libitum* and allowed free access to drinking water in rooms with a controlled 12/12 h light/dark cycle (08:00 a.m. to 20:00 p.m.). All animals were acclimatized for at least 1 week before the experiments.

Preparation of Test Drugs Indomethacin, brewer's yeast, and polysorbate 80 USP (Tween 80) were purchased from Sigma Chemical Co. All test drugs including the crude plant extracts and chromatographic fractions were dissolved or suspended in 2% polysorbate 80 (Tween 80) in normal saline, which was used as a vehicle. Test drugs were administered intraperitoneally (i.p.) with a volume equivalent to 1 ml/100 g body weight of the animals. Control group animals were injected only with 2% Tween 80 in normal saline (vehicle) in the same volume.

Antiinflammatory Activity Antiinflammatory activity was measured according to the method described by Winter C. A. *et al.*[53] Sixty male rats weighing 100—120 g were divided into 12 groups of 5 animals each. The right paw, or control paw, was injected with 2% Tween alone in normal saline (vehicle). Edema was induced on the left hind paw by subplantar injection of 20% brewer's yeast suspension in vehicle at a dose of 1 ml/100 g body weight. Indomethacin 8 mg/kg body weight was injected i.p. 30 min before examining paw swelling. The CH_2Cl_2/MeOH (1 : 1) and MeOH 80% extracts, as well as chromatographic fractions **D**—**K** of the epigeal parts of *D. graveolens* were injected at a dose 300 mg/kg of body weight i.p. 1 h after induction of inflammation. Paw thickness was measured 30, 60, 120, 180, and 240 min after. The percentage of edema and percentage of inhibition were calculated as follows:

$$\%\ \text{edema} = (\text{right paw thickness} - \text{left paw thickness}) \times 100/\text{right paw thickness}$$

$$\%\ \text{inhibition} = V_0 - V_1 \times 100/V_0$$

where V_0 is the average paw thickness in the control group and V_1 is the average paw thickness in the treated group. The antiinflammatory and inhibitory effects of the crude plant extracts and chromatographic fractions as well as the reference drug are shown in Table 4.

Antipyretic Activity The initial rectal temperature of the rats was recorded using an electric thermometer, and animals with a temperature 37 °C were selected for the test. Hyperthermia was induced in the rats by injection of 20% brewer's yeast suspension in vehicle in a dose of 1 ml/100 g body weight subcutaneously (s.c.).[52,54] Then the animals were fasted but allowed access to water until the termination of the experiments performed in a temperature-controlled room. Only rats that developed satisfactory pyrexia (1.0 °C or greater increase in rectal temperature) were used. When temperature was at a peak (20 h after yeast injection), the rectal temperature was again recorded. The rats were randomly divided into 12 groups of 5 animals each. Then the crude plant extracts and chromatographic fractions **D**—**K** of *D. graveolens* (300 mg/kg each), indomethacin (8 mg/kg), and control vehicle were administered s.c. The rectal temperature was recorded at 30-min intervals for 4 h following the administration of test drugs. The results of the antipyretic activity of the crude plant extracts and chromatographic fractions as well as the reference drug are listed in Table 5.

Statistical Analysis Data are expressed as mean±S.E. or as percentages (Tables 4, 5). Differences between the control and the treatment groups were tested for statistical significance using Student's *t*-test. *p* values of less than 0.05 were considered to be statistically significant.

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