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SESQUITERPENE GLYCOSIDES FROM *CALENDULA ARVENSIS*

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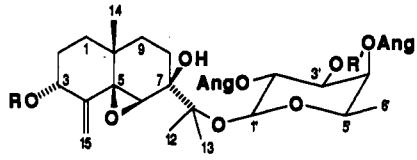
ABSTRACT.—The extract of the aerial parts of *Calendula arvensis* afforded four new sesquiterpene glycosides **1–4** in addition to three known compounds. The structures of the new compounds were established by high field ^{1}H-nmr spectroscopy.

The genus *Calendula* (Compositae, tribe Calendulea) contains about 20 species, which are all common in the Mediterranean region. Extracts of some of these species have been used in folk medicine for treating various diseases (1). The genus has been subjected to numerous chemical investigations, notably for terpenoids (2–6), and from Italian collections of *Calendula arvensis* L. several sesquiterpene and triterpene glycosides have been reported. Our investigation of an Egyptian collection of this same species afforded seven sesquiterpene glycosides, four of which are new.

RESULTS AND DISCUSSION

The polar fraction of the extract of the aerial parts of *C. arvensis* yielded known compounds, the β-D-fucopyranosides of α-bisabolane and α-bisabolone (7) and dihydroactinidiolide (8), in addition to four new eudesmane glycosides **1–4**.

The ^{1}H-nmr spectrum of **1** (Table 1) indicated the presence of a β-D-fucopyranoside moiety by the anomeric proton at δ 4.72 (d, J=8 Hz) and the other sugar protons at δ 5.27 (dd, J=10.5 and 8 Hz, H-2′), 5.15 (dd, J=10.5 and 3.5 Hz, H-3′), 5.32 (dd, J=3.5 and 1 Hz, H-4′), 3.84 (dq, J=6.5 and 1 Hz, H-5′), and 1.21 (d, J=6.5 Hz, H-6′). The downfield chemical shifts of H-2′, H-3′, and H-4′ as well as the presence of two angelates and an acetate suggested that the hydroxyl groups were esterified. The location of the angelates at C-2′, C-4′ and the acetate at C-3′ was evident from COLOC experiments (Table 2). The carbonyl of the acetate group at δ 170.0 showed connection with its methyl at δ 1.94 and H-3′ at δ 5.15. On the other hand, one angelate carbonyl showed connection with H-4′.

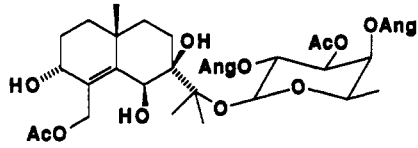


1 R=H, R′=Ac

2 R=H, R′=$\overset{O}{\overset{\|}{C}}CHMe_2$

3 R=H, R′=$\overset{O}{\overset{\|}{C}}CH(Me)CH_2Me$

5 R=R′=Ac



4

The signals of the aglycone established the eudesmane nature of **1**. Thus, H-14 appeared at δ 0.96 and H-15 at δ 4.98 and 5.02. A signal at δ 4.38 could be attributed to a proton on a carbon-bearing oxygen, which spin decoupling placed

TABLE 1. ^{1}H-nmr Spectral Data of Compounds **1–4** (400 MHz, $CDCl_3$).

Proton	Compound				
	1	**5**	**2**[a]	**3**[b]	**4**
H-1α	2.29 ddd (13, 3.5, 3.5)	2.05	2.28	2.29	—
H-1β	1.11 ddd (13, 11, 6.5)	1.21	1.11	1.11	—
H-2α	1.85 m	1.85	1.86	1.85	—
H-2β		2.05			
H-3	4.38 brdd (2.5, 2.5)	5.55	4.29	4.39	4.03 brdd (11, 5)
H-6	3.19 brd (1)	3.09	3.19	3.19	5.17 brd (5)
H-8α	1.68 ddd (14, 14, 3.5)	1.66	1.62	1.66	—
H-8β	1.25 (brddd 14, 3.5, 3.5)	1.33	1.26	1.26	1.71 (1H) 1.35 (3H)
H-9α	1.04 ddd (14, 3.5, 3.5)	1.06	1.06	1.05	—
H-9β	1.51 ddd (14, 14, 3.5)	1.52	1.52	1.53	—
H-12	1.36 s	1.32	1.37	1.36	1.32 s
H-13	1.22 s	1.29	1.23	1.22	1.13 s
H-14	0.96 s	1.00	0.97	0.97	1.17 brs
H-15	5.02 d (2)	5.21	5.03	5.03	5.42 brd (12)
H-15′	4.98 d (2)	5.13	4.99	4.99	4.58 d (12)
H-1′	4.72 d (8)	4.80	4.75	4.74	4.53 d
H-2′	5.27 dd (10.5, 8)	5.27	5.31	5.31	5.00
H-3′	5.15 dd (10.5, 3.5)	5.16	5.16	5.17	5.13
H-4′	5.32 dd (3.5, 1)	5.35	5.35	5.36	5.33
H-5′	3.84 dq (1, 6.5)	3.87	3.87	3.87	3.82
H-6′	1.21 d (6.5)	1.24	1.21	1.20	1.22
OAng	6.12, 6.09 qq (7.5, 1.5)	6.12, 6.06	6.14, 6.11	6.14, 6.12	6.12, 6.12
	2.01, 1.95 dq (7.5, 1.5)	2.02, 1.92	2.02, 1.97	2.02, 1.97	2.00, 1.95
	2.00, 1.83 dq (1.5, 1.5)	1.98, 1.82	2.00, 1.82	2.00, 1.82	2.06, 1.81
OAc	1.94	1.96, 1.95			2.03, 1.92
OH					3.46 brd (11) 3-OH 3.03 d (5) 6-OH

[a]Isobutyrate: 2.42 (qq, J=7 and 7 Hz), 1.06 (d, J=7 Hz), 1.03 (d, J=7 Hz). 2-Methylbutyrate was assigned by H/H COSY.

[b]2-Methylbutyrate: 2.25 (m), 1.24 (m), 1.04 (d, J=7 Hz), 0.76 (t, J=7 and 7 Hz).

at C-3. Acetylation of **1** gave the monoacetate **5** in which H-3 shifted downfield at δ 5.55 (Table 1). The appearance of H-6 at δ 3.19 suggested an epoxide ring. The presence of a 5,6-epoxide and a 7-hydroxyl group was deduced from the chemical shifts of the corresponding carbons (Table 3). A 2D hetero COSY spectrum allowed the unambiguous assignment of all ^{13}C signals. The configuration was deduced from the observed nOe's (Table 4). Clear effects were observed between H-14 and H-9β (6%) and H-2β (4%), between H-8α and H-1α. No nOe's were observed between H-12/13 and H-9β. In the ms spectrum of **1**, $[M-H_2O]^+$ appeared at *m/z* 602 followed by loss of angelic acid to give an ion at *m/z* 502; loss of a second angelic acid moiety produced an ion at *m/z* 402. An ion for sugar moiety appeared at *m/z* 353. Negative fabms showed $[M-H]^-$ at *m/z* 619 for **1**.

The ^{1}H-nmr spectra of **2** and **3** differed from that of **1** by the absence of a

TABLE 2. COLOC of Compound **1**.

Carbon irradiated	Proton connected
C-14	H-1β, H-1α, H-9β
C-8	H-6
C-10	H-14, H-1β, H-1α, H-2 H-8β, H-9β, H-9α
C-5/6	H-8β
C-7	H-12, H-13
C-11	H-12, H-13, H-6, H-1′
C-4	H-2, H-6, H-3, H-15
C=O (Acetate)	Me_3 (Acetate), H-3′
C=O (Angelate)	Me_3 (Angelate), H-4′

TABLE 3. ^{13}C-nmr Spectral Data of Compounds **1**, **2**, and **4** (100.6 MHz, $CDCl_3$).

Carbon	Compound		
	1[a]	**2**[b]	**4**
C-1	28.7	28.8	33.0
C-2	28.7	28.8	28.1
C-3	72.6	72.6	66.8
C-4	147.9	148.1	131.3
C-5	66.3	66.5	146.1
C-6	66.4	66.5	64.5
C-7	71.5	71.4	75.3
C-8	26.0	26.1	27.4
C-9	29.6	29.6	32.2
C-10	34.0	33.9	33.7
C-11	82.4	83.1	83.1
C-12	20.2	20.6	21.5
C-13	21.9	22.0	21.0
C-14	22.5	22.6	29.4
C-15	110.3	110.5	62.8
C-1′	95.4	95.5	95.4
C-2′	68.8	69.0	68.8
C-3′	71.4	71.3	71.1
C-4′	69.7	69.9	69.7
C-5′	69.1	69.4	69.3
C-6′	16.2	16.4	16.3
OAng	167.4, 166.1	167.8, 167.0	167.4, 166.5
	127.4, 127.0	127.9, 126.2	127.4, 127
	139.8, 139.2	139.6, 139.0	140.5, 139.2
	15.7, 15.6	15.8, 15.8	15.8, 15.8
	20.5, 20.4	20.5, 20.3	20.5, 20.4
OAc	170.0, 20.5		171.9, 170.1
			20.8, 20.6

[a]Assigned by 2D-hetero COSY.
[b]Isobutyrate: 175.0, 34.2, 28.6, 18.7, 15.6.

signal for an acetate group and instead exhibited signals for isobutyrate and 2-methylbutyrate groups. These groups were deduced from the signals at δ 1.03 (d, J= 7 Hz), 1.06 (d, J=7 Hz), and 2.42 (qq, J=7 and 7 Hz). The 2D homo-COSY of **3** allowed the assignments of all signals for the butyrate group (Table 1). The ms data of **2** and **3** (see Experimental) and ^{13}C of **2** (Table 3) supported the proposed structures.

Spectral data for **4** was in part similar to that of **1**. The signals of the sugar moiety for **4** clearly showed that a fucopyranoside was present. The exomethylene signals at C-15 observed for **1** were replaced in **4** by signals of an oxymethylene group at δ 5.42 (d, J=12 Hz) and 4.58 (d, J=12 Hz). This finding was confirmed by ^{13}C data which showed C-15 at 62.8 (t). Furthermore, H-6 was shifted downfiield to δ 5.17. Ms and ^{13}C data agreed with the proposed structure for **4**. The formation of **4** can easily be explained by proton-catalyzed epoxide

TABLE 4. NOe's of Compound **1**.

H irradiated	nOe (%)
H-14	H-9β (6), H-2β (4), H-15 (2)
H-12	H-6 (10), H-1′ (10)
H-9β	H-14 (2)
H-8α	H-1α (5)
H-1α	H-8α (4)
OH	H-9β (3)
H-6	H-12 (2), H-13 (2), H-15 (1)
H-3	H-15′ (10)
H-15′	H-3 (10)
H-15	H-6 (4)

ring opening and addition of acetate at C-15.

EXPERIMENTAL

PLANT COLLECTION, EXTRACTION, AND ISOLATION.—The air-dried aerial parts of *C. arvensis* (800 g, collected along the Cairo-El-Fyium road in March 1988, voucher Ahmed 560, deposited in the Department of Botany, El-Minia University, Egypt) was extracted with MeOH-Et_2O-petroleum ether (1:1:1). The extract was separated as previously reported (9) to yield **1** (90 mg), **2** (9 mg), **3** (4 mg), and **4** (15 mg). Known compounds were identified by their ^1H nmr, ms, and ir and by comparison with data reported for authentic samples.

3α,7β-Dihydroxy-5β,6β-epoxyeudesm-4(15)-ene-11-(O-β-D-fucopyranoside-2′4′-diangelate-3′-acetate) [**1**].—Ir ν max $CHCl_3$ cm^{-1} 3600, 3580, 1750, 1720, 1680; ms *m/z* (rel. int.) $[M-H_2O]^+$ 602 (1), $[602-C_4H_7COOH]^+$ 502 (15), $[M-2XC_4H_7COOH]^+$ 402 (10), [sugar moiety]$^+$ 353 (20), $[C_4H_7CO]^+$ 83 (100).

7β-Hydroxy-3β-acetoxy-5β,6β-epoxyeudesm-5(15)ene-11-(O-β-D-fucopyranoside-2′4′-diangelate-3′-acetate) [**5**].—Ir ν max $CHCl_3$ cm^{-1} 3500, 1750, 1700; ms *m/z* (rel. int.) $[M-Ac]^+$ 619 (2), $[M-C_4H_7COOH]^+$ 562 (3), $[562-Ac]^+$ 519 (6), [sugar moiety]$^+$ 353 (60), $[C_4H_7CO]^+$ 83 (100).

3α,7β-Dihydroxy-5-β,6-β-epoxyeudesm-4(15)-ene-11-(O-β-D-fucopyranoside-2′,4′-diangelate-3′-isobutyrate) [**2**].—Ir ν max $CHCl_3$ cm^{-1} 3610, 3570, 1740, 1700, 1660; ms *m/z* (rel. int.) $[M-H_2O]^+$ 630 (3), $[M-2H_2O]^+$ 612 (10), $[M-C_4H_7COOH]^+$ 548 (6), $[548-H_2O]^+$ 530 (4), $[548-C_4H_7COOH]^+$ 448 (10), [sugar moiety]$^+$ 381 (100), $[C_4H_7CO]^+$ 83 (100).

3α,7β-Dihydroxy-5-β,-6β-epoxyeudesm-4(15)-ene-11-(O-β-D-fucopyranoside-2′,4′-diangelate-3′-methylbutyrate) [**3**].—Ir ν max $CHCl_3$ cm^{-1} 3590, 3570, 1730, 1700, 1650; ms *m/z* (rel. int.) $[M-H_2O]^+$ 644 (1), $[M-C_4H_9COOH]^+$ 560 (9), $[M-2XC_4H_7COOH]^+$ 462 (6), [sugar moiety]$^+$ 395 (100), $[C_4H_9CCO]^+$ 85 (30), $[C_4H_7CO]^+$ 83 (90).

3α,7β-Dihydroxy-15-acetoxyeudesm-4(5)-ene-11-(O-β-D-fucopyranoside-2′,4′-diangelate-3′-acetate) [**4**].—Ms *m/z* (rel. int.) $[M-H_2O]^+$ 662 (1.5), $[M-2H_2O-O]^+$ 628 (7), $[M-HOAc-2H_2O]^+$ 584 (2), [sugar moiety]$^+$ 353 (60), [angelate]$^+$ 83 (100).

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