Palmitoleate (=(9*Z*)-Hexadeca-9-enoate) Esters of Oleanane Triterpenoids from the Golden Flowers of *Tagetes erecta:* Isolation and Autoxidation Products[1])

by **Shaheen Faizi*** and **Aneela Naz**

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan (Phone: ++ 92 9243086; fax: ++92-21-9243190-91; e-mail: shaheen@khi.comsats.net.pk)

Dedicated to the fond memory of Professor *Salim-uz-Zaman Siddiqui* (1897–1994), FRS, the founder director of H.E.J. Research Institute of Chemistry, University of Karachi

Six oleanane-type triterpenoid esters were isolated from the golden flowers of *Tagetes erecta*. Spectral studies characterized their structures as 3-*O*-[(9*Z*)-hexadec-9-enoyl]erythrodiol (**1**), 11α,12α:13β,28-diepoxyoleanan-3β-yl (9*Z*)-hexadec-9-enoate (**2**), 13β,28-epoxyolean-11-en-3β-yl (9*Z*)-hexadec-9-enoate (**3**), 28-hydroxy-11-oxoolean-12-en-3β-yl (9*Z*)-hexadec-9-enoate (**4**), 3-*O*-[(9*Z*-hexadec-9-enoyl]-β-amyrin (**5**), and 11-oxoolean-12-en-3β-yl (9*Z*)-hexadec-9-enoate (**6**). Compounds **1**–**4** and **6** are new natural products, while the known **5** was isolated for the first time from the genus *Tagetes*, from which only one triterpenoid has earlier been obtained. Aerial oxidation (autoxidation) converted amyrin **1** into **2**–**4** and transformed amyrin **5** into **6**. The configuration of **1**–**6** and an autoxidation mechanism (*Scheme*) involving the formation of the intermediate 11α-hydroxyolean-12-ene derivatives **1b** and **5b** on thermal decomposition of the labile 11α-OOH derivatives **1a** and **5a**, respectively, under neutral conditions are discussed. For the first time, the reactivity of the allylic H–C(11) bond of triterpenoids of type **1** and **5** toward aerial oxidation was observed. The long-chain ester group at C(3) of **1** and **5** may be responsible for their labile nature, as β-amyrin (**7**), erythrodiol (**8**), and ursolic acid were found to be inert toward autoxidation.

Introduction. – *Tagetes erecta* LINN. (African Marigold), locally known as genda, belongs to the family Asteraceae (Compositae). It is a stout, branching herb, native of Mexico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics. It is very popular as a garden plant and yields a strongly aromatic essential oil (tagetes oil), which is mainly used for the compounding of high-grade perfumes. An infusion of the plant is used against rheumatism, cold, and bronchitis [1]. The flowers of *T. erecta* are used for the cure of eye diseases, colds, conjunctivitis, coughs, and ulcers, and its aqueous extract shows activity against *Gram*-positive bacteria. Internally, it is employed to purify the blood, and the juice was given for the cure of bleeding piles. Leaves of *T. erecta* are used as an application for boils and carbuncles, while their juice is given against earache. The leaves and florets are used as emmenagogue, and the infusion is prescribed as a vermifuge, diuretic, and carminative. An extract of the roots is credited with laxative properties [1]. Moreover, the volatiles from different parts of *T. erecta* are reported to be effective against both larvae and adult mosquitoes [2a]. Recently, the identification of an antimicrobial compound in the volatile oil of leaves of *T. erecta* has been reported [2b]. Phytochemical studies of its

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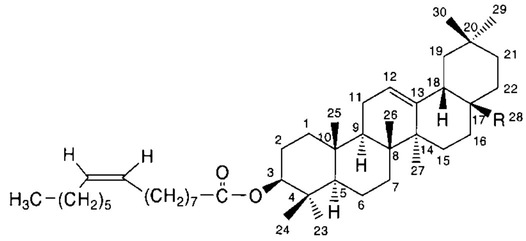
different parts have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, and carotenoids [3a–d].

In continuation of studies on the genus *Tagetes* [3e], the present investigation of the petroleum ether and $CHCl_3$ extracts of the golden flowers of *T. erecta* has led to the isolation of the five new triterpenoids **1**–**4** and **6** and of the known compound **5**, which are all oleanane-type triterpenoids. Compound **5** has previously been obtained from *Wedelia rugosa* Greenm [4]. Earlier, only one triterpenoid (erythrodiol 3-palmitate) has been isolated from the entire *Tagetes* genus [5].



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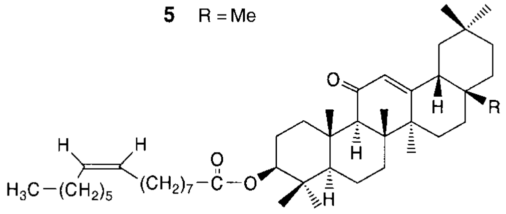
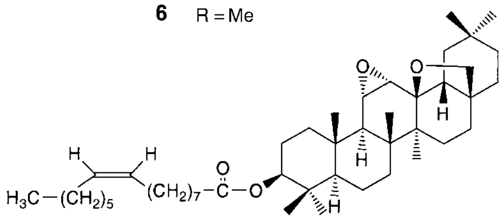
5 R = Me



4 R = CH_2OH
6 R = Me



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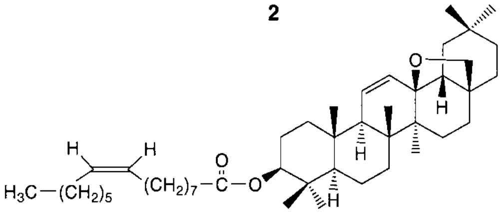

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Results and Discussion. – *Isolated Triterpenoids.* Separation of the petroleum ether and $CHCl_3$ extracts of the flowers of *T. erecta* by prep. TLC and vacuum liquid chromatography (VLC) followed by TLC purification afforded the six chemical constituents **1**–**6** (see *Exper. Part*).

The molecular formula of **1** was determined to be $C_{46}H_{78}O_3$ by HR-EI-MS (*m*/*z* 678.5877), which established the presence of seven double-bond equivalents in the molecule. The structure of **1** was elucidated by spectroscopic means as 3-*O*-[(9*Z*)-hexadec-9-enoyl]erythrodiol, which is a new natural product.

The ^{1}H-NMR spectrum of **1** in $CDCl_3$ (500 MHz, *Table 1*) displayed *s* (δ 1.14, 0.93, 0.92, 0.87, 0.85 (6 H), and 0.83) for seven tertiary Me groups, an *AB* system (δ 3.19 (*d*, *J* = 11.0 Hz) and 3.53 (*d*, *J* = 11.0 Hz)) for a CH_2OH group, a *dd* (δ 4.48 (*J* = 9.0 and 7.0 Hz)) characteristic of the axial H–C(3) of a triterpene geminal to an ester function, and a *t* (δ 5.18 (*J* = 3.5 Hz)) typical for H–C(12), the trisubstituted olefinic proton of olean-12-ene derivatives. These data are compatible with those reported for erythrodiol (= (3*β*)-olean-12-ene-3,28-diol) and its derivatives [5b] [6] [7]. Furthermore, the ^{1}H-NMR spectrum of **1** also showed the characteristic signals at δ 0.86 (*t*, *J* = 7.0 Hz) for a terminal Me group, at 2.27 (*t*, *J* = 7.5 Hz) for a CH_2 group in *α*-position to an ester function, at 5.33 (*m*) for two olefinic protons, suggesting a disubstituted C=C bond, and other signals at δ 1.99, 1.55–1.65, and 1.22–1.29 all related to a monounsaturated long-chain fatty-acid ester moiety [8a], which was identified as palmitoleate (= (9*Z*)-hexadec-9-enoate) ester. This was confirmed by the HR-EI-MS of **1**, which has a fragment at *m*/*z* 424.3691 corresponding to the loss of $C_{16}H_{30}O_2$ from M^+. The HR-EI-MS also gave characteristic fragment ions at *m*/*z* 234.1939 ($C_{16}H_{26}O^+$) and 203.1759 (100%, $C_{15}H_{23}^+$) due to the *retro-Diels–Alder* process; this along with the 7 Me *s* in the ^{1}H-NMR spectrum, confirmed that **1** must be a member of the *β*-amyrin (= (3*β*)-olenan-12-en-3-ol) group having all seven degrees of unsaturation as shown by the molecular formula. Supporting evidence was obtained from the ^{13}C-NMR spectra (broad-band-decoupled and DEPT), which have characteristic downfield signals (*Table 2*) at δ 80.51 (C(3)), 122.23 (C(12)), 144.31 (C(13)), and 69.60 (C(28)) for the triterpene skeleton [6] and signals at 173.65 (C(1′)), 27.27 (C(8′)), 129.54 (C(9′)), 129.99 (C(10′)), and 27.23 (C(11′)), for the palmitoleate moiety with (*Z*)-configuration, the latter being revealed by the characteristic upfield δ(C) values of both the allylic C-atoms C(8′) and C(11′), which are typical for *cis*-olefins as compared to *trans*-olefins [8b]. The position of H–C(18) (δ 1.95) is *β*, as it has axial orientation showing a broad *dd* with $J_{ax,eq}$ = 4.2 Hz and $J_{ax,ax}$ = 14.2 Hz.

The HR-EI-MS of compound **2** displayed the molecular ion at *m*/*z* 692.5811 corresponding to the formula $C_{46}H_{76}O_4$ and a characteristic fragment at *m*/*z* 438.3497 corresponding to the ion $[M - C_{16}H_{30}O_2]^+$. The structure of **2** was determined as 11*α*,12*α*:13*β*,28-diepoxyoleanan-3*β*-yl (9*Z*)-hexadec-9-enoate, which is a new natural product.

The ^{1}H-NMR spectrum of **2** (*Table 3*) showed characteristic resonances at δ 2.91 (br. *s*, $w_{1/2}$ = 2.3 Hz, H_β–C(11) and H_β–C(12)), 3.31 (*dd*, *J* = 1.8, 6.6 Hz, H_a–C(28)), and 3.75 (*d*, *J* = 6.6 Hz, H_b–C(28)) which revealed the presence of a 11*α*,12*α*:13*β*,28-diepoxy functionality in the molecule [9]. Its ^{13}C-NMR spectra (broad-band-decoupled and DEPT) (*Table 2*) exhibited signals at δ 52.33 (C(11)), 59.11 (C(12)), and 77.91 (C(28)) for the diepoxy functionality and characteristic down-field resonances at δ 80.34, 173.56, 129.56, and 129.97 for C(3), C(1′), C(9′), and C(10′), respectively. Other structural features of **2**, except for the diepoxy functionality, were the same as those of **1**, as revealed by their ^{1}H- and ^{13}C-NMR data.

Compound **3** possessed the molecular formula $C_{46}H_{76}O_3$ as shown by the HR-EI-MS and was characterized as 13*β*,28-epoxyolean-3*β*-yl (9*Z*)-hexadec-9-enoate.

The ^{1}H-NMR data (*Table 1*) of **3** were identical to those of **2**, except for protons of ring C (H–C(11) and H–C(12)) which appeared down-field at δ 5.36 (*dd*, *J* = 3.2, 10.4 Hz, H–C(11)) and 5.82 (*dd*, *J* = 1.5, 10.4 Hz, H–C(12)) due to the olefinic bond between C(11) and C(12) instead of the epoxy moiety as in **2**. The molecular formula of **3** showed the presence of nine double-bond equivalents, of which seven were accounted for by the five rings of the triterpenoid skeleton and two for the 13*β*,28-epoxy-11-ene functionality. The remaining two were taken for a C=O of an ester function and for the C=C bond of the palmitoleate side chain. Moreover, important down-field ^{1}H-NMR signals were observed at δ 5.32 (*m*, H–C(9′) and H–C(10′)), 4.49 (*dd*, *J* = 5.5, 11.0 Hz, H–C(3)), 3.68 (*d*, *J* = 6.5 Hz, H_a–C(28)), and 3.26 (*dd*, *J* = 1.8, 6.5 Hz, H_b–C(28)). Supporting evidence was obtained from the ^{13}C-NMR spectra (broad-band-decoupled and DEPT), which have character-

Table 1. *^{1}H-NMR Data* (CDCl$_3$, 500 MHz) *for Triterpenoids* **1**, **3**, *and* **4**. δ in ppm, *J* in Hz

	1	**3**	**4**
CH$_2$(1)	1.01, 1.67 (2*m*)	1.05, 1.58 (2*m*)	0.99 (*m*), 2.77 (*ddd*, *J* = 3.9, 3.9, 12.9)
CH$_2$(2)	1.60, 1.69 (2*m*)	1.63 (*m*)	1.59, 1.67 (2*m*)
H–C(3)	4.48 (*dd*, *J* = 7.0, 9.0)	4.49 (*dd*, *J* = 5.5, 11.0)	4.50 (*dd*, *J* = 5.0, 11.0)
H–C(5)	0.81 (*m*)	0.82 (*m*)	0.82 (*m*)
CH$_2$(6)	1.51 (*m*)	1.51 (*m*)	1.54 (*m*)
CH$_2$(7)	1.19, 1.43 (2*m*)	1.21, 1.51 (2*m*)	1.50 (*m*)
H–C(9)	1.53 (*m*)	1.87 (*m*)	2.35 (*s*)
CH$_2$(11) or H–C(11)	1.57, 1.89 (2*m*)	5.36 (*dd*, *J* = 3.2, 10.4)	–
H–C(12)	5.18 (*t*, *J* = 3.5)	5.82 (*dd*, *J* = 1.5, 10.4)	5.56 (br. *s*)
CH$_2$(15)	0.91, 1.45 (2*m*)	1.78, 1.11 (2*m*)	0.92 (*m*), 1.78 (*ddd*, *J* = 5.1, 14.0, 14.0)
CH$_2$(16)	1.02, 1.81 (2*m*)	1.99, 1.13 (2*m*)	1.13 (*m*), 1.94 (*ddd*, *J* = 5.1, 14.0, 14.0)
H–C(18)	1.95 (br. *dd*, *J* = 4.9, 14.2)	1.62 (*m*)	2.15 (br. *dd*, *J* = 4.3, 13.4)
CH$_2$(19)	1.11, 1.72 (2*m*)	1.31, 1.74 (2*m*)	1.09 (*m*), 1.69 (*dd*, *J* = 13.4, 13.4)
CH$_2$(21)	1.15, 1.21 (2*m*)	1.14, 1.23 (2*m*)	1.15, 1.21 (2*m*)
CH$_2$(22)	1.24, 1.36 (2*m*)	1.31, 1.40 (2*m*)	1.24, 1.36 (2*m*)
Me(23)	0.85 (*s*)	0.85 (*s*)	0.82 (*s*)
Me(24)	0.92a) (*s*)	0.92 (*s*)	0.99 (*s*)

	1	**3**	**4**
Me(25)	0.93a) (*s*)	1.01 (*s*)	1.15 (*s*)
Me(26)	0.83 (*s*)	1.07 (*s*)	1.10 (*s*)
Me(27)	1.14 (*s*)	0.96 (*s*)	1.37 (*s*)
CH$_2$(28)	3.19 (*d*, *J* = 11.0), 3.53 (*d*, *J* = 11.0)	3.26 (*dd*, *J* = 1.8, 6.5), 3.68 (*d*, *J* = 6.5)	3.21 (*d*, *J* = 10.9), 3.46 (*d*, *J* = 10.9)
Me(29)	0.85 (*s*)	0.88 (*s*)	0.89 (*s*)
Me(30)	0.87 (*s*)	0.91 (*s*)	0.91 (*s*)
CH$_2$(2′)	2.27 (*t*, *J* = 7.5)	2.28 (*t*, *J* = 7.5)	2.27 *t*, *J* = 7.5)
CH$_2$(3′)	1.55 – 1.65 (*m*)	1.61 (*m*)	1.61 (*m*)
CH$_2$(4′), CH$_2$(5′), CH$_2$(6′)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)
CH$_2$(7′)	1.55 – 1.65 (*m*)	1.61 (*m*)	1.61 (*m*)
CH$_2$(8′)	1.99 (*m*)	2.01 (*m*)	2.00 (*m*)
H–C(9′), H–C(10′)	5.33 (*m*)	5.32 (*m*)	5.32 (*m*)
CH$_2$(11′)	1.99 (*m*)	2.01 (*m*)	2.00 (*m*)
CH$_2$(12′)	1.55 – 1.65 (*m*)	1.61 (*m*)	1.61 (*m*)
CH$_2$(13′)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)
CH$_2$(14′)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)
CH$_2$(15′)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)	1.22 – 1.29 (*m*)
Me(16′)	0.86 (*t*, *J* = 7.0)	0.85 (*t*, *J* = 7.0)	0.86 (*t*, *J* = 7.0)

a) Assignments may be reversed.

Table 2. *^{13}C-NMR Chemical Shifts* ($CDCl_3$, 125 MHz) *of Compounds* **1**–**4** *and* **6**. δ in ppm.

	1	**2**	**3**	**4**	**6**		**1**	**2**	**3**	**4**	**6**
C(1)	38.26	38.29	38.19	39.40	39.21	C(24)	28.10	28.09	28.11	28.09	28.12
C(2)	23.52	23.49	23.64	23.61	23.36	C(25)	15.55	16.38	18.41	16.61	16.67
C(3)	80.51	80.34	80.52	80.34	80.53	C(26)	16.72	19.41	19.38	18.62	18.66
C(4)	37.73	37.93	37.81	37.51	37.82	C(27)	25.99	19.83	19.80	23.52	23.55
C(5)	55.24	55.19	55.01	55.93	55.89	C(28)	69.60	77.91	77.26	69.70	28.68
C(6)	18.23	17.87	18.32	17.48	17.92	C(29)	33.18	33.22	33.21	33.13	23.54
C(7)	32.47	33.94	32.51	32.64	32.53	C(30)	23.27	23.31	23.34	23.51	33.11
C(8)	39.79	40.81	41.61	45.47	45.25	C(1′)	173.65	173.56	173.66	173.77	173.75
C(9)	47.58	49.74	53.12	61.69	62.98	C(2′)	34.83	34.80	34.81	34.90	34.94
C(10)	36.93	36.73	37.21	37.48	37.54	C(3′)	25.15	25.17	25.17	24.76	25.09
C(11)	23.58	52.33	132.13	198.91	201.60	C(4′)	29.02–29.67	29.02–29.69	29.01–29.71	29.12–29.74	29.01–29.71
C(12)	122.23	59.11	130.80	128.13	129.43	C(5′)	29.02–29.67	29.02–29.69	29.01–29.71	29.12–29.74	29.01–29.71
C(13)	144.31	83.09	84.51	169.25	171.61	C(6′)	29.02–29.67	29.02–29.69	29.01–29.71	29.12–29.74	29.01–29.71
C(14)	41.71	43.44	43.13	43.44	45.43	C(7′)	29.02–29.67	29.02–29.69	29.01–29.71	29.12–29.74	29.01–29.71
C(15)	25.54	25.31	25.21	25.51	26.88	C(8′)	27.27	27.28	27.29	27.21	27.19
C(16)	22.62	25.52	25.47	21.70	27.12	C(9′)	129.54	129.56	129.58	129.58	129.56
C(17)	36.81	41.30	41.47	37.07	32.51	C(10′)	129.99	129.97	129.96	129.91	129.92
C(18)	42.35	50.38	50.99	42.50	49.11	C(11′)	27.23	27.22	27.25	27.21	27.16
C(19)	46.44	36.84	36.91	44.85	45.11	C(12′)	29.02–29.67	29.02–29.69	29.01–29.71	29.12–29.74	29.01–29.71
C(20)	31.05	31.77	31.68	31.08	30.91	C(13′)	29.02–29.67	29.02–29.69	29.01–29.71	29.12–29.74	29.01–29.71
C(21)	34.74	34.50	34.99	32.97	34.64	C(14′)	31.89	31.91	31.78	31.97	31.89
C(22)	31.91	31.67	31.12	30.15	35.98	C(15′)	22.77	22.78	22.81	22.74	23.10
C(23)	15.73	15.81	15.69	16.08	16.31	C(16′)	14.10	14.10	14.11	14.18	14.16

Table 3. *^{1}H-NMR Data* ($CDCl_3$, 500 MHz) *for Triterpenoids* **2** *and* **6**. δ in ppm, J in Hz.

	2	**6**
H–C(3)	4.50 (*dd*, $J = 5.4$, 10.8)	4.47 (*dd*, $J = 5.5$, 10.5)
H–C(9)	n.d.[a])	2.34 (*s*)
H–C(11)	2.91[b]) (br. *s*, $w_{1/2} = 2.3$)	
H–C(12)	2.91[b]) (br. *s*, $w_{1/2} = 2.3$)	5.68 (*s*)
H–C(18)	n.d.[a])	2.12 (*dd*, $J = 4.5$, 13.8)
H_a–C(28)	3.31 (*dd*, $J = 1.8$, 6.6)	–
H_b–C(28)	3.75 (*d*, $J = 6.6$)	–
Me	1.06	1.36
Me	1.00	1.15
Me	0.99	1.11
Me	0.94	0.96
Me	0.92	0.92
Me	0.89	0.86
Me	0.85	0.83
Me	–	0.82
CH_2(2′)	2.28 (*t*, $J = 7.5$)	2.27 (*t*, $J = 7.5$)
CH_2(3′)	1.60 (*m*)	1.61 (*m*)
CH_2(4′), CH_2(5′), CH_2(6′)	1.22–1.30 (*m*)	1.22–1.29 (*m*)
CH_2(7′)	1.60 (*m*)	1.61 (*m*)
CH_2(8′)	2.02 (*m*)	2.00 (*m*)
H–C(9′), H–C(10′)	5.33 (*m*)	5.32 (*m*)
CH_2(11′)	2.02 (*m*)	2.00 (*m*)
CH_2(12′)	1.60 (*m*)	1.61 (*m*)
CH_2(13′), CH_2(14′), CH_2(15′)	1.22–1.30 (*m*)	1.22–1.29 (*m*)
Me(16′)	0.86 (*t*, $J = 7.0$)	0.86 (*t*, $J = 7.0$)

[a]) n.d. = Not detected. [b]) δ 2.80 (*m*) for H–C(11) and 3.08 (*d*, $J = 3.8$ Hz) for H–C(12) in C_6D_6.

istic down-field signals (*Table 2*) at δ 80.52 (C(3)), 132.13 (C(11)), 130.80 (C(12)), 84.51 (C(13)), 77.26 (C(28)), and 173.66 (C(1′)). These NMR values are in good agreement with those reported for model compounds [6][8][10].

It is noteworthy in this context that triterpenoids **2** and **3** were initially obtained as a mixture showing nearly a single spot on prep. TLC plates (silica gel *GF_{254}*, petroleum ether/AcOEt 8.5 : 1.5); however, they could be separated on TLC aluminium sheets (silica gel *$60\ F_{254}$*, petroleum ether/AcOEt 9.3 : 0.7), showing two distinct spots with very close R_f values.

The HR-EI-MS of **4** showed the molecular-ion peak at *m/z* 692.5728 ($C_{46}H_{76}O_4^{+}$) revealing that it is an isomer of **2**. The structure of **4** was elucidated as 28-hydroxy-11-oxoolean-12-en-3*β*-yl (9*Z*)-hexadec-9-enoate, which is a new compound.

The ^{1}H-NMR spectrum of **4** showed characteristic peaks at δ 5.56 (br. *s*, H−C(12)), 3.21 (*d*, *J* = 10.9 Hz, H_a−C(28)), and 3.46 (*d*, *J* = 10.9 Hz, H_b−C(28)) [10][11]. In the COSY-45° spectrum, δ 3.46 correlated with 3.21, while the ^{13}C-NMR spectrum (*Table 2*) showed peaks for C(11), C(12), C(13), and C(28) at δ 198.91, 128.13, 169.25, and 69.70, respectively [6b][12]. An enone moiety in ring C of **4** was confirmed by its characteristic UV maximum at 246 nm [6b][10] and by the HR-EI-MS, which showed two important fragments at *m/z* 289.2139 ($C_{19}H_{29}O_2^{+}$) and 248.1777 ($C_{16}H_{24}O_2^{+}$), in accordance with the well-known fragmentation pattern [13]. Moreover, the EI-MS also exhibited a significant peak at *m/z* 438 arising from M^+ by the loss of $C_{16}H_{30}O_2$. The remaining ^{1}H- and ^{13}C-NMR features were the same as those of **1**.

Compound **5** was analyzed for $C_{46}H_{78}O_2$ by HR-EI-MS (*m/z* 662.5981), and the mass and ^{1}H-NMR data (see *Exper. Part*) established its structure as 3-*O*-[(9*Z*)-hexadec-9-enoyl]-*β*-amyrin [4].

The mass spectrum of **6** gave a molecular-ion peak at *m/z* 676.5749 (HR-EI-MS) corresponding to the molecular formula $C_{46}H_{76}O_3$. The structure of **6** was elucidated as 11-oxoolean-12-en-3*β*-yl (9*Z*)-hexadec-9-enoate, also a new compound.

In the ^{1}H-NMR spectrum (*Table 3*), all the structural features of **6** were the same as those of **5**, except for a *t* at δ 5.17 (H−C(12) of **5**), which was replaced by a *s* at δ 5.68 [10][11], revealing that there must be a ketone function at C(11) in **6**, as in case of **4**. This was confirmed by the ^{13}C-NMR spectrum (*Table 2*) [12]. The *α*,*β*-unsaturated-ketone moiety was also substantiated by the HR-EI-MS, which showed two important fragments at *m/z* 273.2201 ($C_{19}H_{29}O^{+}$) and 232.1820 ($C_{16}H_{24}O^{+}$) and by the UV maxima at 247 nm.

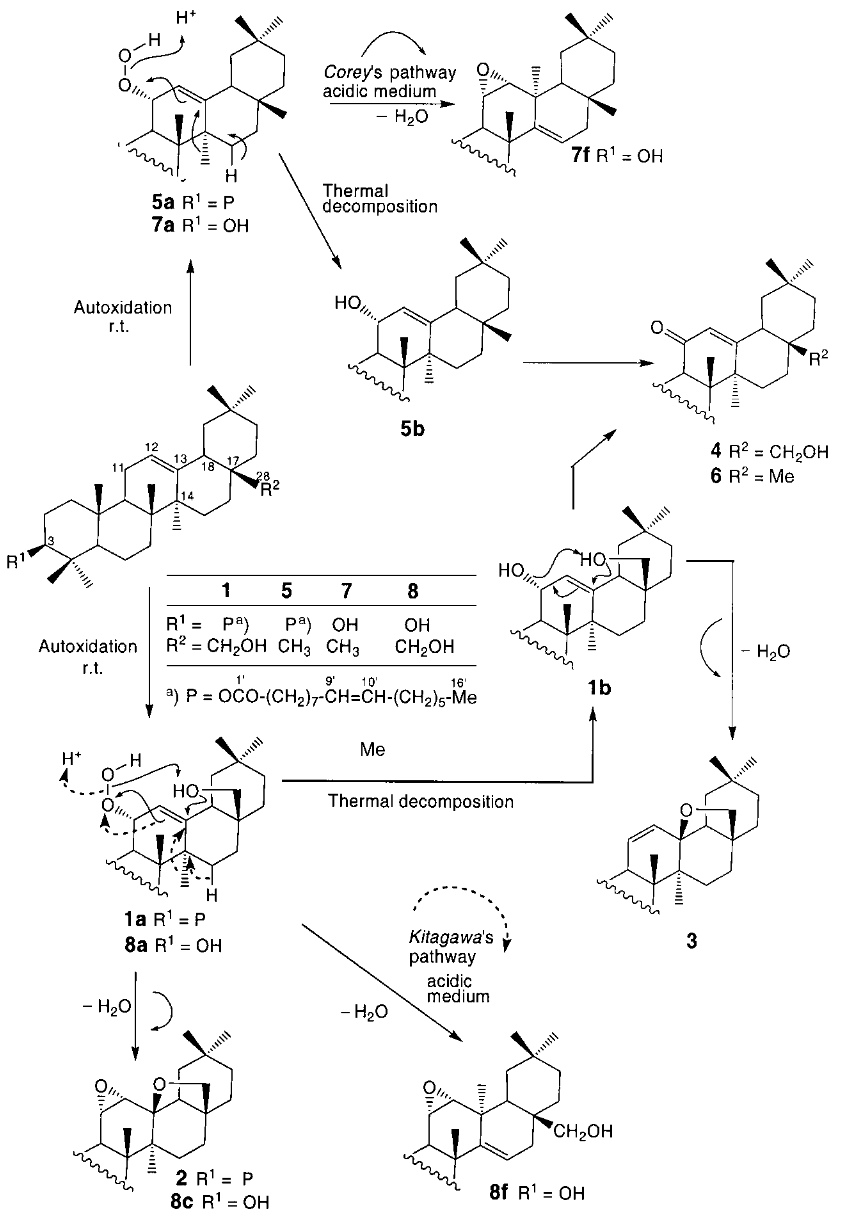
Autoxidation Products of **1** *and* **5**. The 2D-NMR spectra run for further strengthening of the structure of the new natural product **1** revealed its decomposition to a mixture of compounds by autoxidation (aerial oxidation). The ^{1}H-NMR spectrum of the 2D-NMR sample of **1** confirmed this observation, which showed traces of peaks for H−C(12), H−C(28), C(11), C(12), C(13), and C(28) for structure **1**, besides prominent signals at δ 2.91, 3.21, 3.26, 3.31, 3.46, 3.68, 3.75, 5.36, 5.56, and 5.82. The mixture was separated by prep. TLC (silica gel, petroleum ether/AcOEt 8.5 : 1.5), affording three bands *I*–*III*. Their spectral analysis along with the 2D-NMR data of the mixture disclosed that band *I* was a mixture of **2** and **3**, *II* was unreacted **1**, and *III* was **4**. In the light of these findings, the ^{1}H-NMR spectrum of **5** was retaken revealing that it was also autoxidized but to only one product, **6**. Following these studies, other Δ^{12}-amyrins including *β*-amyrin (**7**), and erythrodiol (**8**) (see *Scheme*) were kept in $CHCl_3$ and ursolic acid in MeOH solutions at room temperature and exposed to air and daylight for several weeks. However, they remained unaffected and did not give their respective autoxidation products. Probably the long-chain ester moiety at C(3) of both the compounds **1** and **5** play an important role in the autoxidation process, possibly by providing medium due to the gummy nature of these compounds.

It is important to note that these autoxidation products **2**–**4** and **6** were also obtained from the extract of the plant as described above.

Mechanism of Autoxidation of **1** *and* **5**. Amyrins (Δ^{12}) are well documented as stable compounds [14], and the phenomenon of autoxidation of these triterpenoids has not been described in the literature. However, photooxidation of β-amyrin (**7**) was observed by *Agata*, *Corey*, and co-workers [15]. They reported the formation of 11α,12α-epoxytaraxerene derivative **7f** in acidic medium (see *Scheme*), which involved a novel skeletal rearrangement (C(14) → C(13) Me migration and shift of the C=C bond). On the other hand, *Kitagawa et al.* [9a] described the photooxidation of erythrodiol (**8**) in acidic medium, leading to the formation of 11α,12α-epoxytaraxerene derivative **8f** and 11α,12α:13β,28-diepoxyoleanan-3β-ol (**8c**). In autoxidation, the molecular O_2 reacts with organic molecules under very mild conditions. It is generally accepted that autoxidation at the allylic position proceeds *via* the allyl radical, which can be formed with or without initiator [16]. This initially formed radical reacts with triplet (ground-state) molecular O_2 to give a peroxide radical, which in turn abstracts an allylic proton of another substrate molecule to initiate the radical chain mechanism [16][17]. For the autoxidation of **1** and **5**, a noninitiated radical process is suggested, because there were no initiator or photoexcited ketonic impurities in the sample of either compounds [17]. It is interesting to note that the allylic positions in the palmitoleate ester moiety of **1** and **5** remained unaffected during the whole autoxidation process. It may be due to the higher stability of open-chain allylic C–H bond towards allylic radical formation, as compared to cyclic allylic positions and those of conjugated open-chain dienes [17].

The proposed pathways for the conversion of both **1** and **5** to their autoxidation products **2**–**4** and **6**, respectively, are shown in the *Scheme*. Initially, autoxidation took place at C(11) of **1** and **5** to form 11α-hydroperoxy-12-ene derivatives **1a** and **5a**, respectively, due to the attack of triplet molecular O_2 from the less-hindered α face of the amyrin system, as the β face was sterically hindered by angular Me groups. The 11α-configuration of **5a** was strongly supported by the initial ^{1}H-NMR spectrum of **5** exhibiting distinct peaks of very low intensity at δ 5.45 (d, $J = 3.0$ Hz) for H–C(12) and δ 4.51 (dd, $J = 3.0, 10.0$ Hz) for H_β–C(11) [14]. Furthermore, the characterization of **2** as 11α,12α-epoxy derivative but not its 11β,12β-isomer also confirmed the exclusive formation of the 11α-hydroperoxy-12-ene derivative **1a**. The 11α,12α-epoxy moiety of **2** could be formed by the nucleophilic attack of OH–C(28) at C(13) of **1a** along with the attack of the π electrons of the C=C bond at the peroxy O-atom and elimination of H_2O, to form the 11α,12α-epoxy group as already established by *Kitagawa et al.* [9a]. Autoxidized product **3** may emerge from **1a** by its thermal decomposition under neutral conditions *via* intermediate **1b**, which was sufficiently labile to yield **3** and **4** by the S_N2'-type neighboring-group participation and thermal decomposition, respectively. On the other hand, the hydroperoxy derivative **5a** of **5** gave a thermally decomposed product **6** *via* intermediate **5b**, similar to the formation of **4** from **1b** [18]. It is noteworthy that 11α,12α-taraxerene derivatives [9a][15] of **1** and **5** like **8f** and **7f**, respectively, could not be obtained during their autoxidation due to the absence of acidic conditions.

Scheme



	1	5	7	8
R^1 =	P[a]	P[a]	OH	OH
R^2 =	CH_2OH	CH_3	CH_3	CH_2OH

[a] $P = O\overset{1'}{C}O\text{-}(CH_2)_7\text{-}\overset{9'}{C}H{=}\overset{10'}{C}H\text{-}(CH_2)_5\text{-}\overset{16'}{Me}$

Hence, the weak but sufficient acidic conditions for the conversion of intermediates **1a** to **2**, and of **1b** to **3**, might be achieved by $CDCl_3$, which, however, was insufficient for the rearrangement of **1a** and **5a** into their respective 11α,12α-epoxytaraxerene derivatives.

Conclusions. The formation of the autoxidized products **2**–**4** and **6** from **1** and **5**, respectively, and the inertness of other triterpenoids such as **7**, **8**, and ursolic acid towards autoxidation revealed that the Δ^{12}-amyrins esterified at C(3) with a long-chain fatty acid group are labile constituents or prone to autoxidation, possibly due to their gummy nature. Furthermore, the nature of the medium, either acidic or neutral, and the type of groups at C(17) (Me, CH_2OH) highly influence product formation in the autoxidation of Δ^{12}-amyrin derivatives of type **1** and **5**. In the light of the above findings, compounds **2**–**4** and **6** isolated from *T. erecta* may be regarded as artifacts formed during the isolation processes in the presence of light and air. It is worth mentioning that many natural products of these types have been cited in the literature [6][9b][10–12][19][20][21a] as genuine compounds; however, there is one report that demonstrates artifact formation during the extraction procedure for the isolation of triterpenes [21b]. It is important to note that triterpenoids bearing an α,β-unsaturated-ketone moiety in ring C have important biological activities [12][21]. Moreover, triterpenoid esters like **1**–**6** have also been reported to possess significant pharmaceutical properties [22][23].

Experimental Part

General. Vacuum liquid chromatography (VLC): silica gel *60 PF*$_{254}$. Prep. TLC: silica gel *GF*$_{254}$; separation of **2/3** on aluminium sheets of silica gel *60 F*$_{254}$. $[\alpha]_D$: *Schmidt & Haansch Polartronic-D* polarimeter. UV Spectra: *Hitachi U-3200* spectrophotometer; λ_{max} in nm. IR Spectra: *Jasco A-302* spectrophotometer, $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker Aspect-AM-500* spectrometer; at 500 (^1H) and 125 MHz (^{13}C); broad-band-decoupled and DEPT spectra for ^{13}C, and assignments partly by DEPT, HMQC, and HMBC and partly by comparison with reported values of similar compounds [6][8b][9b][10][12][20]; $CDCl_3$ solns; δ in ppm (referenced to residual solvent signals) and coupling constants J in Hz. EI- and HR-MS: *Finnigan MAT-112* and *JMS-HX-110* spectrometers; in m/z (rel. %).

Plant Material. Flowers of *Tagetes erecta* were collected from the Karachi University Campus in January 1998, the plant was authenticated by Dr. *Rubina Dawar*, Department of Botany, University of Karachi, and a voucher specimen (No. 67281 KUH) was deposited in the same department.

Extraction and Isolation. Fresh, undried, and uncrushed golden flowers of *T. erecta* (250 g) were percolated with petroleum ether, followed by $CHCl_3$ for 3 days at r.t. After filtration, the petroleum ether and $CHCl_3$ extracts were evaporated to give *Fractions I* (1.9 g) and *II* (3.5 g), resp. *Fr. I* was partitioned between petroleum ether and 80% MeOH/H_2O. A portion (30 mg) of the petroleum ether phase was subjected to prep. TLC (silica gel, petroleum ether/AcOEt 9.9 : 0.1): pure **5** (16.5 mg).

In another run, the 80% MeOH/H_2O phase and the petroleum ether phase of *Fr. I* and *Fr. II* were combined and evaporated to a thick mass (4.7 g) which was subjected to VLC (silica gel *60PF*$_{254}$, petroleum ether/AcOEt of increasing polarity); 70 fractions. Both the petroleum ether/AcOEt 9.8 : 0.2 and 9.9 : 0.1 eluates (58.9 and 31.2 mg, resp.) were subjected to prep. TLC (silica gel, petroleum ether/AcOEt 8.5 : 1.5): **1** (23.7 mg), **2/3** (34.2 mg), and **6** (13.1 mg). The petroleum ether/AcOEt 9.7 : 0.3 eluate (44.6 mg) yielded **4** (21.5 mg). The mixture **2/3** was separated by TLC (silica gel *60 F*$_{254}$ (aluminium sheets), petroleum ether/AcOEt 9.3 : 0.7): **2** (12.2 mg) and **3** (14.9 mg).

3-O-[(9Z)-Hexadec-9-enoyl]erythrodiol (=*(3β)-28-Hydroxyolean-12-en-3-yl (9Z)-Hexadec-9-enoate*; **1**): Gum. $[\alpha]_D^{26} = +51.1$ ($c = 0.12$, $CHCl_3$). UV (MeOH): 203. IR ($CHCl_3$): 3400, 2908, 1725, 1603, 1465, 1368, 1304, 1172, 1009, 915. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. HR-EI-MS: 678.5877 (2.16, M^+; $C_{46}H_{78}O_3^+$), 660.5782 (1.0, $[M - H_2O]^+$, $C_{46}H_{76}O_2^+$), 647.5732 (1.0, $[M - CH_2OH]^+$, $C_{45}H_{75}O_2^+$), 424.3691 (2.71, $[M - C_{16}H_{30}O_2^+$), $C_{30}H_{48}O^+$), 410.3480 (1.18, $[M - C_{16}H_{30}O_2 + Me - H]^+$, $C_{29}H_{46}O^+$), 395.3336 (0.96, $C_{28}H_{43}O^+$), 234.1939 (19.66, $C_{16}H_{26}O^+$), 203.1759 (100, $C_{15}H_{23}^+$), 190.1649 (21.46, $C_{14}H_{22}^+$).

(3β,11α,12α)-11,12:13,28-Diepoxyoleanan-3-yl (9Z)-Hexadec-9-enoate (**2**): Gum. $[\alpha]_D^{26} = +41.4$ ($c = 0.10$, $CHCl_3$). UV (MeOH): 203. IR ($CHCl_3$): 2951, 2849, 1728, 1465, 1356, 1262, 1130. ^1H- and ^{13}C-NMR: *Tables 3* and *2*. EI-MS: 692 (2, M^+), 439 (6), 438 (8), 423 (12), 271 (15), 239 (18), 208 (11), 207 (64), 205 (15), 201 (20), 191 (16), 190 (27), 189 (58), 188 (31), 177 (45), 176 (13), 175 (24), 163 (24), 111 (10), 109 (30), 107 (22), 105

(18), 71 (46), 69 (75), 57 (100), 55 (64). HR-EI-MS: 692.5811 (M^+, $C_{46}H_{76}O_4^+$), 438.3497 ($[M - C_{16}H_{30}O_2]^+$, $C_{30}H_{46}O_2^+$), 271.2058 ($C_{19}H_{27}O^+$), 190.1651 ($C_{14}H_{22}^+$), 177.1349 ($C_{12}H_{17}O^+$), 125.0975 ($C_8H_{13}O^+$), 107.0813 ($C_8H_{11}^+$).

(3β)-13,28-Epoxyolean-11-en-3yl (9Z)-Hexadec-9-enoate (**3**): Gum. $[\alpha]_D^{26} = +48.4$ ($c = 0.10$, $CHCl_3$). UV (MeOH): 203. IR ($CHCl_3$): 2945, 2851, 1731, 1462, 1373, 1264. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*. EI-MS: 676 (16, M^+), 422 (13), 391 (16), 276 (6), 271 (10), 255 (18), 253 (10), 239 (18), 235 (25), 217 (13), 215 (15), 213 (14), 208 (11), 207 (59), 205 (17), 204 (17), 203 (42), 202 (12), 201 (16), 199 (9), 191 (15), 190 (26), 189 (48), 188 (24), 187 (23), 177 (25), 173 (11), 163 (23), 161 (18), 159 (13), 149 (21), 135 (28), 133 (16), 121 (22), 119 (20), 111 (12), 109 (25), 107 (24), 105 (18), 97 (22), 95 (44), 77 (18), 71 (49), 67 (18), 57 (100), 55 (69). HR-EI-MS: 676.5750 (M^+, $C_{46}H_{76}O_3^+$), 422.3473 ($[M - C_{16}H_{30}O_2]^+$, $C_{30}H_{46}O^+$), 273.2211 ($C_{19}H_{27}O^+$), 217.1647 ($C_{15}H_{21}O^+$), 205.1631 ($C_{14}H_{21}O^+$), 201.1506 ($C_{15}H_{21}^+$).

(3β)-28-Hydroxy-11-oxoolean-12-en-3-yl (9Z)-Hexadec-9-enoate; (**4**): Gum. $[\alpha]_D^{26} = +59.3$ ($c = 0.10$, $CHCl_3$). UV (MeOH): 246. IR ($CHCl_3$): 3405, 2953, 2875, 1377, 1350, 1260, 1145, 895. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 692 (2, M^+), 439 (34), 438 (15), 437 (3), 423 (13), 398 (21), 383 (7), 355 (6), 299 (9), 290 (20), 289 (92), 271 (15), 250 (21), 248 (100), 239 (10), 217 (15), 203 (11), 191 (18), 190 (21), 189 (29), 175 (22), 57 (25), 55 (20). HR-EI-MS: 692.5728 (M^+, $C_{46}H_{76}O_4^+$), 289.2139 ($C_{19}H_{29}O_2^+$), 271.2028 ($C_{19}H_{27}O^+$), 248.1777 ($C_{16}H_{24}O_2^+$), 217.1651 ($C_{15}H_{21}O^+$).

*3-*O*-[(9Z)-Hexadec-9-enoyl]-β-amyrin* (= *(3β)-Olean-12-en-3-yl (9Z)-Hexadec-9-enoate* (**5**): Gum. $[\alpha]_D^{26} = +75.4$ ($c = 0.17$, $CHCl_3$). UV (MeOH): 202. IR ($CHCl_3$): 2954, 2852, 1721, 1603, 1154. EI-MS: 662 (2.08, M^+), 409.2 (5.94, $[M - C_{16}H_{29}O_2]^+$, $C_{30}H_{49}^+$), 408.2 (3.19, $[M - C_{16}H_{30}O_2]^+$, $C_{30}H_{50}^+$). HR-EI-MS: 662.5981 (3.17, M^+, $C_{46}H_{78}O_2^+$), 218.2027 (94.37, $[M - C_{16}H_{26}]^+$), 189.1626 (8.37, $C_{14}H_{21}^+$), 57.0669 (100, $C_4H_9^+$).

(3β)-11-Oxoolean-12-en-3-yl (9Z)-Hexadec-9-enoate (**6**): Gum. $[\alpha]_D^{26} = +79.9$ ($c = 0.11$, $CHCl_3$). UV (MeOH): 247. IR ($CHCl_3$): 2952, 2849, 1725, 1658, 1618. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*. HR-EI-MS: 676.5749 (4, M^+, $C_{46}H_{76}O_3^+$), 422.3531 (12, $[M - C_{16}H_{30}O_2]^+$, $C_{30}H_{46}O^+$), 273.2201 (100, $C_{19}H_{29}O^+$), 232.1820 (99, $C_{16}H_{24}O_3^+$).

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