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GERMACRANOLIDES FROM *VIGUIERA MICROPHYLLA*

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Key Word Index—*Viguiera microphylla*, Asteraceae; Heliantheae; sesquiterpene lactones; germacranolides; germacrolide; heliangolides; niveusin C-2′,3′-epoxide; 1,2-dehydroniveusin C-2′,3′-epoxide; 3β-hydroxy-8β-epoxyangeloyloxycostunolide-1β,10α-epoxide.

Abstract—Three new germacranolides, including two heliangolides (niveusin C-2′,3′-epoxide and 1,2-dehydroniveusin C-2′,3′-epoxide) and a germacrolide (3β-hydroxy-8β-epoxyangeloyloxycostunolide-1β,10α-epoxide) were isolated from *Viguiera microphylla*. Their structures were established by spectroscopic analyses, including extensive ^{1}H NMR and ^{13}C NMR decoupling experiments and chemical transformations. X-ray diffraction analysis confirmed the structure of niveusin C-2′,3′-epoxide.

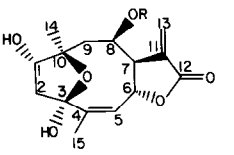
INTRODUCTION

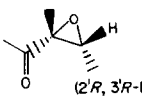
Our interests in the bioactive constituents and chemotaxonomy of *Helianthus* have led us to investigate the closely related genus *Viguiera* (Asteraceae: Heliantheae) [1]. Only about 20 of the 150 species of *Viguiera* [2] have been investigated chemically, and sesquiterpene lactones have been reported from about half of these. Heliangolides with a 3,10-furanone ring are quite common (*V. angustifolia* [3], *V. buddleiaeformis* [4], *V. eriophora* [5], *V. hemsleyana* [5], *V. hypochlora* [5], *V. pinnatilobata* [6] and *V. schultzii* [5]), but simple heliangolides (*V. eriophora* [5], *V. pinnatilobata* [6], *V. procumbens* [7], *V. sphaerocephala* [9] and *V. stenoloba* [6, 8, 10]) and their derivatives (*V. linearis* [11]) are also known, as well as germacrolides (*V. buddleiaeformis* [4] and *V. sphaerocephala* [9]). We report here the isolation and structure determination of three new germacranolides, two heliangolides (**1**, **5**) and one germacrolide (**9**), from *Viguiera microphylla* Vasey and Rose.

RESULTS AND DISCUSSION

Aerial parts of *V. microphylla* were extracted and worked up by standard procedures [12]. Three sesquiterpene lactones (**1**, **5** and **9**) were isolated by silica gel column chromatography and preparative TLC. Compound **1** was obtained as the main component (0.25% yield from dry plant material). The mass spectrum showed a weak molecular ion peak at *m/z* 394 which had a formula of $C_{20}H_{26}O_8$ (HRMS: measured 394.1629, calculated 394.1627). A band at 3450 cm^{-1} in the IR spectrum and the disappearance of two one-proton ^{1}H NMR signals (δ 3.10 and 3.62) following D_2O addition indicated the presence of two hydroxyl groups. A pair of narrowly split doublets at 6.25 (J = 2.5 Hz) and 5.64 (J = 2.0 Hz) in the ^{1}H NMR spectrum (Table 1) and an IR band at 1753 cm^{-1} demonstrated that **1** contained an α-methylene-γ-lactone function. The presence of an epoxyangelate ester side chain on the skeleton was shown by an additional IR absorbance in the carbonyl region (1727 cm^{-1}), and by ^{1}H NMR and ^{13}C NMR data very similar to that reported for other epoxyangelate side chains [13–16]. The remainder of the ^{1}H NMR and ^{13}C NMR signals (Tables 1 and 2) were strikingly similar to those of niveusin C (**2**) [17–19] and were assigned analogously. Compound **1** differed from niveusin C only in the nature of its C-8 ester side chain.

When **1** was acetylated with acetic anhydride in pyridine, a monoacetate **8** was produced whose ^{1}H NMR and ^{13}C NMR spectra showed that a major skeletal change had taken place during reaction, similar to those observed when tagitinin B and niveusin A were acetylated under these conditions [17–20]. The hemiacetal linkage had been cleaved, the resulting C-10 hydroxyl group ac-

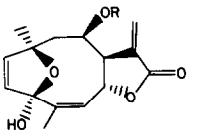


1 R =  (2′*R*, 3′*R*-Epoxyang)

2 R = Ang*

3 R = iBut

4 R = MeAcr



5 R = 2′*R*, 3′*R*-Epoxyang

6 R = Sar

7 R = iBut

*Ang = angelate
i-But = *iso*-butyrate
MeAcr = α-methylacrylate
Sar = sarracinate (5′OH-angelate)
2-MeBut = 2-methylbutanoate

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Table 1. ^{1}H NMR spectra of the sesquiterpene lactones of *V. microphylla**

	1		5	8	9
	($CDCl_3$)	(C_5D_5N)	($CDCl_3$)	($CDCl_3$)	(acetone-d_6)
H-1	4.06 *br dd*	4.44 *br dd*	5.84 *d*	7.01 *d*	2.87 *dd*
H-2	α 2.38 *br d*	α 2.58 *br d*	6.38 *d*	6 27 *d*	α 2.27 *ddd*
	β 2.49 *dd*	β 2.85 *dd*	—	—	β 1.52 *ddd*
H-3	—	—	—	—	4.62 *br dd*
H-5	5.63 *dq*	5 92 *dq*	5.67 *dq*	5 83 *dq*	5 59 *br dq*
H-6	5 32 *tq*	5.68 *tq*	5.92 *br dd*	5.41 *dd*	5.28 *t*
H-7	4.22 *ddt*	4.50 *ddt*	3.46 *br dt*	3.33 *m*	3.30 *dddd*
H-8	5.72 *ddd*	6.09 *ddd*	5.21 *br t*	5.50 *ddd*	5.84 *dt*
H-9	†	α 2.08 *dd*	2.32 *dd*	α 2 77 *dd*	α 1.50 *dd*
	†	β 2.36 *dd*	2.44 *dd*	β 2.01 *dd*	β 2.67 *dd*
H-13a	6 25 *d*	6.42 *d*	6.31 *d*	6.35 *d*	6.17 *d*
H-13b	5.64 *d*	5.82 *d*	5.72 *d*	5.79 *d*	5.58 *d*
H-14	1.54 (3H) *s*	1.72 (3H) *s*	1.53 (3H) *s*	1 74 (3H) *s*	1.28 (3H) *s*
H-15	1.86 (3H) *dd*	2.03 (3H) *dd*	1.94 (3H) *br d*	1.96 (3H) *d*	1.93 (3H) *d*
H-3′	2.99 *q*	2 97 *q*	3.02 *q*	3.02 *q*	3.06 *q*
H-4′	1.22 (3H) *d*	1 31 (3H) *d*	1 17 (3H) *d*	1 22 (3H) *d*	1.16 (3H) *d*
H-5′	1.43 (3H) *s*	1.48 (3H) *s*	1 42 (3H) *s*	1.46 (3H) *s*	1.58 (3H) *s*
1-OH	3 10 *d*	†			
3-OH	3.62 *s*	6.82 *s*			3.02 *s*
Ac				2.15 (3H) *s*	

*Run at 200 MHz with TMS as an internal standard.

†Signal obscured.

Coupling constants, *J* (Hz): **1**: 1β, 2α = ~ 1, 1β, 2β = 3.5; 1β, 1-OH = 7; 2α, 2β = 15; 5, 15 = 1.5; 5, 6β = 4; 6β, 15 = 2; 6β, 7α = 4; 7α, 13a = 2.5, 7α, 13b = 2, 7α, 8α = 4; 8α, 9α = 6; 8α, 9β = 9 5; 9α, 9β = 14; 3′β, 4′ = 5; **5**: 1, 2 = 6; 5, 6β = 7; 5, 15 = 1.5; 6β, 7α = 2; 6β, 15 = ~ 1; 7α, 8α = ~ 1; 7α, 13a = 2 5, 7α, 13b = 2; 8α, 9 = 3 5; 8α, 9′ = 3.5, 9, 9′ = 17; 3′β, 4′ = 5. **8**· 1, 2 = 17, 5, 6β = 9; 5, 15 = 1.5; 6β, 7α = 2, 7α, 13a = 2; 7α, 13b = 2; 7α, 8α = 4; 8α, 9α = 6; 8α, 9β = 10; 9α, 9β = 15; 3′β, 4′ = 5; **9**: 1α, 2α = 3; 1α, 2β = 11 5; 2α, 2β = 13; 2α, 3α = 5; 2β, 3α = 11; 3α, 5 = ~ 1; 5, 6β = 9.5, 5, 15 = 1.5; 6β, 7α = 9 5; 7α, 8α = 1.5; 7α, 13a = 3.5; 7α, 13b = 3.0; 8α, 9α = 5.5, 8α, 9β = 1.5, 9α, 9β = 15, 3′β, 4′ = 5.5.

etylated, and a dehydration had occurred at C-1 and C-2. The ^{1}H NMR of **8** showed an acetate methyl signal at δ 2.15 and a pair of doublets at 7.01 and 6.27 (J = 17 Hz) supporting the presence of a *trans* 1,2 double bond conjugated with a carbonyl group at position 3. The formation of **8** confirmed the close structural similarities of **1** to tagitinin B and niveusin C, all of which share a 3,10 ether linkage and 1α- and 3α-hydroxyl groups.

The final problem in the structural determination of **1** was deducing the configuration at positions 2′ and 3′ in the ester side chain. Since the NMR spectra of the two epoxyangelate diastereomers differ only very slightly [16] an X-ray diffraction analysis was undertaken (Table 3). Figure 1 is a stereoscopic drawing of the molecule. The bond distances (Table 4) and angles in the ten-membered, hemiketal 3,10-bridged ring system are not unusual and show good agreement with corresponding fragments in other germacranolides [21–23]. The geometry of the C-8 side chain also compares well with those compounds [21, 22], and the epoxide ring dimensions are in the range of values given by Foces-Foces *et al.* [24]. The C-17:C-20 and C-18:C-19 methyl bonds show the characteristic shortening, mainly due to high thermal motion of the methyl carbons.

The α-methylene-γ-lactone ring is very nearly planar, as evidenced by the sum of the endocyclic torsion angle moduli (18°, see Table 5) This is exceedingly rare in *trans*-fused lactones, and is in fact well below the values usually noted in *cis* fusions [21–26]. The crystal structure shows the lactone chromophore to have right-handed chirality (positive C:O–C:C torsion angle), and the CD spectrum shows a positive band at 270 nm, indicative of a positive Cotton effect of the $n \rightarrow \pi^*$ transition. The correspondence of sign between these two parameters follows Beecham's rule [27]. The empirical rule of Stocklin, Waddell and Geismann [28], however, is violated since it would predict a negative torsion angle for this C-6-*trans*-fused lactone. Fortunately, violations of this 'rule' are quite common, such as in heliangine [29]. Cox and Sim [30] have shown that the exocyclic C:O–C:C and endocyclic C–C–C–O torsion angles are highly correlated (both 4.8° in the present case), and they suggest that the latter may be a more sensitive indicator of the sign of the Cotton effect. The most probable configuration for our molecule is that shown in Fig. 1.

The packing of the molecules in the unit cell is shown in Fig. 2. There appears to be a weak hydrogen bond between O-1 and O-7 *via* H-21 which binds pairs of molecules together such that an infinite spiral chain is formed along the *b* axis. The hydrogen bonding parameters are O-1 . . . O-7 = 2.85 Å, H-21 . . . O-7 = 1.87 Å, and O-1--H-21 . . . O-7 = 168°.

Compound **5** was isolated as a pale yellow gum less polar than **1** on TLC The mass spectrum showed a weak

Table 2. ^{13}C NMR spectra of the sesquiterpene lactones of *V microphylla**

	1	5	8	9
C-1	77.5 *d*†	130.8 *d*	157.7 *d*	64.5 *d*
C-2	44.9 *t*†	139.4 *d*	128.8 *d*	33.8 *t*
C-3	106.7 *s*	108.5 *s*	195.9 *s*	69.4 *d*¶
C-4	140.7 *s*	139.9 *s*	139.2 *s*§	149.2 *s*
C-5	128.3 *d*	128 1 *d*	135.8 *d*	120.9 *d*
C-6	75.4 *d*†	74.6 *d*‡	75 6 *d*	74 5 *d*¶
C-7	49.9 *d*	47.7 *d*	47 5 *d*	51 7 *d*
C-8	73.2 *d*†	77.8 *d*‡	73 9 *d*	73 5 *d*¶
C-9	39.6 *t*†	43.9 *t*	47 5 *t*	42 5 *t*
C-10	86.2 *s*	87.0 *s*	79.6 *s*	60 3 *s*
C-11	136.0 *s*	138.8 *s*	135.8 *s*§	136.7 *s*
C-12	170.0 *s*	169 5 *s*	170 0 *s*‖	169.8 *s*
C-13	123.5 *t*	124.5 *t*	124.9 *t*	121 6 *t*
C-14	22 1 *q*†	31 5 *q*	24.6 *q*	20.1 *q*
C-15	22 3 *q*†	20 6 *q*	20.0 *q*	12.8 *q*
C-1′	169.0 *s*	168 6 *s*	168.6 *s*	168.9 *s*
C-2′	59.6 *s*	59.7 *s*	59.4 *s*	59.9 *s*
C-3′	60.2 *d*	60 2 *d*	60.3 *d*	59.7 *d*
C-4′	13.7 *q*	13.2 *q*	13.3 *q*	13.9 *q*
C-5′	19 1 *q*	19.0 *q*	18.9 *q*	19.1 *q*
$\underline{C}H_3$–COOH			21.7 *q*	
CH_3–$\underline{C}$OOH			169.2 *s*‖	

*Run at 22.6 MHz in $CDCl_3$ with TMS as internal standard, except for **9** which was run in DMSO-d_6. Assignments made by analogy with model compounds (**1** and **5** [17, 18], **8** [7, 17, 44], **9** [45, 46]) and by using off-resonance decoupling experiments.

†Assignments confirmed by single frequency off-resonance decoupling experiments.

‡, §, ‖, ¶Assignments interchangeable.

M^+ peak at *m/z* 376 in accord with a $C_{20}H_{24}O_7$ formula (HRMS: measured 376.1522, calculated 376.1521). The spectral data for **5** were on the whole similar to those for **1** and indicated that this compound was a heliangolide with a 12,6-*trans*-fused lactone ring, an 8β-epoxyangelate ester side chain and a 3,10 ether linkage with a 3α-hydroxyl group. Two new signals for olefinic protons which were spin coupled to each other appeared at δ 5.84 (*d*, J = 6 Hz) and 6.38 (*d*, J = 6 Hz) in place of the H-1 double doublet at 4.06 and the signals for the methylene group at C-2 in **1**. These changes demonstrated the presence of a 1,2 double bond, with the J value specifying a *cis* configuration. The ^{13}C NMR spectrum of **5** showed parallel differences, with new doublets at 130.8 and 139.4 replacing the doublet at 75.2 and the triplet at 44.9 in **1**. Final confirmation for the structure of **5** came from the results of chemical interconversion. When **1** was treated with HIO_4, instead of the expected fission product [17], a dehydrated derivative identical to **5** in all respects was the only compound that could be isolated from the reaction mixture. Spectral data for compound **5** are very similar to those for **6** [31] and **7** [32], differing only in the signals for the side chain.

There are some significant ^{1}H NMR differences between compounds **1** and **5** in the chemical shifts and coupling constants of H-6, H-7, H-8, H-9α and H-9β. Among other 3,10-ether-linked, 3-hydroxy heliangolides, these differences are also apparent when comparing compounds like **1** with a 1α-hydroxyl group (**2** [17–19], **3** [33], **4** [33]) to compounds like **5** with a 1,2 double bond (**6** [31], **7** [32]). The downfield shift of H-6 in **5** and other $\Delta^{1(2)}$ compounds (relative to **1** and other 1α-hydroxyl compounds) can be explained by the deshielding effect of the 1,2 double bond. Using Dreiding models, the other ^{1}H NMR differences between the two types can be rationalized by assuming a conformational difference

Table 3. Data collection and processing parameters

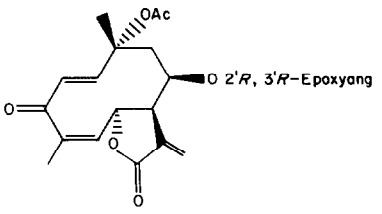
Space group	$P2_1$, monoclinic
Cell constants	a = 8.784(2) Å
	b = 10 300(4)
	c = 11.924(10)
	β = 109.86(3)
	V = 1014.7 Å^3
Molecular formula	$C_{20}H_{26}O_8$
Molecular weight	394.4
Molecules per cell	Z = 2
Density	ρ = 1.29 g/cm^3
Absorption coefficient	μ = 0.6 cm^{-1}
Radiation (MoK$_\alpha$)	λ = 0.71073 Å
Collection range	$4° \leqslant 2\theta \leqslant 60°$
Scan width	$\Delta\theta = (1.00 + 0.35 \tan\theta)°$
Maximum scan time	240 sec
Total data collected	3104
Independent data, $I > 3_\sigma(I)$	1736
Total variables	319
$R = \sum\|\|F_o\| - \|F_c\|\| / \sum\|F_o\|$	0.044
$R_w = [\sum_w(\|F_o\| - \|F_c\|)^2 / \sum_w\|F_o\|^2]^{1/2}$	0.033
Weights	$w = \sigma(F)^{-2}$
Goodness-of-fit	1.5

Table 4. Selected torsion angles (°)

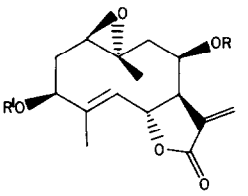
C12-O4-C6-C7	−2.7
O4-C6-C7-C11	4.8
C6-C7-C11-C12	−5.5
C7-C11-C12-O4	4.1
C11-C12-O4-C6	−0.7
O5-C12-C11-C13	4.8
O3-C3-C2-C1	29.8
O3-C10-C1-C2	30.0
O6-C16-C17-C20	−27.4
C1-C2-C3-C4	151.4
C2-C3-C4-C5	−76.1
C3-C4-C5-C6	−0.5
C3-C2-C1-C10	−36.4
C4-C5-C6-C7	−64.7
C5-C6-C7-C8	123.3
C6-C7-C8-C9	−42.7
C7-C8-C9-C10	−62.5
C8-C9-C10-C1	157.2
C8-O6-C16-C17	−172.9
C19-C18-C17-C20	154.3

involving the orientation of the 8β-ester side chain. The side chain appears to have an equatorial orientation in the 1α-hydroxyl compounds and an axial orientation in the $\Delta^{1(2)}$ compounds. In both cases, the inter-proton angles seen in the models are in good agreement with the observed coupling constants.

The third sesquiterpene lactone (**9**) was a minor component of the extract that was more polar than **1** or **5**. Its chemical ionization mass spectrum showed an $[M+1]^+$ peak at *m/z* 379 in accord with a $C_{20}H_{26}O_7$ formula (HRMS: measured 378.1678, calculated 378.1678). Spectral data showed the presence of a 12,6-α-methylene-γ-lactone ring which was *trans*-fused by Samek's rule (J_7, 13 ⩾ 3 Hz) [34] and the occurrence of an epoxyangelate ester side chain at C-8, which was β-oriented because of the low chemical shift of H-8 and the small 7,8 coupling constant [35]. ^{1}H NMR decoupling experiments established the location of protons at positions 5, 6, 7, 8, 9 and



8



9 R = 2'R, 3'R-Epoxyang, R^1 = H

10 R = Ang, R^1 = Ac

11 R = Ang, R^1 = 2-MeBut

15 (partial structure **A**). As in **1** and **5**, a $\Delta^{4(5)}$ double bond is present, but the coupling constants $J_{5,6}$, $J_{6,7}$ and $J_{7,13}$ indicated that its geometry is *trans* rather than *cis*, making **9** a germacrolide [36]. The chemical shift of H-7 was at considerably higher field than in **1** or **5** suggesting the absence of a 3,10 oxygen bridge. However, C-10 was still shown to be fully substituted because irradiation at H-8 (5.84) collapsed two double doublets at 2.67 and 1.50 (H-9α and H-9β) into a simple geminal AB system (J = 15 Hz). These structural features accounted for five of the seven oxygen atoms and seven of the eight degrees of unsaturation required by the molecular formula.

IR and ^{13}C NMR spectral data pointed to the presence of another epoxide function (two unassigned ^{13}C NMR resonances at δ 60.32 *s* and 64.5 *d*) and a hydroxyl group (IR absorbance at 3528 cm^{-1} and unassigned ^{13}C NMR doublet between 69 and 75). The hydroxyl group appeared to be both secondary and allylic because the ^{13}C NMR resonance at the point of attachment was a doublet and there was an unassigned ^{1}H NMR signal at δ 4.62 (1H, *br dd*). Irradiation at 5.59 (H-5), besides simplifying signals for H-6 and H-15, sharpened the signal at 4.62 dem-

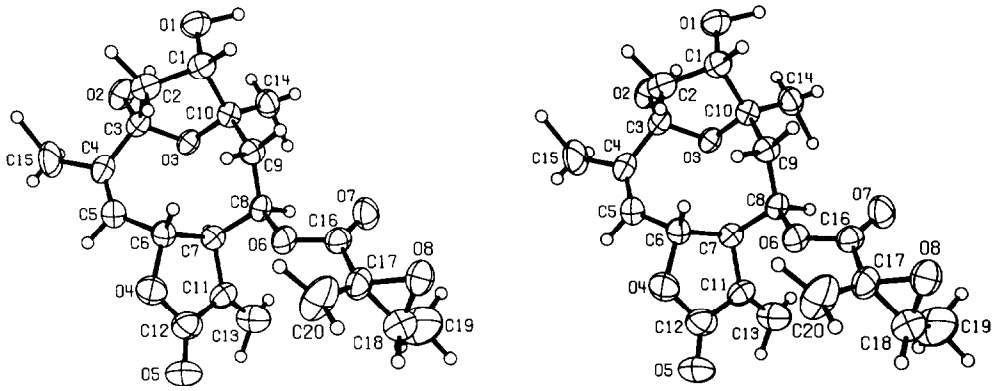
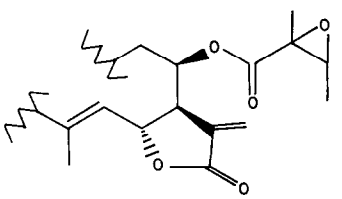


Fig. 1. Stereoscopic view of the molecule showing the atom labeling scheme. The thermal ellipsoids are 40% equiprobability evelopes, with hydrogens as spheres of arbitrary diameter Hydrogens are numbered the same as the atom to which each is attached, and are shown in the refined positions before rigid body methyl groups were employed.

Table 5. Intramolecular bond distances (A)

O1-C1	1.455 (5)	C4-C5	1.323 (6)
O2-C3	1.441 (5)	C4-C15	1.510 (6)
O3-C3	1.432 (4)	C5-C6	1.515 (6)
O3-C10	1.435 (4)	C6-C7	1.550 (5)
O4-C6	1.484 (5)	C7-C8	1.525 (5)
O4-C12	1.352 (5)	C7-C11	1.483 (5)
O5-C12	1.193 (5)	C8-C9	1.538 (6)
O6-C8	1.476 (5)	C9-C10	1.543 (5)
O6-C16	1.345 (5)	C10-C14	1.507 (5)
O7-C16	1.197 (5)	C11-C12	1.461 (6)
O8-C17	1.440 (5)	C11-C13	1 333 (6)
O8-C18	1.437 (5)	C16-C17	1.503 (6)
C1-C2	1.487 (6)	C17-C18	1.464 (6)
C1-C10	1.555 (5)	C17-C20	1.485 (6)
C2-C3	1.516 (6)	C18-C19	1.500 (7)
C3-C4	1.507 (5)		



A

onstrating the hydroxyl group was probably at the 3 position. Additional decoupling experiments showed that the C-3 proton was coupled to two geminally-coupled methylene protons (1.52 and 2.27) which were in turn both coupled to a double doublet at 2.87. This evidence plus the chemical shift of the remaining methyl group (1.28) placed the epoxide function at C-1:C-10. The spectral data for compound **9** are very similar to those for **10** [37] and **11** [38] with the exceptions of the side chain and the chemical shift of H-3. As in **10** and **11**, the $J_{1,2}$ and $J_{2,3}$ values show a β-orientation for the 3-oxygen substituent.

The sesquiterpene lactone chemistry of *Viguiera microphylla* is similar to that of other *Viguiera* species studied. Heliangolides with 3,10 ether linkages like **1** and **5** are known from *V. angustifolia* [3], *V. buddleiaeformis* [4] and *V. pinnatilobata* [6], while a germacrolide-1,10-epoxide like **9** has been isolated from *V. sphaerocephala* [19]. Similar structural types are also found in *Helianthus* supporting the idea that these genera have a close relationship. Niveusin C (**2**), which differs from **1** only in the presence of a double bond in the side chain instead of an epoxide, has been isolated from three different species of *Helianthus* [17–19]. Further taxa of both *Viguiera* and *Helianthus* are currently under investigation.

EXPERIMENTAL

Air dried and ground leaves and stems (350 g) of *Viguiera microphylla*, collected in Baja California Sur, Mexico, 34 miles north of San Ignacio by Dr. M. Dillon on 29 March, 1981 (Dillon # 1994, voucher on deposit in the Herbarium of the Field Museum, Chicago, Illinois) were extracted with CH_2Cl_2 (4 × 3.5 l.) and worked up in the usual manner [12]. When the crude syrup (2.5 g) was dissolved in hot toluene in preparation for column chromatography and then allowed to cool, crystals of **1** formed (780 mg). Recrystallization from EtOAc–*iso*-Pr_2O (1:1) gave 550 mg colorless prisms. The remaining extract was charged on a silica gel column (220 g) which was eluted with a toluene–EtOAc gradient with increasing amounts of EtOAc. Twenty-three 50 ml fractions were collected. From fractions 10–12 (toluene–EtOAc, 3:1) an additional portion of **1** was obtained (98 mg). Fractions 8–9 (170 mg, toluene–EtOAc, 4:1) were combined and subjected to preparative TLC on silica gel (0.5 mm), developed with $CHCl_3$–MeOH (25:1). A pale yellow gum (96 mg, impure **5**) was obtained and passed through a Sephadex LH-20 column, eluted with EtOAc, and then repurified by preparative TLC to give 55 mg of pure **5**. Fraction 18 (toluene–EtOAc, 3:1) gave 11 mg of **9**, which was recrystallized from EtOAc–*iso*-Pr_2O (1:1) to give 7 mg of crystals. All three compounds appeared bright pink on TLC after the plate was sprayed with acidified *p*-dimethylaminobenzaldehyde reagent and heated [39].

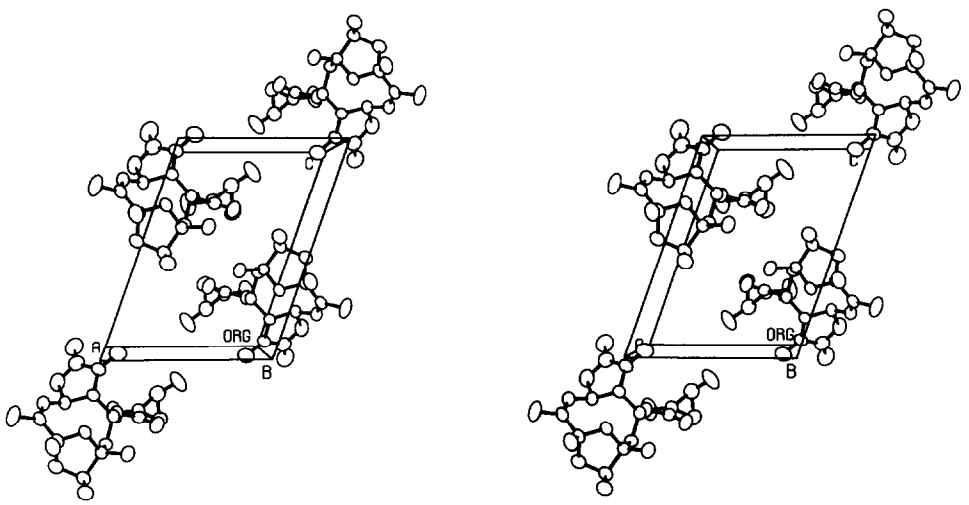


Fig. 2. Stereoscopic view of the molecular packing in the unit cell, as viewed into the *b* axis.

Niveusin C-2',3'-epoxide (**1**). Colorless prisms, mp 190–192° (EtOAc–*iso*-Pr_2O), $[\alpha]_D^{25} - 108°$ ($CHCl_3$, *c* 0.315); UV λ_{max}^{EtOH} nm (log ε): 209 (4.13). IR ν_{max}^{Nujol} cm^{-1}: 3450 (OH), 1753 (lactone C=O), 1727 (side chain ester C=O), 1656, 1274, 1173, 1139, 1117, 989, 877. MS (probe) 70 eV, *m/z* (rel. int.). 394 (0 5) $[M]^+$, 376 (4) $[M - H_2O]^+$, 305 (10), 278 (8) $[M - C_5H_8O_3]^+$ McLafferty rearrangement and cleavage of side chain, 260 (8) $[278 - H_2O]^+$, 205 (25), 71 (45) $[C_4H_7O]^+$ side chain acylium ion − CO, 55 (45), 43 (100).

Acetylation of **1**. Compound **1** (300 mg) was dissolved in 2 ml pyridine, mixed with 4 ml Ac_2O and left for 36 hr at room temp. After standard work-up, the crude product was separated on a silica gel column (20 g) eluted with a CH_2Cl_2–*iso*-PrOH gradient begun with 1% *iso*-PrOH Seventeen 50 ml fractions were collected Fractions 9–13 (4% *iso*-PrOH) were combined and then purified by repeated preparative TLC ($CHCl_3$–MeOH, 15:1) to give pure **8** (95 mg) obtained as a gum. IR ν_{max}^{film} cm^{-1}: 1751 (lactone C=O), 1734 (side chain ester C=O and acetate C=O). 1685 (α,β-unsatd ketone), 1266, 1152, 1134, 1117, 1085, 1044, 966. MS (probe) 70 eV, *m/z* (rel. int.): 418 (0.4) $[M]^+$, 390 (14) $[M - CO]^+$, 376 (12), 358 (15) $[M - HOAc]^+$, 260 (82), 242 (87) $[M - C_5H_8O_3 - HOAc]^+$ McLafferty rearrangements and cleavage of ester side chain and acetate, 97 (100).

HIO_4 *oxidation of* **1** Compound **1** (102 mg) was treated with 555 mg of H_5IO_6 in 5 ml of dry THF for 1 hr. The reaction mixture was partitioned between H_2O and Et_2O and the Et_2O layer washed with H_2O and satd NaCl (aq) and finally dried over Na_2SO_4. Removal of Et_2O gave 112 mg of oily material which was separated on a silica gel column (22 g) eluted with a $CHCl_3$–MeOH gradient, initiated with 1% *iso*-PrOH Thirty-six 10 ml fractions were collected. Fractions 3–4 (1% *iso*-PrOH) were combined and repeatedly purified by preparative TLC (CH_2Cl_2–*iso*-PrOH, 15:1) to give 11 mg of **5**

X-Ray crystallography of **1**. For all X-ray measurements, an Enraf–Nonius CAD-4 automatic diffractometer was used with MoK_α radiation monochromatized by a dense graphite crystal. Final cell constants, as well as other pertinent information, are available on request to the authors. The Laue symmetry was determined to be 2/m, and from the systematic absences noted the space group was shown to be either $P2_1$ or $P2_1/m$. Intensities were measured using the $\theta - 2\theta$ scan technique, with the scan rate depending directly on the net count obtained in rapid pre-scans. Two standard reflections were monitored every two hours, and no abnormalities were noted. In reducing the data, Lorentz and polarization factors were applied, but no corrections for absorption were made

The structure was solved using MULTAN [40], under the assumption that the space group was the non-centrosymmetric $P2_1$ The usual sequence of isotropic and anisotropic refinement was followed, after which most of the hydrogens were located in difference density Fourier maps. The methyl hydrogens could not all be found, and those that could refined poorly, so in the final cycles of full-matrix least squares all methyl groups were treated as rigid bodies After all shift/esd ratios were less than 0 5, the refinement converged to the agreement factors $R = 0.044$ and $R_w = 0.033$. The atomic scattering factors for C and O were computed from numerical Hartree-Fock wave functions [41]; for hydrogen those of Stewart *et al.* [42] were used. All calculations were made with the SHELX-76 series of crystallographic programs [43] No unusually high correlations were noted between any of the independent variables Final positional and thermal parameters, bond angles and torsion angles based on these positions, and a listing of observed and calculated structure factors are available from the authors on request.

1,2-Dehydroniveusin C-2',3'-*epoxide* (**5**) Colorless oil, UV λ_{max}^{EtOH} nm (log ε): 220 (4.32). IR $\nu_{max}^{CCl_4}$ cm^{-1} 3470 (OH), 1752 (lactone C=O), 1743 (side chain ester C=O), 1660, 1279, 1265, 1149, 1085, 979, 895. MS (probe) 70 eV, *m/z* (rel. int) 376 (0 4) $[M]^+$, 277 (3) $[M - C_5H_7O_2]^+$ α-cleavage of ester side chain, 260 (4) $[M - C_5H_8O_3]^+$ McLafferty rearrangement and cleavage of side chain, 242 (4) $[260 - H_2O]^+$, 71 (35) $[C_4H_7O]^+$ side chain acylium ion − CO, 55 (45), 43 (100)

3β-Hydroxy-8β-epoxyangeloyloxycostunolide-1β,10α-epoxide (**9**). Colorless needles, mp 193–195° (EtOAc–*iso*-Pr_2O), UV λ_{max}^{EtOH} nm (log ε): 213 (4.34); IR ν_{max}^{Nujol} cm^{-1} · 3530 (OH), 1752 (lactone C=O), 1724 (side chain ester C=O), 1660, 1269, 1245, 1155, 1123, 966, 946, 886. CIMS (*iso*-butane) *m/z*. 379 $[M + H]^+$, 361 $[M + H - H_2O]^+$, 279 $[M - C_5H_7O_2]^+$, 263 $[M - C_5H_7O_3]^+$, 254, 146, 117 $[C_5H_9O_3]^+$, 99 $[C_5H_7O_2]^+$, 76, 71 $[C_4H_7O]^+$

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