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SESQUITERPENE LACTONES FROM A TEXAS POPULATION OF *HELIANTHUS MAXIMILIANI*

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Key Word Index—*Helianthus maximiliani*; Heliantheae; Asteraceae; sunflowers; sesquiterpene lactones, guaianolides; germacrolides; diterpene; labdane; intraspecific chemical variability.

Abstract—A population of *Helianthus maximiliani* from south-central Texas was shown to have a sesquiterpene lactone chemistry which was completely different from that reported for collections of this species from Kansas and north-central Texas. A series of five new guaianolides, two germacrolides (*trans, trans*-1(10),4(5)-germacradienolides), one of which is new, and a known labdane diterpene acid were isolated from a chloroform extract. Structures were determined by spectral and chemical methods. The structure of one guaianolide had been previously determined by X-ray crystallography.

INTRODUCTION

The increasing agricultural importance of cultivated sunflower (*Helianthus annuus*) has stimulated interest in the terpenoid chemistry of the entire genus *Helianthus*. A variety of terpenoid compounds has been found in species of *Helianthus*, with sesquiterpene lactones and diterpenes being the principal types reported [1–15, see ref. 4 for earlier work].

The terpenoid chemistry of *H. maximiliani* Schrader, a widespread perennial sunflower native to the Great Plains of the United States [16], has been previously investigated by Herz and Kumar [8], who isolated a group of eight closely-related heliangolide sesquiterpene lactones from a Kansas collection of this species. Chromatographic surveys and ^{1}H NMR analyses of extracts from leaf samples of Texas *H. maximiliani* populations, however, showed that these had sesquiterpene lactone profiles that were completely different from that of the Kansas population studied. Two distinct sesquiterpene lactone patterns were noted in the Texas material which appear to represent separate chemical races with different geographical ranges [Gershenzon, J., Stewart, E. and Mabry, T. J., unpublished results].

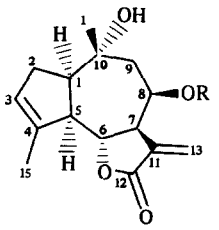
Collections representing both of these races have now been chemically analysed. A series of 2α-hydroxy-8β-acyloxy-*trans,trans*-1(10),4(5)-germacradienolides has been characterized from a north-central Texas population of *H. maximiliani* [Stewart, E., Gershenzon, J. and Mabry, T. J., unpublished results]. In this report, we describe the terpenoid constituents isolated from a south-central Texas population. A series of five new 10α-hydroxy-$\Delta^{3,4}$-guaian-12,6-olides with varying 8β-ester side chains (**1–4** and **6**) were found. Also isolated were two *trans, trans*-1(10),4(5)-germacradienolides (germacrolides), a new compound **13** and the known compound **15** [10, 17], and a known *ent*-labdane diterpene acid, **17** [2, 12, 18–21]. This is the first report of guaianolides in *Helianthus*.

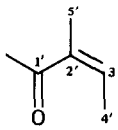
RESULTS AND DISCUSSION

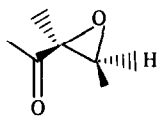
The mass spectrum of the first guaianolide purified from this extract, compound **1**, exhibited a weak molecular ion peak at *m/z* 346 (relative intensity 1%), which had a formula of $C_{20}H_{26}O_5$ (HRMS: 346.1780 measured, 346.1780 calculated). Spectral data showed that this compound was clearly an α-methylene-γ-lactone (IR: 1740, 1660 cm^{-1}; ^{13}C NMR: δ 121.7 *t*, 170.0 *s*; ^{1}H NMR: two narrowly-split doublets at δ 5.48 and 6.29) with an angelate side chain (IR: 1705 cm^{-1}; MS: *m/z* 83, 100% relative intensity; ^{1}H NMR: a spin system consisting of two vinylic methyl groups at δ 1.80, *br s* and 1.92, *br d*, J = 7 Hz and one olefinic proton at 6.08, *br q*, J = 7 Hz).

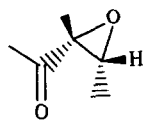
The remaining oxygen in the molecule was part of a hydroxyl group (IR: 3300 cm^{-1}), which was considered to be tertiary because **1** could not be acetylated with acetic anhydride in pyridine under standard conditions [22]. Two more methyl groups were present in the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). One was adjacent to the hydroxyl group (^{1}H NMR: δ 1.32, *br s*), while the other seemed to be on a double bond (1.94, *br s*). The ^{13}C NMR spectrum showed the presence of one more double bond, which was trisubstituted. Since there were no further resonances for sp^2-hybridized carbons, compound **1** had to have two rings, in addition to the lactone ring, as required by the degrees of unsaturation calculated from the molecular formula.

With the exception of the signals of the ester side chains, the ^{1}H NMR data for **1** (Table 1) were very similar to those reported for cumambrin A (**9**) [23] and the 8α-cumambranolides (**10–12**) [24, 25], differing somewhat only in the signals for H-6, H-7 and H-8. However, the magnitude of the coupling constants $J_{6,7}$ (8.5 Hz) and $J_{7,13a,b}$ (3.5, 4 Hz) and extensive decoupling experiments on both **1** and its principal hydrolysis product **8** indicated that, like **9–12**, compounds **1** and **8** had 12,6-lactone rings which were *trans*-fused [26]. Therefore, the main skeleton



1 R =  (Ang)*

2 R = 
(2′*S*, 3′*S*−Epoxyang)

3 R = 
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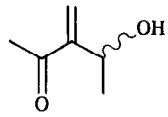
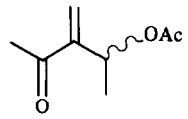
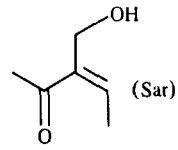
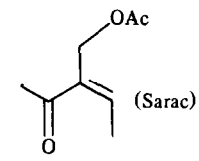
4 R = 
[(2−OH−Et)Acr]

5 R = 
[(2−OAc−Et)Acr]

6 R =  (Sar)

7 R =  (Sarac)

8 R = H

*Ang = angelate
Epoxyang = epoxyangelate
iBut = isobutyrate
Meacr = α-methylacrylate
Sar = sarracinate
Sarac = 5′-acetoxysarracinate
Tigl = tiglate
(2-OH-Et)Acr = (2α-hydroxyethyl)acrylate
(2-OAc-Et)Acr = (2α-acetoxyethyl)acrylate

Table 1. ^{1}H NMR spectra of the guaianolides **1–8***

	1	2 and 3† (*ca* 1:1)	4	5	6	7	8
H-3	5.54 *m*	5.55‡	5.55	5.54	5.56	5.51	5.52
H-6	4.50 *dd*	4.51	4.51	4.48	4.53	4.46	4.39
H-7	3 95 *m*	3.99	3.98	3.96	4.07	3 93	3.60
H-8	5.76 *dt*	5.78, 5.82	5.76	5.76	5.77	5.80	4.21
H-13a	6.29 *d*	6.27, 6.33	6 27	6.25	6.14	6.26	6.15
H-13b	5.48 *d*	5.45, 5.51	5 46	5.44	5.47	5.48	5.57
H-14	1.32 (3H) *s*	1.30 (3H)	1.30 (3H)	1.30 (3H)	1.28 (3H)	1.30 (3H)	1.11 (3H)
H-15	1 94 (3H) *br s*	1.94 (3H)	1.93 (3H)	1.92 (3H)	1.90 (3H)	1.92 (3H)	1 82 (3H)
H-3′	6.08 *br q*	3.03 *q*	4.59	5.64	6.34	6 43	—
H-4′	1.92 (3H) *br d*	1.24, 1.27 (3H) *d*	1.33 (3H) *d*	1.33 (3H) *d*	1 96 (3H)	2 04 (3H)	—
H-5′	1.80 (3H) *br s*	1 42, 1.48 (3H) *s*	5.83 *br s* 6.07 *br s*	5.80 *br s* 6 18 *br s*	4.13 (2H) *br s*	4.64 (2H) *br s*	—
Acetate methyl	—	—	—	2.04 (3H) *s*	—	2.00 (3H) *s*	—

*Run at 100 MHz in $CDCl_3$ with TMS as an internal standard except for **6** which was run in Me_2CO-d_6 and **8** which was run in DMSO-d_6.

†Where two sets of signals are visible in this mixture, they are listed separately.

‡Multiplicities of **2–8** are similar to **1** unless otherwise noted. Coupling constants for **1**, *J* (Hz): 5α, 6β = 10; 6β, 7α = 8.5; 7α, 8α = 4; 7α, 13a = 4; 7α, 13b = 3.5; 8α, 9 = 8, 8; 3′, 4′ = 7 Values for compounds **2–8** where these differ from **1** are: **2** and **3**—3′, 4′ = 5.5, **4** and **5**— 3′, 4′ = 6.

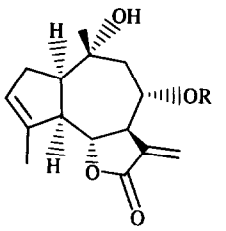
Table 2. ^{13}C NMR spectra of the guaianolides **1–4**, **6** and **7***

	1	2 and 3† *ca* 1:1	4	6	7
C-1	55.0 *d*‡	54.9 *d*‡	55.0 *d*‡	55.0 *d*‡	55.0 *d*‡
C-2	34 1 *t*§	34.1 *t*§	34.1 *t*§	34.0 *t*§	34.1 *t*§
C-3	125.4 *d*	125.5 *d*	125.4 *d*	125.5 *d*	125.4 *d*
C-4	143.5 *s*	143.3, 143.4 *s*	143.5 *s*	143.4 *s*	143.5 *s*
C-5	55.2 *d*‡	54.9 *d*‡	55.2 *d*‡	55.1 *d*‡	55.2 *d*‡
C-6	80.7 *d*	80.4 *d*	80.6 *d*	80.8 *d*	80.5 *d*
C-7	46.8 *d*‡	46.5 *d*‡	46.7 *d*‡	46.6 *d*‡	46.8 *d*‡
C-8	67.1 *d*	68.8, 69.0 *d*	68.0 *d*	67.9 *d*	67.5 *d*
C-9	38.5 *t*§	38.0, 38.4 *t*§	38.0 *t*§	38.6 *t*§	38.6 *t*§
C-10	73.2 *s*	73.1 *s*	73.1 *s*	73.1 *s*	73.4 *s*
C-11	135.3 *s*	135.1, 135.2 *s*	135.1 *s*	135.3 *s*	135 2 *s*
C-12	170.0 *s*	170.0 *s*	170.0 *s*	170.2 *s*	169.9 *s*
C-13	121.7 *t*	121.4, 121.8 *t*	121.7 *t*	121.9 *t*	121.7 *t*
C-14	33.0 *q*	32.7, 32.8 *q*	32.9 *q*	32.9 *q*	32.8 *q*
C-15	17.7 *q*	17.6 *q*	17.6 *q*	17.7 *q*	17.6 *q*
C-1′	167 3 *s*	169.3 *s*	166.0 *s*	166.6 *s*	165.2 *s*
C-2′	127.5 *s*	59.8 *s*	124.6 *s*	131.6 *s*	127.4 *s*
C-3′	138.8 *d*	60.0 *d*	66.9 *d*	140.5 *d*	145.4 *d*
C-4′	15.9 *q*	13.9 *q*	22.4 *q*	15.8 *q*	15.9 *q*
C-5′	20.6 *q*	19.3 *q*	121.7 *t*	64.4 *t*	65.4 *t*
Acetate	—	—	—	—	20.8 *q*
	—	—	—	—	170.7 *s*

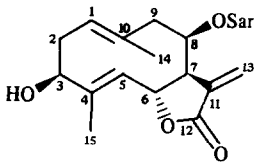
*Run at 22.6 MHz in $CDCl_3$ with TMS as an internal standard. Assignments made using off-resonance decoupling experiments and by analogy with model compounds [30, 31, 35–37, 55].

†Where two sets of signals are visible in this mixture, they are both listed.

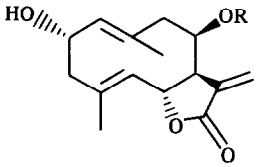
‡§Assignments interchangeable.



9 R = Ac
10 R = iBut
11 R = Meacr
12 R = Tigl



13



14 R = Sar
15 R = 2′*S*,3′*S* — Epoxyang
16 R = 2′*S*,3′*R* — Epoxyang

of **1** appeared to differ from those of **9–12** only in its configuration at C-8.

A survey of the ^{1}H NMR spectra of 10-oxygenated guaian-12,6-olides with ester side chains at C-8 showed that compounds with 8β-side chains [27–31] had H-6 (δ 4.5–4.8) and H-8 (5.5–5.8) chemical shifts at lower field than compounds with C-8α-oriented side chains (H-6: 3.8–4.1, H-8: 5.2) [23–25, 32, 33] and had a completely different pattern of coupling constants between H-7, H-8 and H-9 (8β-side chain compounds: $J_{7,8}$ = 3.5–4 Hz and $J_{8,9}$ = 7–8 Hz; 8α-side chain compounds: $J_{7,8}$ = 9–10 Hz and $J_{8,9}$ = 5.5 Hz). These correlations suggested that **1** had an 8β-side chain, a supposition that was confirmed by hydrolysis of **1** to **8**. The ^{1}H NMR spectrum of **8** (Table 1) lacked a paramagnetic shift for H-13a and showed no evidence of geminal coupling between H-13a and H-13b, indicating that the 8-hydroxyl group was β-oriented [34] and, therefore, that the parent compound **1** had a β-oriented side chain.

As in compounds **9–12**, the C-10 hydroxyl group in **1** appeared to have an α-configuration and an axial conformation based on the unusual downfield ^{1}H NMR shift of H-7. 10α-Hydroxyl groups in guaianolides have been previously reported to significantly deshield H-7 [24, 29], which is also on the α-face of the molecule. Models show the close proximity of H-7 to an axial 10α-hydroxyl function. Additional support for the axial orientation of the hydroxyl group at C-10 comes from the ^{13}C NMR shift of C-14 (δ 33.0) (Table 2) which suggests that this methyl group occupies an equatorial position because of the lack of 1,3-diaxial interactions [35]. Similar shifts are seen in other 10α-hydroxyl-12,6-lactonized guaianolides [36, 37]. In guaian-12,6-olides with 10β-hydroxyl groups, by contrast, C-14 appears at higher field [37, 38], presumably because it is axially-positioned. The structure of **1** was recently confirmed by single crystal X-ray crystallography [39].

The spectral data for compounds **2**, **3**, **4** and **6** showed that these differed from **1** only in the nature of their ester side chains (Tables 1 and 2). All were readily converted to **8** on hydrolysis with potassium hydroxide. The diastereomers **2** and **3** were isolated as a 1:1 mixture. Herz and Kumar [10] isolated an analogous 1:1 mixture of 2α-hydroxy-costunolide derivatives bearing these side chains from *H. pumilus*.

Spectral data for another sesquiterpene lactone isolated in this study (**13**) showed that it was not a guaianolide but a germacra-1(10),4(5)-dienolide with an 8β-sarracinoyl side chain. The mass spectrum gave a molecular ion at *m/z* 362, consistent with a molecular formula of $C_{20}H_{26}O_6$. The presence of an α-methylene-γ-lactone was clear from IR (1740, 1650 cm^{-1}), ^{13}C NMR (δ 169.2 *s*, 120.8 *t*) and ^{1}H NMR data (δ 5.81, *d*, J = 3.0 Hz and 6.45, *d*, J = 3.5 Hz) (Tables 3 and 4). Irradiation at 6.45 in pyridine-d_5 (H-13a) located H-7 (3.18) which was coupled to a *dd* at 5.64 (J = 8.5, 10.5 Hz) and weakly-coupled to a *br dd* at 6.10. The signal at 5.64 was assigned to H-6 since its appearance was characteristic for H-6 in 12,6-*trans*-lactonized germacrolides, a class of compounds common in *Helianthus* [9, 10, 15, 17; Gershenzon, J., Mabry, T. J., Pearce, J. and Stewart, E. S., unpublished results]. This meant that the signal at 6.10 was that for H-8. Further spin-decoupling experiments allowed the placement of a double bond between C-4 and C-5; the signal at 5.64 (H-6) was spin-coupled to an olefinic proton at H-5 (5.08), which was in turn coupled to a vinylic methyl group at C-4 (2.01). Irradiation at H-8 (6.10), meanwhile, transformed signals at 2.42 and 2.98 into an AB pattern (J = 15 Hz) indicating that position 10 was blocked. The chemical shift of the C-10 methyl group (1.66) and the fact that it was spin-coupled to a *br dd* at 5.07 (H-1) placed a double bond between C-1 and C-10.

The presence of a sarracinoyl ester side chain in **13** was apparent from the IR spectrum (1715), MS (*m/z* 99, 100% relative intensity) and ^{1}H NMR spectra (a spin system consisting of a vinylic methyl group, 2.09, *br d*, J = 7 Hz, an olefinic proton, 6.55, *br q*, J = 7 Hz, and a geminally coupled pair of protons at 4.56 and 4.67, both *br d*, J = 14 Hz). The chemical shift of the signal assigned to H-8 (6.10) suggested attachment at that position. The magnitude of $J_{7,8}$ (~ 1 Hz) and the chemical shift of H-8 showed that the side chain was probably β-oriented [40]. The lactone ring itself was considered to be *trans*-fused based on the values of $J_{7,13}$ (3, 3.5 Hz) [26].

At this point, the partial structure of **13** (**A**) was identical to the corresponding portion of desacetyleupasserin (**14**), a germacrolide previously isolated from several *Helianthus* species [2, 10, 17, 41; Gershenzon, J., Mabry, T. J., Pearce, J. and Stewart, E. S., unpublished results]. ^{1}H NMR and ^{13}C NMR data for this fragment of desacetyleupasserin are very similar to those discussed for **13** (Tables 3 and 4). Irradiation at H-1 of **13** (5.07), however, did not decouple a proton adjacent to an oxygen as in **14**, but altered two methylene signals at 2.60 and 2.69, which were in turn both coupled to a *dd* at 4.64. Since the IR spectrum of **13** indicated the presence of two hydroxyl groups in the molecule and the ^{13}C NMR spectrum had an unassigned resonance for an sp^3-hybridized carbon attached to an oxygen atom (76.7, *d*), it seemed likely that there was a hydroxyl function at position 3.

Table 3. ^{1}H NMR spectra of the germacrolides **13** and **14***

	14 C_5D_5N	**13** C_5D_5N	**13** DMSO-d_6
H-1	5.39 *br d* (10)	5.07 *br dd* (4, 11)	~ 4.95†
H-2α	—	2.69 *ddd* (4, 6, 13)	2.15
H-2β	5.01 *ddd* (6, 10)	2.60 *ddd* (10, 11, 13)	2.35†
H-3α	2.40 *dd* (10, 10.5)	4.64 *dd* (6, 10)	4.15
H-3β	2.91 *dd* (6, 10.5)	—	—
H-5	5.19 *br d* (10.5)	5.08 *br d* (10.5)	~4.90†
H-6	5.54 *dd* (8, 10.5)	5.64 *dd* (8.5, 10.5)	5.25
H-7	3.22 *br ddd* (3, 3 5, 8)	3.18 *br ddd* (3, 3.5, 8.5)	3.22
H-8	6.10 *br dd* (2, 5)	6.10 *br dd* (2, 4.5)	5.73
H-9α	2.98 *dd* (5, 14)	2.98 *dd* (4.5, 15)	2.63
H-9β	2.45 *dd* (2, 14)	2.42 *dd* (2, 15)	2.38
H-13a	6.43 *d* (3.5)	6.45 *d* (3.5)	6.14
H-13b	5.78 *d* (3)	5.81 *d* (3)	5.68
H-14	1.66 (3H) *br s*	1.66 (3H) *br s*	1.66 (3H)
H-15	1.77 (3H) *br s*	2.01 (3H) *br s*	1.86 (3H)
H-3′	6.54 *br q* (7)	6.55 *br q* (7)	6.30
H-4′	2.08 (3H) *br d* (7)	2.09 (3H) *br d* (7)	1.93 (3H)
H-5′a	4.58 (2H) *br s*	4.56 *br d* (14)	~4.0 (2H)†
H-5′b		4.67 *br d* (14)	

*Run at 200 MHz with TMS as an internal standard. Numbers in parentheses are coupling constants in Hz. Coupling constants for **13** in DMSO-d_6 were essentially the same as in C_5D_5N except for H-5′.

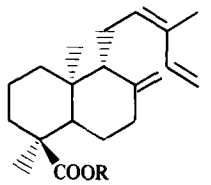
†Obscured due to overlapping signals.

Table 4. ^{13}C NMR spectra of the germacrolides **13** and **14**

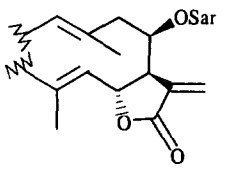
	13*	**14†**
C-1	129.0 *d*	135.0 *d*
C-2	35.4 *t*	67.8 *d*
C-3	76.7 *d*	48.7 *t*
C-4	144.4 *s*	142.3 *s*
C-5	123.2 *d*	128.7 *d*
C-6	75.3 *d*	75.5 *d*
C-7	51.0 *d*	51.9 *d*
C-8	71.7 *d*	71.5 *d*
C-9	43.1 *t*	43.2 *t*
C-10	132.4 *s*	132.7 *s*
C-11	137.3 *s*	137.2 *s*
C-12	169.2 *s*	169.0 *s*
C-13	120.8 *t*	120.3 *t*
C-14	18.6 *q*	19.4 *q*
C-15	11.9 *q*	18.3 *q*
C-1′	165.3 *s*	165.2 *s*
C-2′	133.8 *s*	132.3 *s*
C-3′	137.3 *d*	137.6 *d*
C-4′	15.0 *q*	15.1 *q*
C-5′	61.7 *t*	61.9 *t*

*Run at 22.6 MHz in DMSO-d_6 with TMS as an internal standard. Assignments made using off-resonance decoupling experiments and by analogy with model compounds [10, 17, 47].

†From ref. [47]. Run at 67.09 MHz in DMSO-d_6.



17 R = H
18 R = Me



A

The principal ^{13}C NMR differences between compounds **13** and **14** (35.4 *t* vs. 48.7 *t*, 76.7 *d* vs. 67.8 *d*, 11.9 *q* vs. 18.3 *q*) (Table 4) support this assignment. Placing the hydroxyl group at C-3 instead of C-2 should move the resonance for the carbon bonded to the hydroxyl group significantly downfield because of the added number of proximal atoms (carbon or oxygen atoms 1 or 2 bonds away) and should move the methylene resonance nearby significantly upfield because of the reduced number of proximal atoms. The C-15 methyl signal should shift upfield because of the γ-effect of the oxygen atom at C-3 [42].

Referring to molecular models, the coupling constants between the H-2 protons and H-3 (J = 6, 10 Hz) seem to

require the 3-hydroxyl function to be β-oriented. A number of germacra-1(10),4(5)-dien-12,6-olides with 3β-oxygen substituents have been previously characterized [27,43–46]. All have $J_{2,3}$ values very similar to those in **13**. Unfortunately, **13** proved to be quite unstable at room temperature and decomposed before any chemical transformations could be attempted.

Another germacra-1(10),4(5)-dien-12,6-olide isolated from this collection of *H. maximiliani* proved to be the known compound **15**, 2α-hydroxy-8β-(2′*S*,3′*S*-epoxyangeloyloxy)costunolide. Comparison of its spectral data and optical rotation to those reported for **15** and **16** [10, 17, 47; Stewart, E., Gershenzon, J. and Mabry, T. J., unpublished results] showed that we had clearly isolated the 2′*S*,3′*S*-diastereomer (**15**) rather than the 2′*R*,3′*R* compound (**16**). The biggest differences in the spectral data of these two compounds are found in the ^{1}H NMR shifts of protons at C-6, C-8, C-9, C-13, C-3′ and C-4′. The high field ^{1}H NMR spectrum of **15** indicated that **16** was a trace contaminant.

The results of this investigation along with the unpublished data previously cited show that there is sharp intraspecific variation in the sesquiterpene lactone chemistry of *H. maximiliani*. None of the compounds isolated in this study (germacrolides and guaianolides) were reported from the Kansas population previously investigated, which only contained heliangolides [8]. The germacrolides **15** and **16** are also constituents of the north-central Texas population currently under study, but this population has not yet yielded any heliangolides or guaianolides. Such variability in the sesquiterpene lactone chemistry of a widespread species is not unusual. Species of *Ambrosia*, *Artemisia* and *Iva*, for example, synthesize different structural types of sesquiterpene lactones in different parts of their ranges [48].

Within the genus *Helianthus*, the isolation of 2α-hydroxy-germacradienolides from *H. maximiliani* suggests close relationships between this species and *H. decapetalus* [2], *H. mollis* [17], *H. pumilus* [10], *H. divaricatus* and *H. resinosus* [Pearce, J., Gershenzon, J. and Mabry, T. J., unpublished results], all of which also produce compounds of this type. These species are all classified in section *Divaricati*, series *Corona-solis* of *Helianthus*, except for *H. pumilus* which is placed in section *Ciliares* [49]. The *ent*-labdane (−)-*cis*-ozic acid has been previously reported from *H. angustifolius* [12], *H. decapetalus* [2], *H. occidentalis* [21] and *H. tuberosus* [2]. The investigation of several other species of *Helianthus* is currently underway.

A number of wild species of *Helianthus* are resistant to the major insect pests of cultivated sunflower (*H. annuus*) [50] and several terpenoids isolated from these plants have been tested against sunflower insects and found to have significant activity as antifeedants and growth inhibitors [7, 51]. *Helianthus maximiliani* is one of these resistant species and tests of the effects of compound **6**, the most abundant sesquiterpene lactone isolated in this study, on the sunflower moth, *Homoeosoma electellum*, are in progress.

EXPERIMENTAL

Air dried and ground leaves of *Helianthus maximiliani* (2 kg) collected along Barton Creek, Travis Co., Texas, 1.5 miles south of Barton Springs by J. Gershenzon and E. E. Schilling on 24 Sept. 1978 (JG #1, voucher on deposit in the Herbarium of the University of Texas) were extracted with $CHCl_3$ and worked up in the usual manner [52]. The crude syrup (50 g) was dissolved in a minimum amount of toluene–EtOAc (9:1) and applied to a silica gel column (1.2 kg) packed in the same solvent mixture. The column was eluted with a toluene–EtOAc gradient, with increasing amounts of EtOAc. Eighty 500 ml fractions were collected. Fractions 8–14 (toluene–EtOAc, 4:1) were combined (1 4 g) and separated on a silica gel column (60 g) eluted with $CHCl_3$–MeOH (25:1). Nine 100 ml fractions were collected. A precipitate from fraction 4 was recrystallized from MeOH to give 45 mg of **17** as colorless needles. Treatment of the mother liquor (67 mg) with 3 mmol CH_2N_2 gave 46 mg **18** as a colorless oil. UV, IR, MS, ^{1}H NMR and ^{13}C NMR data and mps for **17** and **18** were very similar to those previously reported [2, 12, 18–21].

Fractions 17–22 from the main column (toluene–EtOAc, 4:1) were combined (1.5 g) and applied to a silica gel column (55 g) eluted with toluene–EtOAc (4:1). Twenty-one 100 ml fractions were collected. Fractions 7–13 (400 mg) were recrystallized from hot EtOAc to give 138 mg **1** as colorless needles.

Main column fractions 29–37 (toluene–EtOAc, 2:1) were combined (7 g) and charged on a silica gel column (200 g) eluted with a $CHCl_3$–MeOH gradient, starting with a 25:1 mixture. Twelve 50 ml fractions were collected Fractions 2–5 contained 3 g of an oily mixture of **2** and **3** (*ca* 1:1) which could not be cleanly separated by prep TLC on silica gel in several solvent systems.

Fraction 39 (350 mg) from the main column (toluene–EtOAc, 2:1) was separated on a small silica gel column (30 g) eluted with toluene–EtOAc (3:1) Sixteen 50 ml fractions were collected. Fractions 8–16 were combined (200 mg) and purified by prep. TLC on silica gel (2 mm, $CHCl_3$–MeOH, 15.1) to give 75 mg **4** as a pale gum.

Main column fractions 43–56 contained principally **4** and **6**. These fractions were combined (12 g) and applied to a silica gel column (400g) eluted with a toluene–EtOAc gradient, initiated with a 7:3 mixture. Seventy 50 ml fractions were collected. Fractions 25–37 were triturated with *iso*-Pr_2O and the resulting powdery crystals recrystallized from hot *iso*-Pr_2O–MeOH to give 2 g of **6** as colorless needles

Fractions 57–66 from the main column (toluene–EtOAc, 1:1) contained **6**, **13** and other constituents. These fractions were combined (6.7 g) and separated on a silica gel column (150 g) eluted with a toluene–EtOAc gradient, initiated with a mixture of 7.3. Twenty-five 100 ml fractions were collected. Fractions 29–40 (toluene–EtOAc, 1:1) were purified by repeated prep. TLC (2 mm silica gel, $CHCl_3$–MeOH, 15:1 and toluene–EtOAc, 5:6) to give 95 mg **13** which was recrystallized from hot EtOAc yielding 35 mg colorless needles.

Compound **15** was isolated as part of an effort to extract large quantities of compound **6** for insect testing. A second collection of *H maximiliani* leaves (2.5 kg) was made at the same site as before on 11 Sept. 1979. Extraction of this material yielded 33 g crude syrup which was separated on a silica gel column (750 g) eluted with a CH_2Cl_2–*iso*-PrOH gradient, initiated with pure CH_2Cl_2 Thirty-eight 1 l. fractions were collected. In addition to 3 g of **6**, obtained by prep TLC (2 mm silica gel, CH_2Cl_2–*iso*-PrOH, 10:1) of fractions 16–19 (5.6 g, CH_2Cl_2–*iso*-PrOH, 50.1), repeated prep TLC of fraction 11 (400 mg, CH_2Cl_2–*iso*-PrOH, 50 1) using toluene–EtOAc (1:1) and CH_2Cl_2–*iso*-PrOH (25:1) with multiple developments yielded 12 mg of **15** as a pale gum contaminated with a small amount of **16**, $[\alpha]_D^{22}$ + 22° ($CHCl_3$, *c* 0.40), pure sample of **15** from *H gracilentus*: $[\alpha]_D^{25}$ + 15° ($CHCl_3$, *c* 0.20), **16** [47]: + 67.7° ($CHCl_3$, *c* 0 204)

The compounds in this study were visualized on silica gel TLC plates using acidified vanillin [53] and 20% H_2SO_4 sprays. The guaianolides **1**–**8** turned dark blue with vanillin and bright pink

with H_2SO_4 [54], while the germacrolides **13** and **15** appeared blue-green with vanillin and brown with H_2SO_4.

8β-Angeloyloxycumambranolide (**1**). Mp 160–161° (EtOAc). CD: (*c* 5.2×10^{-3}, MeOH) $[\theta]_{236} + 1350$, $[\theta]_{265} - 1400$. IR ν_{max}^{Nujol} cm^{-1}: 3300, 1740 (lactone $\gtrless$C=O), 1705 (side chain $\gtrless$C=O), 1660, 1250, 1150, 1140, 1010, 950, 820. MS (probe) 70 eV, *m/z* (rel. int.). 346 (1) $[M]^+$ $C_{20}H_{26}O_5$, 328 (3) $[M - H_2O]^+$, 300 (2) $[M - H_2O - CO]^+$, 263 (1) $[M - C_5H_7O]^+$ α-cleavage of side chain (HRMS: $C_{15}H_{19}O_4$, 263.1276 meas., 263.1283 calc.), 246 (10) $[M - C_5H_8O_2]^+$ McLafferty rearrangement and cleavage of side chain (HRMS: $C_{15}H_{18}O_3$, 246.1257 meas., 246.1256 calc.), 228 (35) $[246 - H_2O]^+$, 213 (11) $[246 - H_2O - Me]^+$, 203 (8) $[246 - CO - Me]^+$, 200 (8) $[246 - H_2O - CO]^+$, 188 (30), 133 (30), 107 (25), 83 (100) $[C_5H_7O]^+$ side chain acylium ion, 55 (75) $[83 - CO]^+$.

8β-(2'S,3'S-Epoxyangeloyloxy)cumambranolide (**2**) *and 8β-(2'R,3'R-epoxyangeloyloxy)cumambranolide* (**3**), *a* ca 1:1 *mixture.* IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1750 (lactone $\gtrless$C=O), 1735 (side chain $\gtrless$C=O), 1660, 1260, 1150, 1080, 1010, 935, 885, 815. MS (probe) 70 eV, *m/z* (rel. int.): 362 (< 0.5) $[M]^+$, 344 (< 0.5) $[M - H_2O]^+$, 316 (0.5) $[M - H_2O - CO]^+$, 246 (3) $[M - C_5H_8O_3]^+$, 228 (12) $[246 - H_2O]^+$, 213 (3) $[246 - H_2O - Me]^+$, 203 (3) $[246 - CO - Me]^+$, 200 (4) $[246 - H_2O - CO]^+$, 188 (17), 133 (15), 107 (26), 81 (33) $[C_5H_5O]^+$ side chain acylium ion $- H_2O$, 43 (100)

8β-(2α-Hydroxyethyl)acryloyloxycumambranolide (**4**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1755 (lactone $\gtrless$C=O), 1710 (side chain $\gtrless$C=O), 1660, 1640, 1215, 1155, 1140, 1085, 1010, 905, 870, 840. MS (probe) 70 eV, *m/z* (rel. int.): 362 (< 0.5) $[M]^+$ (HRMS: $C_{20}H_{26}O_6$, 362.1734 meas, 362.1729 calc), 246 (6) $[M - C_5H_8O_3]^+$, 228 (16) $[246 - H_2O]^+$, 213 (8) $[246 - H_2O - Me]^+$, 203 (6) $[246 - CO - Me]^+$, 200 (5) $[246 - H_2O - CO]^+$, 188 (21), 133 (23), 107 (30), 99 (28) $[C_5H_7O_2]^+$ side chain acylium ion, 84 (95) $[99 - Me]^+$, 81 (65) $[99 - H_2O]^+$, 43 (100)

8β-Sarracinoyloxycumambranolide (**6**). Mp 129–130° (*iso*-Pr_2O–MeOH). IR ν_{max}^{Nujol} cm^{-1}. 3550, 3450, 1745 (lactone $\gtrless$C=O), 1710 (side chain $\gtrless$C=O), 1660, 1260, 1140, 1010, 945, 855, 825 MS (probe) 70 eV, *m/z* (rel. int.) 362 (< 0.5) $[M]^+$ (HRMS: $C_{20}H_{26}O_6$, 362.1736 meas., 362.1729 calc), 344 (< 0.5) $[M - H_2O]^+$, 246 (7) $[M - C_5H_8O_3]^+$ (HRMS: $C_{15}H_{18}O_3$, 246.1262 meas., 246.1256 calc), 228 (21) $[246 - H_2O]^+$, 213 (8) $[246 - H_2O - Me]^+$ (HRMS. $C_{14}H_{13}O_2$, 213.0922 meas., 213.0915 calc), 203 (8) $[246 - CO - Me]^+$, 200 (5) $[246 - H_2O - CO]^+$, 188 (28) (HRMS. $C_{12}H_{12}O_2$, 188.0840 meas., 188.0837 calc.), 133 (22), 107 (25), 99 (88) $[C_5H_7O_2]^+$ side chain acylium ion, 81 (48) $[99 - H_2O]^+$, 43 (100).

Acetylation of **4**. Slightly impure **4** (170 mg) was acetylated in 7 ml Ac_2O and 15 mg K_2CO_3 for 4 hr at 65° and the reaction worked-up in the usual manner [17] (No reaction occurred at room temp.) The crude product was purified by prep. TLC ($CHCl_3$–MeOH, 15:1) to give 46 mg **5** as a pale gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1760 (lactone $\gtrless$C=O), 1735 (acetate $\gtrless$C=O), 1715 (side chain $\gtrless$C=O), 1660, 1640, 1250, 1155, 1080, 1040, 1015, 955, 920, 845, 815. MS (probe) 70 eV, *m/z* (rel. int.): 404 (< 0.5) $[M]^+$, 344 (< 0.5) $[M - HOAc]^+$, 246 (8) $[M - C_7H_{10}O_4]^+$, 228 (22) $[246 - H_2O]^+$, 213 (10) $[246 - H_2O - Me]^+$, 203 (10) $[246 - CO - Me]^+$, 188 (38), 141 (30) $[C_7H_9O_3]^+$ side chain acylium ion, 133 (28), 123 (31), 107 (34), 99 (31), 81 (62) $[141 - HOAc]^+$, 43 (100).

Acetylation of **6** Compound **6** (150 mg) was acetylated in 6 ml Ac_2O and 13 mg K_2CO_3 for 2 hr at room temp. and worked up in the usual manner. The crude product was purified by prep. TLC (toluene–EtOAc, 1:1) to give 84 mg of **7**, which upon recrystallization from EtOAc gave 72 mg crystals, mp 130–131°, IR ν_{max}^{Nujol} cm^{-1}: 3400, 1750 (lactone $\gtrless$C=O), 1730 (acetate $\gtrless$C=O), 1710 (side chain $\gtrless$C=O), 1660, 1250, 1150, 1025, 1010, 960, 855, 820. MS (probe) 70 eV, *m/z* (rel. int.): 404 (4) $[M]^+$, 386 (1) $[M - H_2O]^+$, 344 (1) $[M - HOAc]^+$, 326 (2) $[344 - H_2O]^+$, 298 (1) $[326 - CO]^+$, 258 (5), 246 (20) $[M - C_7H_{10}O_4]^+$ McLafferty rearrangement and side chain cleavage, 228 (50) $[246 - H_2O]^+$, 213 (23) $[246 - H_2O - Me]^+$, 203 (23) $[246 - CO - Me]^+$, 188 (57), 141 (56) $[C_7H_9O_3]^+$ side chain acylium ion, 133 (37), 123 (37), 107 (45), 99 (45), 81 (77) $[141 - HOAc]^+$, 43 (100)

Hydrolysis of **1**, **2**, **3**, **4** *and* **6** *to* **8**. (Reaction of **6** presented as an example.) Compound **6** (100 mg) was hydrolysed in 6 ml 5% KOH–MeOH for 12 hr at room temp. and the reaction worked up in the usual manner [17]. Compound **8** (14 mg) crystallized out of the reaction mixture, mp 91–92°, IR ν_{max}^{Nujol} cm^{-1}: 3450, 3300, 1750, 1675, 1245, 1140, 1050, 1010, 950, 840, 820. MS (probe) 70 eV, *m/z* (rel. int.): 264 (0.5) $[M]^+$, 246 (27) $[M - H_2O]^+$, 228 (17) $[M - H_2O - H_2O]^+$, 218 (4) $[M - H_2O - CO]^+$, 213 (6) $[M - H_2O - H_2O - Me]^+$, 202 (10) $[M - H_2O - CO_2]^+$, 133 (22), 121 (33), 107 (92), 93 (58), 81 (56), 43 (100). Two minor products of this reaction appeared to be the 11,13 methanol adduct (7 mg) and the 6α-hydroxyl-12,8-lactonized isomer of **8** (6 mg) from their ^1H NMR spectra

3β-Hydroxyl-8β-sarracinolyoxycostunolide (**13**) Mp 80–81°. IR ν_{max}^{Nujol} cm^{-1}: 3460, 3430, 1740 (lactone $\gtrless$C=O), 1715 (side chain $\gtrless$C=O), 1650, 1245, 1235, 1150, 1105, 1080, 1055, 990, 965, 945, 892, 878, 815. MS (probe) 70 eV, *m/z* (rel. int.). 362 (0.1) $[M]^+$, 344 (0.1) $[M - H_2O]^+$, 247 (4) $[M - C_5H_7O_3]$, 246 (6) $[M - C_5H_8O_3]^+$, 236 (11), 228 (3) $[246 - H_2O]^+$, 218 (18) $[246 - CO]^+$, 213 (3) $[246 - H_2O - Me]^+$, 207 (23), 203 (12) $[246 - CO - Me]^+$, 189 (23), 173 (32), 147 (24), 119 (36), 105 (67), 99 (100) $[C_5H_7O_2]^+$ side chain acylium ion, 81 (88) $[99 - H_2O]^+$, 43 (82).

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