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SESQUITERPENE LACTONES AND DITERPENE CARBOXYLIC ACIDS FROM *HELIANTHUS DIVARICATUS*, *H. RESINOSUS* AND *H. SALICIFOLIUS*

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Key Word Index—*Helianthus divaricatus*; *H. resinosus*; *H. salicifolius*; Asteraceae; sunflowers; sesquiterpene lactones; germacranolides; diterpenes; trachylobane; kaurane.

Abstract—Ten similar germacranolide sesquiterpene lactones were isolated from *Helianthus divaricatus*, *H. resinosus* and *H. salicifolius*. Three of these compounds are new and two of the angelate-derived ester side chains are previously unreported. An *ent*-kaurane and an *ent*-trachylobane diterpene were also isolated from *H. salicifolius*. The results of this study support previous proposals that (*a*) *H. divaricatus*, *H. resinosus* and *H. salicifolius* are closely related, (*b*) that *H. mollis* may be a progenitor of the hexaploid *H. resinosus* and (*c*) that the unique features of *H. divaricatus* populations on the western edge of its range may be due to introgression with *H. mollis*.

INTRODUCTION

As part of a chemosystematic study of North American sunflowers, *Helianthus* (Asteraceae) [1–6], we have investigated the terpenoid constituents of three perennial species native to the eastern and central United States, *H. divaricatus* L., *H. resinosus* Small and *H. salicifolius* A. Dietr. [7]. The terpenoid chemistry of about one-third of the *ca* 50 species of *Helianthus* has been studied in some detail [6]. The principal non-volatile terpenoid compounds found are germacranolide sesquiterpene lactones and diterpene carboxylic acids with labdane, kaurane, atisirane or trachylobane carbon skeletons. In this paper, we report the isolation of a series of ten 2α-hydroxy-8β-acyloxy-*trans*,*trans*-1(10),4(5)-germacradienolides (**1–8**, **11** and **13**), which includes three new compounds **7**, **8** and **13**. *Helianthus divaricatus* contained compounds **3** and **4**, *H. resinosus* contained **1–3**, **5–8**, **11** and **13** and *H. salicifolius* yielded **3** and **6**. Two previously characterized diterpene carboxylic acids, the *ent*-kaurane **15** [4] and the *ent*-trachylobane **17** [8], were also isolated from *H. salicifolius*.*

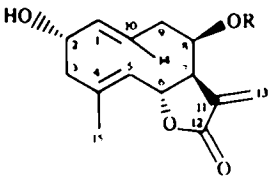
RESULTS AND DISCUSSION

Comparison of ^{1}H NMR (Table 1), ^{13}C NMR (Table 2) and mass spectral data indicated that compounds **7** and **8** differed from the known lactones **1–6** [10–12] only in their 5-carbon ester side chains. The molecular formula of **7**, $C_{20}H_{26}O_7$ (confirmed by HRMS of the molecular ion), suggested that its side chain contained, in addition to the carbonyloxy group, two oxygen atoms and one ring or one double bond. The ^{13}C NMR spectrum lacked side chain sp^2 signals (besides C-1′) but did provide evidence for a methyl group and three oxygen-bearing sp^3 carbon atoms (δ81.3 *s*, 59.6 *d*, 65.3 *t*) in the acyl function. Thus, one of the oxygen atoms was in a heterocyclic ring and the other must be part of a hydroxyl group. A methyl doublet at δ1.36 coupled to a one-proton quartet at 4.40 in the ^{1}H NMR spectrum showed that the hydroxyl and methyl groups were attached to the tertiary carbon atom. A pair of geminally-coupled methylene doublets near δ3.5 was consistent with the presence of a terminal epoxide, which accounted for the remaining carbon and oxygen atoms in the ester side chain. Formula **7** incorporates these substructures into an angelate-derived ester analogous to **6**, with the vinylidene moiety now epoxidized.

The acetylation product **10** furnished indirect support for the structure deduced for **7**. ^{1}H NMR and ^{13}C NMR data for **10** indicated that it was a triacetate. The 1.2 ppm paramagnetic shift of the H-2 signal in **10** relative to the H-2 signal in **7** demonstrated acetylation at C-2. Comparison of the ^{1}H NMR side chain signals with those of **7** revealed that the one-proton quartet assigned to H-3′ was unaltered, while the pair of H-5′ methylene doublets had shifted *ca* 1.3 ppm downfield. Thus, C-5′ was also acetylated, and the third acetate was placed at the only remaining position, namely C-2′. The resulting side chain for **10** thus implies a transformation of **7** involving opening of the epoxide ring, with the tertiary hydroxyl group at C-2′ ending up acetylated (possibly involving an acyl shift).

Compound **8**, the most polar component isolated from *H. resinosus*, did not give a molecular ion under EIMS, but chemical ionization provided an $[M+H]^+$ of *m/z* 397, indicating a probable molecular formula of $C_{20}H_{28}O_8$. ^{13}C NMR signals for a methyl group and three oxygen-bearing sp^3 carbons (δ81.8 *s*, 68.6 *d*, 64.7 *t*) correlated closely with those for the side chain of **7**. Comparison of the molecular formulae for **8** and **7** revealed differences which, due to clear spectral evidence for a common main skeleton, were necessarily confined to their side chains.

*After our work on *H. salicifolius* had been completed, a paper by W. Herz, S. V. Govindan and K. Watanabe appeared [9] which described the isolation of **15**, **17** and a 16-hydroxykaurane from *H. salicifolius*. However, these authors did not report the presence of any sesquiterpene lactones.



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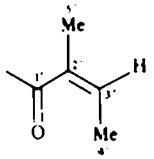

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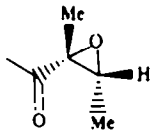

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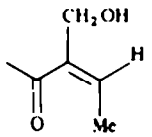

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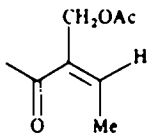

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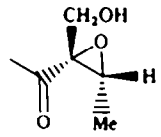

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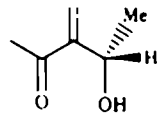

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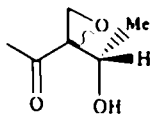

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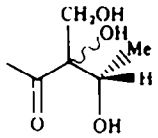

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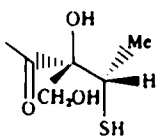
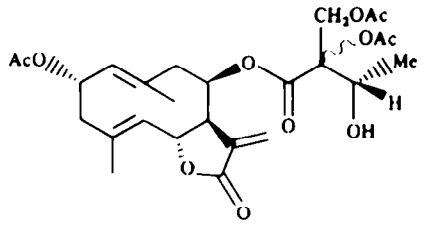


Specifically, it was inferred that the non-carbonyl portion of the side chain of **8** lacked a ring (or double bond) and contained, relative to **7**, additional mass which was equivalent to a molecule of water. Thus, an obvious candidate structure for **8** could be generated from **7** by hydrolysis of the 2',5' epoxide ring to the corresponding diol, giving a 2',3',5' trihydroxy ester side chain. The ^{1}H NMR spectrum of **8** in DMSO-d_6 did not clearly support this proposal, as several of the predicted side chain signals were either obscured or shifted anomalously upfield from expected values. However, a subsequent spectrum in $CDCl_3$ (with CD_3OD added for solubility) contained signals consistent with the 2',3',5'-trihydroxyangelate structure: an AB pattern near δ3.7 assigned to the hydroxymethylene H-5' protons, and the H-3' quartet at 3.85 coupled to the H-4' methyl doublet at 1.12 (the latter shift in accord with values reported for H-4' in analogous 2',3'-dihydroxyangelates [13]). Additional support for the proposed structure was provided by a comparison of ^{13}C NMR data for **8** and the 3'-sulphydryl analogue **9** [12]. The only notable difference between these two spectra was a 9 ppm downfield shift of the C-3' signal of **8** relative to **9**, attributable to the inductive effect of the more electronegative oxygen substituent on this carbon atom [14].

The absolute configurations of the congeners **1–8** and **10** were established by correlation with compound **9**, whose structure was derived from X-ray data [12]. In particular, the stereochemistry at C-3' in **7**, **8** and **10** was tentatively designated as *S* by analogy with **6** and in accord with a biosynthetic scheme assuming nucleophilic attack at C-3' of the 3'*R*-epoxides **2** or **5** [12, 15]. The orientation of the hydroxyl group at C-2' in **8** could not be deduced solely on mechanistic grounds since both **5** and **7** are plausible precursors.

Spectral data for the sesquiterpene portion of compounds **11** and **13** were similar to those for **1–10** (Tables 1–3) with the exception of the signals for the C-14 methyl group, which were replaced by a pair of geminally-coupled methylene doublets at δ3.75 and 4.25 in their ^{1}H NMR spectra and by a triplet at δ60 in their ^{13}C NMR spectra. These changes indicated the presence of a hydroxyl group at the C-14 position in **11** and **13**, a fact further supported by the allylic coupling observed between H-1 and the H-14 methylene signals, by the 0.5 ppm downfield shift of the β-oriented H-9a compared to those in the 14-methyl analogues **1–10**, and by the *ca* 5.5 ppm ^{13}C NMR upfield shift of C-9 compared to those in compounds **1–10**. The latter effect is presumably due to the interaction of C-9 with the γ-situated oxygen atom at C-14 [14]. ^{13}C NMR and ^{1}H NMR data for the ester side chain signals of **11** and **13** were virtually identical to those obtained from **1** and **2** and those reported for an 8β-angelate and epoxyangelate, respectively, from *H. pumilus* [10]. The stereochemistry at C-2' and C-3' of **13** was assumed to be the same as that in **2** and **5**. Acetylation of **11** and **13** yielded the diacetates **12** and **14**, whose ^{1}H NMR spectra exhibited typical acetate methyl signals and paramagnetic



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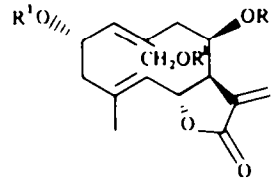
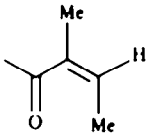
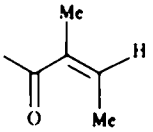
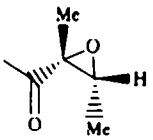
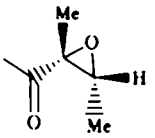
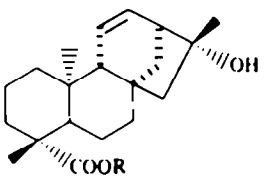


11 R =  R' = H

12 R =  R' = Ac

13 R =  R' = H

14 R =  R' = Ac



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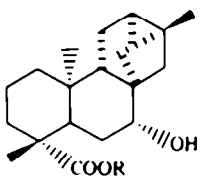
16 R = Me



17 R = H
18 R = Me

shifts of the protons at the acetylated positions C-2 and C-14. While **13** is apparently new, compound **11** appears to be identical to a compound previously characterized in the form of its diacetate [16].

The sesquiterpene lactone profiles of the three species of *Helianthus* examined in this study are clearly very similar to each other. All three species produce only *trans,trans*-1(10),4(5)-germacradienolides (germacrolides) with 2α-hydroxyl groups and 8β-angelate or angelate-derived ester side chains. These compounds differ only in the exact nature of the ester function and in the presence of oxygenation at C-14. Compound **3** was found in all three species.

Helianthus divaricatus L., *H. resinosus* Small and *H. salicifolius* A. Dietr. have all been placed in section *Divaricati*, series *Corona-solis*, based on morphological characters and the results of crossing studies [17]. The sesquiterpene lactone data lend support to this classification, since 2α-hydroxy-8β-acyloxygermacrolides (2-OH-8-ACGs) have also been isolated from four other members of this group: *H. decapetalus* L. [18], *H. mollis* Lam. [1], *H. hirsutus* Raf. [19] and one chemical race of *H. maximiliani* Schrader [20]. Accumulation of 2-OH-8-ACGs is not restricted to members of series *Corona-solis* however, as these compounds have also been isolated from *H. pumilus* Nutt. [10] and *H. gracilentus* A. Gray [21], currently placed together in a different section of the genus [17].

Since the collection of *H. divaricatus* studied came from

Table 1. ^{1}H NMR spectra of compounds 6–8 and 10*

	6 DMSO-d_6	7 DMSO-d_6	7 C_5D_5N	8 DMSO-d_6	8 $CDCl_3$-CD_3OD (3:1)	10 $CDCl_3$
H-1	5.00 *br d*	4.96	5.42	4.97	5.04	5.02
H-2	4.57 *ddd*	4.56	5.08	4.58	4.73	5.72
H-3a	2.56 *dd*	2.5§	2.91	2.5§	2.71	2.77
H-3b	1.95 *dd*	1.93	2.44	1.93	2.10	2.19
H-5	5.05‡	4.96 *br d*	5.18	4.97	4.98	4.99
H-6	5.05‡	5.21 *dd*	5.60	5.24	5.23	5.11
H-7	3.3§	3.23 *br ddd*	3.28	3.18	3.04	2.98
H-8	5.71 *br dd*	5.77	6.26	5.78	5.93	6.05
H-9a	2.66 *dd*	2.5§	2.98	2.5§	2.81	2.82
H-9b	2.40 *dd*	2.42	2.52	2.38	2.43	2.42
H-13a	6.13 *d*	6.08	6.52	6.07	6.30	6.38
H-13b	5.64 *d*	5.64	5.89	5.64	5.73	5.70
H-14†	1.42 *br s*	1.52	1.79	1.55	1.63	1.77
H-15†	1.72 *br s*	1.74	1.94	1.72	1.82	1.86
H-3′	4.47 *q*	4.40	4.78	3.67	3.85	4.40
H-4′†	1.14 *d*	1.36	1.62	0.94	1.12	1.53
H-5′a	6.10 *br s*	3.56 *d*	4.22	3.4§	3.74	4.95
H-5′b	5.90 *br s*	3.45 *d*	4.13		3.59	4.65
acetate†	-		—		—	2.07 *s*
methyls		-		—		2.10 *s*
		-	—	-	—	2.10 *s*

*Run at 200 MHz with TMS as an internal standard. Multiplicities are similar to those in the previous column unless otherwise noted. Coupling constants for 7, J (Hz): 1, 2 = 10; 1, 14 = 1.5; 2, 3a = 6; 2, 3b = 10; 3a, 3b = 10.5; 5, 6 = 10; 5, 15 = 1.5; 6, 7 = 8.5; 7, 8 < 1.5; 7, 13a = 3.5; 7, 13b = 3.2; 8, 9a = 5.5; 8, 9b = 2.5; 9a, 9b = 14; 3′, 4′ = 6.5; 5′a, 5′b = 11. Values for 6, 8 and 10 are similar to those of 7 except in 10: 5′a, 5′b = 12.5.

†Intensity three protons.

‡Not first-order.

§Partially obscured by overlapping signals.

a site on the Ozark Plateau at the western edge of its range, several additional small collections of this species from elsewhere in its range were subsequently analysed by TLC for comparison. Surprisingly, neither compounds 3 nor 4 were detected in any of these supplementary collections, indicating that these western populations of *H. divaricatus* may have chemically diverged from the rest of the species. Heiser [7] states that the *H. divaricatus* growing on the Ozark Plateau appears to be geographically isolated from the remainder of the species and, based on certain distinctive morphological features of these plants, he hypothesizes that introgression with *H. mollis* has occurred in this area. As mentioned above, *H. mollis* also produces a series of 2-OH-8-ACGs, two of which (3 and 4) are identical to those found in *H. divaricatus*.

Helianthus resinosus is a hexaploid species that is thought to have originated from hybridization between the diploid species *H. giganteus* and *H. mollis* [7]. The sesquiterpene lactone data provide some support for this suggestion, since *H. mollis* also synthesizes 2-OH-8-ACGs. *H. giganteus* apparently does not accumulate 2-OH-8-ACGs, but it does contain analogous 1,2-secogermacranolides, which have been shown to co-occur with 2-OH-8-ACGs in a related tetraploid, *H. hirsutus* [19]. Further terpenoid constituents of *H. giganteus* and other species of *Helianthus* are currently under study.

EXPERIMENTAL

Extraction of H. divaricatus. Leaves (1.4 kg) were collected from plants at the U.S.D.A. research center, Bushland, Texas on 2 August 1980 (J.G. # 83, voucher on deposit at the Herbarium of the University of Texas). These plants had grown from rootstock originally collected in Leflore Co., Oklahoma, along highway 270, 5 miles south of Wister (C. E. Rogers and T. E. Thompson, #830). Leaves were air-dried, washed with CH_2Cl_2 for 5 min and the residue remaining after evaporation of the solvent worked up by standard procedures [22]. Intact rather than ground leaves were extracted, since, in many species of *Helianthus*, sesquiterpene lactones appear to be localized in surface glands [Kreitner, G., Gershenzon, J. and Mabry, T. J., unpublished results] and a rapid surface wash has been shown to give a greater absolute yield of sesquiterpene lactones and reduced amounts of other plant constituents than does an extraction of ground material.

The crude syrup (18.9 g) was separated on a silica gel column (500 g) eluted with a CH_2Cl_2 *iso*-PrOH gradient. Fractions that eluted with 1.5% *iso*-PrOH gave crystals on standing. Recrystallization from *iso*-Pr_2O–EtOAc gave 520 mg of compound 4 as large plates, mp 145–146° (lit. 153 154° [11]), identified by comparison of its spectral data with those in the literature [1, 11] and with those obtained from an authentic specimen isolated from *H. mollis* [1].

Table 2. ^{13}C NMR spectra of compounds 7–11 and 13*

	7 DMSO-d_6	8 DMSO-d_6	9 DMSO-d_6	10 $CDCl_3$	10 DMSO-d_6	11 $CDCl_3$	13 $CDCl_3$
C-1	134.5 *d*	134.5 *d*	134.5 *d*	131.8 *d*§	131.9 *d*§	136.4 *d*	136.4 *d*
C-2	68.1 *d*	67.9 *d*‡	68.1 *d*	76.1 *d*	76.1 *d*	68.1 *d*	68.0 *d*†
C-3	48.8 *t*	48.7 *t*	48.7 *t*	46.2 *t*	45.9 *t*	48.5 *t*	48.4 *t*
C-4	142.3 *s*	141.7 *s*	142.2 *s*	142.6 *s*	142.0 *s*	142.6 *s*	142.9 *s*
C-5	129.4 *d*	129.4 *d*	128.3 *d*	131.4 *d*§	131.6 *d*§	129.5 *d*	129.5 *d*
C-6	75.1 *d*	75.2 *d*	75.0 *d*	75.2 *d*	76.1 *d*	75.6 *d*	75.2 *d*†
C-7	51.7 *d*	51.9 *d*	51.7 *d*	53.7 *d*	52.5 *d*	53.3 *d*	52.9 *d*
C-8	73.3 *d*	72.3 *d*	73.2 *d*	72.6 *d*	72.6 *d*	70.8 *d*	72.6 *d*†
C-9	43.7 *t*	43.9 *t*	42.7 *t*	45.4 *t*	44.8 *t*	38.8 *t*	38.4 *t*
C-10	133.5 *s*	133.3 *s*	133.4 *s*	137.5 *s*	137.6 *s*	136.5 *s*‡	136.4 *s*‡
C-11	136.5 *s*	136.7 *s*	136.2 *s*	138.1 *s*	138.0 *s*	138.3 *s*‡	138.4 *s*‡
C-12	169.4 *s*	169.4 *s*	169.2 *s*	170.3 *s*	170.2 *s*	169.6 *s*	169.5 *s*
C-13	121.4 *t*	121.2 *t*	121.3 *t*	123.4 *t*	123.2 *t*	121.6 *t*	121.4 *t*
C-14	19.5 *q*‡	19.6 *q*	19.5 *q*	21.3 *q*‡	21.2 *q*‡	61.3 *t*	60.4 *t*
C-15	18.6 *q*‡	18.3 *q*	18.6 *q*‡	19.8 *q*‡	19.6 *q*‡	18.3 *q*	18.4 *q*
C-1′	171.5 *s*	172.9 *s*	171.3 *s*	166.3 *s*	166.4 *s*	166.5 *s*	168.5 *s*
C-2′	81.3 *s*	81.8 *s*	81.3 *s*	83.0 *s*	82.9 *s*	126.9 *s*	60.0 *s*
C-3′	59.6 *d*	68.6 *d*‡	59.5 *d*	56.1 *d*	56.8 *d*	140.3 *d*	60.5 *d*
C-4′	19.0 *q*‡	17.7 *q*	19.9 *q*‡	20.2 *q*‡	20.4 *q*‡	20.5 *q*	13.8 *q*
C-5′	65.3 *t*	64.7 *t*	65.2 *t*	61.9 *t*	61.4 *t*	15.8 *q*	18.7 *q*
Acetate	—	—	—	170.5 *s*	170.8 *s*	—	—
groups	—	—	—	171.2 *s*	171.4 *s*	—	—
	--	—	—	171.6 *s*	171.4 *s*	—	—
	—	—	—	21.9 *q*	21.9 *q*	—	—
	-	--	—	21.9 *q*	21.9 *q*	—	—
	—	—	—	22.3 *q*	22.3 *q*	—	—

*Run at 22.6 MHz with TMS as an internal standard (9, 11, 13), DMSO-d_6 as an internal standard (7, 8, 10-DMSO-d_6) and D_2O as an external standard (10-$CDCl_3$). Assignments made using off-resonance decoupling experiments and by analogy with model compounds [10, 12, 25]. Data for 9 is from ref. [12], run at 67.1 MHz.

†Assignment made using single-frequency off-resonance decoupling experiments.

‡§Assignments interchangeable.

Fractions that eluted with 5% *iso*-PrOH were purified by repeated prep. TLC (silica gel, CH_2Cl_2–*iso*-PrOH, 8:1 and EtOAc–MeOH, 15:1) to give 85 mg of compound 3 as an oil. Seeding with crystals of an authentic specimen isolated from *H. mollis* [1] gave 35 mg powdery crystals, mp 132–135° (lit. 134–135° [23]). Spectral data for 3 were nearly identical to those in the literature [1, 11, 12, 23].

Other collections of *H. divaricatus* analysed by TLC (CH_2Cl_2–*iso*-PrOH, 15:1 and toluene–EtOAc, 1:1) for the presence of compounds 3 and 4 were from Tennessee (J.G. # 116 and 124), North Carolina (# 174 and 177) and New York (# 191). Neither compound 3 nor 4 was detected in extracts of any of these collections.

Extraction of H. resinosus. Leaves (3.5 kg) collected in Chatham Co., North Carolina 7–10 miles north of Pittsboro on U.S. Hwys. 15–501 by J. Gershenzon and R. M. Pfeil on 7 Sept. 1980 (J.G. # 157) were air-dried, washed with CH_2Cl_2 and worked up by standard procedures [22]. The crude syrup (14.0 g) was separated on a silica gel column (400 g) eluted with a CH_2Cl_2–*iso*-PrOH gradient in 0.5 l. fractions collected as follows: fractions 1–9 (CH_2Cl_2), 10–13 (CH_2Cl_2–*iso*-PrOH, 99:1), 14–44 (CH_2Cl_2–*iso*-PrOH, 98:2), 45–59 (CH_2Cl_2–*iso*-PrOH, 97:3), 60–71 (CH_2Cl_2–*iso*-PrOH, 19:1), 72–73 (CH_2Cl_2–*iso*-PrOH, 9:1), 74 (CH_2Cl_2–*iso*-PrOH, 3:1), 75 (Me_2CO).

Fraction 19 was purified by prep. TLC (CH_2Cl_2–*iso*-PrOH, 15:1 and toluene–EtOAc, 5:6) to yield 10 mg of 1, identified by comparison of spectral data with those in the literature [10]. Fraction 25 was purified in the same manner to give 12 mg of 2. ^1H NMR spectra (200 MHz) of 2 and an authentic sample of the 2′*S*,3′*S* diastereomer [1], run consecutively under identical conditions, showed chemical shift differences consistent with those reported previously [10].

Fractions 29–30 were purified by prep. TLC (CH_2Cl_2–*iso*-PrOH, 15:1 and EtOAc–hexane, 7:3) to give 65 mg of 3 and 36 mg of 6. Spectral data for 3 were similar to those reported previously [1, 11] and to those obtained from an authentic specimen from *H. mollis* [1]. Spectral data and the optical rotation ($[\alpha]_D + 89°$, lit. + 80.5°) for 6 were also similar to literature values [12]. Fractions 54–59 were combined and purified by prep. TLC (CH_2Cl_2–*iso*-PrOH, 15:1) to yield 175 mg of 5 and 52 mg of 7. Spectral data, mp (168–170°, lit. 170–172°) and optical rotation ($[\alpha]_D + 100°$, lit. + 97.8°) for 5 were similar to those in the literature [12].

Fractions 62–64 were separated by prep. TLC (CH_2Cl_2–*iso*-PrOH, 15:1, and EtOAc–MeOH, 50:1) to give 100 mg of 11. Prep. TLC (CH_2Cl_2–*iso*-PrOH, 10:1 and EtOAc–MeOH, 35:1 and 15:1) of fractions 65–73 afforded 150 mg of 13 and 25 mg of 8, which was further purified on a Sephadex LH-20 column eluted with CH_2Cl_2–MeOH (3:1).

Extraction of H. salicifolius. Leaves (1.7 kg) collected at the U.S.D.A. research center, Bushland, Texas on 14 October 1978 (C. E. Rogers and T. E. Thompson # 617; J. G. # 31) were air-

Table 3. ^{1}H NMR spectra of compounds 11–14*

	11 $CDCl_3$	11 C_5D_5N	12 $CDCl_3$	13 $CDCl_3$	13 C_5D_5N	14 $CDCl_3$
H-1	5.15 *br d*	5.55	5.22	5.15	5.53	5.25
H-2	4.84 *ddd*	5.27	5.71	4.80	5.23	5.76
H-3a	2.71 *dd*	2.92	2.78	2.71	2.92	2.81
H-3b	2.13 *dd*	2.47	2.23	2.13	2.46	2.24
H-5	5.03‡	5.28 *br d*	5.08‡	5.00‡	5.25 *br d*	5.08‡
H-6	5.07‡	5.45 *dd*	5.08‡	5.03‡	5.45 *dd*	5.08‡
H-7	2.99 *br ddd*	3.29	2.97	3.04	3.30	3.01
H-8	5.82 *br dd*	6.10	5.82	5.82	6.07	5.81
H-9a	3.37 *dd*	3.91	3.32	3.35	3.92	3.29
H-9b	2.17 *dd*	2.28	2.22	2.19	2.29	2.28
H-13a	6.33 *d*	6.47	6.33	6.32	6.48	6.33
H-13b	5.64 *d*	5.78	5.62	5.61	5.71	5.57
H-14a	4.26 *br d*	4.72	4.79	4.25	4.73	4.98
H-14b	3.75 *br d*	4.13	4.24	3.75	4.12	4.19
H-15†	1.71 *br s*	2.01	1.79	1.74	1.81	1.81
H-3′	6.13 *dq*	5.93	6.18	3.06 *q*	3.01	3.03
H-4′†	1.97 *br d*	2.01	2.00	1.26 *d*	1.27	1.23
H-5′†	1.86 *br s*	1.96	1.82	1.56 *s*	1.73	1.50
Acetate methyls†	—	—	1.96 *s* 2.07 *s*	—	—	2.06 *s* 2.08 *s*

*Run at 200 MHz with TMS as an internal standard. Multiplicities are similar to those in the previous column unless otherwise noted. Coupling constants for **11**, *J* (Hz): 1, 2 = 10; 1, 14a = 1, 14b = 1.5; 2, 3a = 6; 2, 3b = 10; 3a, 3b = 11; 5, 6 = 9.5; 5, 15 = 1.5; 6, 7 = 9; 7, 8 < 1.5; 7, 13a = 3.5; 7, 13b = 3.2; 8, 9a = 5.5; 8, 9b = 2; 9a, 9b = 14.5; 3′, 4′ = 7; 3′, 5′ = 2. Values for **12–14** are similar to those of **11** except in **13** and **14**: 3′, 4′ = 5.5.

†Intensity three protons.

‡Not first-order.

dried, washed with CH_2Cl_2 and worked up by standard procedures [22]. The crude syrup (8.1 g) was separated on a silica gel column (250 g) eluted with a toluene–*iso*-PrOH gradient. 500 fractions of 10 ml each were collected with a fraction collector.

The first 270 fractions were eluted with 5% *iso*-PrOH. Crystals of **15** (88 mg) formed in fractions 98–106 and crystals of **17** (45 mg) formed in fractions 112–127. Methylation of **15** and **17** with CH_2N_2 gave **16** and **18**, respectively. These compounds were identified by comparison of their physical properties and spectral data with those in the literature [3, 4, 8] and with those obtained from authentic specimens isolated from other species of *Helianthus* [3, 5]. Crystals (30 mg) of another diterpene formed in fractions 131–144 and appeared to be those of a kaurane diol, whose structure is still under investigation.

Fractions 321–350 (eluted with 20% *iso*-PrOH) were purified by prep. TLC (CH_2Cl_2–*iso*-PrOH, 20:1 and 10:1) to give 21 mg of a mixture of **3** and **6** and 40 mg of pure **3** as an oil which crystallized when seeded with an authentic specimen (mp 133–135°, lit. 134–135° [21]).

The compounds in this study were visualized on silica gel TLC plates using an acidified vanillin spray reagent [24]. The 2-hydroxy (and acetoxy) costunolides **1–8** and **10** turned blue-green, while the 2,14-dihydroxy (and acetoxy) costunolides **11**–**14** appeared violet with this reagent.

2α-Hydroxy-8-β-3′-hydroxy-2′,5′-epoxyangeloyloxycostunolide (**7**). Gum. IR ν_{max}^{Nujol} cm^{-1}: 3434, 3229, 1752 (lactone >C=O), 1745 (side chain >C=O), 1660, 1300, 1248, 1198, 1148, 1113, 1027, 965, 905, 825. EIMS (probe) 70 eV, *m/z* (rel. int.): 378 [M]$^+$ (0.2) (HRMS: $C_{20}H_{26}O_7$, 378.1675 meas., 378.1678 calc.), 363 [M – Me]$^+$ (0.4), 333 (M – C_2H_5O]$^+$ (0.8), 265 (2.0), 264 (2.4), 263 (1.5), 247 [M – $C_5H_7O_4$]$^+$ (29), 246 [M – $C_5H_8O_4$]$^+$ (13) McLafferty rearrangement and alpha cleavage of side chain, 229 (19), 228 (9), 203 (48), 202 (30), 187 (15), 175 (40), 163 (80), 135 (60), 117 (35), 107 (90), 91 (100).

Acetylation of compound **7**. Compound **7** (30 mg) was stirred in 1.4 ml pyridine and 0.7 ml Ac_2O for 18 hr at 25°. Subsequent heating at 50° for 1 hr produced no change as judged by TLC except for the addition of a non-migrating spot. After evaporation to dryness, prep. TLC (toluene–EtOAc, 5:6 and CH_2Cl_2–*iso*-PrOH, 15:1) of the crude product yielded 18 mg of **10** as a pale gum. IR ν_{max}^{Nujol} cm^{-1}: 3484, 1750 (lactone >C=O), 1735 (side chain and acetate >C=O), 1663, 1305, 1288, 1243, 1215, 1171, 1141, 1088, 1046, 1019, 973, 947, 907, 814. EIMS (probe) 70 eV, *m/z* (rel. int.): 480 [M – CH_2CO]$^+$ (20), 438 [M – $2CH_2CO$]$^+$ (6), 437 (8), 402 [M – 2HOAc]$^+$ (3), 360 [402 – CH_2CO]$^+$ (3), 288 [M – $C_9H_{14}O_6$]$^+$ (24) McLafferty rearrangement and alpha cleavage of side chain, 246 [288 – CH_2CO]$^+$ (42), 235 (45), 228 (100), 213 (55), 207 (30), 200 (48), 165 (55). CIMS (isobutane, probe) 70 eV, *m/z* (rel. int.): 505 [M + H – H_2O]$^+$ (0.4), 481 [M + H – CH_2CO]$^+$ (0.6), 463 [M + H – HOAc]$^+$ (0.4), 439 [M + H – $2CH_2CO$]$^+$ (0.2), 421 [M + H – CH_2CO – HOAc]$^+$ (0.5), 403 [M + H – 2HOAc]$^+$ (0.3), 289 [M + H – $C_9H_{14}O_5$]$^+$ (7), 271 (8), 235 (42), 229 [289 – HOAc]$^+$ (100), 211 (30).

2α-Hydroxy-8β-2′,3′,5′-trihydroxyangeloyloxycostunolide (**8**). Mp 166–168° (CH_2Cl_2–MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3469, 3230, 1750 (lactone >C=O), 1735 (side chain >C=O), 1653, 1574, 1410, 1339, 1293, 1245, 1197, 1153, 1113, 1082, 1054, 1026, 987, 966, 945, 905, 879, 859, 819. EIMS (probe) 70 eV, *m/z* (rel. int.):

246 $[M - C_5H_{10}O_5]^+$ (8) McLafferty rearrangement and alpha cleavage of side chain (HRMS: $C_{15}H_{18}O_3$, 246.1257 meas., 246.1256 calc.), 228 (2), 202 (3), 187 (1), 175 (1), 163 (2), 103 (26), 43 (100). CIMS (isobutane, probe) 70 eV, *m/z* (rel. int.): 397 $[M + H]^+$ (1.2), 379 $[M + H - H_2O]^+$ (0.3), 265 (10), 247 $[M + H - C_5H_{10}O_5]^+$ (100), 229 (97), 204 (40), 203 (80), 185 (22).

2α,14-Dihydroxy-8β-angeloyloxycostunolide (**11**). Gum. Spectral data for **11** were not reported previously [16]. IR ν_{max}^{Nujol} cm^{-1}: 3390, 1755 (lactone >C=O), 1716 (side chain >C=O), 1653, 1305, 1247, 1148, 1083, 1038, 969, 913, 890, 852, 814. EIMS (probe) 70 eV, *m/z* (rel. int.): 279 $[M - C_5H_8O]^+$ (4.3), 244 $[M - C_5H_8O_2 - H_2O]^+$ (2), (medium-mass ion series obscured in this spectrum), 83 $[C_5H_7O]^+$ (70) side chain acylium ion, 55 $[83 - CO]^+$ (70), 43 (100). CIMS (isobutane, probe) 70 eV, *m/z* (rel. int.): 363 $[M + H]^+$ (12), 345 $[M + H - Me]^+$ (10), 263 (24), 245 $[M + H - C_5H_8O_2 - H_2O]^+$ (100), 227 (20), 217 (20), 199 (11).

Acetylation of compound **11**. Compound **11** (44 mg) was stirred in 1.6 ml pyridine and 0.8 ml Ac_2O at 25° for 12 hr, then evaporated to dryness. Prep. TLC (CH_2Cl_2–*iso*-PrOH, 50:1) of the crude product yielded 35 mg of **12**, which upon recrystallization from EtOAc gave 20 mg of colorless prisms, identified as eriofertin diacetate by comparison of mp [158–160° (EtOAc), lit. 161–163° (EtOAc)] and spectral data with literature values [16]. Detailed ^1H NMR data previously unreported for **12** are listed in Table 3. IR ν_{max}^{Nujol} cm^{-1}: 1763 (lactone >C=O), 1737 (side chain >C=O), 1715 (acetate >C=O), 1663, 1647, 1367, 1300, 1292, 1249, 1231, 1153, 1143, 1086, 1044, 1026, 972, 944, 881, 849, 815. EIMS (probe) 70 eV, *m/z* (rel. int.): 446 $[M]^+$ (0.2), 404 $[M - CH_2CO]^+$ (0.1), 387 (1.3), 386 $[M - HOAc]^+$ (0.9), 344 $[M - HOAc - CH_2CO]^+$ (8), 326 $[M - 2HOAc]^+$ (12), 286 $[M - C_5H_8O_2 - HOAc]^+$ (8), 244 (42), 226 (55), 211 (32), 198 (30), 183 (20), 153 (22), 83 $[C_5H_7O]^+$ (100) side chain acylium ion, 55 $[83 - CO]^+$ (70).

2α,14-Dihydroxy-8β-(2'R,3'R)-2',3'-epoxyangeloyloxycostunolide (**13**). Gum. IR ν_{max}^{Nujol} cm^{-1}: 3411, 1760 (lactone >C=O), 1748 (side chain >C=O), 1659, 1310, 1290, 1266, 1148, 1084, 1023, 969, 914, 815. EIMS (probe) 70 eV, *m/z* (rel. int.): 378 $[M]^+$ (0.5) (HRMS: $C_{20}H_{26}O_7$, 378.1680 meas., 378.1678 calc.), 363 $[M - Me]^+$ (0.5), 360 $[M - H_2O]^+$ (0.3), 296 (0.4), 294 (0.5), 262 $[M - C_5H_8O_3]^+$ (3.8) McLafferty rearrangement and alpha cleavage of side chain, 244 $[262 - H_2O]^+$ (3), (medium-mass ion series obscured in this spectrum).

Acetylation of compound **13**. Compound **13** (50 mg) was stirred in 1.0 ml Ac_2O and 2.0 ml pyridine at 25° for 12 hr, then evaporated to dryness. Prep. TLC (CH_2Cl_2–*iso*-PrOH, 50:1 and toluene–EtOAc, 1:1) of the crude product yielded 35 mg of **14**, as a pale gum. IR ν_{max}^{Nujol} cm^{-1}: 1760 (lactone >C=O), 1740 (side chain and acetate >C=O), 1662, 1350, 1320, 1265, 1234, 1139, 1082, 1028, 974, 951, 932, 916, 886, 837, 821. EIMS (probe) 70 eV, *m/z* (rel. int.): 462 $[M]^+$ (3.6), 402 $[M - HOAc]^+$ (4), 360 $[M - HOAc - CH_2CO]^+$ (16), 342 $[M - 2HOAc]^+$ (42), 304 (7), 287 (8), 286 $[M - C_5H_8O_3 - HOAc]^+$ (11), 262 $[M - C_5H_8O_3 - 2CH_2CO]^+$ (9), 244 (85), 226 (100), 211 (70), 198 (60), 183 (22), 153 (10).

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