SESQUITERPENE LACTONES AND FLAVONOIDS FROM *HELIANTHUS* SPECIES[1]

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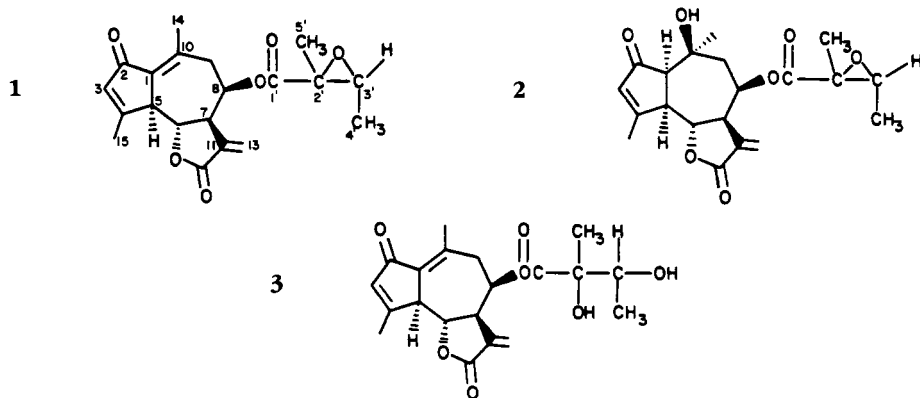*Department of Botany, University of Texas at Austin, Austin, Texas 78713*

ABSTRACT.—From the leaves and the flowers of *Helianthus glaucophyllus* and *Helianthus microcephalus*, three new guaianolides [1-3] were isolated. *H. microcephalus* afforded another two new guaianolides [4 and 5], the highly unsaturated guaianolide 6, the flavane 7, the flavone 8, and three flavonols [9-11]. Structures of all the compounds were determined by spectral analysis.

Since the late 1970s, the sunflower genus *Helianthus* (Compositae) has been a major system in our laboratory for investigating the role of terpenoids in plant-insect interactions. Up to now, we have reported on the chemistry of 18 species of the more than 50 species in this North American genus (1-3 and references cited therein). Moreover, we have described the behavioral and physiological responses of the specialist herbivore *Homoeosoma electellum* (sunflower moth) to the antifeedant secondary compounds in *Helianthus* (4-7). In a continuation of these studies, we report here the chemistry of two additional closely related species *Helianthus glaucophyllus* D.M. Smith and *Helianthus microcephalus* T.&G. Both species afforded the same three new guaianolide-type sesquiterpene lactones while the latter species yielded additional compounds.

RESULTS AND DISCUSSION

The concentrate of the CH_2Cl_2 extract of both *H. glaucophyllus* and *H. microcephalus* yielded the same three guaianolides [1-3]. The latter species also yielded another two guaianolides [4 and 5], the fully conjugated guaianolide 6, 5-hydroxy-7,4'-dimethoxyflavane [7], and four flavonoids [8-11]. The sesquiterpene lactones 1-5 were previously unreported. Compound 6, malaphyllidin, was previously isolated from *Ferula macrophylla* (8), and the flavane 7 was reported from chemical transformation (9), but apparently this is the first report of its natural occurrence. Compounds 8-11 are known.

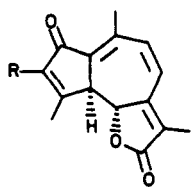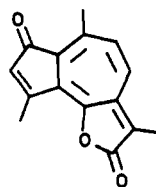Compound 1 was the major constituent of both plants. The eims of 1 showed a

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4   R=H
5   R=OH

6
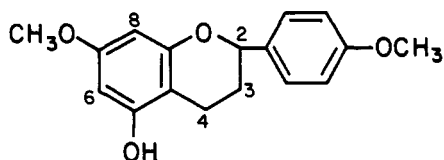
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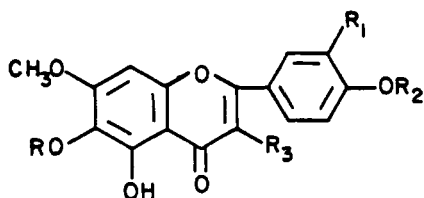
	R	R$_1$	R$_2$	R$_3$
8	H	H	H	H
9	CH$_3$	OH	CH$_3$	OH
10	CH$_3$	OH	CH$_3$	OCH$_3$
11	CH$_3$	H	CH$_3$	OH

prominent peak at m/z 358 (100%) corresponding to a molecular formula of $C_{20}H_{22}O_6$. A pair of narrowly split doublet signals (δ 6.20, J=3.2 Hz and 5.47, J=3 Hz) in its ^{1}H-nmr spectrum and the carbonyl absorption signal at 1760 cm^{-1} in its ir spectrum indicated that **1** exhibited an α-methylene γ-lactone functional group. An ir peak at 1670 cm^{-1}, a vinylic proton signal at δ 6.19, and two vinylic methyl signals at δ 2.37 and 2.31 (see Table 1) suggested the presence of another conjugated ketone carbonyl group. Additional ir peaks at 1730, 1140 cm^{-1} and ^{1}H-nmr signals at δ 2.97 (1H, q, J=5.4 Hz), 1.17 (3H, d, J=5.4 Hz), and 1.41 (3H, s) indicated the presence of an

TABLE 1. ^{1}H-nmr Data of Compounds **1-3**[a]

	Compounds			
Proton	**1** (500 MHz)	**2** (200 MHz)	**2** (C$_6$D$_6$) (500 MHz)	**3** (200 HMz)
H-1	—	2.69 d (6.5)	1.99	—
H-3	6.19 brs	6.08	5.75	6.23
H-5	3.50 brd (11)	3.21 dd (6.5,10)	2.38	3.54 brd (11)
H-6	4.06 t (11)	5.19 t (10)	4.94	4.07 t (11)
H-7	3.14 dq (3,11)	3.12 dq (4,10)	2.12	3.16 dq (3,11)
H-8	5.78 dt (3,6)	5.72 q (4)	5.23	5.71 dt (3,6)
H-9	2.75 brd (16)	1.98 dd (4,16)	1.18	2.78 brd (16)
H-9	2.80 dd (6,16)	2.34 dd (3.5,16)	1.89	2.92 dd (6,16)
H-13a	6.20 d (3.2)	6.32	6.17	6.26
H-13b	5.37 d (3)	5.55	5.12	5.57
H-14	2.37 s[b]	1.54	1.27	2.47 brs
H-15	2.31 brs	2.35	1.92	2.35
H-3'	2.97 q (5.4)	3.01	2.51	3.72 q (6.4)
H-4'	1.17 d (5.4)	1.23	1.01	1.16 d (6.4)
H-5'	1.41 s	1.49	1.24	1.21

[a]Coupling patterns and coupling constants are not repeated if identical with the preceding column. Spectra were recorded in CDCl$_3$ unless otherwise indicated.
[b]Very slightly broadened.

epoxyangelate (or epoxytiglate) side chain (1,10,11). In the ^{13}C-nmr spectrum of **1**, two characteristic carbon signals (δ 59.05 singlet and 59.44 doublet) attributable to an epoxy ring system were in accord with an epoxyangelate or epoxytiglate side chain. One ketone carbonyl signal at δ 194.71 (singlet), carbonyl signals for one lactone carbonyl, and one epoxyangelate or epoxytiglate ester (δ 169.02 and 167.67) and other carbon signals all supported the structure of **1**. In the ^{1}H-nmr spectrum of **1**, couplings between H-3 and H-15, H-5 and H-14 were observed. Double resonance experiments also confirmed all signal assignments. The chiral center at C-8 could be deduced to have the β-configuration based on the small coupling constants of H-8 (δ 5.78 dt, J=3, 6 Hz). An epoxyangelate was confirmed by nOe difference spectra which also supported the C-8β orientation of the angelate (500 MHz, CDCl$_3$): irradiation of the signal for H-3' (δ 2.97) enhanced a signal for H-5' (δ 1.41); irradiation of the signal for H-5' gave nOe to the signals for H-3', H-6, and H-14 but not the signal for H-4'. Irradiation at the signal for H-8 (δ 5.78) gave nOe to H-13b and H-7. Irradiation of the signal for H-7 (δ 3.14) enhanced the signals for H-5 and H-8. Other irradiations also supported the above assignments. Since a mixture of $2'R, 3'R$ and $2'S, 3'S$ epoxyangelate was reported from *Helianthus maximiliani* (1) and *Helianthus pumilus* (10), we could conclude that compound **1** (and also **2** and **3**) appeared to be in pure form in the present study. However, we could not assign an absolute stereochemistry to the epoxy side chain based on the nmr data (1,10). Although nOe was observed on H-6 and H-14 upon irradiation at the signal for H-5' (see the above discussion), models indicated that both diastereoisomers might give the same nOe. Therefore, a crystal sample of **1** was submitted for X-ray investigation and the results[4] proved the structure of **1** with a $2'R,3'R$ stereochemistry assignment to the epoxyangelate side chain as depicted.

Spectral properties of compound **2** indicated that it was also a guaianolide bearing an epoxyangelate side chain. The ms of **2** gave the same observable highest molecular weight fragment as **1** at *m/z* 358. However, comparison of the ^{1}H-nmr data of **1** and those of **2** indicated that one of the two vinylic methyl signals (δ 2.37) in **1** shifted upfield to 1.54 in **2**. At the same time, the signal for H-5 changed from a broadened doublet in **1** to a double doublet in **2**. Another doublet signal at δ 2.69 (J=6.5 Hz) in **2** was not observed in **1**. Comparison of the ^{13}C-nmr data of **2** with those of **1** indicated that there were two sp^2 carbon signals less in **2** than as in **1**. In contrast, another two sp^3 carbon signals appeared at δ 73.73 and 59.61. All evidence indicated that the double bond between C-1 and C-10 in **1** was opened to yield **2**. The C-1 α-proton could be deduced from the H-1 coupling with H-5 (models). A hydroxyl group at C-10 could be assigned based on the ir absorption peak at 3500 cm^{-1}, the ^{1}H-nmr signal at δ 1.54 (3H, singlet), and the ^{13}C-nmr signal at δ 73.73. The compound could be expected to lose a molecule of H$_2$O, thereby yielding the more stable structure **1** during ion formation, and thus no molecular ion was observed in its electron impact mass spectrum. The β-orientation of the C-10 hydroxyl function was assigned from nOe experiments recorded at 500 MHz in C$_6$D$_6$: irradiation of the signal for H-6 exhanced only, to some extent, a signal for H-15. Irradiation of the signal for H-5 enhanced the signal for H-1 and H-7, and irradiation of the signal for H-14 enhanced the signal for H-1 and H-9β (models indicated spatial proximity of a C-10 methyl and H-9β). Irradiation of the signal for H-1 enhanced signals for H-5, H-9α and, to some extent H-14. The spectral data for the epoxyangelate side chain are very similar to those for **1**. Therefore, we assigned a $2'R,3'R$-epoxyangelate side chain in **2** as in **1**.

Spectral analysis indicated that **3** had a very similar structure to **1**. While ^{1}H-nmr

data of **3** showed that it exhibited the same α-methylene γ-lactone function and two conjugated vinylic methyl groups as **1** had, the signals for the ester side chain in **3** were different from those in **1** (Table 1). These data together with the ir band at 3460 cm^{-1} and the mass spectral data at m/z 376 ($C_{20}H_{24}O_7$) (22%) suggested a dihydroxyangelate group in **3**. This assignment could be confirmed by analysis of the ^{13}C-nmr data of **3** in which two free hydroxy-bearing carbon signals were evident at δ 77.40 (C-2') and δ 71.43 (C-3') (see Table 2). Other signals were close to those for the relevant signals in **1**. Therefore, **3** was deduced as depicted.

TABLE 2. ^{13}C-nmr Data of Compounds **1-3**[a]

Carbon	Compounds		
	1	**2**	**3**[b]
C-1	134.16 s	59.61	133.50 N
C-2	194.71 s	206.18	195.10 N
C-3	135.53 d	134.43	135.89 P
C-4	168.70 s	178.57	168.35 N
C-5	54.50 d	48.74	55.20 P
C-6	78.37 d	76.94	78.76 P
C-7	52.61 d	53.35	53.04 P
C-8	65.36 d	67.69	66.42 P
C-9	40.65 t	46.10	40.81 N
C-10	145.35 s	73.73	145.67 N
C-11	132.41 s	132.23	132.75 N
C-12	169.02 s	168.74	169.43 N
C-13	119.99 t	121.78	121.45 N
C-14	22.76 q	31.23	23.13 P
C-15	18.67 q	18.80	19.76 P
C-1'	167.67 s	168.87	175.66 N
C-2'	59.05 s	59.61	77.40 N
C-3'	59.44 d	59.96	71.43 P
C-4'	13.33 q	13.64	16.48 P
C-5'	19.32 q	19.55	21.78 P

[a]**1** was recorded at 22.6 MHz in CDCl$_3$. Assignments were made by SFORD. **3** was recorded at 125.7 MHz and assignments were made by Attached Proton Test spectrum. **2** was recorded at 125.7 MHz. Assignments were made by comparison with data for **1** and **3**.
[b]N and P are attached proton test results:
N=negative
P=positive.

The structure of **6** (malaphyllidin) was previously reported (4). Unfortunately, the ^{1}H-nmr signal for H-3 was not reported. Signals for H-8 and H-9 appeared at different chemical shifts (δ 6.88 and 7.18 for malaphyllidin, δ 6.68 and 6.96 for **6**) while signals for the vinylic methyls appeared at δ 2.12, 2.64, and 2.70 which were very close to the relevant signals of **6**. Because solvents and instruments used to obtain the ^{1}H-nmr data for malaphyllidin were not reported, no explanation is possible here to account for the difference of the H-8 and H-9 vinylic proton signals. Therefore, decoupling experiments were conducted for **6** to confirm its structure; the ms, ir, and mp data were the same as those previously reported for malaphyllidin (8).

The structure of compound **4** was deduced based on spectral data: ms at m/z 242 (100%) corresponding to $C_{15}H_{14}O_3$, ir γ max (KBr) at 1740 (α,β-unsaturated-γ-lactone), 1670 (conjugated ketone), 3070, 1640, 1620, 1600, 1560 (conjugated double bond), and 1430 cm^{-1}. A guaianolide skeleton could be assigned when the ^{1}H-nmr

data of **4** were compared with those of **1-3**, as well as those of malaphyllidin. Its structure could be confirmed by decoupling experiments: irradiation of the broad doublet signal at δ 3.39 (H-5) collapsed the vinylic methyl signals at δ 2.46 (H-14) into a sharp singlet and δ 2.32 (H-15) into a doublet. Irradiation of the signal at δ 4.84 (H-6) collapsed signals at δ 3.39 (H-5) into a broadened singlet, and vinylic methyl signals at δ 2.01 (H-13) into a singlet. Irradiation of the signal at δ 6.59 collapsed the doublet at δ 6.25 (J = 12 Hz). Irradiation of the signal at δ 6.28 (H-3; however, this irradiation may also affect the δ 6.25 signal for either H-8 or H-9) collapsed signals at δ 6.59 (J = 12 Hz), and also collapsed the vinylic methyl signal at δ 2.32 (H-15) into a doublet. The *trans*-relationship of H-5 and H-6 followed from their large coupling (11.5 Hz). Therefore, the structure of **4** could be assigned as depicted.

[1]H-nmr data for compound **5** indicated that **5** had a similar guaianolide skeleton to that of **4** (see Table 3). The H-3 signal exhibited by **4** was not observed for **5**, but in contrast, a hydroxyl absorption band at 3300 cm^{-1} in the ir spectrum of **5** was observed. These data suggested that H-3 in **4** was substituted by a hydroxyl group to yield **5**. This suggestion could be confirmed by the ms data which had a base peak at m/z 258 for $C_{15}H_{14}O_4$. Complete decoupling experiments were conducted on **5** which confirmed its structure and all signal assignments.

TABLE 3.   [1]H-nmr Data of Compounds **4-6**[a]

Protons	Compounds		
	4	**5**	**6**
H-3	6.28 q (1.3)	—	6.21 q (1.3)
H-5	3.39 brd (11.6)	3.28	—
H-6	4.84 dq (1.8, 11.5)	4.77	—
H-8,9	6.25 d (12)	6.25	6.68
	6.59 d (12)	6.62	6.96
H-13	2.01 d (1.8)	2.01	2.66 s
H-14	2.46 d (0.9)	2.20	2.09 s
H-15	2.32 t (1)	2.51 s	2.63 d (1.4)

[a]Recorded at 200 MHz, CDCl$_3$ with TMS as internal standard. Coupling patterns and coupling constants (in parenthesis) are not repeated if identical with the preceding column.

[1]H-nmr, ir, and ms data of **7** indicated that **7** was a flavane with a hydroxyl group at C-5 and methoxyl groups at the C-7 and the C-4' positions. The double resonance experiments confirmed the signals at δ 2.15 and 2.00 attributed to H-3, the signals at δ 2.75 and 2.62 to H-4, and the signal at δ 4.91 assignable to H-2. The coupling constants of H-2 indicated that the B-ring was equatorially substituted at C-2. In our survey of the literature, we found two papers (9, 12) describing different preparations of (2S)-5-hydroxy-7,4'-dimethoxyflavane. Unfortunately, the mp for the same (2S)-flavane reported were quite different [134° (9) and 167-169° (12), respectively]. In any case, our compound exhibited a mp of 134°. Structures of the known flavonoids of ladanetin [**8**], eupatin [**9**], casticin [**10**], and mikanin [**11**] were determined by standard procedures (13).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on an Electrothermal Melting Point Apparatus (capillary) and are uncorrected. Ir spectra were measured on a Beckman Acculab-8 Infrared Spectrometer. Mass spectra were obtained with a Dupont model 21-490 mass spectrometer

operating at 70 eV. The ^{1}H-nmr spectra were recorded with GN-500 (500 MHz) or NT-200 (200 MHz) machines. The ^{13}C-nmr data were recorded on a GN-500 (operating at 125.7 MHz) or a Bruker (22.6 MHz) nmr instrument. Merck silica gel (70-230 mesh) was used for column chromatography and Merck tlc silica gel 60 F254 was used for tlc.

PLANT MATERIAL.—*H. glaucophyllus* was collected by Dr. Jim Wallace and Dr. J. Dan Pittillo in Cullowhee, North Carolina on North Country Club Dr., one mile from the campus of Western Carolina University in July 1983. A voucher prepared by Alan Whittemore in June 1986, is deposited in the Plant Resources Center of The University of Texas at Austin. *H. microcephalus* was collected by Dr. Jonathan Gershenzon on August 29, 1982, by State Highway 46, east of Bloomington, Indiana, 0.3 mile west of West Entrance Road to Brown County State Park, 2.5 miles west of Nashville, south side of road. A voucher specimen (J.G. #246) is deposited in the Herbarium of The University of Texas at Austin.

ISOLATION AND PURIFICATION OF THE COMPOUNDS.—The air-dried leaves and flowers of *H. glaucophyllus* (357 g) were extracted with CH_2Cl_2 twice (4 liters, 20 min). The combined extracts were evaporated to yield a dark-brown residue. The waxy substance was precipitated by Me_2CO. By filtering through celite to remove the precipitate, the solution was evaporated to yield 6.4 g crude material. The crude material was passed through a Si gel column eluted with a hexane/EtOAc gradient solvent system starting with 100% hexane with increasing amounts of EtOAc to 100%; the column was finally washed with 10% MeOH in EtOAc. The sesquiterpene lactones (in the eluate containing 15% EtOAc to 30% EtOAc) were further separated and purified by Sephadex LH-20 columns using cyclohexane-CH_2Cl_2-MeOH (7:4:1) as eluent. Compounds 1 (600 mg), 2 (8 mg), and 3 (15 mg) were obtained.

The air-dried leaves and flowers of *H. microcephalus* (2100 g) were extracted with CH_2Cl_2 (12 liters × 2). The same workup procedures and silica gel column chromatography as described above were employed. After repeated column chromatography over Sephadex LH-20, the following compounds were obtained: 1 (2.5 g), 2 (45 mg), 3 (36 mg), 4 (11 mg), 5 (7 mg), 6 (18 mg), 7 (180 mg), 8 (130 mg), 9 (160 mg), 10 (9 mg), 11 (79 mg).

Compound 1.—Colorless prisms from CH_2Cl_2/cyclohexane, mp 176-177°; ir ν (KBr) 1760 (γ-lactone), 1730 and 1140 (COOR, side chain), 1670 (C=O), 1620, 1610 (C=C) cm^{-1}; eims (probe) 70 eV m/z (rel. int.) 358 [M]$^+$ ($C_{20}H_{22}O_6$) (100), 343 [M-Me]$^+$ (2), 243 [M-$C_5H_7O_3$]$^+$ (57), 242 [M-$C_5H_8O_3$]$^+$ (55), 43 (80); ^{1}H-nmr data in Table 1 and ^{13}C-nmr data in Table 2).

Compound 2.—Ir ν max (KBr) 3500 (OH), 1760 (br. 2×C=O, γ-lactone and ester side chain), 1690 (C=O), 1640, 1630 (C=C), 1160 cm^{-1}; eims (probe) 70 eV m/z (rel. int.) 358 [M-H_2O]$^+$ ($C_{20}H_{24}O_7$=376) (17), 250 [M-$C_5H_8O_3$]$^+$ (8), 242 [M-H_2O-$C_5H_8O_3$]$^+$ (46), 43 (100); nmr data in Tables 1 and 2).

Compound 3.—Ir ν max (KBr) 3460 (OH), 1770 (γ-lactone), 1750 and 1150 (COOR, side chain), 1690 (C=O), 1640, 1620 (C=C) cm^{-1}; eims (probe) 70 eV m/z (rel. int.) 376 [M]$^+$ ($C_{20}H_{24}O_7$) (22), 358 [M-H_2O]$^+$ (18), 244 [M-$C_5H_8O_4$]$^+$ (97), 243 [M-$C_5H_9O_4$]$^+$ (86), 242 [M-$C_5H_{10}O_4$]$^+$ (34), 229 [244-Me]$^+$ (53), 43 (100); nmr data in Tables 1 and 2.

Compound 4.—Ir ν max (KBr) 1740 (α,β-unsaturated-γ-lactone), 1670 (conjugated ketone), 3070, 1640, 1620, 1600 and 1560 (conjugated double bonds), 1430, 1380 (methyls), 1320, 1280, 1240, 1200, 1080, 1050, 1010, 750 cm^{-1}; ms m/z 242 (M$^+$) (100%, $C_{15}H_{14}O_3$), 227 (M$^+$-Me); ^{1}H-nmr data in Table 3.

Compound 5.—Ir ν max (KBr) 3300 (OH), 1740 (γ-lactone), 1680 (conjugated ketone), 3000, 1650, 1630, 1600, 1560 cm^{-1} (conjugated double bonds); eims m/z 258 (M$^+$) (100%, $C_{15}H_{14}O_4$) 243 (M$^+$-Me); ^{1}H-nmr data in Table 3.

5-Hydroxy-7,4'-dimethoxyflavane [7].—Colorless needles from hexane/CH_3Cl, mp 134°; ir ν max (KBr) 3380, 3010, 1620, 1600, 1520, 1500, 1470, 1450, 1430, 1380, 1350, 1270, 1230, 1200, 1140, 1110, 1080, 1050, 840, 830, 800 cm^{-1}; eims m/z 286 (M$^+$) (100%) ($C_{17}H_{18}O_4$), 269 (M$^+$-OH), 255 (M$^+$-OMe); ^{1}H-nmr (200 MHz, CDCl$_3$) δ 7.30 (2H, d, J=8.7 Hz, H-2',6'), 6.84 (2H, d, J=8.4 Hz, H-3',5'), 6.10 (2H, q, J=2.3 Hz, H=6,8), 4.91 (1H, dd, J=2.5, 10 Hz, H-2 axial), 2.75 (1H, m, H-4 equatorial), 2.62 (1H, m, H-4 axial), 2.15 (1H, m, H-3 equatorial), 2.00 (1H, m, H-3 axial), 3.79 and 3.75 (3H each, OMe).

ACKNOWLEDGMENTS

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