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GERMACRANOLIDES IN *HELIANTHUS MOLLIS*

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Key Word Index—*Helianthus mollis*; Heliantheae; Compositae; sesquiterpene lactones; germacranolides; mollisorin-A; mollisorin-B; eupaserrin; desacetyleupaserrin; ^{1}H NMR; ^{13}C NMR.

Abstract—Desacetyleupaserrin, eupaserrin and two new germacranolides, mollisorin-A and mollisorin-B, were isolated from *H. mollis*. ^{1}H NMR and ^{13}C NMR data and chemical interconversions of the four germacranolides are described.

INTRODUCTION

In connection with a broad biochemical systematic investigation of the genus *Helianthus* (Tribe Heliantheae, Family Compositae), we describe here the isolation and characterization of four sesquiterpene lactones from *H. mollis*. Two of the lactones were identified as desacetyleupaserrin (**1**)[1] and eupaserrin (**2**)[1], the latter being also recently isolated from *H. pumilus* [2]. The other two germacronolides, 2α-hydroxy-8β-tiglyloxycostunolide (mollisorin-A, **3**) and 2α-hydroxy-8β-epoxyangelyloxy-costunolide (mollisorin-B, **8**), are new. Previous studies of sesquiterpene lactones from species of *Helianthus* include the germacranolides heliangine [3, 4] and ciliarin [5] from *H. tuberosus* and *H. ciliaris*, respectively.

RESULTS AND DISCUSSION

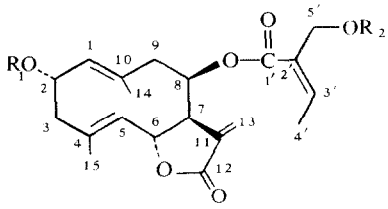
From the $CHCl_3$ extract of *H. mollis* [6], four sesquiterpene lactones were isolated by Si gel column chromatography and repeated PLC (Si gel). The most polar and most abundant compound ($C_{20}H_{26}O_6$, M^+ = 362; 0.12% yield from dried plant material) was a relatively unstable oil at room temperature which crystallized only when seeded with an authentic sample of desacetyleupaserrin. ^{1}H NMR data including extensive decoupling experiments (Table 1) as well as IR, mp and mmp against an authentic specimen confirmed that **1** was desacetyleupaserrin.

The spectroscopic data for a slightly less polar compound indicated that it was eupaserrin (**2**)[1]. This was confirmed when acetylation of **1** in Ac_2O/K_2CO_3 gave **2** along with a second monoacetate (**5**) and a diacetate (**6**).

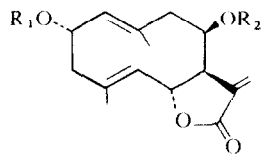
The ^{1}H NMR spectrum in $CDCl_3$ of the third compound (**3**, *m/e* 346.177; calculated for $C_{20}H_{26}O_5$, 346.178) showed a characteristic pair of doublets at δ 6.32 and 5.63 for H-13a, b. An IR absorption at 1755 cm^{-1} also supported the presence of an α-methylene-γ-lactone moiety. The ^{1}H NMR spectrum in C_6D_6 was especially useful since it exhibited well separated and assignable signals (Table 1). The presence of a tiglic ester side chain was indicated by the signals at δ 6.82 (1H, *brq*, J = 7.0, ~1), 1.24 (Me, *dq* J = 7.0, ~1) and 1.45 (Me, *brs*) as well as IR (1720 cm^{-1}). In addition, the base peak in the MS came at *m/e* 83.049 in agreement with the fragment $\dot{O}\equiv C-C(Me)=CHMe$ (calculated 83.049). The broadened double doublet at δ 5.68 (J = 6.0, 3.0) was typical for H-8 in germacranolides which have a C-8β-acyloxyl group, e.g. epitulipinolide [7] and eupatoriopicrin [8]. The small coupling (~2 Hz) between H-7 and H-8 was also indicative of the β-orientation of the C-8 acyloxyl group. The presence of a hydroxyl group in **3** was indicated by an IR adsorption at 3450 cm^{-1}.

From the spectral data discussed above, it appeared that **3** differed from **1** and **2** in the nature of the side chain ester function. This was confirmed when the desacyl derivative obtained from **3** by alkaline hydrolysis was found to be identical with 2α,8β-dihydroxycostunolide obtained by the hydrolysis of desacetyleupaserrin (**1**). The absence of geminal coupling between H-13a and H-13b in **4** also supported a β-orientation for the C-8 hydroxyl group [9]. The ^{13}C NMR spectrum of **3** was consistent with having a tiglic ester function at C-8 (Table 2) [10–14]. From these spectral data and chemical transformations, **3** was confirmed to be 2α-hydroxy-8β-tiglyloxycostunolide (mollisorin-A).

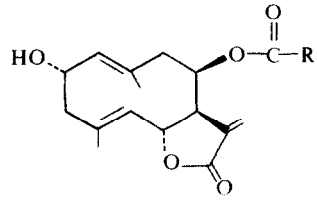
The fourth compound, mollisorin-B (**8**) (found for $C_{20}H_{26}O_6$, M^+, *m/e* = 362.174; calculated 362.173) gave a typical ^{1}H NMR spectrum for a germacranolide skeleton with 2α-hydroxyl and 8β-acyloxyl groups (Table 1). The nature of the ester side chain was also evident from the ^{1}H NMR data: a three-proton doublet at δ 1.26 exhibiting coupling with a one-proton quartet at δ 3.06 and a three-proton singlet at δ 1.52 were typical for either an epoxyangelic or epoxytiglic ester moiety. The MS of **8** showed abundant fragments at *m/e* 116.046 (calculated for $C_5H_8O_3$, 116.047) and *m/e* 99.045 (calculated for $C_5H_7O_2$, 99.045) in accord with the presence of the ester side chain. The ^{13}C NMR spectrum also exhibited signals for five carbon atoms whose chemical shifts coincided with published data for an epoxyangelic ester [15]. Therefore, on the basis of these data, mollisorin-B was assigned structure **8**. This assignment for the epoxy ester was favored since, with the exception of maculatin whose

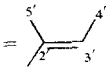


1 $R_1 = R_2 = H$
2 $R_1 = H$; $R_2 = Ac$
5 $R_1 = Ac$; $R_2 = H$
6 $R_1 = R_2 = Ac$



4 $R_1 = R_2 = H$
7 $R_1 = R_2 = Ac$



3 R =  (mollisorin-A)

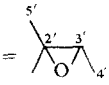
8 R =  (mollisorin-B)

Table 1. ^1H NMR data (ppm) for compounds **1–8***

Compound		**1**	**2**	**3**		**4**	**5**	**6**	**7**	**8**	
Solvent		$CHCl_3$	$CDCl_3$	$CDCl_3$	C_6D_6	Me_2CO-d_6	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	C_6D_6
1-H	*bd*†	5.05	5.06	5.06	4.76	5.04	5.04	5.04	5.02	5.08	4.66
2-H	*dt*	4.84	4.78	4.77	4.39	4.74	5.71	5.70	5.70	4.79	4.33
4-CH_3	*bs*	1.53	1.65	1.52	1.30*‡	1.70	1.63	1.63	1.66	1.59	1.37‡
5-H	*bd*	5.12	5.00	5.03	4.64	4.95	5.04	5.04	5.02	5.08	4.56
6-H	*dd*	5.20	5.16	5.16	5.06	5.24	5.20	5.18	5.13	5.15	5.02
7-H	*m*	2.95	3.02	3.0	2.74	2.96	3.00	3.00	2.92	2.98	2.50
8-H	*m*	5.88	5.90	5.82	5.63	4.72	5.90	5.91	5.77	5.85	5.50
9-Ha	*dd*	2.76	2.78	2.78	2.47	2.66	2.79	2.78	2.78	2.77	2.49
9-Hb	*dd*	2.38	2.40	2.33	1.83	2.31	2.38	2.40	2.32	2.09	§
10-CH_3	*bs*	1.86	1.82	1.81	1.64‡	1.73	1.84	1.85	1.84	1.82	1.47‡
13-Ha	*d*	6.33	6.35	6.32	6.27	6.19	6.36	6.35	6.34	6.36	6.22
13-Hb	*d*	5.65	5.65	5.63	5.31	5.66	5.66	5.64	5.63	5.65	5.20
3′-H	*bq*	6.42	6.57	6.83	6.82		6.44	6.58		*q* 3.06	*q* 2.57
3′-CH_3	*bd*	2.02	2.15	1.81	1.36		2.05	2.15		*d* 1.26	*d* 0.97
5′-Ha	*bd*	4.39	4.89				4.34	4.90			
5′-Hb	*bd*	4.22	4.55				4.20	4.56			
2′-CH_3				*bs* 1.81	*bs* 1.45‡					*s* 1.52	*s* 1.28
5′-OAc	*s*		2.00					2.07‡			
2-OAc	*s*						2.07	1.95‡	2.08		
8-OAc	*s*								2.08		
Coupling constants (Hz)											
$J_{1,2}$		10.0	9.0	10.0	10.0	10.0	10.0	10.0	8.0	10.0	9.5
$J_{2,3a}$		10.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.5
$J_{2,3b}$		6.0	5.5	6.0	6.0	5.0	6.0	6.5	5.5	7.0	5.5
$J_{5,6}$		10.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.5
$J_{6,7}$		7.0	7.0	7.0	8.0	7.0	6.0	6.0	6.5	7.0	8.0
$J_{7,8}$		~2	~2	~2	~2	~2	~2	~2	~2	~2	~2
$J_{7,13}$		3.5	3.5	3.5	4.0	3.5	3.5	3.5	3.5	3.5	3.5
$J_{8,9a}$		6.0	5.0	5.5	6.0	5.5	6.0	6.0	6.0	5.0	6.0
$J_{8,9b}$		3.0	3.0	2.0	3.0	2.0	2.0	2.5	3.0	2.0	§
$J_{9a,b}$		13.0	12.5	13.5	10.0	13.0	13.0	12.0	13.0	10.5	11.0
$J_{3',4'}$		8.0	7.0	6.5	6.5		7.0	7.0		6.0	5.0
$J_{5'a,b}$		13.0	10.5				13.0	13.0			

* Recorded at 100 MHz with TMS as internal standard. Signal assignments were confirmed by extensive decoupling experiments on compound **1**; the other assignments were interpreted by analogy to **1**.

† Multiplicities of the signals are virtually the same for **1–8** unless otherwise specified.

‡ Assignments can be interchanged.

§ Indistinguishable due to overlap with 3-H signals.

Table 2. ^{13}C NMR data (ppm) for compounds **1**–**4** and **8***

Carbon No.	Compounds 1	2	3†	4	8
C-1	*d*‡ 129.4	*d* 129.6	129.6	*d* 130.8	*d* 129.3
2	*d* 69.3	*d* 69.4	69.3	*d* 69.4	*d* 69.3
3	*t* 48.8	*t* 48.8	48.8	*t* 49.8	5 48.8
4	135.1	135.1	135.4	142.7	134.6
5	*d* 134.2	*d* 134.3	134.1	*d* 134.2	*d* 134.6
6	*d* 75.9	*d* 75.5	75.7	*d* 75.6	*d* 75.3
7	*d* 53.1	*d* 53.2	53.3	*d* 54.7	*d* 52.9
8	*d* 71.8	*d* 71.5	71.4	*d* 72.1	*d* 72.6
9	*t* 44.0	*d* 44.2	44.1	*t* 48.4	*t* 44.8
10	136.6	136.6	136.6	136.4	136.6
11	141.1	143.1	138.9	140.3	143.2
12	169.9	170.7	169.5	170.6	169.3
13	*t* 121.6	*t* 121.3	121.5	*t* 119.9	*t* 121.5
14	*q* 18.8§	*q* 18.8§	20.7§	*q* 20.8§	*q* 20.0§
15	*q* 15.9§	*q* 16.1§	18.7§	*q* 18.7§	*q* 19.4§
1′	166.0	169.5	166.7		169.3
2′	131.6	126.9	128.1		59.6
3′	*d* 143.1	*d* 147.8	142.7		*d* 60.0
4′	*q* 19.8	*q* 19.9	12.2		*q* 13.9
5′	*t* 64.3	*t* 65.5	14.6		*q* 18.7
6′		164.5			
7′		*q* 20.8			

* Recorded at 22.6 MHz in $CDCl_3$ (except for **4** which was run in Me_2CO-d_6) with TMS as internal standard. Assignments were made by comparing chemical shifts to published data and confirmed by off-resonance partially decoupled spectra.

† Not confirmed by off-resonance analysis due to the small quantity of the compound. Signals were assigned by analogy to the others.

‡ These are multiplicities determined by off-resonance partially decoupled spectra. Signals without indication appeared as singlets.

§ Assignments can be interchanged.

side chain ester was reported to be epoxytiglic ester [16], all the known C-5 epoxy esters of germacranolides are epoxyangelic esters [15, 17–20]. However, since *H. mollis* contains compounds bearing both types of side chains (i.e. **1**, **2** and **3** whose side chain esters are either sarracinate (angelate) or tiglate types), it cannot be rigorously established that **8** was derived from an angelate precursor.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were measured at 100 and 22.6 MHz, respectively. Mps were determined on a Fischer–Johns melting point block and are uncorr. Analytical TLC were run on Si gel 60-GF_{254}, PLC were done on Si gel 60-GF_{254} (1.0 mm). Si gel 60 (70–230 mesh, Merck) was used for CC separations. MS were recorded by direct inlets with 70 eV ionization.

Extraction and separation. Air-dried *Helianthus mollis* Lam. (1260 g) (collected by Lowell Urbatsch in Lamar County, Mississippi, 1 mile S of jct of Hwy 98 and 509, Voucher No. U-2749 deposited in the L.S.U. Herbarium) was extracted with $CHCl_3$. The $CHCl_3$ extract was worked up by standard procedures [6] to give 10.5 g of crude syrup. 9.80 g of the syrup were chromatographed on a Si gel column (250 g) using a $CHCl_3$–EtOAc gradient elution system. The $CHCl_3$–EtOAc (1:1) fractions gave crystals on concn which, when purified on PLC ($CHCl_3$–MeOH, 15:1), afforded 22.9 mg of eupaserrin (**2**). The oily portions of the above fractions were purified by repeated PLC to give 28.9 mg of mollisorin-A (**3**) and 10.5 mg of mollisorin-B (**8**). The $CHCl_3$–EtOAc (1:4) fractions gave desacetyleupaserrin (**1**) as a colorless oil (*ca* 1.5 g) which crystallized when seeded with an authentic specimen. Eupaserrin (**1**) was unstable on standing at room temp.

Desacetyleupaserrin (**1**). Mp and mmp 132–133° (lit. 134–135°). MS: *m/e* (rel. int.): 362 (0.1), 347 (0.2), 246 (10), 163 (45), 135 (35), 99 (100). IR ν_{max}^{Nujol} cm^{-1}: 3300, 3200, 1750, 1720, 1690, 1660, 1240 and 820; identical with the spectrum of an authentic specimen.

Eupaserrin (**2**). Mp and mmp 154–155° (lit. 153–154°). MS: *m/e* (rel. int.): 404 (0.1), 389 (0.1), 163 (35), 141 (100). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1755, 1735, 1680, 1620 and 1225; identical to spectrum of an authentic specimen prepared from **1**.

Mollisorin-A (**3**). Colorless oil (M^+ *m/e* = 346.1769 calcd for $C_{20}H_{26}O_5$: 346.1780, 0.3%) and *m/e* (rel. int.): 246 (12), 218 (10), 163 (13), 135 (13), 83 (100), 55 (40). IR ν_{max}^{Film} cm^{-1}: 3450, 1755, 1720, 1680, 1670, 1250, 1130 and 820.

Mollisorin-B (**8**). Mp 165–166°. IR ν_{max}^{Nujol} cm^{-1}: 3350, 1760, 1740, 1680, 1250, 955 and 815. MS: *m/e* (rel. int.): 362.1737 (M^+, calcd for $C_{20}H_{26}O_6$: 362.1729, 0.9), 263.1293 (calcd for $C_{15}H_{19}O_4$: 263.1283, 1), 246.1259 (calcd for $C_{15}H_{18}O_3$: 246.1256, 38), 116.0461 (calcd for $C_5H_8O_3$: 116.0473, 22), 99.0449 (calcd for $C_5H_7O_2$: 99.0446, 11.7), and 347 (0.9), 163 (88.3), 135 (62.4), 117 (37.1), 95 (100), 91 (96.6), 71 (88.3).

2α,8β-Dihydroxycostunolide (**4**). 275 mg of **1** was hydrolysed in 5% KOH–MeOH (5 ml) at room temp. for 15 hr. MeOH was removed *in vacuo* and the residue was partitioned between H_2O and Et_2O. The aq. layer was acidified to pH 1 with 5% HCl and extracted 2× with EtOAc. The combined EtOAc layer was washed with cold 2% aq. NaOH and dried over dry Na_2SO_4. Removal of the solvent gave 123 mg of an oily material

which was purified on PLC ($CHCl_3$–MeOH, 10:1) to give 58 mg of **4**, mp 157–159°. MS: *m/e* (rel. int.): 264 (M^+ = $C_{15}H_{20}O_4$, 10) 202 (12), 152 (70), 137 (42), 113 (90), 107 (100), 91 (42), 84 (50), 69 (68). IR ν_{max}^{Nujol} cm^{-1}: 3200, 1750, 1680, 1225, 998 and 810.

Acetylation of desacetyleupaserrin (**1**). 170 mg of **1** was acetylated with Ac_2O (5.7 ml) and K_2CO_3 (dry powdered, 11.3 mg) at room temp. for 2 hr. The mixture was poured into ice-H_2O and stirred at room temp. for 3 hr, then extracted 3 × with $CHCl_3$. The combined $CHCl_3$ extract was washed with H_2O, 5% K_2CO_3 and H_2O successively and dried over dry Na_2SO_4. Removal of the solvent under a reduced pres. gave 163.5 mg of yellow gum. The gummy material was purified on PLC ($CHCl_3$–MeOH, 15:1) to give 4 bands. The most non-polar band gave the diacetate (**6**) (33 mg). The second band gave the monoacetate (**5**) (22 mg). The third band gave eupaserrin (**2**, 46.5 mg). The most polar band gave the starting material (**1**, 31.5 mg). Compound **2** was recrystallized from MeOH, mp 154–154.5° (lit. 153–154°), identical by NMR, IR, mp and mmp with an authentic sample.

Eupaserrin acetate (**6**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1730, 1680, 1250, 1050 and 810.

2-Acetyl-desacetyleupaserrin (**5**). Mp 173–174° (lit. 174–176°). IR ν_{max}^{Nujol} cm^{-1}: 3500, 1765, 1735, 1720, 1680, 1250 and 1050.

2α,8β-Diacetoxycostunolide (**7**). 16.2 mg of **4** was acetylated by the usual procedure (Ac_2O–Py) to give 20.8 mg of the diacetate **7** after PLC purification ($CHCl_3$–MeOH, 10:1) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1735, 1240 and 816.

Hydrolysis of mollisorin-A (**3**). 9.9 mg of mollisorin-A (**3**) was hydrolysed in 5% KOH–MeOH at room temp. for 14 hr. The MeOH was removed *in vacuo* and the residue was worked up in the same manner as described for the hydrolysis of desacetyleupaserrin; yield: 1.0 mg of crystals identical by IR, TLC and mp with **4** prepared from desacetyleupaserrin (**1**).

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