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3-GERMACREN-6,12-OLIDES FROM AN UNDESCRIBED *LIATRIS* TAXON

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Key Word Index—*Liatris* species; *L. gracilis*; Compositae; Eupatorieae; 3-germacren-6,12-olides; eudesmanolide; sesquiterpene lactones; X-ray analysis.

Abstract—A previously undescribed variant of *Liatris gracilis* whose taxonomic status is still uncertain furnished a series of 3-germacren-6,12-olides different from the chapliatrin analogues found in authentic *L. gracilis*. The stereochemistry of one of the new sesquiterpene lactones was established by X-ray diffraction. A eudesmanolide previously isolated from *L. gracilis* was also found in the variant.

INTRODUCTION

In the course of the years one of us (RKG) has noted the occurrence on the upper slopes and rims of the so-called 'steepheads' west of Tallahassee of a *Liatris* variant somewhat similar to but different from typical *Liatris gracilis* Pursh.‡ As part of our study [1, 2] of *Liatris* species which give rise to a variety of cytotoxic and antitumour sesquiterpene lactones, it was of interest to investigate the chemistry of this variant and we now describe the isolation and structure determination of the new 3-germacren-6,12-olides **1a–c**, **2** and **3a**, **b** and the eudesmanolide **5**. The only sesquiterpene lactone common to this previously undescribed taxon and authentic *L. gracilis* collected near Tallahassee two days later [3] was the eudesmanolide **5**.

RESULTS AND DISCUSSION

We commence with **1b**, $C_{24}H_{32}O_9$, which was the major lactone constituent of the steephead material and the only one which was crystalline. It was a β-methylene-α,β-unsaturated lactone (IR bands at 1755 and 1650 cm^{-1}, narrowly split one-proton doublets at δ 6.26 and 5.65) containing an acetylsarracenyl ester side chain (partial structure **a**) as evidenced by the mass spectrum and the ^1H and ^{13}C NMR spectra (Tables 1 and 2), one additional acetate (NMR spectra) and a tertiary hydroxyl group [IR band at 3490 cm^{-1}, appearance of a carbamate resonance at δ 8.37 on addition of trichloroacetylisocyanate (TAI) unaccompanied by a paramagnetic shift of another signal in the δ 3.5–5 region].

Partial structure **b** where the carbon atoms are numbered as in the final structure was established by the usual decoupling sequence, beginning with irradiation at the frequency of the vinylic protons to establish the location of H-7. The lactone ring was closed to C-6 and an ester function was placed on C-8 because of the chemical shifts of H-6 (δ 4.81) and H-8 (δ 5.73). Partial structure **c** followed from irradiation of one of the H-2 protons at δ 2.34 which collapsed the H-3 triplet at δ 5.95 to a doublet and rendered evident the AB part of an ABX system due to H-1a and H-1b. That H-3 was vinylic was apparent from the ^{13}C NMR spectrum. Partial structure **d**, deduced from the presence of a methyl singlet of δ 1.32 and a carbon singlet at δ 71.84, was confirmed by the shift of the methyl singlet to δ 1.70 on addition to TAI. The remaining carbon atom of the basic skeleton had to be of type **e** because of the presence, in the proton spectrum, of a second AB system near δ 4.5 and, in the carbon spectrum, of a second triplet in the δ 65–70 region.

Biogenetic considerations made it logical to combine these structures as in **1b**, with the distribution of ester functions rendered probable by analogy with other germacranolides from *Liatris*. The values of $J_{6,7}$ (7 Hz) and $J_{7,8}$ (1.5 Hz) indicated that H-6 and H-7 were *trans* and H-7 and H-8 *cis*, as in the other germacranolides, although the stereochemistry at C-10 remained uncertain. To ascertain this point, to confirm the other deductions and to gain information on the conformation of 3-germacren-6,12-olides in general, an X-ray analysis of **1b** was undertaken.

Figure 1a is a stereoscopic drawing of the molecule which shows that the distribution of ester functions is as postulated, that the 3,4-double bond is *Z* and that the lactone possesses the relative stereochemistry shown in formula **1b** ($6R^*$, $7S^*$, $8R^*$, $10S^*$). Figure 1b is a side view of the atom frame work. Tables 3–5 list bond lengths, bond angles and selected torsion angles. (The lists of final atomic and final anisotropic thermal parameters are deposited at the Cambridge Crystallographic Data Centre.) The conformations of the cyclodecene ring system in the solid state and solution are probably not significantly different, to judge from the coupling constants listed in Table 1 which conform to those estimated from the figure. The five-membered lactone ring is not quite planar, with $\Sigma/\omega/$, the sum of the internal torsion angles, being 43°; it is a flattened half-chair, not an envelope, with C-6 below and C-7 above the plane of C-11, C-12 and O-2. The sign of the O-3–C-12–C-11–C-13

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‡The steephead material whose ultimate status is still uncertain is included in a general study of *Liatris* section Graminifoliae now being undertaken by R. Pilatowski, Ohio State University.

Table 1. ^1H NMR spectra (270 MHz, $CDCl_3$, TMS as int. standard unless specified otherwise*)

H	**1a**	**1a** (+TAI)	**1b**	**1b**(C_6D_6)	**1b** (+TAI)	**1c**	**1c** (+TAI)	**2**	**2**(C_6D_6)	**3a**	**3b**	**3b**(C_6D_6)	**3b** (+TAI)	**3b**(C_6D_6) (+TAI)	**4**
1a	†	†	*ca* 1.80	1.25 *m*	†	†	†	1.85§	1.57 *d* (*br*) (17, ~ 2)	†	3.83 *t* (5)	3.81 *br*	5.50 *m*	5.6 *m*	—
1b	†	†	*ca* 1.55	1.06 *m*	†	1.54 *dt* (15,4)	†		1.46 *dd* (17,9)	†	—	—	—	—	—
2a	*ca* 2.30 *m*	†	2.34 *m*	*ca* 1.90	†	2.31 *m*	†	4.80 *ddd* (9,7.5,4)	4.44 *dd* (*br*) (10,9,2)	†	*ca* 2.05	†	3.05 *dd* (17,4)	2.93 *dd* (16.5,4)	6.75 *d* (16)
2b	†	†	*ca* 1.60	1.43 *m*	†	†	†	— —	— —	†	*ca* 2.05	†	*ca* 2.4	1.96 *dd* (16.5,6)	—
3	5.95 *t* (8.5)	6.19 *dd* (11,5.5)	5.95 *t* (9)	5.50 *t*	6.01 *dd* (11,7)	5.97 *t* (8.5)	6.0 *m*	5.73 *d* (9)	5.46 *d* (10)	5.41 *t* (6.5)	5.52 *t* (7)	5.62 *t*	5.5 *m*	5.6 *m*	6.99 *dd* (16,2)
5a	2.89 *dd* (15,4.5)	3.09 *dd* (16,5)	2.93 *dd* (15.5,4)	2.48§	3.01 *dd* (16,5)	2.92 *dd* (15.5,4)	3.00 *dd* (15,5)	2.87 *dd* (15,8)	2.42 *dd* (14.5,8)	5.78 *d* (9.5)	5.76 *d* (10)	5.58 *m*	5.92 *d* (*br*) (10)	5.78 *d*	5.66 *d* (*br*) (2)
5b	2.81 *dd* (15,4)	2.81 *dd* (16,2)	2.81 *dd* (15.5,4)		2.78 *dd* (16,2)	2.80 *dd* (15.5,4.5)	2.75 *dd* (15,2.5)	2.69 *d* (*br*) (15)	2.53 *d* (*br*) (14.5)	—	—	—	—	—	—
6	4.81 *m*	4.97 *m*	4.79 *dt*	4.72 *dt*	4.89 *m*	4.82 *ddd* (7,4.5,4)	4.89 *m*	4.52 *dd* (8,6)	4.63 *m*	5.16 *dd* (9.5,4)	5.57 *dd* (10,4)	5.58 *m*	5.61 *dd* (10,4)	5.38 *dd*	5.97 *br*
7	3.68 *m*	3.54 *m*	3.66 *ddt* (7,1.5,3.5)	3.51 *dddd* (7,3.5,2.5,1.5)	3.55 *m*	3.66 *m*	3.53 *m*	3.86 *dddd* (6,3.5,2.5,1.5)	3.66 *m*	3.43 *dddd* (6,4,2.5,2)	3.34 *m*	3.21 *br*	3.50 *m*	2.34 *m*	3.34 *dt* (*br*) (3.5,2)
8	5.73 *d* (*br*) (12,2,1.5)	5.82 *d br* (11)	5.71 *dt* (12,1.5)	5.76 *ddd* (12,2,1.5)	5.82 *d* (*br*) (9.5)	5.63 *m*	5.74 *m* (11)	5.52 *ddd* (12,2.5.1.5)	5.56 *ddd* (12,2.5,2)	5.54 *ddd* (9,6,4.5)	5.60 *ddd* (8,5,4)	5.57 *dt* (7.5,4)	5.50 *m*	5.50 *m*	5.52 *dt* (10.5,3.5)
9a	†	†	*ca* 1.65	1.34 *dd* (15,12)	†	1.65 *d* (*br*) (14)	†	1.54 *dd* (15,2.5)	1.32 *dd* (14.5,2.5)	1.96 *dd* (14,4.5)	†	†	†	†	2.27 *dd* (15,3.5)
9b	2.34 *dd* (14,12)	†	2.29 *dd* (14.5,12)	1.92 *dd* (15,12)	†	2.27 *dd* (14.5,12)	†	2.27 *dd* (15,12)	2.12 *dd* (14.5,12)	2.31 *dd* (14,9)	2.26 *dd* (15.5,8)	†	*ca* 2.4	†	2.41 *dd* (15,10.5)
13a	6.26 *d* (3.5)	6.29 *d* (3)	6.28 *d* (3.5)	6.20 *d*	6.28 *d* (3)	6.29 *d*	6.28 *d*	6.30 *d* (2.5)	6.28 *d* (3)	6.32 (2.5)	6.25 *d* (3)	6.25 *d* (2)	6.39 *d*	6.25 *d* (2.5)	6.25 *d* (2)
13b	5.65 *d* (3)	5.69 *d*	5.64 *d* (3.5)	6.33 *d* (2.5)	5.63 *d* (3)	3.63 *d* (2.5)	5.64 *d*	5.68 *d* (2.5)	5.42 *d* (3.5)	5.82 *d* (2)	5.85 *d* (2.5)	5.47 *d* (2)	5.88 *d*	5.62	5.68 *d* (2)
14‡	1.32	1.69	1.32	0.87	1.70	1.33	1.67	1.46	1.03	1.26	1.22	1.06	1.80	1.62	1.43
15a	4.06 *br*§	4.66 *d* (*br*) (13)	4.53 *d* (*br*) (12.5)	4.47 *d* (*br*) (13)	4.49 *d* (*br*) (12)	4.52 *d* (*br*) (12)	4.47 *d* (*br*)	4.66 *d* (*br*) (14)	4.59 *d* (*br*) (12)	4.06 *br*	4.68 *d* (*br*) (12)	4.51 *d* (*br*) (13)	4.69 *d* (*br*) (12)	4.73 *d* (*br*) (13)	4.71 *d* (*br*) (13.5)
15b		4.59 *d* (*br*) (13)	4.45 *d* (*br*) (12.5)	4.40 *d* (*br*) (13)	4.42 *d* (*br*) (12)	4.45 *d* (*br*) (13)	4.40 *d* (*br*)	4.57 *d* (*br*) (14)	4.47 *d* (*br*) (12)		4.59 *d* (*br*) (12)	4.44 *d* (*br*) (13)	4.58 *d* (*br*) (12)	4.63 *d* (*br*) (13)	4.60 *d* (*br*) (13.5)

3′	6.44 *q* (7)	6.45 *q*	6.44 *q*	5.94 *q*	6.44 *q*	6.05 *qq* (7,1)	6.01 *dq*	6.43 *q*	5.92 *q*	6.46 *q* (7)	6.48 *q*	6.02 *q*	6.51 *q*	6.07 *q*	6.46
4′‡	2.04 *d* (7)	2.02 *d*	2.04 *d*	1.87 *d*	2.03 *d*	1.90 *dq* (7,1)	1.87 *dq*	2.01 *d*	1.86	2.04 *d* (7)	2.06 *d*	1.89 *d*	2.08 *d*	1.88 *d*	2.05 *d*
5′a	4.69 *d* (15)	4.78 *d*	4.66 *d*	4.72 *d*	4.66 *d*	1.78 quint‡ (1)	1.74 quint‡	4.69 *d*	4.66 *d* (12)	4.67 *d* (13)	4.61 *br*§	4.64 *d* (13)	4.61 *br*§	4.81 *d*	4.71 *d*
5′b	4.58 *d* (15)	4.69 *d*	4.61 *d*	4.55 *d*	4.61 *d*			4.61 *d*	4.55 *d* (12)	4.58 *d* (13)		4.70 *d* (13)		4.69 *d*	4.53 *d*
Ac‡	2.02	2.01	2.03 2.02	1.85 1.79	2.02 2.02	2.03	2.00	2.11 2.00	1.84 1.78	2.10 2.05	2.11 2.11 2.06	1.85 1.79 1.74	2.15 2.08 2.08 8.49 *br* (NH) 8.47 *br* (NH)	1.85 1.78 1.77 8.57 *br* (NH) 8.37 *br* (NH)	2.09 2.08

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*Unmarked signals are singlets. Figures in parentheses are coupling constants in Hz and are not listed if they correspond to those in the preceding column.

†In unresolved multiplets.

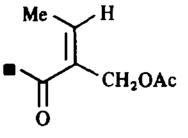
‡Intensity three protons.

§Intensity two protons (AB system).

Table 2. ^{13}C NMR spectra (67.89 MHz, $CDCl_3$, TMS as int. standard)*

C	**1a**	**1b**	**1c**	**3a**	**3b**
1	39.62 *t*‡	39.53 *t*‡	39.55 *t*‡	39.06 *t*‡	71.91 *d*†
	22.04 *t*	22.31 *t*	22.26 *t*	28.26 *t*	39.20 *t*‡
3	130.88 *d*	135.26 *d*	135.28 *d*	128.45 *d*	130.81 *d*
4	133.89	129.37	129.42	139.29	134.96
5	33.51 *t*	34.54 *t*†	34.19 *t*	72.36 *t*§	70.55 *d*§
6	77.97 *d*	77.42 *d*	77.40 *d*	73.61 *d*§	73.37 *d*§
7	46.64 *d*	46.34 *d*	46.39 *d*	48.28 *d*	48.21 *d*
8	66.89 *d*	69.22 *d*†	68.84 *d*	71.53 *d*§	71.68 *d*§
9	39.20 *t*‡	39.12 *t*‡	38.87 *t*‡	34.82‡	36.45 *t*‡
10	72.03	71.84	72.19	71.70	73.72
11	136.71	136.87	136.79	135.34	135.26
12	169.42	169.15	169.23	169.26	169.15
13	122.13 *t*	121.88 *t*	122.02 *t*	123.84 *t*	124.28 *t*
14	31.93 *q*	32.06 *q*	32.12 *q*	30.94 *q*	23.02 *q*
15	66.89 *t*	69.01 *t*†	59.16 *t*	66.39 *t*	67.23 *t*
1′	164.79	164.76	167.00	164.93	164.71
2′	127.42	127.53	127.42	127.05	126.96
3′	145.39 *d*	145.03 *d*	138.83 *d*	145.83 *d*	145.70 *d*
4′	15.86 *q*	15.82 *q*	15.83 *q*	15.88 *q*	15.83 *q*
5′	65.41 *t*	65.27 *t*†	20.79 *q*§	65.00 *t*	64.97 *t*
Ac	170.78	170.62	170.73	170.68	170.90
	20.79 *q*	170.51	20.49 *q*§	170.16	170.75
		20.73 *q* (2)		21.17 *q*	169.64
				20.92 *q*	21.03 *q*
					20.37 *q* (2)

*Unmarked signals are singlets.
†Assignment by selective decoupling.
‡§Assignments interchangeable.



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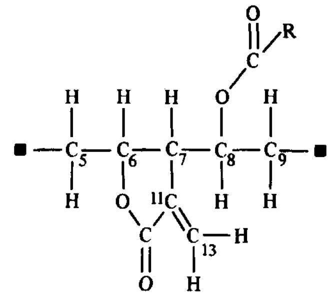

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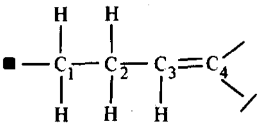

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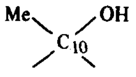

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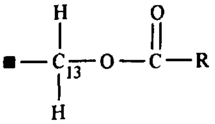

e

torsion angle (ω_2) is paired with the sign of the O-2–C-6–C-7–C-11 torsion angle (ω_3) [4] and corresponds to the sign of the weakly negative Cotton effect associated with the *n*, π^*-transition of the γ-lactone which **1b** exhibits in solution [5, 6]. This suggests that Fig. 1 also corresponds to the absolute configuration of the molecule; this is reasonable since in all sesquiterpene lactones from higher plants with established absolute configuration, H-7 is α. The torsion angles (Table 5) show that the conformation of **1b** differs from that of the silver nitrate adduct of *cis*-cyclodecene [7] and vicolide B (**6**) [8] in that the C-9–C-10 bond is twisted in the opposite direction so that C-9 lies above the plane of the molecule. This forces C-1 and C-10 into the 'waist'.

Comparison of the ^{1}H and ^{13}C NMR spectra of lactones **1a** and **1c**, isolated in much smaller amounts from the *Liatris* variant, with those of **1b** showed that the C-15 hydroxyl of **1a** was not acetylated and that the acid esterifying C-8 of **1c** was angelic acid. The molecular formula and analysis of the ^{1}H NMR spectrum of a third minor constituent **2** and spin decoupling showed that it was a derivative of **1b** with an additional hydroxyl group on C-2. H-2 at δ 4.80 (in $CDCl_3$), the X part of an ABXY system where $J_{AY} = J_{BY} = O$, was coupled to H-1a and H-1b at δ 1.85 ($J_{AX} = 7.5$, $J_{AY} = 4$ Hz) and to H-3 at δ 5.73 ($J_{XY} = 9$ Hz). Since the chemical shifts in the ^{1}H NMR spectrum of **2** are not significantly different from those in **1b**, we assume that the hydroxyl group of **2** is α-orientated and quasi-equatorial†. The coupling constants would be satisfied by either α- or β-orientation of the hydroxyl group, the model being very flexible.

The empirical formulae, analysis of the ^{1}H and ^{13}C NMR spectra and spin decoupling of the two remaining minor lactones **3a** and **3b** revealed that each carried an acetoxy group on C-5, the H-6 signal now being reduced to a *dd* and the C-5 methylene triplet being replaced by a C–O doublet. Lactone **3a** corresponded to **1a** in other respects; however, **3b** in which C-15 was acetoxylated contained an additional secondary hydroxyl group. This was demonstrated by replacement, in the ^{13}C NMR spectrum, of a second methylene triplet by a C–O doublet and by reaction with TAI which resulted in the appearance of two NH-frequencies at δ 8.57 and δ 8.37. As neither H-8 (*dt* at δ 5.75 in C_6H_6) nor H-3 (*t* at δ 5.52 in $CDCl_3$) was

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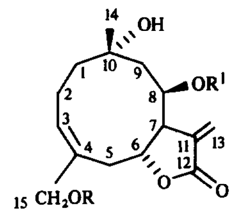
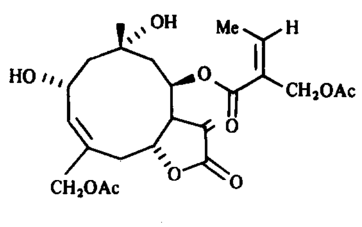
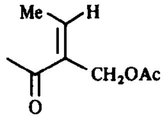
†The signals of H-3, H-6 and H-8 are *ca* 0.2 ppm upfield of H-3, H-6 and H-8 of **1b**, whereas those of H-7 and H-14 are *ca* 0.2 ppm downfield of H-7 and H-14 of **1b**. A β-orientated C-3 hydroxyl would be expected to deshield one of the H-5 and H-9 signals if the conformation of **2** resembled that of **1b**. On the whole this appears to be the case, the only significant difference in the *J* values being those involving H-5 and H-6 (see Table 1).





2

1a R=H,R^1=



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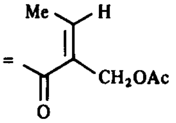

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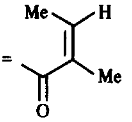
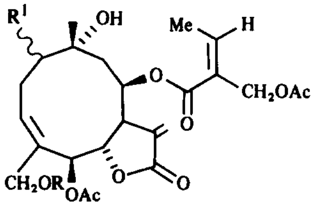
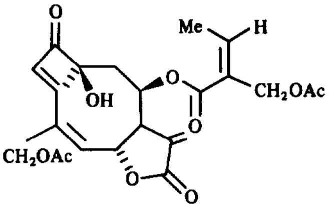




3a R,R^1=H

3b R=Ac,R^1=OH



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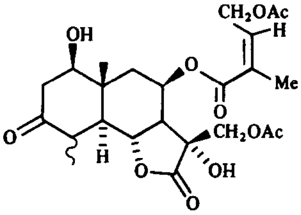

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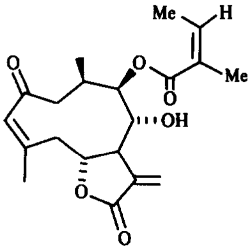

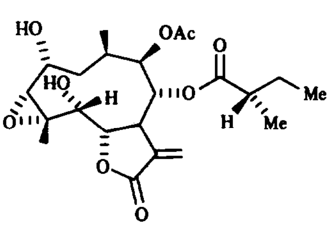

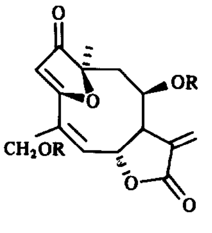

6 **7** **8**

coupled to the proton under the new hydroxyl group (*t* at δ 3.83 in $CDCl_3$), the hydroxyl had to be placed at C-1. This was confirmed as follows: (1) As a result of the reaction with TAI, the signals of H-2a, b experienced paramagnetic shifts sufficiently great to permit their observation in the ^1H NMR spectrum. Irradiation at the frequencies of H-1 and H-3 which overlapped collapsed these signals to an AB system; (2) Oxidation of **2b** with Jones reagent afforded a dienone **4**, λ_{max} 254 nm, as the result of double bond migration and elimination of the elements of acetic acid. In the ^1H NMR spectrum of **4** (Table 1) the signals of the now single and vinylic H-2

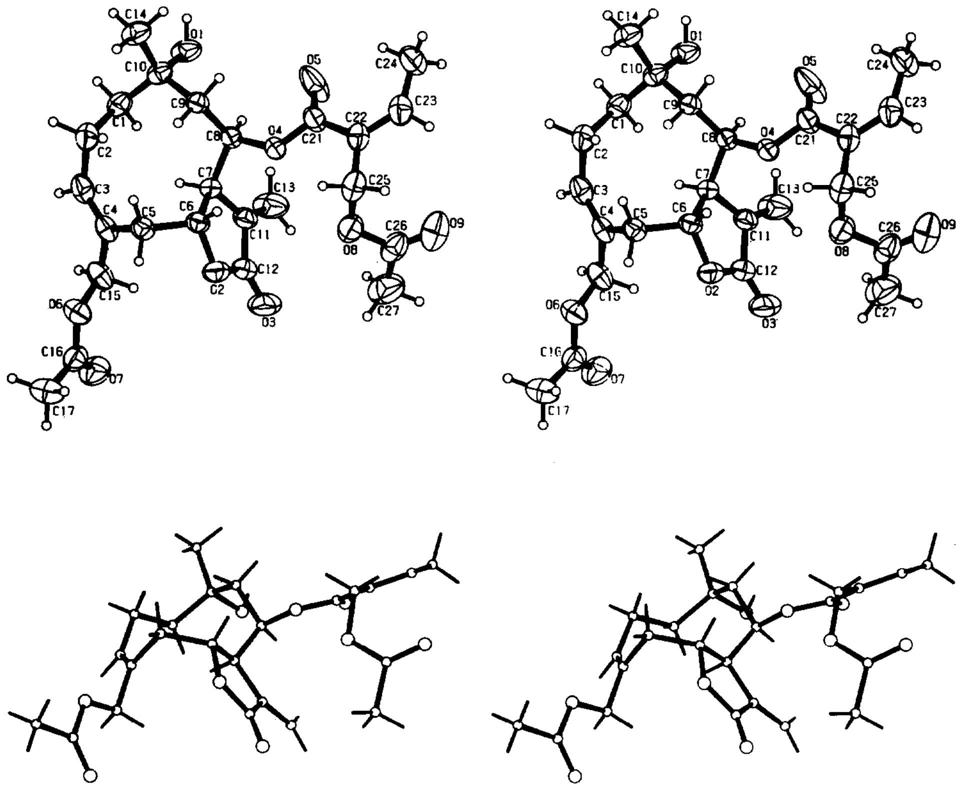

Fig. 1. Top: Stereoscopic view of **1b** molecule with ellipsoids of thermal motion; Bottom: Side view of atomic framework.

proton at δ6.75 and H-3 at δ6.99 constituted an AB system with H-3 being additionally coupled to H-5 ($J_{3,5}$ = 2 Hz). The magnitude of $J_{2,3}$ (16 Hz) indicated that the newly formed 2,3-double bond was *trans*; hence **4** is an 'open' form of a heliangolide type (**8**) frequently encountered in Helianthinae and Neurolaeninae. However the greater flexibility of the open form manifests itself in coupling constants which differ in the C-5 through C-9 portion of the ten-membered ring.*

As the H-6 signals of **3a** and **3b** are nearly 0.9 ppm downfield from the H-6 signals of **1a–c**, the acetoxyl group on C-5 must be *cis* to H-6 or β. The interaction introduced in this fashion must result in a conformational change which is reflected not only in considerably altered values of $J_{5\alpha,6\beta}$, $J_{6\beta,7\alpha}$, $J_{7\alpha,8\alpha}$, $J_{8\alpha,9\alpha}$ and $J_{8\alpha,9\beta}$ (9.5, 4, 6, 9 and 4.5 Hz vs. 4, 7, 1.5, 12 and 1.5 Hz in **1b**), but in inversion of the lactone CD from weakly negative to weakly positive. The 0.5 ppm diamagnetic shift of H-3 in **3a, b** is attributable to the 6-acetoxyl in such a changed conformation (model).† Verification of this by X-ray crystallography was unfortunately not possible as neither **3a** nor **3b** could be obtained in crystalline form. The magnitudes of $J_{1,2}$ in **3b** and the observation that the C-10 methyl is not deshielded by the hydroxyl at C-2 are compatible with either α- and β-orientation of the hydroxyl group.

Table 3. Bond lengths (A) in **1b** with standard deviations in parentheses

O(1)-C(10)	1.436(4)	C(4)-C(5)	1.515(5)
O(2)-C(6)	1.474(4)	C(4)-C(15)	1.486(5)
O(2)-C(12)	1.343(6)	C(5)-C(6)	1.527(6)
O(3)-C(12)	1.204(5)	C(6)-C(7)	1.538(5)
O(4)-C(8)	1.459(5)	C(7)-C(8)	1.520(5)
O(4)-C(21)	1.327(5)	C(7)-C(11)	1.512(5)
O(5)-C(21)	1.203(5)	C(8)-C(9)	1.523(5)
O(6)-C(15)	1.448(5)	C(9)-C(10)	1.542(5)
O(6)-C(16)	1.341(5)	C(10)-C(14)	1.522(5)
O(7)-C(16)	1.192(5)	C(11)-C(12)	1.471(6)
O(8)-C(25)	1.462(5)	C(11)-C(13)	1.294(7)
O(8)-C(26)	1.346(7)	C(16)-C(17)	1.481(7)
O(9)-C(26)	1.202(7)	C(21)-C(22)	1.482(6)
C(1)-C(2)	1.542(6)	C(22)-C(23)	1.330(7)
C(1)-C(10)	1.537(6)	C(22)-C(25)	1.495(5)
C(2)-C(3)	1.501(6)	C(23)-C(24)	1.490(7)
C(3)-C(4)	1.325(5)	C(26)-C(27)	1.485(8)

EXPERIMENTAL

Extraction of L. gracilis *variant.* Godfrey #78179 (above ground parts, wt 0.45 kg), collected on September 26, 1980, on

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*For some examples of compounds of type **8** see ref. [9].

†The conformation appears to be similar to that of vicolide C(7) [8].

Table 4. Bond angles (°) in **1b** with standard deviations in parentheses

C(6)-O(2)-C(12)	112.1(3)
C(8)-O(4)-C(21)	118.2(3)
C(15)-O(6)-C(16)	116.6(3)
C(25)-O(8)-C(26)	116.1(4)
C(2)-C(1)-C(10)	115.8(3)
C(1)-C(2)-C(3)	112.2(4)
C(2)-C(3)-C(4)	127.0(4)
C(3)-C(4)-C(5)	122.9(3)
C(3)-C(4)-C(15)	120.0(4)
C(5)-C(4)-C(15)	117.0(3)
C(4)-C(5)-C(6)	116.3(3)
O(2)-C(6)-C(5)	107.6(3)
O(2)-C(6)-C(7)	105.1(3)
C(5)-C(6)-C(7)	118.0(3)
C(6)-C(7)-C(8)	115.6(3)
C(6)-C(7)-C(11)	103.7(3)
C(8)-C(7)-C(11)	113.5(3)
O(4)-C(8)-C(7)	104.1(2)
O(4)-C(8)-C(9)	108.9(3)
C(7)-C(8)-C(9)	117.3(3)
C(8)-C(9)-C(10)	116.6(3)
O(1)-C(10)-C(1)	104.6(3)
O(1)-C(10)-C(9)	107.6(3)
O(1)-C(10)-C(14)	110.2(3)
C(1)-C(10)-C(9)	115.1(3)
C(1)-C(10)-C(14)	110.6(4)
C(9)-C(10)-C(14)	108.6(3)
C(7)-C(11)-C(12)	107.6(3)
C(7)-C(11)-C(13)	130.2(4)
C(12)-C(11)-C(13)	122.2(4)
O(2)-C(12)-O(3)	121.8(4)
O(2)-C(12)-C(11)	109.8(3)
O(3)-C(12)-C(11)	128.5(4)
O(6)-C(15)-C(4)	107.6(3)
O(6)-C(16)-O(7)	123.0(4)
O(6)-C(16)-C(17)	110.5(4)
O(7)-C(16)-C(17)	126.5(4)
O(4)-C(21)-O(5)	122.7(4)
O(4)-C(21)-C(22)	111.6(3)
O(5)-C(21)-C(22)	125.7(4)
C(21)-C(22)-C(23)	121.3(4)
C(21)-C(22)-C(25)	118.5(4)
C(23)-C(22)-C(25)	120.2(4)
C(22)-C(23)-C(24)	130.3(4)
O(8)-C(25)-C(22)	112.3(3)
O(8)-C(26)-O(9)	123.1(5)
O(8)-C(26)-C(27)	111.6(5)
O(9)-C(26)-C(27)	125.3(5)

deciduous woodland on the upper slopes and rim of a steephead near county road 270, between its junction with Florida Rt. 12 and the Sweetwater community, Liberty Co., Florida, was extracted with $CHCl_3$ and worked up in the usual fashion [10]. The crude extract (25.4 g) was absorbed on 50 g silica gel (Merck, particle size 0.063–0.2 mm) and chromatographed over 400 g of the same absorbent packed in *n*-hexane, 300 ml fractions being collected as follows: Fr. 1–3 (hexane), 4–7 (hexane–EtOAc, 19:1), 8–11 (hexane–EtOAc, 9:1), 12–15 (hexane–EtOAc, 4:1), 16–19 (hexane–EtOAc, 1:1), 20–23 (hexane–EtOAc, 1:3), 24–27 (EtOAc), 28–31 (EtOAc–MeOH, 19:1), 32–35 (EtOAc–MeOH, 3:1), 36–40 (MeOH).

Table 5. Torsion angles (°) in **1b** with standard deviations in parentheses

C(10)-C(1)-C(2)-C(3)	−132.7(4)
C(1)-C(2)-C(3)-C(4)	102.4(5)
C(2)-C(3)-C(4)-C(5)	1.8(7)
C(3)-C(4)-C(5)-C(6)	−95.9(5)
C(4)-C(5)-C(6)-C(7)	37.0(4)
C(5)-C(6)-C(7)-C(8)	102.5(4)
C(6)-C(7)-C(8)-C(9)	−63.2(4)
C(7)-C(8)-C(9)-C(10)	−82.8(4)
C(8)-C(9)-C(10)-C(1)	66.1(5)
C(9)-C(10)-C(1)-C(2)	56.8(5)
C(6)-C(7)-C(11)-C(12)	10.7(4)
C(7)-C(11)-C(12)-O(2)	−4.5(5)
C(11)-C(12)-O(2)-C(6)	−4.2(5)
C(12)-O(2)-C(6)-C(7)	11.0(4)
O(2)-C(6)-C(7)-C(11)	−12.7(4)
O(3)-C(12)-C(11)-C(13)	−2.8(8)

Fr. 10–11 were combined and gave 0.71 g lupeol. Combination of fr. 18–23 and purification by prep. TLC ($CHCl_3$–MeOH, 97:3 and 19:1) gave 0.10 g **1c**. Fractions 26–32 were combined and separated by prep. TLC ($CHCl_3$–MeOH, 19:1 or 9:1, and $CHCl_3$–Me_2CO, 7:3) to give 0.38 g **1a**, 3.5 g **1b**, 0.27 g **3b** and 0.16 g **5** which was identical with material isolated from authentic *L. gracilis* [3]. Fr. 33–36 were combined and separated by prep. TLC ($CHCl_3$–MeOH, 9:1, and $CHCl_3$–Me_2CO, 1:1) to give 30 mg of **3a** and 10 mg of **2**.

Lactone **1a** was a gum, $[\alpha]_D^{26}$ −75.1° (*c* 1.55, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1765, 1725; MS *m/z* (rel. int.): 422 $[M]^+$ (0.05), 404 (0.27), 362 (0.24), 281 (0.25), 141 (58.6), 99 (75.1) and 81 (100); ^1H and ^{13}C NMR: Tables 1 and 2. [Calc. for $C_{22}H_{30}O_8$: MW, 422.1938. Found: MW (MS, peak matching), 422.1925.]

Lactone **1b**, mp 138–138.5° (EtOAc), $[\alpha]_D^{26}$ −101.0° (*c* 1.07, $CHCl_3$); CD curve (EtOH) $[\theta]_{280}$ 0, $[\theta]_{253}$ −533 (neg. min), $[\theta]_{245}$ −480, $[\theta]_{228}$ −49500 (neg. min), $[\theta]_{223}$ −38900 (last reading); IR ν_{max}^{KBr} cm^{-1}: 3480, 2950, 1755, 1740, 1715; MS (CI) *m/z*: 465 $[M+H]^+$, 405 $[M+H^+-C_2H_4O_2]^+$; ^1H and ^{13}C NMR: Tables 1 and 2. [Calc. for $C_{24}H_{32}O_9$: MW, 464.2043. Found: MW (MS, peak matching), 464.2043].

Lactone **1c** was a gum, $[\alpha]_D^{26}$ −112.1° (*c* 0.33, $CHCl_3$); CD curve (EtOH) $[\theta]_{310}$ +110 (broad max), $[\theta]_{283}$ 0, $[\theta]_{258}$ −275 (neg. min), $[\theta]_{244}$ 0, $[\theta]_{236}$ +166 (max), $[\theta]_{206}$ −39600 (last reading); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1765, 1740; MS *m/z* (rel. int.): 406 $[M]^+$ (0.4), 388 (0.1), 346 (14.6), 246 (46) and 83 (100); ^1H and ^{13}C NMR: Tables 1 and 2. [Calc. for $C_{22}H_{30}O_7$: MW, 406.1989. Found: MW (MS, peak matching), 406.1999.]

Lactone **2** was a gum; MS *m/z* (rel. int.): 481 $[M+H]^+$ (2.3), 463 (4.0), 420 (6.2), 361 (3.3), 305 (5.8), 262 (12.8), 245 (17.8), 165 (57.9), 141 (80.7), 123 (26.4), 99 (34.4) and 81 (100); ^1H NMR: Table 1. [Calc. for $C_{24}H_{33}O_{10}$: $MW+H^+$, 481.2071. Found: $MW+H^+$ (MS), 481.2071. Calc. for $C_{24}H_{32}O_{10}$: MW, 480.1992. Found: MW (MS), 480.2022.]

Lactone **3a** was a gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1770, 1740; MS *m/z* (rel. int.): 481 $[M+H]^+$ (0.05), 421 (0.2), 360 (0.3), 323 (0.4), 322 (0.4), 305 (0.6), 141 (77.9), 99 (47.0) and 91 (100). The $[M]^+$ was too weak to be observed in the high resolution MS. ^1H and ^{13}C NMR: Tables 1 and 2.

Lactone **3b** was a gum, $[\alpha]_D^{26}$ −62.4° (*c* 1.04, $CDCl_3$), CD curve (EtOH) $[\theta]_{254}$ +385 (sh), $[\theta]_{233}$ +995, $[\theta]_{227}$ 0; $[\theta]_{223}$ −38900 (last reading); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1770, 1745; MS (EI) *m/z* (rel. int.): 539 $[M+H]^+$ (0.1), 479 (2.7), 381 (5.0), 278 (13.7), 260 (27.6), 141 (8.4.3), 99 (62.3) and 81 (100); MS (CI) *m/z* (rel. int.): 539

(12.8), 479 (100). The $[M]^+$ was too weak to be observed in the high resolution MS. ^1H and ^{13}C NMR: Tables 1 and 2.

Oxidation of **2b**. A soln of 10.2 mg **2b** in 0.75 ml of Me_2CO and one drop of Jones' reagent was allowed to stand at 0° for 5 min. Decomposition of excess reagent with 3 days of *iso*-PrOH, addition of $NaHCO_3$, filtration, dilution with a small amount of EtOH, and evaporation at red. pres. was followed by prep. TLC ($CHCl_3$–Me_2CO, 7:3 and 4:1) to give 2.4 mg of gummy **4**; UV λ_{max}^{EtOH} nm: 254 (sh, ε3260), 207 (ε20200, last reading); MS *m/z* (rel. int.): 477 $[M+H]^+$ (0.07), 416 (0.7), 373 (0.6), 357 (0.8), 258 (9.1), 172 (11.2), 141 (18.7), 99 (8.8), 81 (29) and 43 (100). Peaks $[M+H]^+$ and $[M]^+$ were too weak to be detected by high resolution MS.

X-Ray analysis of **1b**. Crystals of **1b**, prepared by slow crystallization from C_6H_6–EtOAc, were monoclinic, space group $P2_1$, with $a = 7.724$ (2), $b = 22.737$ (7), $c = 6.867$ (3) Å, $\beta = 94.66$ (3)°, and $d_{calcd} = 1.283$ g/cm^3 for $Z = 2$ ($C_{24}H_{32}O_9$, M = 464.51). The intensity data were measured on a Hilger–Watts diffractometer (Ni-filtered Cu Kα radiation, θ–2θ scans, pulse-height discrimination). The size of the crystal used for data collection was *ca* 0.50 × 0.75 × 1.0 mm. A total of 1614 independent reflections were measured for $\theta < 57°$, of which 1608 were considered to be observed $[I > 2.5\sigma(I)]$. The structure was solved by a multiple solution procedure [10] and was refined by full-matrix least squares. Six reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement, anisotropic thermal factors were used for the nonhydrogen atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are $R = 0.039$ and $wR = 0.052$ for the remaining 1602 observed reflections. The final difference map has no peaks greater than ± 0.2 eÅ^{-3}.

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