Provincialin, a Cytotoxic Germacradienolide from *Liatris Provincialis* Godfrey with an Unusual Ester Side Chain[1]

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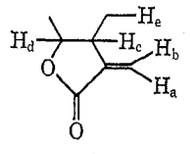
The isolation and structure determination of provincialin (1), a new cytotoxic germacradienolide from *Liatris provincialis* Godfrey, is reported. Provincialin belongs to the class of *trans*-$\Delta^{1,10}$,*cis*-$\Delta^{4,5}$-germacranolides and possesses an unusual C_{10} ester side chain made up of two C_5 acyl units. A revised stereochemistry is suggested for eucannabinolide.

In an earlier communication we reported the isolation of a new dihydrobenzofuran[2] from a chloroform extract of *Liatris provincialis* Godfrey, tribe Eupatoriae, family Compositae, a plant native to a few counties in north Florida.[3] The main component of the extract, however, was a new cytotoxic sesquiterpene lactone, provincialin, whose structure has now been elucidated as **1**.[4]

Provincialin (**1**), $C_{27}H_{34}O_{14}$, $[\alpha]D$ $-85°$, was a gum and various attempts to convert it into crystalline form proved unsuccessful. It was a conjugated γ-lactone (ir bands at 1760 and 1655 cm^{-1}; strong uv end absorption at 210 nm). The nmr spectrum exhibited the typical two doublets of H_a and H_b of partial structure A at 6.35 and 5.78 ppm, respectively. Spin-decou-



A

pling experiments involving H_a and H_b established the location of the narrowly split H_c multiplet at 2.91 ppm. Irradiation at the frequency of H_c collapsed a doublet of doublets at 5.93 ppm (J = 11.1 and 2.4 Hz) to a doublet and caused a change in the region around 5.3 ppm, where overlapping signals due to four hydrogens were located. Thus, H_d and H_e are at 5.93 and 5.3 ppm, respectively, or the reverse.

One narrowly split and one broadened three-proton signal at 1.83 and 1.77 ppm, respectively, in the nmr spectrum of provincialin indicated the presence of two vinylic methyl groups, both of which were coupled to protons giving rise to signals in the complex 5.3-ppm region. Epoxidation of **1** yielded a monoepoxide **2**, $C_{27}H_{34}O_{11}$, in whose nmr spectrum the signal at 1.77 ppm was replaced by a three-proton singlet at 1.47 ppm and one of the low-field signals was shifted upfield to 2.9 ppm.

The ir spectrum of provincialin had absorptions due to ester groups (1740 and 1730 cm^{-1}). One of the ester functions was obviously an acetate (nmr peak at 2.11 ppm). Provided that **1** was a sesquiterpene lactone, the molecular formula would require that the other ester substituent(s) comprised ten carbon atoms altogether.

Hydrolysis of **1** using methanol and K_2CO_3 afforded as the main component **3**, $C_{18}H_{26}O_6$. The nmr spectrum of **3** indicated the loss of one ester side chain; simultaneously one of the signals in the 5.3-ppm region had moved upfield to 4.07 ppm. The acetate group was still present, but the presence of a new three-proton singlet at 3.39 ppm and a two-proton signal centered at 3.67 ppm together with the disappearance of the signals due to H_a and H_b demonstrated that **3** was an 11,13-methanol adduct. These observations were supported by the mass spectrum, which displayed prominent peaks at m/e 338 (M), 306 (M − MeOH), 296 (M − ketene), 278 (M − HOAc), 260 (M − HOAc − H_2O), 246 (M − HOAc − MeOH), and 228 (M − HOAc − MeOH − H_2O). In view of the empirical formula of **3** the hydrolysis involved loss of one C_{10} side chain. Since the nmr spectra of **1** and **3** were similar in other respects, it was concluded that the hydrolysis had occurred without rearrangement.

Treatment of **3** with *m*-chloroperbenzoic acid afforded a monoepoxide **4**, $C_{18}H_{26}O_7$, whose nmr spectrum was amenable to extensive spin-decoupling experiments. Since **4** was an 11,13-methanol adduct, unambiguous identification of the signal due to H-7 (H_c) now had to be accomplished in the following manner. Irradiation at the frequency of the two H-13 protons (3.66 ppm) collapsed the H-11 multiplet to a doublet at 2.74 ppm. In turn, irradiation at the frequency of H-11 affected not only the signals due to the H-13 protons but also a multiplet located at 2.28 ppm, which could therefore be identified as the signal of H-7. In accordance with its now nonallylic nature, it was shifted upfield compared with its shift in the spectra of **1** and **2**.

In conformity with the results for **1** and **2** H-7 of **4** was coupled to a low-field doublet of doublets at 5.95 ppm, H-6 (H_d), and to a multiplet at 4.01 ppm, H-8 (H_e), ascribed to the proton on the carbon carrying the newly generated hydroxyl group. Hence, the C_{10} ester side chain was attached to C-8 in **1**. Conversely, irradiation at the frequency of H-8 collapsed the H-7 multiplet to a doublet of doublets and converted two doublets of doublets centered at 2.48 (J = 14.6 and 4.8 Hz) and 1.29 ppm (J = 14.6 and 1.9 Hz) to doublets. This indicated that H-8 was adjacent to a $-CH_2$ group, H-9a and H-9b.

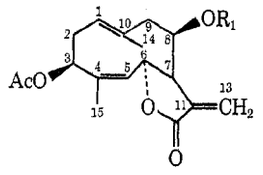
Spin-tickling experiments involving H-6, the lactone hydrogen, of **4** caused changes in another low-field doublet of doublets, H-5 (5.55 ppm; J = 11.0 and 1.2 Hz). The latter signal was collapsed into a doublet on irradiation at the frequency of the vinylic methyl doublet. In turn, the methyl doublet was

(1) (a) Part III in a series "Constituents of *Liatris* Species;" for part II, see W. Herz and I. Wahlberg, *Phytochem.*, in press. (b) This work was supported in part by U. S. Public Health Service Research Grant No. CA-13121 from the National Cancer Institute.

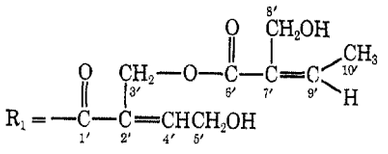
(2) W. Herz and I. Wahlberg, *Phytochem.*, **12**, 429 (1973).

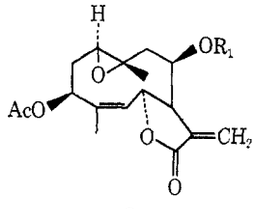
(3) R. K. Godfrey, *Amer. Midland Naturalist*, **66**, 466 (1966).

(4) Provincialin showed significant cytotoxicity (ED_{50} = 3.5 μg/ml) against cells derived from the human carcinoma of the nasopharynx (KB). Cytotoxicity was assayed under the auspices of the National Cancer Institute.



1





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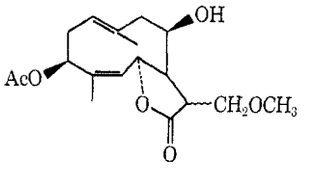

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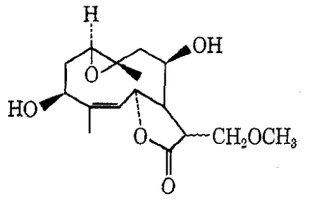

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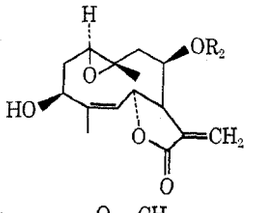
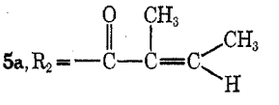
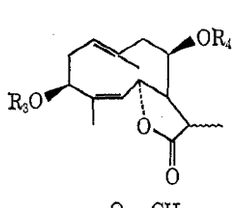
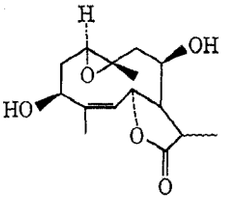


5a, R_2 =



b, R_2 =

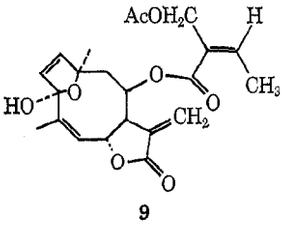




6, R_3 = Ac; R_4 =

7, R_3, R_4 = H

8



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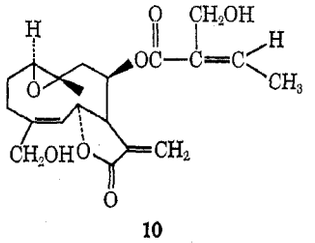

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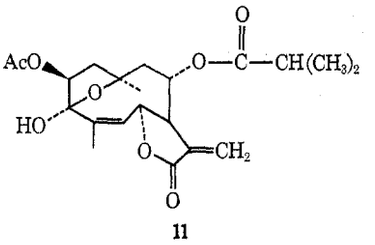

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converted into a singlet on irradiation at the frequency of H-5. Hence, **4** incorporates a 4,5 double bond and H-5 is a vinylic proton allylically coupled to the methyl group on C-4. On the basis of these spin-decoupling experiments it was concluded that the conversion of **3** into **4** involved epoxidation of the 1,10 double bond and that the 4,5 double bond, also adjacent to a methyl group, was not attacked.

The remaining low-field signal in the nmr spectrum of **4**, a narrowly split doublet of doublets at 5.23 ppm, was ascribed to a proton on a carbon carrying the acetate group. The multiplicity of the signal alone suggested that the proton was adjacent to two other protons, a requirement met if the acetate was attached to C-3 and not to C-2. This assignment was confirmed by further double irradiation experiments. Thus, the proton under the acetate grouping was coupled to two protons (H-2a and H-2b), which were represented by multiplets centered at 2.40 and 1.62 ppm. Irradiation at the frequency of each of these affected the other as well as the H-3 doublet of doublets and a doublet of doublets at 2.86 ppm due to H-1. Conversely, irradiation at the frequency of H-1 affected the signals ascribed to H-2a and H-2b and not that of H-3.

Information on the stereochemistry of the centers at C-3 and C-5 in **4** was obtained from NOE experiments. Thus, irradiation at the frequency of the vinylic methyl group at C-4 in **4** produced a 15% increase in the strength of the H-5 signal and a 9% increase in the strength of the H-3 signal. Hence, the 4,5 double bond is cis and H-3 is α oriented and cis to the methyl group at C-4, reminiscent of the stereochemistry of the corresponding centers in heliangine (**5a**)[5,6] and eriofloriin (**5b**).[7]

In fact a comparison of the nmr spectral data revealed that the coupling constants of H-3 and H-5 as well as those of H-6, H-7, and H-8 in **4**, **5a**, and **5b** were very similar, indicating that these compounds had identical stereochemistry at all these positions.

Chemical evidence for the structure and stereochemistry of the sesquiterpene portion of provincialin was finally obtained as follows. Treatment of **1** with $NaBH_4$ resulted in the reduction of the 11,13 double bond and the formation of **6**, which incorporates a modified ester side chain (*vide infra*). Hydrolysis of **6** afforded in low yield the diol **7**, $C_{15}H_{22}O_4$, which on epoxidation furnished **8**. The latter compound was identical in all respects with dihydroheliangino1, prepared from eriofloriin (**5a**) on $NaBH_4$ reduction followed by hydrolysis.

Hence, the lactone ring in provincialin is trans-fused, the ester side chains attached to C-3 and C-8 are β oriented and the 4,5 double bond is cis, whereas the 1,10 double bond is trans.

We now turn to the structure of the ester side chain attached to C-8. On the basis of the results presented earlier it was concluded that the side chain incorporated ten carbon atoms. The nmr data of pro-

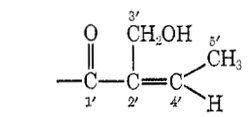
(5) H. Morimoto, Y. Sanno, and H. Oshio, *Tetrahedron*, **22**, 3173 (1966).

(6) M. Nishikawa, K. Kamiya, A. Takabatake, H. Oshio, Y. Tomiie, and I. Nitta, *Tetrahedron*, **22**, 3601 (1966). Contrary to its depiction as a *trans*-Δ^4-germacrenolide, this substance has a cis 4,5 double bond [*cf.* S. Neidle and D. Roberts, *Chem. Commun.*, 140 (1972)].

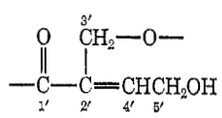
(7) S. J. Torrance, T. A. Geissman, and M. R. Chedekel, *Phytochem.*, **8**, 2381 (1969). The correlation of eriofloriin with heliangine carried out by these authors requires that eriofloriin be re-formulated as a *cis*-Δ^4-germacrenolide.

vincialin implied that these could be conveniently divided into two C_5 units. Thus, one of the units comprised a vinylic methyl group coupled to a vinylic proton (6.96 ppm, J = 7.3 Hz). This in turn was long range coupled to a two-proton broadened singlet at 4.32 ppm, which was assigned to a carbinol group, indicating that the first unit was of type B. In fact, the chemical shifts of all these protons agreed well with those published for the cis isomer of sarracinic acid.[8]

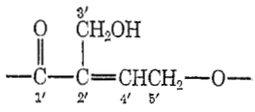
The other C_5 moiety was represented in the nmr spectrum of **1** by a two-proton doublet at 4.43 ppm coupled to a vinylic triplet at 7.02 ppm, which in turn showed a peak coupling to a two-proton signal centered at 4.93 ppm. It could therefore be formulated as C or C′. However, the relative chemical shifts of the two two-proton signals favored structure C, since the signal of the carbinol group is upfield from the signal of the hydrogens on the carbon carrying the ester group. The side chain was consequently of type D consisting of unit B esterified on C-3′ of unit C.



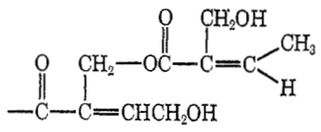
B



C

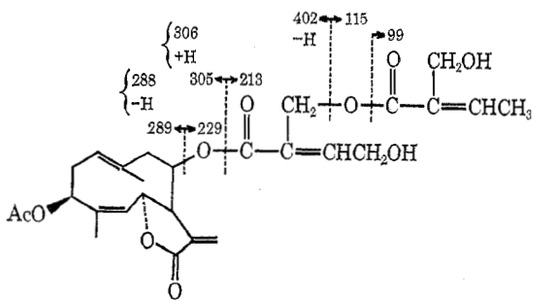


C′



D

The mass spectral fragmentation of provincialin strongly supported this formulation. The base peak in the spectrum was due to a $C_5H_7O_2$ fragment of mass 99. Furthermore, abundant ions of masses 115 ($C_5H_7O_3$) and 402 (M − $C_5H_8O_3$) confirmed that unit B of the side chain was terminal (*cf.* molecular structure below



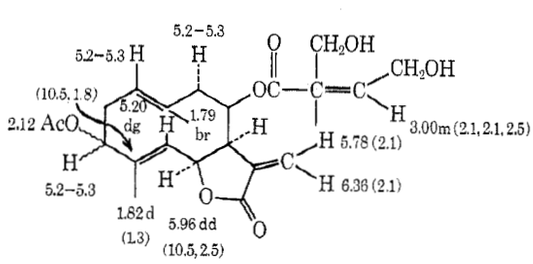
which summarizes the principal fragmentation reactions). The fragmentation also resulted in the formation of diagnostically important ions of masses 229.0711 ($C_{10}H_{13}O_6$) and 213.0788 ($C_{10}H_{13}O_5$) comprising the side chain or part of it, while charge retention on the sesquiterpene portion of the molecule led to fragments of masses 288, 289, 305, and 306. The latter ions decomposed further by elimination of ketene or acetic acid, giving rise to species of masses 228, 229, 246, 247, 264, 342, and 360.

Evidence for the structure of the side chain in **1** was also obtained by treatment of **1** with $NaBH_4$, which gave **6**, $C_{22}H_{30}O_7$. The reaction involved not only reduction of the 11,13 double bond but also simultaneous reduction of unit C and elimination of unit B of the side chain in a manner analogous to that reported for the acetylsarracinoyl residue in liatrin (**9**),[9] a conclusion which was confirmed by spectral data. The mass spectrum of **6** displayed peaks at m/e 99 ($C_5H_7O_2$), 307 (M − $C_5H_7O_2$), and 291 (M − $C_5H_7O_3$) demonstrating that the new side chain had the elemental composition $C_5H_7O_3$. Furthermore, the presence of peaks at m/e 230, 231, 248, 249, 290, and 291, shifted by two mass units compared with **1**, revealed the uptake of two hydrogen atoms by the sesquiterpene portion of the molecule. In harmony with these findings the nmr spectrum of **6** displayed a two-proton triplet at 3.70 ppm, assigned to the terminal carbinol group, now adjacent to a $-CH_2-$ group, whereas the terminal vinylic methylene group was represented by two narrowly split doublets at 6.23 and 5.72 ppm and the methyl group at C-11 by a new methyl doublet.

To our knowledge provincialin is the only sesquiterpene lactone encountered so far which possesses a C_{10} ester side chain comprising two C_5 acyl units. It is also noteworthy that one of these units is the cis isomer of sarracinic acid, since sarracinoyl type moieties are present in liatrin (**9**)[9] and punctatin (**10**)[1] as well as other sesquiterpene lactones[10] isolated from various *Liatris* species.

In conclusion, we wish to comment briefly on the values of $J_{7,13}$ in provincialin and related sesquiterpenoids which contain a trans-fused lactone ring closed to C-6 and incorporate a cis 4,5 double bond. In these compounds $J_{7,13}$ is less than 3 Hz, *e.g.*, for heliangin (**5a**, structure established by X-ray analysis[3,4]), $J_{7,13a}$ = $J_{7,13b}$ = 2 Hz; for erioflorin (**5b**, correlated with **5a**), $J_{7,13a}$ = $J_{7,13b}$ = 2 Hz; for liatrin (**9**, X-ray analysis[9]), $J_{7,13a}$ = $J_{7,13b}$ = 2.3 Hz; for punctatin (**10**),[1] $J_{7,13a}$ = 2.1, $J_{7,13b}$ = 1.4 Hz; for provincialin (**1**), $J_{7,13a}$ = 2.1, $J_{7,13b}$ = 1.9 Hz; and for woodhousin (**11**),[11] $J_{7,13a}$ = 2.4, $J_{7,13b}$ = 2.0. Thus, such compounds appear to constitute an important exception to the rule formulated by Samek[12] that in trans-fused lactones $J_{7,13} > 3$ Hz.

Samek's rule has been used in several instances to assign lactone ring stereochemistry in germacranolides, a procedure which in view of the observations recorded in the previous paragraph may be of dubious validity if the stereochemistry of the double bonds is not established with certainty. A particularly interesting example is eucannabinolide (**12a** or mirror image) where the assignment of stereochemistry was based solely on



12a

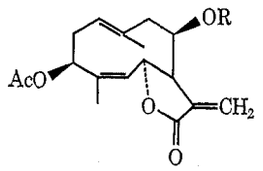
(8) J. D. Edwards, T. Matsumoto, and T. Hase, *J. Org. Chem.*, **32**, 244 (1967).

(9) S. M. Kupchan, V. H. Davis, T. Fujita, M. R. Cox, and R. F. Bryan *J. Amer. Chem. Soc.*, **93**, 4916 (1971).

(10) W. Herz and I. Wahlberg, unpublished results.

(11) W. Herz and S. V. Bhat, *J. Org. Chem.*, **37**, 906 (1972).

(12) Z. Samek, *Tetrahedron Lett.*, 671 (1970).



12b

nmr data.[13] The similarities of chemical shifts and coupling constants in the nmr spectra of eucannabinolide (taken from ref 13) and provincialin (1) (see Table I) are so striking that it is tempting to formulate eucannabinolide as **12b** rather than **12a**.

Experimental Section[14]

The isolation of crude provincialin from a chloroform extract of *L. provincialis* Godfrey has been described elsewhere.[2] Portions of this material were rechromatographed over silica gel (hexane–ethyl acetate 1:4) to give a gum, which was homogeneous on tlc. Various attempts to induce crystallization were unsuccessful. Provincialin (1) had $[\alpha]_D$ −85° (*c* 0.6, $CHCl_3$); ir 3420 (br), 1760, 1740, 1730, and 1655 cm^{-1}; strong uv end absorption at 210 nm; *m/e* (%, composition) 518 (M, 3, $C_{27}H_{34}O_{10}$), 402 (1, $C_{22}H_{26}O_7$), 360 (2, $C_{20}H_{24}O_6$), 342 (0.5, $C_{20}H_{22}O_5$), 306 (0.5, $C_{17}H_{22}O_5$), 305 (0.4, $C_{17}H_{21}O_5$), 289 (7, $C_{17}H_{21}O_4$), 288 (1.6, $C_{17}H_{20}O_4$), 264 (1, $C_{15}H_{20}O_4$), 247 (9, $C_{15}H_{19}O_3$), 246 (22, $C_{15}H_{18}O_3$), 229 (38, $C_{15}H_{17}O_2$), 229 (0.4, $C_{10}H_{13}O_6$), 228 (89, $C_{15}H_{16}O_2$), 213 (9, $C_{10}H_{13}O_5$), 115 (25, $C_5H_7O_3$), 99 (100, $C_5H_7O_2$), and 81 (30, C_5H_5O).

Anal. Calcd for $C_{27}H_{34}O_{10}$: mol wt 518.2149. Found: mol wt (mass spectrum) 518.2119.

Epoxyprovincialin (2).—A solution of 66 mg of 1 in 2 ml of chloroform was allowed to stand with 35 mg of *m*-chloroperbenzoic acid at room temperature for 6 hr. The reaction mixture was diluted with chloroform, washed, and evaporated. Chromatography over silica gel (hexane–ethyl acetate 1:1 → 1:3) gave 53 mg of epoxyprovincialin (2) as a gum, ir 3460 (br), 1750, 1740, 1720, and 1655 cm^{-1}.

Anal. Calcd for $C_{27}H_{34}O_{11}$: C, 60.67; H, 6.41; O, 32.92; mol wt 534. Found: C, 61.05; H, 6.72; O, 32.10; mol wt (mass spectrum) 534.

Hydrolysis of Provincialin.—A solution of 107 mg of 1 in 10 ml of aqueous methanol (80%) was stirred with 403 mg of potassium carbonate at room temperature under nitrogen for 3.5 hr. The solvents were evaporated *in vacuo* and the residue was diluted with water. Extraction with chloroform and chromatography over silica gel afforded as the main component 86 mg of **3**, which on recrystallization from isopropyl ether had mp 139–141.5°; ir 3520, 1770, and 1720 cm^{-1}; *m/e* (%) 338 (M, 2), 306 (0.1), 296 (2), 278 (3), 260 (2.5), 246 (4), 228 (7), and 31 (100).

Anal. Calcd for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74; O, 28.37. Found: C, 63.53; H, 7.82; O, 28.19.

Epoxidation of 3.—A solution of 35 mg of **3** in 1.5 ml of chloroform was allowed to stand with 33 mg of *m*-chloroperbenzoic acid for 3 hr. The mixture was diluted with chloroform, washed with aqueous sodium hydrogen carbonate, and evaporated. Chromatography over silica gel yielded 18 mg of **4**, which on recrystallization from hexane–ethyl acetate had mp 165–169°: ir 3550, 1770, and 1730 cm^{-1}; *m/e* (%) 354 (M, 0.1), 339 (1), 312 (5), 294 (8), 279 (4), 123 (92), 69 (75), 45 (98), and 43 (100). Scarcity of material prevented elemental analysis, but the high-resolution mass spectrum was in accord with the postulated empirical formula.

Anal. Calcd for $C_{16}H_{24}O_6$: M − C_2H_2O 312.1573. Found: 312.1584. Calcd for $C_{16}H_{22}O_5$: M − $C_2H_4O_2$ 294.1467. Found: 294.1466.

Sodium Borohydride Reduction of 1.—A solution of 1.6 g of 1 in 25 ml of methanol was stirred with 630 mg of sodium borohydride at 0° for 2 hr. The mixture was acidified and the solvents

TABLE I.—NMR SPECTRA OF PROVINCIALIN DERIVATIVES[a]

Compd	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-9	H-13	H-14[b]	H-15[b]	H-3′	H-4′	H-5′[c]	H-8′[c]	H-9′	H-10′[b]	Ac
1	~5.3[d]	*d*	~5.3[d]	5.21 dd (11.1, 1.2)	5.93 dd (11.1, 2.4)	2.91 m $W_{1/2}$ ~ 8	~5.3[d]	*d*	6.35 d (2.1) 5.78 d (1.9)	1.77 br	1.83 d (1.2)	4.90 d (~12) 5.01 d (~12)	7.02 t (5.8)	4.43 d (5.8)	4.32 br	6.96 q (7.3)	1.90 d (7.3)	2.11
2	~2.9[d]	*d*	~5.3[d]	5.28 dd (11.1, 1.2)	6.18 dd (11.1, 2.4)	2.9[d]	~5.3[d]	*d*	6.35 d (2.1) 5.80 (1.9)	1.47	1.91 d (1.2)	4.90 d (~12) 5.01 d (~12)	7.03 t (5.8)	4.43 d (5.8)	4.31 br	6.96 q (7.3)	1.91 d (7.3)	2.16
3	~5.2[d]	*d*	~5.2[d]	5.39 dd (10.8, 1.2)	5.72 dd (10.8, 3.9)	*d*	4.07 m $W_{1/2}$ ~ 11	*d*	3.67 m[e] (3.39)[b]	1.83 br	1.81 (1.2)							2.06
4	2.86 (4.5, 10.3)	2.40 m 1.62 m	5.23 dd (2.2, 4.7)	5.55 dd (11.0, 1.2)	5.95 dd (11.0, 3.1)	2.28 m	4.01 m $W_{1/2}$ ~ 11	2.48 dd (14.6, 4.8) 1.29 dd (14.6, 1.9)	3.66 m[e] (3.39)[b]	1.58	1.88 d (1.2)							2.10
6	~5.2[d]	*d*	~5.2[d]	5.41 dd (11.0, 1.2)	5.86 dd (11.0, 2.2)	*d*	~5.2[d]	*d*	1.15 d (7)	1.79 br	1.86 d (1.2)	6.23 d (~1) 5.72 d (~1)	*d*	3.70 t (6.5)				2.09

[a] Run at 90 MHz in $CDCl_3$ solution on a Bruker nmr spectrometer using TMS as internal standard. Values are in ppm. Multiplicities are indicated by the usual symbols: d, doublet; t, triplet; q, quartet; br, broadened singlet; m, multiplet whose center is given. Unmarked signals are singlets. Figures in parentheses are coupling constants (*J*, in Hz) measured by spin decoupling. [b] Intensity three protons. [c] Intensity two protons. [d] Obscured signal.

(13) B. Drozdz, H. Grabarczyk, Z. Samek, M. Holub, V. Herout, and F. Sorm, *Collect. Czech. Chem. Commun.*, **37**, 1546 (1972).

(14) Melting points are uncorrected. Rotations were run in chloroform on a Perkin-Elmer 141 polarimeter, ultraviolet spectra in methanol on a Cary Model 14 recording spectrophotometer, and infrared spectra on a Perkin-Elmer Model 257 grating spectrophotometer. High-resolution mass spectra were run at 70 eV on a MS-9 mass spectrometer. Analyses were performed by F. Pascher, Bonn, Germany.

were evaporated under reduced pressure. Dilution with water, extraction with chloroform, and chromatography over silica gel (hexane–ethyl acetate 3:2 → 1:1) gave 312 mg of **6** as a gum: ir 3460 (br), 1760, 1740, and 1720 cm^{-1}; *m/e* (%, composition) 406 (M, 6, $C_{22}H_{30}O_7$), 307 (1, $C_{17}H_{23}O_5$), 291 (8, $C_{17}H_{23}O_4$), 290 (2, $C_{17}H_{22}O_4$), 249 (4, $C_{15}H_{21}O_3$), 248 (17, $C_{15}H_{20}O_3$), 231 (21, $C_{15}H_{19}O_2$), 230 (50, $C_{15}H_{18}O_2$), 175 (32, $C_{12}H_{15}O$), 157 (100, $C_{12}H_{13}$), 156 (41, $C_{12}H_{12}$), and 99 (56, $C_5H_7O_2$). The gum could not be purified satisfactorily, but the high-resolution mass spectrum was in accord with the postulated empirical formula.

Anal. Calcd for $C_{22}H_{30}O_7$: mol wt 406.1990. Found: mol wt (mass spectrum) 406.1989.

Hydrolysis of 6.—A solution of 300 mg of **6** in 10 ml of aqueous methanol (80%) was stirred with 229 mg of potassium hydroxide at room temperature under nitrogen for 24 hr. The solvents were removed and the residue was diluted with water. Extraction with chloroform and chromatography over silica gel (hexane–ethyl acetate 3:2) gave 39 mg of **7**: mp 167–171°; ir 3460, 3400, and 1755 cm^{-1}; nmr (acetone-d_6) 1.19 (3 H, d, $J \sim 7$), 1.77 (3 H, d, $J \sim 1$), 1.92 (3 H, br) 4.10 (1 H, dd, $J \sim 2$ and 5), 4.22 (1 H, m), 5.11 (1 H, dd, $J \sim 11$ and 1), and 5.40 ppm (1 H, dd, $J \sim 11$ and 1); *m/e* (%) 266 (M, 11), 248 (26), 230 (18), and 95 (100).

Anal. Calcd for $C_{15}H_{22}O_4$: mol wt 266.1518. Found: mol wt (mass spectrum) 266.1501.

Epoxidation of 7.—A solution of 30 mg of **7** in 2 ml of chloroform was allowed to stand with 22 mg of *m*-chloroperbenzoic acid at room temperature for 1 hr. The reaction mixture was evaporated and chromatographed over silica gel (hexane–ethyl acetate 3:2) to give 10 mg of **8**, mp 222–225° dec, undepressed on admixture with a sample of dihydroheliangino1 (**8**) prepared from eriodictyol; the ir, nmr, and mass spectra were identical with those of the authentic sample.

Preparation of Dihydroheliangino1 (8) from Eriodictyol (5b).—A solution of 391 mg of eriodictyol in 10 ml of methanol was stirred with 390 mg of sodium borohydride at 0° for 0.5 hr. The reaction mixture was acidified, evaporated at reduced pressure, diluted with water, and extracted with chloroform. The crystalline residue obtained was dissolved in 20 ml of aqueous methanol (80%) and heated with 200 mg of potassium hydroxide on a steam bath under nitrogen for 4 hr. The reaction mixture was acidified and the solvents were removed under reduced pressure. Dilution with water and continuous extraction with ether for 48 hr afforded 264 mg of dihydroheliangino1 (**8**), which on recrystallization from ethyl acetate had mp 224–225° dec (reported mp 219–220° dec,[7] 202–203°[5]). The nmr spectrum was identical with that of an authentic sample, whereas the ir spectrum of our sample (KBr) differed in a few minor details from the spectrum recorded by Torrance, *et al.*[7] These discrepancies can probably be ascribed to differences in crystal forms of the two samples.

Registry No.—**1**, 40328-96-9; **2**, 40386-87-6; **3**, 40328-97-0; **4**, 40386-88-7; **6**, 40328-98-1; **7**, 40328-99-2.

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The Total Synthesis of *dl*-Avenaciolide[1]

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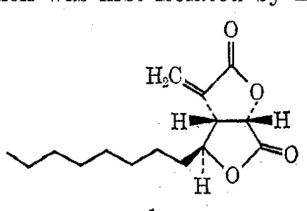
The total synthesis of the mold product avenaciolide (**1**) is reported using the following sequence. Acylative decarboxylation of tricarballylic acid with nonanoic anhydride afforded the dilactone (**26**) of 3-(1,1-dihydroxynonyl)glutaric acid. Reduction of this dilactone by means of alkaline borohydride then led to *trans*-tetrahydro-2-octyl-5-oxo-3-furanacetic acid (**16**) which was converted *via* its acid chloride to the pyrrolidine amide **32**. Carbomethoxylation of the latter compound afforded the amidolactonic ester **33**. Treatment of this material with sodium hypochlorite solution followed by boiling both the neutral and acidic products with aqueous hydrobromic acid led to the dilactone **35**. Carboxylation of **35** with methyl methoxymagnesium carbonate provided the dilactonic acid **40** which when treated with formaldehyde and diethylamine in buffered acetic acid yielded *dl*-avenaciolide (**1**).

Avenaciolide (**1**) is a naturally occurring antifungal compound which was first isolated by Brookes, Tidd,



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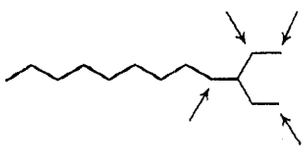
and Turner[2a] from *Aspergillus avenaceus* H. Smith. It was also subsequently obtained from cultures of *Aspergillus fischeri* var. glaber[2b] by investigators at the U. S. Department of Agriculture. The unique bislactonic structure **1**, assigned to avenaciolide by Brookes, *et al.*,[2a] was later confirmed by a more detailed nmr study.[3] More recently 4-isoavenaciolide has been isolated[4] in small quantity during the large-scale preparation of **1**. In addition, the same authors[4] have isolated ethisolide, the 4-isoethyl lower homolog of **1**, from an unidentified species of *Penicillium*.

For the purposes of synthesis, the skeleton of avenaciolide can be looked upon as that of a γ-nonylpentane (**2**), which is oxygenated to varying degrees at the



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points indicated by the arrows but which is lacking the methylene carbon atom of the second lactone ring. With the objective of simplifying the synthesis in its initial stages, we elected to introduce this latter group, last of all. Our initial synthetic attempts were geared therefore to the synthesis of a suitably oxygenated derivative of 3-nonylglutaric acid. An early attempt

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(3) D. Brookes, S. Sternhell, B. K. Tidd, and W. B. Turner, *Aust. J. Chem.*, **18**, 373 (1965).

(4) D. C. Aldridge and W. B. Turner, *J. Chem. Soc.*, 2431 (1970).