Subsequent difference Fourier maps indicated the presence of additional areas of residual electron density which were assigned to two molecules of water and one partial molecule of 2-propanol. The remaining H atom coordinates were calculated at optimum positions for the cyclic peptide. The H atoms were added in the final stages of least-squares refinement and structure factor calculation processes, but were not refined. The cyclic peptide structure, in addition to the two solvate oxygen atoms from water and the three carbon atoms of 2-propanol, were refined in a full-matrix least-squares process with CRYSTALS, using the Robust-Resistant (Tukey and Prince) weighting method (Scheme 15 in CRYSTALS). Anomalous dispersion effects were included in F_c. The final cycle of refinement included 498 variable parameters (anisotropic refinement on all peptide non-hydrogen atoms and isotropic refinement on the five solvate atoms) and converged with unweighted and weighted standard crystallographic residuals of $R = 0.093$ and $Rw = 0.119$.

Final bond distances and angles were all within acceptable limits, except for the isopropyl group of leucine. During the whole refinement process, the C_{39}–C_{41} bond (1.41 Å) was consistently observed to be shorter than its supposedly, chemically equivalent neighbor, the C_{39}–C_{40} (1.58 Å). No logical explanation for this observation seems readily apparent.

The absolute stereochemical assignment for stylostatin 1 was based upon the known absolute stereochemistry determined (see above) for (*S*)-Ile. Thus, the absolute stereochemistry at the eight chiral centers of stylostatin 1 using the numbering shown in Figure 2[15] are as follows: 2*S*, 5*S*, 8*S*, 11*S*, 14*S*, 17*S*, 20*S*, and 45*S*. A final difference Fourier map showed some residual electron density in regions not associated with the main peptide molecule. These areas are believed to be due to additional, highly disordered solvate molecules. The higest peak in the final difference Fourier had a height of 1.13 e/Å^3. A computer-generated perspective drawing depicting the absolute configuration of stylostatin 1 is shown in Figure 2.

(15) Preparation of Figure 2 was accomplished with SHELXTL-PLUS: G. Sheldrick, Siemens Analytical X-ray Instruments, Inc., Madison WI 53719.

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Supplementary Material Available: A more detailed description of the X-ray data collection and refinement, X-ray crystallographic tables of atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for stylostatin 1 (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Herboxidiene: A Potent Phytotoxic Polyketide from *Streptomyces* sp. A7847

Barbara G. Isaac,* Stephen W. Ayer,[1] Robert C. Elliott, and Richard J. Stonard

Monsanto Agricultural Company, A Unit of Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, Missouri 63198

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Investigation of a secondary metabolite produced by *Streptomyces* sp. A7847 (ATCC 49982) has resulted in the identification of herboxidiene (1, $C_{25}H_{42}O_6$), a novel polyketide with exceptional phytotoxicity to several annual weed species. Synthetic modification studies have defined the permissible structural alterations of 1 which maintain biological activity. Significant structural change (see compounds 12 and 13) resulted in a loss of activity as did opening of the epoxide ring (see compounds 5–8) or oxidation of the secondary alcohol at carbon 18 (3).

Microorganisms have demonstrated a remarkable potential for producing secondary metabolites which are toxic to plants. These metabolites offer opportunities for controlling noxious weeds as products per se or by providing agricultural researchers with new structural models for analog synthesis.[2] In the continuation of a screening program to discover novel natural product herbicides from microbial sources,[3] *Streptomyces* sp. A7847 (ATCC 49982) was found to produce herboxidiene[4] (1), a novel polyketide which effectively and selectively controls several annual weed species at application rates as low as 7 g per acre. *Streptomyces* sp. A7847 was previously shown to produce the phytotoxic amino acid (2*R**,3*S**)-β-methyltryptophan.[5] This paper describes the isolation, structure determination, synthetic modification, and biological activity of herboxidiene and its derivatives.

Isolation and Characterization. Herboxidiene was produced in shake flask fermentations of *Streptomyces* sp. A7847. The sterile culture filtrate (1 L) was extracted with *n*-butanol, and the extract was purified by reversed-

(1) Present address: National Research Council, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada B3H 3Z1.

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(4) Isaac, B. G. Presented at the 201st National Meeting of the American Chemical Society, Atlanta, GA, April 1991; paper AGFD 62.

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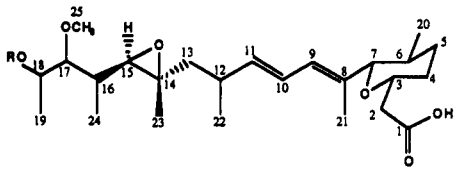
0022-3263/92/1957-7220$03.00/0

phase C18 flash and HPLC chromatographies (water/methanol) to give 0.8 mg of herboxidiene (1). The molecular formula, $C_{25}H_{42}O_6$, was determined by HRFAB-MS ($(M - H)^-$ m/z 437.2902 for $C_{25}H_{41}O_6$, Δ −0.1 mmu). ^1H and ^{13}C NMR examination of 1, as discussed in the following section, revealed the presence of a carboxylic acid carbonyl (as supported by IR absorptions at 1570 and 1400 cm^{-1}, consistent with the anion of a carboxylic acid), two olefins, eight methines (five bearing oxygen atoms), four methylenes, and seven methyl groups (one methoxy methyl). Multiplicities of the ^{13}C signals were determined by APT[6] and DEPT[7] experiments. The ultraviolet spectrum of 1 in methanol, λ_{max} 238 nm (ϵ 21 060), suggested the presence of a diene chromophore.[8]

The molecular formula indicated five sites of unsaturation. Therefore, in addition to the diene and carboxylic acid functionalities, the molecule was required to have two rings.

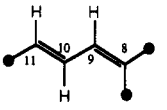


1 R=H
2 R=Ac

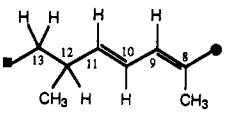
Utilization of NMR Techniques in the Structure Determination. Extensive one- and two-dimensional NMR experiments were carried out on herboxidine (1) and proved to be very effective in elucidating the structure. Data from a ^1H–^1H COSY experiment,[9] in conjunction with one-dimensional ^1H and ^{13}C NMR data, enabled the formulation of partial structures **A**–**G**. An HMQC spectrum,[10] which established all of the one-bond ^1H–^{13}C connectivities, facilitated the assignment of all of the carbons bearing hydrogens. The two- and three-bond ^1H–^{13}C correlations as determined by an HMBC experiment[10] established the connectivities of partial structures **A**–**G** through oxygen atoms and quaternary carbons. Finally, the geometries of the trisubstituted double bond and the two rings were determined by cross peaks observed in a NOESY experiment.[11]

The following discussion on the formulation and connectivities of partial structures **A**–**G** leading to the assignment of the structure of herboxidine (1) is largely based on the combination of these NMR analyses. ^1H, ^{13}C, and HMBC NMR experimental results are presented in Table I.

Partial Structure A. The ^1H NMR, proton-decoupled ^{13}C NMR, DEPT, and HMQC spectra of herboxidiene facilitated the assignment of the four sp^2-hybridized carbons at δ 136.52 (s), 129.51 (d), 126.59 (d), and 140.46 (d) to C-8–C-11, respectively, and the three olefinic protons at δ 5.90 (d), 6.29 (dd), and 5.45 (dd) to H-9–11, respectively. The COSY spectrum demonstrated coupling from H-10 to both H-11 and H-9, but not from H-11 to H-9. The magnitude of the H-10 to H-11 coupling (15 Hz) indicated a trans substitution for the disubstituted olefin, thus providing the diene unit shown below.



The COSY spectrum contained a further correlation between H-9 and a methyl group at δ 1.68 (H_3-21). Additional coupling observed between H-11 and a proton at δ 2.44 (m, H-12) indicated a vicinal relationship between these protons. Finally, H-12 was coupled to a methyl group at δ 1.03 (H_3-22) and to two protons at δ 1.91 (H_a-13) and ~1.19 (H_b-13). The above data were consistent with the presence of partial structure **A**.



A

Partial Structure B. The chemical shifts of H-3 (δ 3.76) and C-3 (δ 76.99) indicated the attachment of this methine carbon to oxygen. In the COSY spectrum, H-3 showed coupling to each proton of a methylene at δ 2.45 and 2.25 ($H_{a,b}$-2). The H-2 protons each appeared as doublets of doublets, with typical 14.1 Hz geminal coupling and ~7 Hz vicinal coupling to H-3. No further couplings to $H_{a,b}$-2 were detectable. The ^1H and ^{13}C (δ 46.35) chemical shifts of this methylene indicated attachment to a deshielding group such as a carbonyl carbon.

Table I. ^1H and ^{13}C NMR Chemical Shifts (ppm) of Herboxidiene (1) and Protons to which a Long-Range Connectivity Was Observed in the HMBC Experiment[a]

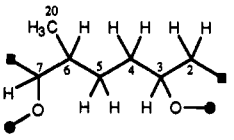
position	H	C	HMBC (^1H)
1		179.77 s	$H_{a,b}$-2
2_a	2.45 dd, J = 14.1, 6.6[b]	46.35 t	
2_b	2.25 dd, J = 14.1, 7.5		
3	3.76 m	76.99 d	H-7, $H_{a,b}$-2
4_a	1.68–1.86 m	33.11 t	$H_{a,b}$-2
4_b	1.30 m		
5_a	1.68–1.86 m	33.69 t	
5_b	1.12–1.26 m		
6	1.55 m	33.48 d	H-7, H_3-20
7	3.34 d, J = 9.9	92.18 d	H-9, H_3-20, H_3-21
8		136.52 s	H-7, H-10, H_3-21
9	5.90 d, J = 11.1	129.51 d	H-7, H-10, H-11, H_3-21
10	6.29 dd, J = 15.0, 10.8	126.59 d	H-9
11	5.45 dd, J = 15.0, 9.0	140.46 d	H-9, $H_{a,b}$-13, H_3-22
12	2.44 m	36.52 d	H-10, H-11, $H_{a,b}$-13, H_3-22
13_a	1.91 dd, J = 13.1, 4.3	48.12 t	H-11, H-15, H_3-22, H_3-23
13_b	1.12–1.26 m		
14		62.55 s	$H_{a,b}$-13, H-15, H_3-23
15	2.65 d, J = 9.6	67.82 d	$H_{a,b}$-13, H-17, H_3-23, H_3-24
16	1.45 m	36.44 d	H-15, H-17, H-18, H_3-24
17	2.96 dd, J = 6.0, 4.5	88.58 d	H-15, H-18, H_3-19, H_3-24, H_3-25
18	3.78 dq, J = 6.6, 6.3	69.78 d	H-17, H_3-19
19	1.11 d, J = 6.6	19.88 q	H-17, H-18
20	0.66 d, J = 6.6	18.19 q	H-7
21	1.68 s	12.08 q	H-7, H-9
22	1.03 d, J = 6.6	22.73 q	H-11, $H_{a,b}$-13
23	1.27 s	16.80 q	$H_{a,b}$-13
24	0.83 d, J = 6.9	11.71 q	H-17
25	3.52 s	61.87 q	H-17

[a] ^1H and ^{13}C NMR data were collected at 300 and 75 MHz, respectively, in CD_3OD; HMBC data were collected at 500 MHz in CD_3OD. [b] $J_{(H,H)}$ in Hz.

(6) Patt, S. L.; Shoolery, J. N. *J. Magn. Res.* **1982**, *46*, 535–539.
(7) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Res.* **1982**, *48*, 323–327.
(8) Kobayashi, J.; Ishibashi, M.; Walchi, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Am. Chem. Soc.* **1988**, *110*, 490–494.
(9) Piantini, U.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.
(10) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.
(11) Kumar, A.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Comm.* **1980**, *95*, 1–6.

The COSY spectrum also showed coupling of H-3 to one of the H-4 methylene protons (H_b-4, δ 1.30). The absence of a correlation between H-3 and H_a-4 ($\sim\delta$ 1.77) suggested that these protons were associated with one of the rings that must be present in the molecule. Further examination of the COSY spectrum identified couplings between the $H_{a,b}$-4 and $H_{a,b}$-5 ($\sim$1.77, 1.19) protons. These couplings were partially obscured by the complexity of the cross peaks in the $\sim\delta$ 1.15–1.80 region and by the partial overlap of the H_a-4 and H_a-5 signals.

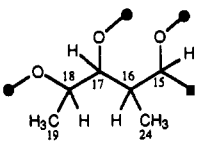
Additional coupling was observed from the H-5 methylene protons to a complex methine resonance at δ 1.55 (H-6), which was in turn coupled to both a methyl group at δ 0.66 (H_3-20) and to a deshielded methine proton (H-7). The chemical shifts of H-7 (δ 3.34) and C-7 (δ 92.18) indicated attachment of this carbon to oxygen. No further coupling to the C-7 proton was observed. The combination of these relationships provided evidence for partial structure **B**.



B

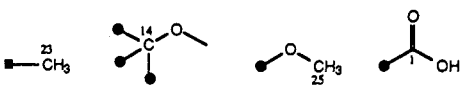
Partial Structure C. The chemical shift of H-18 (δ 3.78) and C-18 (δ 69.78) indicated the attachment of this methine carbon to oxygen. The COSY spectrum demonstrated coupling of H-18 to H-17 which, by its chemical shift (δ 2.96) and the shift of C-17 (δ 88.58), was also attached to a carbon-bearing oxygen. H-18 showed additional coupling to a methyl group at δ 1.11 (H_3-19).

The COSY spectrum also indicated coupling from H-17 to H-16 (δ 1.45) and from H-16 to H-15 (δ 2.65). Although the H-15 doublet was relatively upfield for a methine proton attached to a carbon-bearing oxygen, the chemical shift of C-15 (δ 67.82) strongly indicated this attachment. The H-16 methine proton was also coupled to a methyl group at δ 0.83 (H_3-24). The preceding correlations are summarized in partial structure **C**.



C

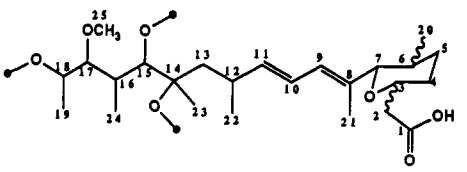
Partial Structures D–G. Four additional, smaller partial structures were deduced from the one-dimensional ^1H and ^{13}C NMR experiments. These included an isolated methyl group **D** (H_3-23, δ 1.27; C-23, δ 16.80), a quaternary carbon bearing oxygen **E** (C-14, δ 62.55), a methoxy methyl group **F** (H_3-25, δ 3.52; C-25, δ 61.87), and a carboxylic acid moiety **G** (C-1, δ 179.77).



D E F G

Formulation of Structure. The two- and three-bond proton–carbon connectivities determined by the HMBC experiment were essential to the formulation of three possible structures for herboxidine (1). The proton–carbon correlations at positions 7, 8, 9, and 21 (Table I) established the attachment of partial structure **A** to **B** via carbons 7 and 8. Furthermore, a correlation between H-7 and C-3 established a linkage through an oxygen atom to form a six-membered cyclic ether, thus accounting for one of the two rings present in the molecule. A correlation between C-1 and the methylene protons at C-2 determined the position of the carboxylic acid moiety **G**.

The $H_{a,b}$-13, C-15 and H-15, C-13 correlations observed in the HMBC experiment established connectivity between units **A** and **C**. The chemical shift of the H-13 methylene protons (δ 1.91, $\sim$1.19), and the absence of coupling from these protons to H-15 in the COSY spectrum, indicated that **A** and **C** were attached through the quaternary carbon (C-14) of **E**. This connectivity was further established by correlations between the protons at positions 13 and 15 and C-14. An additional HMBC correlation between the C-23 methyl protons and C-14 indicated the attachment of **D** to the C-14 quaternary center. Finally, the HMBC experiment indicated relationships between H_3-25 and C-17 and H-17 and C-25, thus establishing the position of the methoxy group **F** at C-17. The combination of these correlations allowed the formulation of partial structure **H**.



H

As indicated by the molecular formula of 1, structure **H** was required to have one fewer oxygen, one hydroxyl proton, and one additional ring in order to satisfy the five degrees of unsaturation. Consequently, three structural possibilities could be drawn. A tetrahydropyran ring could be formed as a result of connecting C-14 and C-18 through an oxygen, a tetrahydrofuran ring by connecting C-15 and C-18 through an oxygen, or an epoxide by connecting C-14 and C-15 and the associated oxygen. Due to the absence of HMBC correlations between C-14 and H-18, C-15 and H-18, or C-18 and H-15, a derivatization was performed in order to establish the position of the hydroxyl group and the resultant size and position of the second ring.

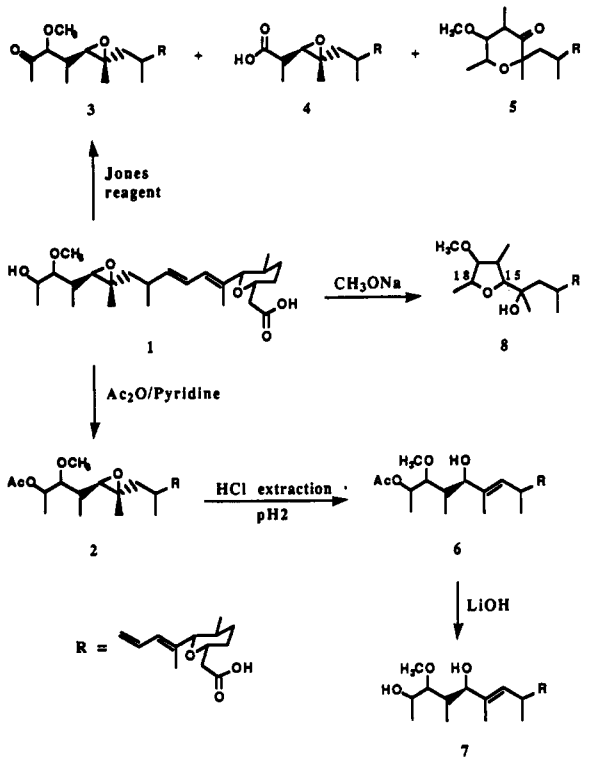
Derivatization of 1. Herboxidiene (15 mg) was reacted with acetic anhydride in pyridine to form the monoacetate derivative **2** in 89% yield. The ^1H NMR spectrum of **2** showed a downfield shift of H-18 from δ 3.78 in **1** to δ 4.95 and a smaller downfield shift of H-17 from δ 2.96 in **1** to δ 3.20. Also present in the ^1H NMR spectrum of **2** was a new three proton singlet at δ 2.01 ppm, assignable to an acetoxy methyl group. The formation of the monoacetate was further supported by a new carbonyl carbon (δ 172.2) and a new methyl group (δ 16.9) in the ^{13}C NMR spectrum of **2**. Additionally, derivative **2** exhibited an ester carbonyl absorbance at 1740 cm^{-1} in the IR spectrum and a molecular weight of 480 (addition of 42 mass units) as indicated by FAB-MS.

From the above data, it was concluded that acetylation occurred at the free hydroxyl located on C-18. Therefore, the second ring of compound **1** was established as an epoxide encompassing C-14 and C-15. Verification was provided by a ^1H-coupled carbon experiment. The one-bond proton–carbon coupling constant at C-15 was measured at 167 Hz, a typical value for an epoxide proton–carbon coupling constant and much larger than the value for similar methines in larger rings and acyclic systems.[12]

Stereochemistry of Selected Sites. The geometry of the C-8, C-9 double bond and the relative stereochemistries

(12) Breitmaier, E.; Voelter, W. In *Carbon-13 NMR Spectroscopy*; VCH Verlagsgesellschaft mbH: D-6940 Weinheim (Federal Republic of Germany), 1987; pp 133–147.

Scheme I



of the two heterocyclic rings of herboxidine (1) were established by the enhancements observed in a NOESY experiment. Nuclear Overhauser effects (NOE's) were observed between H-7 and H-9, and between H-10 and H_3-21, thus indicating a trans relationship between H-9 and the C-21 methyl group. Regarding the epoxide ring, NOE's were observed between H-15 and H_b-13 and between H-16 and H_3-23, establishing a trans configuration of H-15 and the C-23 methyl group at position 14.

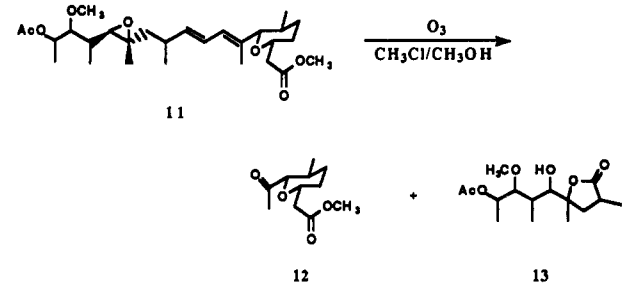
The magnitude of the coupling constant for H-7 (9.9 Hz) required that this proton and H-6 be positioned in a trans-diaxial orientation on the six-membered ring. This was confirmed by an NOE between H-7 and the equatorial methyl at C-6 (H_3-20). An additional NOE between H-7 and H-3 established the axial nature of H-3. The NOESY experiment also allowed the assignment of the H-4 and H-5 methylene protons. NOE's were observed between H-3 and H_a-4, between H_3-20 and H_b-5, and between H-6 and H_a-5. It was concluded from these observations that H_a-4 and H_a-5 were equatorial and H_b-4 and H_b-5 were axial. These relationships, combined with the conclusions discussed in previous sections, established the structure of herboxidiene as 1. The relative stereochemistries of C-7, C-12, C-16, C-17, and C-18, and the absolute stereochemistry, remain to be determined.

Synthetic Modification. Synthetic modification of herboxidiene was performed in order to determine whether it was feasible to reduce its structure to a simplified subunit retaining potent phytotoxic properties.[13] Additionally, derivatives which closely resembled 1 were targeted in order to provide an understanding of the influence of individual functional groups on biological activity.

The polyoxygenated tail (C-14–C-19) of 1 proved to be the most readily altered portion of the molecule. Oxidation of the secondary alcohol at C-18 with Jones reagent afforded the ketone 3 in 37% yield. Two minor products

(13) Synthetic modifications of 1 were carried out on 0.33 g of 3.36 g isolated from a total of 112 L of optimized fermentation broth.

Scheme II

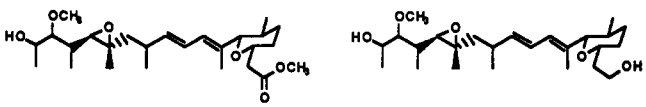


were isolated from this reaction, 4, an overoxidation product of 3, and the cyclic ether, 5, in 5% and 6% yield, respectively[14] (Scheme I).

The epoxide ring was found to be quite labile, opening under both electrophilic and nucleophilic reaction conditions. As discussed above, acetylation with acetic anhydride/pyridine resulted in formation of the acetate 2. However, when 2 was subjected to a single extraction with THF/HCl (aqueous, pH 2), a 1:1 mixture of compounds 2 and 6 (16% isolated yield) resulted.

Reaction of the epoxide ring under nucleophilic conditions (sodium methoxide in methanol) resulted in the formation of a single product in 61% yield. This unknown possessed the same molecular weight (FAB-MS) as 1, indicating that a rearrangement had occurred. ^1H NMR, ^{13}C NMR, COSY, and HETCOR[15] experiments indicated that the original carbon skeleton was intact, the substitution of hydrogen and oxygen atoms on the carbon skeleton was unchanged, and the diene and tetrahydropyran acetic acid units had not undergone reaction. The identity of the unknown product was established as compound 8 by a long-range HETCOR experiment in which a three-bond coupling was observed between C-18 and H-15.

Two reactions aimed at modifying the carboxylic acid group of 1 were carried out. Reaction with diazomethane gave the methyl ester 9, and reduction with lithium aluminum hydride gave the primary alcohol 10 in 53% and 86% yield, respectively.



Selective hydrogenation of the diene was attempted with Wilkinson's catalyst ($RhCl(PPh_3)_3$), $K_3[Co(CN)_5H]$, diimide, and 10% Pd/C. Reduction occurred only with 10% Pd/C, but discrete reaction products could not be identified.

Compound 11 was ozonolyzed in order to ascertain whether the tetrahydropyran acetic acid moiety might be responsible for the biological activity of 1. This reaction afforded the expected product, 12, in 77% yield and an unexpected product, 13, in 40% yield[16] (Scheme II).

Biological Activity.[17] In an agar-based assay,[5] in which sample was applied to the surface of 1-mL solidified

(14) Compound 5 apparently results from electrophilic opening of the epoxide followed by capture of the incipient tertiary carbonium ion by the alcohol at C-18 and subsequent oxidation of the newly formed alcohol at C-15.

(15) Bax, A. D.; Morris, G. A. *J. Magn. Res.* 1981, *42*, 501–505.

(16) Compound 13 possibly results from oxidation of carbon-11 to the carboxylic acid followed by capture of the carbonium ion at C-14 during electrophilic opening of the epoxide ring.

(17) In agar-based assays, 1 was inactive against several microorganisms, including *Schizosaccharomyces pombe*, *Pyricularia oryzae*, *Candida albicans*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Escherichia coli*, and *Micrococcus luteus* but inhibited *Phytophthora megasperma* at rates as low as 0.1 μg/well.

agar in wells of a 96-well tissue culture plate and allowed to dry prior to seed addition, herboxidiene completely inhibited the germination of *Arabidopsis thaliana* at 5 μg/well. When tested at 35 g acre^{-1}, post plant emergence in a pot assay,[18] herboxidiene exhibited excellent control (≥90%) of the broadleaf annual weeds oilseed rape (*Brassica napus*), wild buckwheat (*Polygonum convolvulus*), morning glory (*Ipomoea* sp.), and hemp sesbania (*Sesbania exaltata*). At 7 g acre^{-1}, 1 retained good inhibition (75%) of *B. napus*, *P. convolvulus*, and *S. exaltata*. Of significance is the selective control of *P. convolvulus* and *B. napus* at a rate of 35 g acre^{-1}, coplanted with wheat (*Triticum aestivum*) for which no phytotoxicity was detected.

Each of the herboxidiene analogs was tested for postemergence herbicidal activity on *T. aestivum* and key weed species. Derivatives that decreased the polarity of **1** without changing the basic structure (compounds **2**, **9**, **10**, and **11**) had slightly increased activity. This may be due to increased penetration into the plant or differences in compound distribution within the plant. The fact that compound **10** retained a significant level of activity indicated that the carboxylic acid functionality was not critical for biological activity. In contrast, the integrity of the polyoxygenated end (C-14–C-19) of **1** proved to be essential for maintenance of activity. A minor modification such as oxidizing the free alcohol to the corresponding ketone, **3**, resulted in a marked decrease in activity[19] as did oxidation to the acid **4**. The loss of activity in compound **7** and all other compounds in which the epoxide had been opened indicated that the intact epoxide ring was required for herbicidal activity. Ozonolysis provided access to two greatly simplified subunits of **1**. Compounds **12** and **13** proved to be devoid of activity.

The mechanism of action by which herboxidiene causes plant cell death has not been established. However, it has been shown that **1** does not interfere with electron transfer in mung bean hypocotyl mitochondria at 1 and 100 μM concentrations.[20]

Conclusion

An examination of the structures of previously identified microbial polyketides which possess phytotoxic properties reveals that the majority of these compounds are macrocyclic.[21] In contrast, herboxidiene, the novel polyketide produced by *Streptomyces* sp. A7847, represents an uncommon example of an essentially linear phytotoxic polyketide.[22] In soil based assays, herboxidiene exhibits phytotoxicity against several important annual weed species at use rates comparable to current commercial herbicides.[23] Structural alterations of herboxidiene did not significantly increase herbicidal activity, and the apparent obligate nature of much of the functionality of this compound suggests that the identification of a simple subunit possessing significant biological activity is unlikely. Nevertheless, the unique biological properties of herboxidiene provide further substantiation of the ability of microorganisms to produce secondary metabolites with qualities relevant to agricultural pest control research.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD or $CDCl_3$. *J* values are given in Hz. RP-HPLC analyses for compounds **3**–**13** were performed using as standard conditions a Perkin–Elmer 0.46- × 3.3-cm 3-μm C-18 cartridge column (linear gradient from 80:20 H_2O/CH_3CN/1% AcOH to 100% CH_3CN/1% AcOH over 15 min, held at final conditions for 5 min, flow rate 2 mL/min). Thin-layer chromatography was carried out on precoated silica gel 60 F_{254} plates (5 × 10 cm; Merck art. 5719; 1:9 methanol/chloroform).

Fermentation. *Streptomyces* sp. A7847 was cultured for 3 days at 30 °C and 250 rpm in a 250-mL Erlenmeyer flask containing 50 mL of seed medium consisting of tryptone (0.5%) and yeast extract (0.3%). The inoculum broth was transferred to a 2-L Erlenmeyer flask containing 1 L of production medium consisting of glucose (0.05%), dextrin (0.25%), cottonseed oil (0.5%), yeast extract (0.05%), Proflo (0.25%), soybean flour (0.5%), and $MgHPO_4 \cdot 3H_2O$ (0.05%), pH 7, to which was added 0.1% antifoam. The fermentation was carried out on a rotary shaker at 30 °C and 80 rpm, with 0.5 L/min aeration, for 4 days.

Isolation. The culture filtrate (1 L) was extracted with *n*-butanol (333 mL × 3) to give 1.33 g of active material. Upon standing for 3 days, a white precipitate formed which was identified as desferrioxamine E.[24] The concentrated mother liquor (0.26 g) was fractionated by flash chromatography (3 × 15 cm reversed-phase C18 column) using a 10% step gradient from 100% water to 100% methanol, 250 mL/solvent, batch elution. The active 20:80 water/methanol eluate (10 mg) was purified by reversed-phase C18 HPLC (Vydac 201HS1010, 1 × 25 cm, eluant 42:58 water/methanol, flow rate 5 mL/min, UV detection at 236 nm) collecting 2-mL fractions. Active fractions 15–17 were combined to give 0.8 mg of herboxidiene (**1**, t_R 6.2 min; R_f = 0.43, UV- and *p*-anisaldehyde-positive[25]).

Herboxidiene (1): UV (CH_3OH) λ_{max} 238 nm (ε 21 060); CD (*c* 0.4 mg/mL, CH_3OH) $[\theta]_{199\,nm}$ −23 021 cm^2/dM, $[\theta]_{236\,nm}$ +24 597 cm^2/dM; IR (neat) ν_{max} 1650, 1570, 1500, 1450, 1400 cm^{-1}; ^{1}H NMR (Table I); ^{13}C NMR (Table I); FAB-MS *m/z* 437 $(M - H)^-$, HRFAB-MS *m/z* 437.2902 for $C_{25}H_{41}O_6$ $((M - H)^-$ calcd 437.2903).

6-[6-[3-[3-(Acetyloxy)-2-methoxy-1-methylbutyl]-2-methyloxiranyl]-1,5-dimethyl-1,3-hexadienyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (2). A solution of **1** (15 mg, 34 μmol), acetic anhydride (0.5 mL), and pyridine (0.5 mL) was stirred at room temperature. After 9 h the starting material had reacted to yield two components (RP-HPLC: t_R 8.5 min (25%) and t_R 10.2 min (73%)). Following the removal of volatiles under N_2, NaOH (≈1.1 equiv) was added and the solution stirred until disappearance of the 10.2 min peak. The reaction material was concentrated under N_2, and **2** was isolated as a clear viscous liquid (14.6 mg, 89% yield) by preparative RP-HPLC (Beckman Ultrasphere 10 mm × 25 cm; 20%–100% CH_3CN/1% HOAc over 40 min; 3 mL/min): IR (KBr) ν_{max} 1740, 1610, 1455, 1380, 1250 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 0.68 (3 H, d, *J* = 6.6, H_3-20), 0.84 (3 H, d, *J* = 7.2, H_3-24), 1.04 (3 H, d, *J* = 6.6, H_3-22), 1.14 (3 H, d, *J* = 6.3, H_3-19), 1.10–1.21 (1 H, m, H_a-5), 1.26 (3 H, s, H_3-23), 1.21–1.36 (1 H, m, H_a-4), 1.21–1.36 (1 H, m, H_a-13), 1.45–1.60 (1 H, m, H-6), 1.45–1.60 (1 H, m, H-16), 1.69 (3 H, s, H_3-21), 1.68–1.76 (1 H, m, H_b-4), 1.81–1.90 (1 H, m, H_b-5), 1.91 (1 H, dd, *J* = 13.7, 4.5, H_b-13), 2.01 (3 H, s, CH_3CO), 2.33 (1 H, dd, *J* = 15.0, 6.0, H_a-2), 2.45 (1 H, dd, *J* = 15.0, 6.9, H_b-2), 2.45 (1 H, m, H-12), 2.61 (1 H, d, *J* = 9.3, H-15), 3.20 (1 H, dd, *J* = 6.5, 4.2, H-17), 3.32 (1 H, d, *J* = 9.9, H-7), 3.49 (3 H, s, H_3-25), 3.77 (1 H, m, H-3), 4.95 (1 H, dq, *J* = 6.6, 6.3, H-18), 5.45 (1 H,

(18) Miller-Wideman, M.; Makkar, N.; Tran, M.; Isaac, B.; Biest, N.; Stonard, R. *J. Antibiot.* **1992**, *45*, 914–921.

(19) It is possible that **3** isomerizes via successive enolizations resulting in opening of the epoxide and consequent loss of activity.

(20) Moreland, D. E.; Huber, S. C. *Pestic. Biochem. Physiol.* **1979**, *11*, 247–257.

(21) For example, see: (a) Filipin; Wallen, V. R.; Bell, W. *Plant Dis. Rep.* **1956**, *40*, 129–132. (b) Borrelidin; Dorgerloh, M.; Kretschmer, A.; Schmidt, R. R.; Steffens, R.; Zoebelein, G.; Tietjen, K.; Roeben, W.; Stendel, W.; Salcher, O. (Bayer A.-G.). Ger. Offen. DE 3,607,287, Jan 7, 1988. (c) SI-4155B; Hirota, H.; Kanza, T.; Isogai, A.; Nakayama, M. Jpn. Kokai Tokyo Koho JP 02,215,791, Aug 28, 1990.

(22) Other examples are as follows. (a) PM-toxins: Kono, Y.; Danko, S. J.; Suzuki, Y.; Takeuchi, S.; Daly, J. M. *Tetrahedron Lett.* **1983**, *24*, 3803–3806. (b) T-toxin: Kono, Y.; Daly, J. M. *Bioorg. Chem.* **1979**, *8*, 391–397.

(23) Chemical and Pharmaceutical Press. *Crop Protection Chemicals Reference*; 4th ed.; J. Wiley & Sons: New York, 1988.

(24) Keller-Schierlein, W.; Prelog, V. *Helv. Chim. Acta* **1961**, *44*, 1981–1985.

(25) Touchstone, J. C.; Dobbins, M. F. In *Practice of Thin Layer Chromatography*, 2nd Ed.; John Wiley & Sons: New York, 1983; p 185.

dd, J = 15.2, 9.3, H-11), 5.91 (1 H, d, J = 10.8, H-9), 6.29 (1 H, dd, J = 15.0, 10.8, H-10); ^{13}C NMR (100 MHz, CD_3OD) δ 176.5 (C-1), 172.2 (CH_3CO), 140.6 (C-11), 136.3 (C-8), 129.6 (C-9), 126.6 (C-10), 92.2 (C-7), 86.1 (C-17), 75.9 (C-3), 73.6 (C-18), 67.3 (C-15), 62.3 (C-14), 61.7 (C-25), 48.1 (C-13), 43.4 (C-2), 36.6 (C-12), 36.3 (C-16), 33.5 (C-5), 33.4 (C-6), 32.9 (C-4), 22.7 (C-22), 21.3 (C-19), 18.1 (C-20), 16.90 (CH_3CO), 16.88 (C-23), 12.1 (C-21), 11.6 (C-24); FAB-MS m/z 479 $(M - H)^-$; R_f = 0.52.

Reaction of Herboxidiene (1) with Jones Reagent. To a solution of 1 (30 mg, 69 μmol) and acetone (1 mL) at room temperature was added a stock solution of Jones reagent (200 μmol) over 20 min. The reaction mixture was added to a small plug of silica gel and eluted with 9:1 $CHCl_3/CH_3OH$, and the solvent was evaporated to yield a tan liquid. The crude reaction product was purified by preparative RP-HPLC to give compounds **3** (11.2 mg, 37%), **4** (1.4 mg, 5%), and **5** (1.4 mg, 6%).

Tetrahydro-6-[6-[3-(2-methoxy-1-methyl-3-oxobutyl)-2-methyloxiranyl]-1,5-dimethyl-1,3-hexadienyl]-5-methyl-2*H*-pyran-2-acetic acid (3): ^{1}H NMR (400 MHz, CD_3OD) δ 0.67 (3 H, d, H_3-20), 0.79 (3 H, d, H_3-24), 1.03 (3 H, d, H_3-22), 1.15 (1 H, dd, H_a-13), 1.28 (3 H, s, H_3-23), 1.28 (1 H, br, H_a-5), 1.28 (1 H, br, H_a-4), 1.53 (1 H, br, H-6), 1.68 (3 H, s, H_3-21), 1.70 (1 H, m, H_b-4), 1.70 (1 H, m, H-16), 1.86 (1 H, m, H_b-5), 1.91 (1 H, dd, H_b-13), 2.11 (3 H, s, H_3-19), 2.33 (1 H, dd, H_a-2), 2.46 (1 H, dd, H_b-2), 2.46 (1 H, br, H-12), 2.65 (1 H, d, H-15), 3.33 (1 H, d, H-7), 3.37 (3 H, s, H_3-25), 3.66 (1 H, d, H-17), 3.76 (1 H, br, H-3), 5.46 (1 H, dd, H-11), 5.90 (1 H, d, H-9), 6.29 (1 H, dd, H-10); FAB-MS m/z 435 $(M - H)^-$.

6-[6-[3-(1-Carboxyethyl)-2-methyloxiranyl]-1,5-dimethyl-1,3-hexadienyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic acid (4): ^{1}H NMR (400 MHz, CD_3OD) δ 0.68 (3 H, d, CH_3), 1.04 (3 H, d, CH_3), 1.07 (3 H, d, CH_3), 1.10–1.24 (2 H, m), 1.28 (3 H, s, H_3-23), 1.34 (1 H, m), 1.53 (1 H, br), 1.65 (3 H, s, H_3-21), 1.73 (1 H, br), 1.86 (1 H, br), 1.90 (1 H, dd), 2.15 (1 H, m, H-16), 2.32 (1 H, dd, H_a-2), 2.43 (1 H, dd, H_b-2), 2.43 (1 H, br, H-12), 2.90 (1 H, d, H-15), 3.36 (1 H, d, H-7), 3.75 (1 H, br, H-3), 5.48 (1 H, dd, H-11), 5.91 (1 H, d, H-9), 6.30 (1 H, dd, H-10); ^{13}C NMR (100 MHz, CD_3OD): δ 140.6, 136.4, 129.6, 126.6, 92.2, 76.0, 67.3, 61.4, 47.9, 36.6, 33.5, 33.4, 32.9, 22.7, 18.1, 16.7, 15.5, 12.1 (4 carbons were not observed owing to the poor S/N of this spectrum); FAB-MS m/z 393 $(M - H)^-$.

6-[1,5-Dimethyl-6-(tetrahydro-5-methoxy-2,4,6-trimethyl-3-oxo-2*H*-pyran-2-yl)-1,3-hexadienyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic acid (5): ^{1}H NMR (400 MHz, CD_3OD) δ 0.69 (3 H, d, H_3-20), 0.96 (3 H, d, H_3-22), 1.03 (3 H, d, H_3-24), 1.20 (3 H, s, H_3-23), 1.25 (1 H, br, H_a-4), 1.30 (1 H, m, H_a-5), 1.51 (1 H, br, H-6), 1.54 (1 H, dd, H_a-13), 1.67 (3 H, s, H_3-21), 1.61 (1 H, m, H_b-4), 1.80 (1 H, dd, H_b-13), 1.82 (1 H, br, H_b-5), 2.29 (1 H, dd, H_a-2), 2.44 (1 H, dd, H_b-2), 2.76 (1 H, br, H-12), 2.75 (1 H, m, H-16), 3.17 (1 H, dd, H-17), 3.31 (1 H, d, H-7), 3.42 (3 H, s, H_3-25), 3.75 (1 H, m, H-3), 4.08 (1 H, m, H-18), 5.29 (1 H, dd, H-11), 5.81 (1 H, d, H-9), 6.17 (1 H, dd, H-10); ^{13}C NMR (100 MHz, CD_3OD) δ 217.8, 141.8, 136.0, 129.5, 126.1, 92.1, 86.2, 84.2, 76.1, 69.7, 59.7, 48.7, 47.9, 44.0, 34.0, 33.6, 33.5, 32.9, 23.6, 23.0, 18.1, 16.5, 13.4, 12.2 (C-1 not obsd); FAB-MS m/z 435 $(M - H)^-$.

6-[11-(Acetyloxy)-8-hydroxy-10-methoxy-1,5,7,9-tetramethyl-1,3,6-dodecatrienyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (6). Compound 1 (200 mg, 0.456 mmol) was acetylated to form **2** as described above. The reaction mixture was added to THF (15 mL) and extracted with water (30 mL, pH2), and the organic layer was dried over $MgSO_4$. RP-HPLC indicated the presence of compound **2**. Removal of the solvents in vacuo yielded 0.2387 g of a tan liquid. Product decomposition during evaporation resulted in the formation of a 1:1 mixture of **2** and **6**. Preparative RP-HPLC gave **2** (8.1 mg, 4%) and **6** (34.4 mg, 16%) as a yellow solid.

6: ^{1}H NMR (400 MHz, CD_3OD) δ 0.67 (3 H, d, H_3-20), 0.68 (3 H, d, H_3-24), 1.10 (3 H, d, H_3-22), 1.15 (3 H, d, H_3-19), 1.29 (1 H, m, H_a-5), 1.29 (1 H, m, H_a-4), 1.54 (1 H, br, H-6), 1.59 (3 H, s, H_3-23), 1.67 (3 H, s, H_3-21), 1.70 (1 H, m, H-16), 1.70 (1 H, m, H_b-4), 1.86 (1 H, dd, H_b-5), 2.06 (3 H, s, $OCOCH_3$), 2.37 (1 H, dd, H_a-2), 2.46 (1 H, dd, H_b-2), 3.21 (1 H, m, H-12), 3.33 (1 H, d, H-7), 3.53 (3 H, s, H_3-25), 3.57 (1 H, dd, H-17), 3.76 (1 H, br, H-3), 3.83 (1 H, d, H-15), 4.99 (1 H, m, H-18), 5.21 (1 H, d, H-13), 5.60 (1 H, dd, H-11), 5.92 (1 H, d, H-9), 6.23 (1 H, dd, H-10); ^{13}C NMR (100 MHz, CD_3OD) δ 175.5 (C-1), 172.5 ($OCOCH_3$), 139.6 (C-11), 136.7 (C-8), 135.8 (C-14), 133.7 (C-13), 129.6 (C-9), 125.2 (C-10), 92.0 (C-7), 83.5 (C-17), 80.2 (C-15), 75.6 (C-3), 75.0 (C-18), 61.8 (C-25), 42.5 (C-2), 38.6 (C-16), 36.4 (C-12), 33.47 (C-5), 33.47 (C-6), 32.8 (C-4), 21.36 (C-22), 21.36 ($OCOCH_3$), 18.0 (C-20), 17.2 (C-19), 12.4 (C-21), 10.7 (C-23), 10.3 (C-24); FAB-MS m/z 479 $(M - H)^-$.

6-(8,11-Dihydroxy-10-methoxy-1,5,7,9-tetramethyl-1,3,6-dodecatrienyl)tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (7). A solution of **6** (17 mg, 35 μmol), THF/H_2O (3:2, 0.5 mL), and 1 M LiOH (0.35 mL) was stirred at room temperature for 1.5 h, at which time 1 M AcOH (0.35 mol) was added. Following in vacuo solvent removal, the residue was dissolved in $CHCl_3/CH_3OH$ (9:1) and eluted through a small plug of SiO_2 gel. Solvent evaporation gave a yellow liquid which was purified by preparative RP-HPLC to give **7** as a clear liquid (4.3 mg, 28%): ^{1}H NMR (400 MHz, CD_3OD) δ 0.65 (3 H, d, CH_3), 0.67 (3 H, d, CH_3), 1.10 (3 H, d, CH_3), 1.12 (3 H, d, CH_3), 1.18 (1 H, m, H_a-5), 1.18 (1 H, m, H_a-4), 1.52 (1 H, br, H-6), 1.59 (3 H, s, H_3-23), 1.65 (3 H, s, H_3-21), 1.71 (1 H, br, H-16), 1.71 (1 H, br, H_b-4), 1.85 (1 H, dd, H_b-5), 2.33 (1 H, dd, H_a-2), 2.46 (1 H, dd, H_b-2), 3.20 (1 H, m, H-12), 3.33 (1 H, d, H-7), 3.40 (1 H, d, H-17), 3.58 (3 H, s, H_3-25), 3.75 (1 H, m, H-3), 3.75 (1 H, m, H-18), 3.84 (1 H, d, H-15), 5.21 (1 H, d, H-13), 5.60 (1 H, dd, H-11), 5.91 (1 H, d, H-9), 6.23 (1 H, dd, H-10); ^{13}C NMR (100 MHz, CD_3OD): δ 176.5, 139.6, 137.0, 135.9, 133.4, 129.6, 125.2, 92.0, 86.6, 80.8, 76.0, 71.1, 61.9, 43.4, 39.0, 36.4, 33.54, 33.54, 32.9, 21.4, 20.0, 18.0, 12.3, 10.8, 10.7; FAB-MS m/z 437 $(M - H)^-$.

Tetrahydro-6-[7-hydroxy-7-(tetrahydro-4-methoxy-3,5-dimethyl-2-furanyl)-1,5,7-trimethyl-1,3-heptadienyl]-5-methyl-2*H*-pyran-2-acetic Acid (8). A vial containing **1** (15 mg, 34 μmol), MeOH (1 mL), and NaOMe (102 μmol) was sealed and heated to 60 °C for 79 h. Glacial AcOH (57 μL, 102 μmol) was added, and the solvent was evaporated under N_2. Preparative RP-HPLC afforded **8** as a yellow oil (9.1 mg, 61%): ^{1}H NMR (400 MHz, CD_3OD) δ 0.70 (3 H, d, H_3-20), 1.04 (3 H, d, H_3-24), 1.07 (3 H, d, H_3-22), 1.13 (3 H, d, H_3-19), 1.23 (3 H, s, H_3-23), 1.30 (1 H, m, H_a-5), 1.30 (1 H, m, H_a-4), 1.53 (1 H, m, H-6), 1.58 (2 H, m, H-13), 1.69 (3 H, s, H_3-21), 1.71 (1 H, br, H_b-4), 1.85 (1 H, dd, H_b-5), 2.33 (1 H, dd, H_a-2), 2.37 (1 H, br, H-16), 2.46 (1 H, dd, H_b-2), 2.51 (1 H, m, H-12), 3.32 (3 H, s, H_3-25), 3.34 (1 H, d, H-7), 3.34 (1 H, d, H-17), 3.75 (1 H, br, H-3), 3.78 (1 H, d, H-15), 4.27 (1 H, m, H-18), 5.60 (1 H, dd, H-11), 5.92 (1 H, d, H-9), 6.26 (1 H, dd, H-10); ^{13}C NMR (100 MHz, CD_3OD) 143.0 (C-11), 135.6 (C-8), 129.7 (C-9), 125.3 (C-10), 92.1 (C-7), 91.2 (C-17), 85.4 (C-15), 76.6 (C-18), 75.9 (C-3), 74.9 (C-14), 57.7 (C-25), 46.7 (C-2), 43.2 (C-13), 40.7 (C-16), 34.4 (C-12), 33.6 (C-5), 33.5 (C-6), 32.9 (C-4), 26.2 (C-23), 23.9 (C-22), 18.1 (C-20), 15.3 (C-19), 14.7 (C-24), 12.4 (C-21) (C-1 not obsd); FAB-MS m/z 437 $(M - H)^-$.

Tetrahydro-6-[6-[3-(3-hydroxy-2-methoxy-1-methylbutyl)-2-methyloxiranyl]-1,5-dimethyl-1,3-hexadienyl]-5-methyl-2*H*-pyran-2-acetic Acid Methyl Ester (9). To a solution of **1** (15 mg, 34 μmol), dry MeOH (1.5 mL), and a catalytic amount of oxalic acid at room temperature was added portions of an ethereal solution of diazomethane until the solution remained yellow. After 1 h, solvent was removed under N_2. Preparative RP-HPLC gave **9** as a yellow oil (8.2 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.65 (3 H, d), 0.86 (3 H, d), 1.03 (3 H, d), 1.16 (3 H, d), 1.21 (2 H, m), 1.27 (3 H, s), 1.32 (1 H, m), 1.51 (2 H, m), 1.66 (1 H, m), 1.69 (3 H, s), 1.81 (1 H, m), 1.88 (1 H, dd), 2.39 (1 H, dd), 2.40 (1 H, br), 2.53 (1 H, br), 2.54 (1 H, d), 2.58 (1 H, dd), 2.96 (1 H, t), 3.31 (1 H, d), 3.53 (3 H, s), 3.65 (3 H, s), 3.75 (1 H, m), 3.84 (1 H, br), 5.42 (1 H, dd), 5.88 (1 H, d), 6.22 (1 H, dd); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 139.2, 135.3, 128.1, 125.2, 90.6, 87.6, 73.8, 68.3, 66.0, 61.3, 61.2, 51.5, 46.9, 41.3, 35.3, 35.1, 32.3, 32.1, 31.7, 22.0, 19.0, 17.6, 16.6, 11.9, 11.8; DP/EI-MS m/z 452 $(M)^+$, DP/CI-MS m/z 453 $(M + H)^+$.

Tetrahydro-6-[6-[3-(3-hydroxy-2-methoxy-1-methylbutyl)-2-methyloxiranyl]-1,5-dimethyl-1,3-hexadienyl]-2*H*-pyran-2-acetic Acid (10). A solution of **1** (60 mg, 0.137 mmol), THF (5 mL), and small portions of $LiAlH_4$ was stirred at room temperature for 5 h. The reaction was diluted with 10 mL of THF, filtered, and concentrated to yield a yellow liquid (22 mg) which by RP-HPLC was composed of **10** (86%) and **1** (14%). Preparative RP-HPLC (65:35 CH_3CN/0.2% NH_3OH) yielded **10** as a clear liquid (9.2 mg, 16%): ^{1}H NMR (400 MHz, CD_3OD) δ 0.68

(3 H, d), 0.83 (3 H, d), 1.04 (3 H, d), 1.11 (3 H, d), 1.14–1.38 (3 H, m), 1.28 (3 H, s), 1.51 (2 H, m), 1.64 (3 H, m), 1.70 (3 H, s), 1.85 (1 H, dd), 1.93 (1 H, dd), 2.46 (1 H, br), 2.65 (1 H, d), 2.97 (1 H, dd), 3.32 (1 H, d), 3.50 (1 H, br), 3.52 (3 H, s), 3.64 (2 H, t), 3.78 (1 H, quintet), 5.48 (1 H, dd), 5.92 (1 H, d), 6.31 (1 H, dd); ^{13}C NMR (100 MHz, CD_3OD) δ 140.7, 136.4, 129.5, 126.6, 92.1, 88.5, 76.4, 69.9, 67.9, 62.6, 61.9, 60.1, 48.1, 40.0, 36.52, 36.45, 33.69, 33.69, 33.3, 22.7, 19.8, 18.2, 16.8, 12.2, 11.6; DP/EI-MS m/z 424 $(M)^+$, DP/CI-MS m/z 425 $(M + H)^+$.

6-[6-[3-[3-(Acetyloxy)-2-methoxy-1-methylbutyl]-2-methyloxiranyl]-1,5-dimethyl-1,3-hexadienyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid Methyl Ester (11). A solution of **9** (0.6288 g), pyridine (10 mL), and acetic anhydride (14 mL) was stirred at room temperature for 4.5 h. Solvent was removed in vacuo, and the resulting material was dissolved in ether and extracted with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give **11** as a yellow oil (0.5796 g, 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.67 (3 H, d), 0.85 (3 H, d), 1.04 (3 H, d), 1.19 (3 H, d), 1.19–1.27 (2 H, br), 1.26 (3 H, s), 1.33 (1 H, m), 1.45 (1 H, m), 1.52 (1 H, m), 1.69 (1 H, br), 1.70 (3 H, s), 1.84 (1 H, dd), 1.91 (1 H, dd), 2.05 (3 H, s, $COCH_3$), 2.35–2.45 (1 H, br), 2.40 (1 H, dd), 2.56–2.63 (1 H, br), 2.60 (1 H, dd), 3.23 (1 H, dd), 3.32 (1 H, d), 3.53 (3 H, s), 3.67 (3 H, s, CO_2CH_3), 3.77 (1 H, m), 5.00 (1 H, quintet), 5.44 (1 H, dd), 5.89 (1 H, d), 6.23 (1 H, dd); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 170.5, 139.3, 135.2, 128.2, 125.2, 90.7, 84.4, 73.8, 72.5, 65.9, 61.5, 60.7, 51.6, 46.9, 41.4, 35.3, 35.1, 32.3, 32.1, 31.7, 22.1, 21.4, 17.6, 16.8, 16.7, 11.9, 10.6; DP/CI-MS m/z 495 $(M + H)^+$ (major ions at m/z 435, 403, 265).

Ozonolysis of Compound 11. Ozone gas was bubbled through a solution of **11** (0.4383 g, 0.89 mmol), $CHCl_3$ (30 mL), and MeOH (0.568 g, 8.9 mmol) at −50 °C until a blue color persisted. The reaction was allowed to stir for 40 min during which time it warmed to −10 °C and became clear. A small amount of ozone was added, and the solution was stirred for an additional 5 min. Dimethyl sulfide (0.6 mL) was added at −10 °C and the solution allowed to warm to room temperature. Following extraction with $CHCl_3/H_2O$, drying of the organic layer over $MgSO_4$, and evaporation under reduced pressure, 0.473 g of a clear liquid was obtained. SiO_2 gel chromatography (chromatotron, 2 mm, hexane/EtOAc) afforded 7 fractions. Two compounds (**12** and **13**) were isolated in sufficient quantity and purity to enable further characterization.

6-Acetyltetrahydro-5-methyl-2*H*-pyran-2-acetic Acid Methyl Ester (12). Impure **12** (146.5 mg, 77% yield) was purified by SiO_2 gel chromatography (chromatotron, 2 mm, hexane/$CHCl_3/CH_3OH$ (8:2:1)) to yield **12** in 98% purity (23.5 mg) as a clear liquid: R_f = 0.67; 2:1 hexane/EtOAc; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 (3 H, d, H_3-20), 1.21 (1 H, m, H_a-5), 1.34 (1 H, ddd, H_a-4), 1.47 (1 H, m, H-6), 1.64 (1 H, ddd, H_b-4), 1.81 (1 H, ddd, H_b-5), 2.08 (3 H, s, H_3-21), 2.38 (1 H, dd, H_a-2), 2.51 (1 H, dd, H_b-2), 3.35 (1 H, d, H-7), 3.60 (3 H, s, CO_2CH_3), 3.72 (1 H, m, H-3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 207.8, 171.5, 89.0, 73.8, 51.7, 41.2, 32.2, 31.8, 31.1, 25.8, 16.9; DP/CI-MS m/z 215 $(M + H)^+$.

5-[4-(Acetyloxy)-1-hydroxy-3-methoxy-2-methylpentyl]-dihydro-3,5-dimethyl-2-(3*H*)-furanone (13). Compound **13** (108.7 mg, 40%) was a yellow solid: R_f = 0.13; 2:1 hexane/EtOAc; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (3 H, d, H_3-24), 1.23 (3 H, d, H_3-19), 1.29 (3 H, d, H_3-22), 1.42 (3 H, s, H_3-23), 1.93 (1 H, t, H_a-13), 1.98 (1 H, m, H-16), 2.09 (3 H, s, $COCH_3$), 2.26 (1 H, dd, H_b-13), 2.29 (1 H, m, H-12), 3.48 (1 H, br, OH), 3.53 (3 H, s, H_3-25), 3.55 (1 H, dd, H-17), 3.56 (1 H, d, H-15), 5.12 (1 H, br, H-18); ^{13}C NMR (400 MHz $CDCl_3$) δ 180.2 (C-11), 171.5 ($COCH_3$), 86.8 (C-14), 85.8 (C-17), 80.1 (C-15), 73.0 (C-18), 61.2 (C-25), 40.9 (C-13), 36.3 (C-16), 35.4 (C-12), 22.6 ($COCH_3$), 22.2 (C-23), 18.7 (C-19), 16.6 (C-22), 14.1 (C-24); DP/CI-MS m/z 303 $(M + H)^+$ (major ions at m/z 211, 243).

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Supplementary Material Available: Figures 1–6 consisting of the NOESY, HMQC, and HMBC spectra of **1** and proton NMR spectra of new compounds **1–13** (19 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Synthesis of Inhibitors of 2,3-Oxidosqualene–Lanosterol Cyclase. 2. Cyclocondensation of γ,δ-Unsaturated β-Keto Esters with Imines

Dharmpal S. Dodd and Allan C. Oehlschlager*

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Nafsika H. Georgopapadakou

Department of Chemotherapy, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110

Anne-Marie Polak and Peter G. Hartman

F. Hoffmann-La Roche Ltd., CH-4002 Basel, Switzerland

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Synthesis and the biological evaluation of ammonium ion analogues of the first carbocyclic cationic intermediate **4** presumed to formed during the cyclization of 2,3-oxidosqualene to protosterol, **2**, by 2,3-oxidosqualene–lanosterol cyclase (OSC) is presented. Preparation of the required 4-hydroxy-2,3-substituted-4-piperidine **12** (and its corresponding methiiodide salt **13**) involved, as a key step, cyclocondensation of imine **17** with methyl 2-methyl-3-oxo-4-pentenoate (**16**) to give C-2,C-3-substituted 4-piperidone **15** as a single diastereoisomer. Subsequent elaboration to give **13** was accomplished in six steps in an overall yield of 50%. Analogue **12** inhibited 2,3-oxidosqualene–lanosterol cyclase of *Candida albicans* with an IC_{50} of 0.23 μM.

I. Introduction

2,3-Oxidosqualene cyclases (OSC)[1] comprise a class of enzymes that catalyze the cyclization of (3*S*)-2,3-oxidosqualene (**1**) to a number of sterols. The cyclases catalyze the sequential formation of four new carbon–carbon bonds leading to the tetracyclic protosterol **2**. Backbone rearrangement of **2** by OSC's gives lanosterol **3** (Figure 1), in fungi and mammals, and cycloartenol or β-amyrin in photosynthetic plants. There is considerable circumstantial evidence to suggest the conversion of 2,3-oxido-

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