biomimetic synthesis of an *Amaryllidaceae* alkaloid which features use of an "activating" group instead of a blocking group to direct the regiochemistry of the cyclization step. It is our hope that further investigation of the ideas set forth here will ultimately lead to an even more efficient solution to this problem.

Acknowledgment. We thank the Public Health Service and the Petroleum Research Fund, administered by the American Chemical Society, for providing generous funding for this work.

Comparison of Stereochemistry of Fatty Acid and Cladosporin Biosynthesis in *Cladosporium cladosporioides* Using ^2H Decoupled ^1H, ^{13}C NMR Shift Correlation

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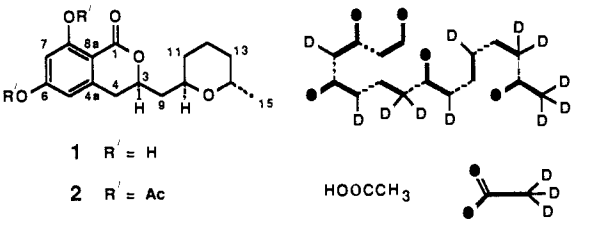
Received June 22, 1987

Extensive experiments employing stable isotope labeling support the idea that partially reduced polyketides are assembled by a process similar to fatty acid biosynthesis.[1] At many sites along carbon chains the correct oxidation state and stereochemistry are believed to appear as a result of the assembly process.[1,2-4] In a few cases intact six- or eight-carbon fatty acids have been employed as chain starter or terminator units.[5] Very recently Hutchinson, Cane, and co-workers demonstrated that functionally and stereochemically correct "diketides" derived from two propionate units can be incorporated intact by *Streptomyces* species into macrolides like tylactone and erythromycin A,[3,4] but this has not yet been achieved with fungi (which generally utilize acetate rather than propionate). A possible approach to examining the relationship of polyketide and fatty acid biosyntheses in fungi involves comparison of the cryptic stereochemistry of acetate-derived hydrogens in both types of metabolites in a single organism.[6] This may be especially useful since the stereochemistry of the last reductive enzyme of fatty acid biosynthesis (enoyl thiol ester reductase) varies with its source.[6a,7] The present report and the following paper[8] employ a new combination of methods, namely incorporation of a $^{13}C,^2H$ doubly labeled precursor and stereochemical analysis by ^2H decoupled $^1H,^{13}C$ heteronuclear shift correlation NMR spectroscopy,[9] to examine this problem.



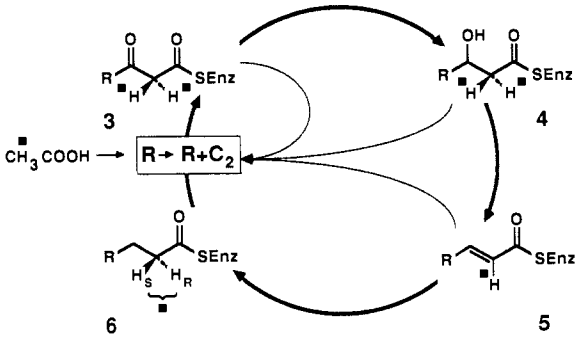
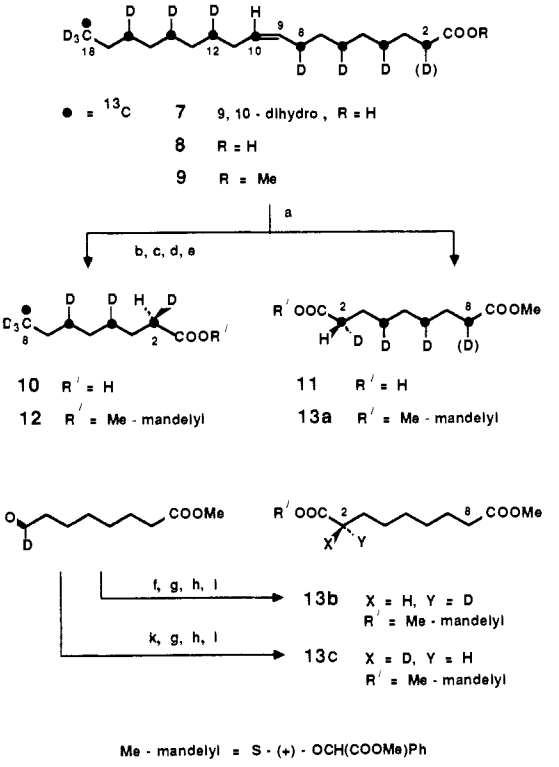


Figure 1. Acetate-derived bonds intact in cladosporin (**1**) and proposed assembly cycle on fatty acid and polyketide synthases.

Scheme I[a]



[a] a. $KMnO_4$, $NaIO_4$; b. CH_2N_2; c. PhMgCl; d. TsOH; e. $NaIO_4$, $RuCl_3$; f. (−)-pinene, 9-BBN; g. TsCl, pyr; h. $Na_2Fe(CO)_4$; l. I_2, methyl *S*-(+)-mandelate; k. (+)-pinene, 9-BBN.

Herein we show that the stereochemistries of cladosporin (**1**) biosynthesis and fatty acid formation by *Cladosporium cladosporioides* NRRL 5507 are *opposite* at several locations.

Cladosporin (**1**) is an antibiotic and plant growth regulator produced by various fungal sources.[10,11] Researchers at Merck

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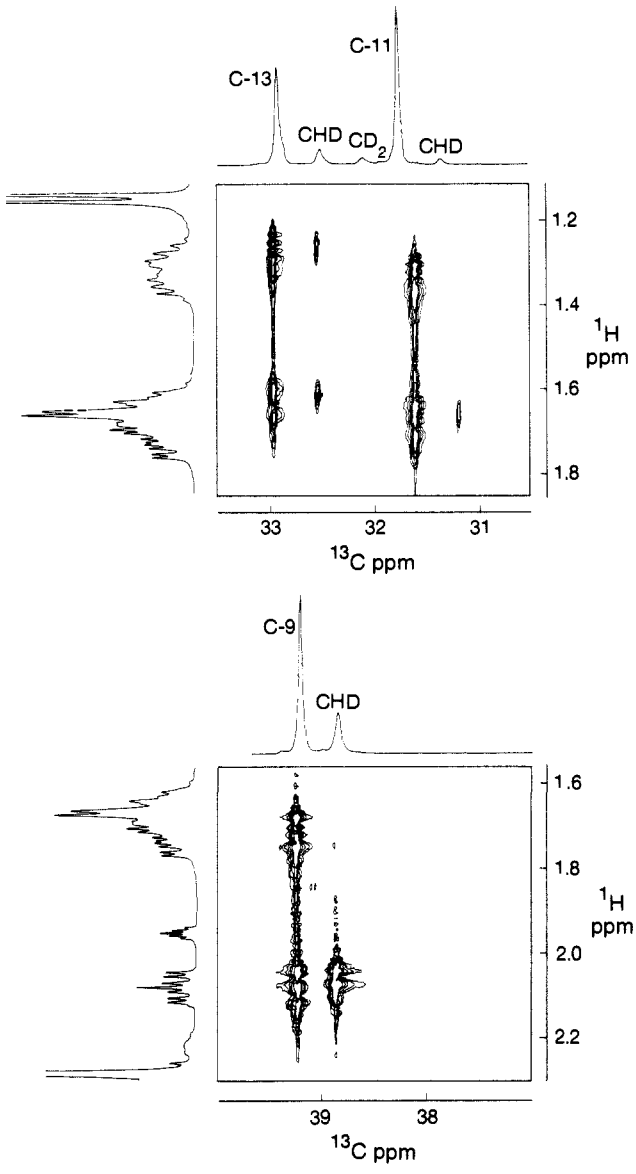

Figure 2. ^2H decoupled $^1H,^{13}C$ chemical shift correlation plots[9] of cladosporin diacetate (**2**) derived from sodium [2-^{13}C,$^2H_3$]acetate. Upfield CHD correlations show C-9 and C-11 are stereospecifically labeled, but C-13 is not. Spectra were obtained on a Bruker WH400 on ca. 0.2 M solutions of **2** in CD_3CN.

have reported its absolute stereochemistry,[11c,12] and Grove and co-workers have previously determined the general features of its biosynthesis in *Aspergillus flavus* by administration of [1-^{13}C]-acetate and [2-^{14}C]malonate.[13] In the present work, separate incorporations of sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,$^2H_3$]-, and [1-^{13}C,$^{18}O_2$] acetates into cultures of *C. cladosporioides* followed by NMR analysis of the diacetates **2** derived from the resulting labeled samples of **1** confirmed the results with *A. flavus* and identified bonds derived intact from acetate as shown in Figure 1.[14] Clearly the number of acetate-derived hydrogens

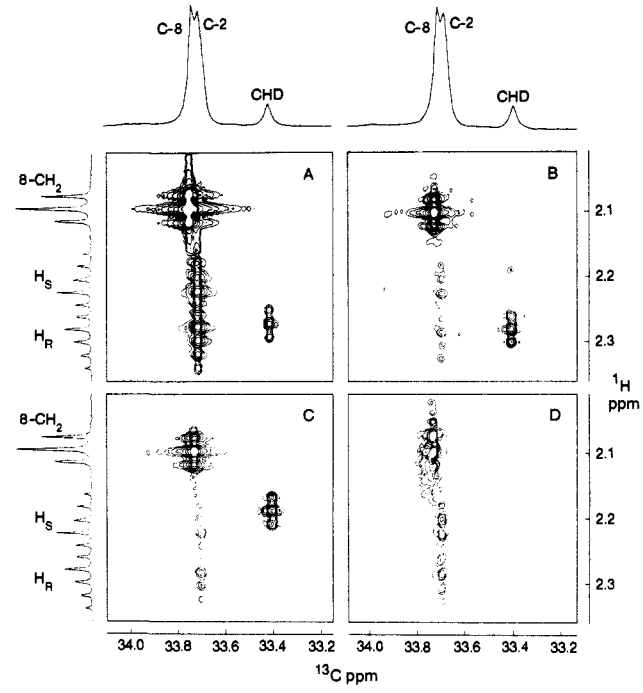

Figure 3. ^2H decoupled $^1H,^{13}C$ chemical shift correlation plots[9] of the C-8 and C-2 regions of **13a** (A), **13b** (B), **13c** (C), and unlabeled material (D). Spectra were measured on a Bruker WH400 with use of ca. 0.3 M solutions in C_6D_6.

at any nonaromatic site corresponds to that predicted from the oxidation state achieved by the *previous* acetate unit (i.e., one closer to the starter) during an assembly process (**3** → **4** → **5** → **6**) akin to fatty acid biosynthesis. Thus the stereochemistry of the single acetate hydrogen at C-11 would be determined by enzymatic reduction of enoyl thiol ester **5** to intermediate **6** enroute to **1**. This is in analogy to the construction of stearic acid (**7**) and its dehydrogenation product,[6a,15] oleic acid (**8**), which *C. cladosporioides* also produces from acetate.

To determine the stereochemistry of these reductions, sodium [2-^{13}C,$^2H_3$]acetate was incorporated (ca. 2%) in a single experiment into both the polyketide cladosporin (**1**) and into fats derived from **7** and **8**. Deuterium-decoupled $^1H,^{13}C$ NMR shift correlation[9] of labeled **2** (Figure 2) derived from **1** shows that the hydrogen of the CHD group at C-11 occupies the downfield pro R position (equatorial).[16] Hence the acetate deuterium is axial at C-11 and the methylene configuration is *S on the growing polyketide chain.* Labeling at C-9 of **1** is also stereospecific although the configuration at that site is presently unknown. Hydrolysis of the fats followed by isolation of the oleic acid (**8**) as its methyl ester **9** and degradative transformation afforded **10** and **11** (Scheme I). These were converted to their (*S*)-(+)-methylmandelyl esters **12** and **13a** for analysis by the same NMR technique[9] since the 2-*pro R* hydrogens are known to appear downfield in such esters.[17] To confirm this for the functionalized ester **13a**, the deuteriated diastereomers **13b** and **13c** were independently synthesized[14] and analyzed by the same method. The spectra (Figure 3) show that deuterium configuration at C-2 of **13a** (corresponding to C-8 of oleate **8**) is *S*, which because of

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(16) The NMR assignment of the axial and equatorial methylene hydrogens at C-11 is based on their respective ~8.8 Hz and ~2.8 Hz coupling to the C-10 axial hydrogen as well as on chemical shift differences. These values are well-precedented for oxygen-containing rings: Kotowycz, G.; Lemieux, R. U. *Chem. Rev.* **1973**, *73*, 669–698.

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priority change corresponds to *R on the growing chain* of the saturated fatty acid **7**. As expected,[7a,d] C-2 of **12** (corresponding to C-12 of **8**) has *R* configuration.

The results show *opposite stereochemistry of acetate-derived deuterium* for fatty acid biosynthesis and polyketide (cladosporin (**1**)) formation. Interestingly, the absolute stereochemistry of single carbon–oxygen bonds of **1** derived intact from acetate at C-14 and C-3 corresponds to *S* on the growing chains (i.e., **4**), again opposite to that expected (*R*) in fatty acid biosynthesis.[7] The same phenomenon can be seen at certain sites in the macrolide antibiotic brefeldin A[6] and has been observed in two other fungal systems in our laboratories.[14]

Acknowledgment. We thank Dr. Heidrun Anke (Universität Kaiserslautern BRD) and Dr. Peter Scott (Banting Research Centre, Ottawa) for generous gifts of cladosporin. Helpful discussions with Dr. Craig Townsend (Johns Hopkins University) and technical assistance by Dr. Laird Trimble are gratefully acknowledged. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada.

Supplementary Material Available: Experimental procedures for production of **1**, **2**, and **9–13c** and for acquisition of NMR spectra (9 pages). Ordering information is given on any current masthead page.

Stereochemical Features of Enoyl Thiol Ester Reductase in Averufin and Fatty Acid Biosynthesis in *Aspergillus parasiticus*

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Received June 22, 1987

Identification of starter units and the mechanism(s) of their homologation, typically by malonylCoA or its alkylated derivatives, are issues central to understanding polyketide biosynthesis. We have described the intact incorporation (3–4%) of [1-^{13}C]hexanoic acid into the side chain of averufin (**2**), accompanied by a 0.5–1%/site secondary incorporation of label throughout the molecule after β-oxidation to [1-^{13}C]acetate.[1,2] Alternative C_4–C_8 acids suffered only degradation to acetate and secondary incorporation at levels comparable to that from hexanoate.[2] On the basis of these findings and the demonstrated roles of aryl[3] and branched chain[4] starters, we proposed, but could not strictly prove, that hexanoylCoA served as the primer of the aflatoxin B_1 biosynthetic pathway (path A, Scheme I). Recently, Simpson has described[5] the incorporation of diethyl [2-^{13}C]malonate into averufin at a uniform level, save a lower rate of incorporation at C-6′. These observations were consistent with an acetate "starter effect"[6] and were interpreted to support path B (Scheme I) to averufin as a

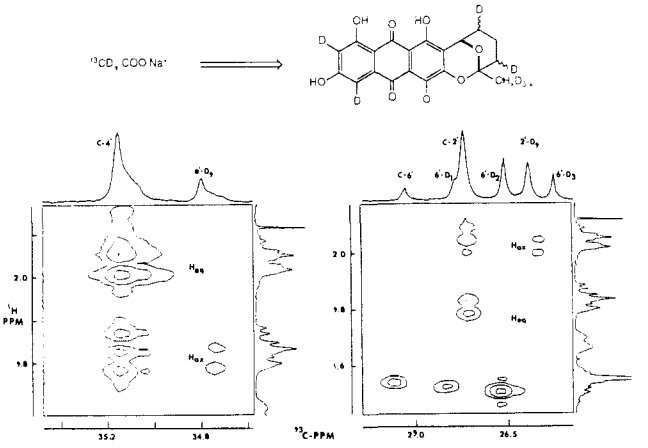
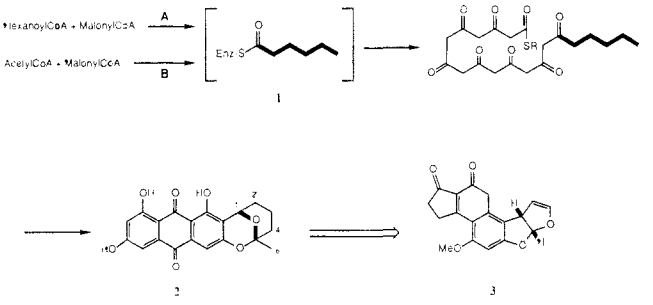


Figure 1. ^{2}H decoupled ^{1}H,^{13}C chemical shift correlation plots of averufin from the incorporation of [2-$^2H_3$,^{13}C]sodium acetate for C-4′ (left) and C-2′ and C-6′ (right).

Scheme I



decaketide rather than as a heptaketide primed by hexanoylCoA (path A). The uniform level of ^{13}C-label seen in this experiment, however, may simply reflect an intracellular pool-size effect and its extent of enrichment by exogeneous labeled malonate affecting polyketide and fatty acid biosynthesis equally. Nonetheless, the possibility cannot be excluded that hexanoylCoA generated in vivo may reversibly exchange with a synthetase that normally produces this segment from acetate and malonate, e.g., **1**. The well-established observation that thioesters can exchange in this manner in fatty acid biosynthesis[7] has been extended in the last year to macrolides[8] and suggests that hexanoate could behave similarly in averufin biosyhthesis.

In this paper we apply the useful technique of ^{2}H decoupled ^{1}H,^{13}C heteronuclear shift correlation spectroscopy[9] to examine the stereochemical fate of [2-$^2H_3$,^{13}C]acetate in oleic acid and averufin **2** biosynthesis. While the second reductive enzyme of fatty acid biosynthesis, enoyl thiol ester reductase, is stereochemically variable across a wide range of organisms,[10] for the imperfect fungi, which are notable producers of polyketide natural products, deuterium from [2-$^2H_3$,^{13}C]acetate assumes the *R* configuration in the growing fatty acid chain in the cases that have been examined to date. For the averufin-accumulating mutant

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