Biosyntheses of Antibiotic A26771B by *Penicillium turbatum* and Dehydrocurvularin by *Alternaria cinerariae*: Comparison of Stereochemistry of Polyketide and Fatty Acid Enoyl Thiol Ester Reductases

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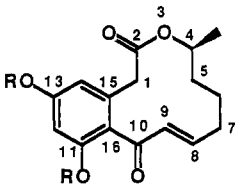
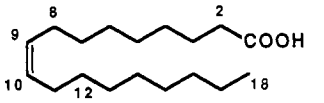
Abstract: The biosyntheses of reduced polyketides and fatty acids were compared by use of stable isotope labeling and NMR spectroscopy. Incorporations of sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,$^{18}O_2$]-, [1-^{13}C,$^{2}H_3$]-, and [2-^{13}C,$^{2}H_3$]acetates into dehydrocurvularin (**1**) by *Alternaria cinerariae* ATCC 11784 followed by ^{13}C NMR analysis provided the pattern of all bonds derived intact from acetate. Similar experiments with *Penicillium turbatum* ATCC 28797 as well as incorporations of $^{18}O_2$ gas and [1,4-$^{13}C_2$]succinic acid afforded the analogous distribution of biosynthetic units in antibiotic A26771B (**3**). Degradations of samples of **1** and **3** derived from [2-^{13}C,$^{2}H_3$]acetate followed by esterification with methyl (*S*)-(+)-mandelate gave **10** and **24**, respectively. Analysis of these esters by ^{2}H-decoupled ^{1}H,^{13}C shift correlation NMR spectroscopy showed that acetate-derived deuterium at C-7 of dehydrocurvularin (**1**) and at C-8 of A26771B (**3**) resides in the *pro-S* positions. Degradation and similar stereochemical analyses of oleic acid (**2**) samples obtained from *A. cinerariae* and *P. turbatum* fermentations with [2-^{13}C,$^{2}H_3$]acetate show that acetate-derived deuterium occupies *pro-R* positions on the growing chains during fatty acid biosynthesis. This indicates that the enoyl thiol ester reductases responsible for polyketide and fatty acid biogenesis operate with different stereochemistry in the same organism. The results are compared to observations with other fungal polyketides.

Numerous experiments have shown that biological formation of polyketide secondary metabolites occurs by a process closely related to biosynthesis of fatty acids.[1-3] However, in certain fungi the stereochemistry of acetate-derived hydrogens is opposite in polyketides and fatty acids.[1,4] This appears to be due to a difference in the reactions catalyzed by the corresponding enoyl thiol ester reductases (Figure 1). During fatty acid production these enzymes, which perform the last reductive step in each cycle of two-carbon (acetate/malonate) addition, are known to operate with different stereochemistry in various organisms.[5] The present study examines the configuration of acetate-derived hydrogens at fully reduced sites in polyketides and fatty acids produced by a particular organism. The purpose is to compare the stereochemical relationship between the two enzyme systems and to provide a possible tool for detecting incorporation of longer (>4 carbons) fatty acid fragments into polyketides.[3d,4d,6] Two additional examples are studied: the biosynthesis of dehydrocurvularin (**1**) and oleic acid (**2**) by *Alternaria cinerariae* ATCC 11784 and the formation of antibiotic A26771B (**3**) and **2** by *Penicillium turbatum* ATCC 28797.

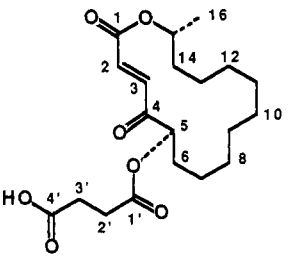




1 R = H **2**

4 R = H 8, 9 - dihydro

5 R = Ac



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The polyketide macrocyclic lactones **1** and **3** possess interesting biological properties.[7] Dehydrocurvularin (**1**) and closely related compounds [e.g., curvularin (**4**)] are produced by a number of fungal species, especially members of the genus *Alternaria* which are potent plant pathogens.[8] This compound has antimicrobial

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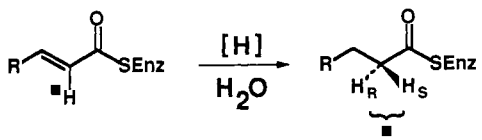

Figure 1. Reaction catalyzed by enoyl thiol ester reductase during polyketide and fatty acid biosynthesis.

Table I. ^{13}C and ^{1}H Chemical Shifts of Dehydrocurvularin (**1**) and Isotope Shifts in ^{13}C NMR Spectra[a]

carbon	^{13}C δ	^{1}H δ	^{2}H isotope shift, ppb (type)[b]	^{18}O isotope shifts, ppb[c] 1	5
4-Me	20.23	1.21	280 (α), 560 (2α), 840 (3α)		
6	24.90	1.70, 1.90	108 (β), 216 (2β)		
7	33.15	2.37, 2.43	390 (α)		
5	34.83	1.67, 1.90	400 (α), 800 (2α)		
1	43.59	3.62, 4.07	280 (α)		
4	73.09	4.70	39 (β), 78 (2β), 117 (3β)	39	38
12	103.16	6.30	none		
14	113.90	6.34	none		
16	116.21				
9	133.11	6.74	none		
15	139.63		*d*		
8	150.35	6.54	31 (β)		
13	163.51		*d*	10	19
11	165.92			10	20
2	172.47		10 (β)	37	36
10	198.11		*d*		

[a] Obtained in $[^{2}H_6]$acetone at 100.6 MHz for ^{13}C and 400 MHz for ^{1}H on a Bruker WH400 spectrometer. [b] Samples of **1** derived from sodium $[1\text{-}^{13}C,^{2}H_3]$acetate (β shifts) and $[2\text{-}^{13}C,^{2}H_3]$acetate (α shifts). All isotope shifts are ±10 ppb. [c] Sample of **1** derived from sodium $[1\text{-}^{13}C,^{18}O_2]$acetate; **5** obtained from **1**. [d] No β-isotope shift observed; see ref 2a.

activity (primarily antifungal) and is quite phytotoxic.[8d,g,i] Synthetic studies on curvularin (**4**),[9] which can be obtained by reduction of **1**,[8g] have established the configuration at C-4 as *S*.[8j,9a] Antibiotic A26771B (**3**) is relatively nontoxic and exhibits moderate antimicrobial activity against Gram-positive bacteria, mycoplasma, and fungi.[7a,10] It has been the target of numerous syntheses;[11] processes beginning from glucose[11b,d] have shown that the absolute configurations of **3** are 5*S*,15*R*. Hence, the stereochemistries of the carbon–oxygen bond at the "starter unit"[2,3] of the fungal 16-carbon polyketide macrocyclic lactones **1** and **3** are opposite. These two antibiotics therefore present an interesting test of possible stereochemical variability in polyketide biosynthesis.

Results

NMR Assignment and Biosynthetic Origin of Atoms in Dehydrocurvularin (1). Complete identification of ^{1}H and ^{13}C NMR signals was essential for the present study. The basic approach was to first assign all carbon resonances by use of 2D INADEQUATE NMR on biosynthetically ^{13}C-enriched samples of **1** and then to use $^{1}H,^{13}C$ shift correlation NMR spectroscopy to obtain hydrogen assignments.[12] Two types of dehydrocurvularin (**1**) samples were generated: the first, derived from sodium $[1,2\text{-}^{13}C_2]$acetate, shows ^{13}C coupling primarily between atoms which are part of the same biosynthetic unit (*intraunit*); the other, derived from a 1:1 mixture of sodium $[1\text{-}^{13}C]$- and $[2\text{-}^{13}C]$acetates, displays couplings only between adjacent units (*interunit*). Because of the increased level of carbon-13 (ca. 3% incorporation per site), 2D INADEQUATE spectra could be obtained on 18-mg (intraunit) and 12-mg (interunit) samples.[13] Together the two spectra give

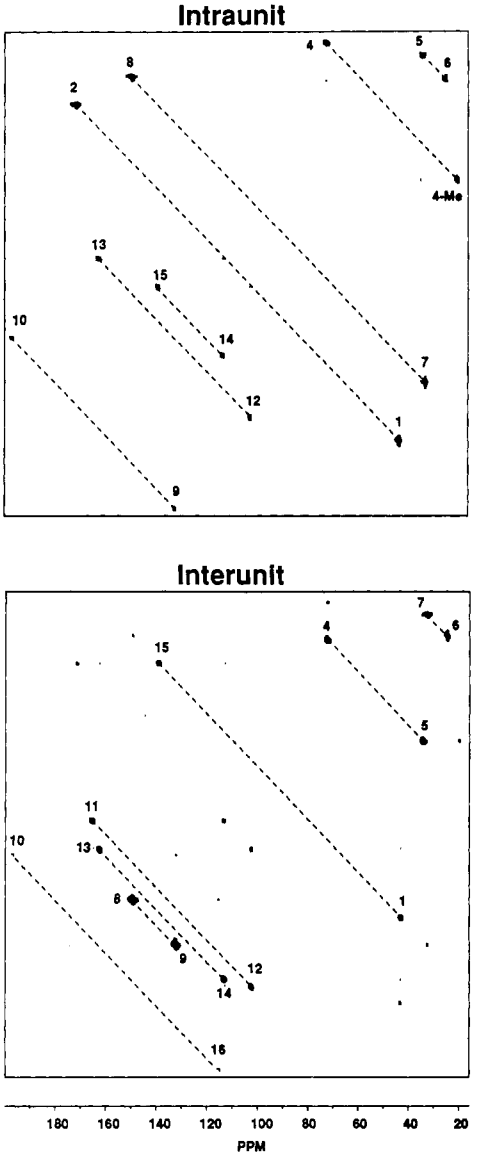

Figure 2. Symmetrized 2D INADEQUATE NMR spectra of dehydrocurvularin (**1**) enriched by incorporation of sodium $[1,2\text{-}^{13}C_2]$acetate (upper spectrum) and a 1:1 mixture of sodium $[1\text{-}^{13}C]$- and $[2\text{-}^{13}C]$-acetates (lower spectrum). Some intraunit coupled carbons can also be seen in the interunit spectrum. The C-11 to C-16 intraunit interaction is present but is not visible at the level shown.

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(13) The intensity of coupled signals in the "interunit" sample is much stronger than expected on purely statistical grounds (~0.09%) because pulse feeding of the labeled precursors momentarily floods the biosynthetic enzymes with carbon-13-labeled substrate.[3d]

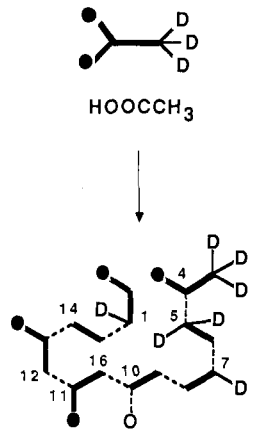

Figure 3. Distribution of biosynthetic units in dehydrocurvularin (**1**) showing acetate bonds remaining intact.

the complete carbon connectivity and assignment (Figure 2). This method has been previously shown to be very effective during biosynthetic studies on the polyene antibiotic fungichromin.[3d] Ensuing assignment of the hydrogen resonances by $^{1}H,^{13}C$ shift correlation employed unlabeled material. Chemical shift values for dehydrocurvularin (**1**) are given in Table I.

Separate incorporations of sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,$^{2}H_3$]-, [2-^{13}C,$^{2}H_3$]-, and [1-^{13}C,$^{18}O_2$]acetates into **1** by *A. cinerariae* were followed by the usual NMR analyses for signal enhancements, ^{13}C-coupled resonances (by 2D INADEQUATE, see above), and ^{2}H- or ^{18}O-induced isotope shifts.[2a,c] The small magnitudes of the ^{18}O isotope shifts at the phenolic carbons made resolution of shifted signals difficult, but this problem could be overcome by examination of the corresponding diacetate (**5**). Attachment of an electron-withdrawing group such as acetyl is known to increase the separation of the $^{13}C-^{16}O$ and $^{13}C-^{18}O$ signals.[2a,14] The pattern of bonds in dehydrocurvularin (**1**) derived intact from acetate is given in Figure 3. Fermentation of *A. cinerariae* in a closed system[15] under an atmosphere containing $^{18}O_2$ (50% isotopic purity) failed to enrich any oxygen atom in **1**, including the one at C-10 which was not labeled by sodium [1-^{13}C,$^{18}O_2$]acetate. The results also show a lack of any acetate deuterium label at certain expected sites, namely, C-9, C-12, and C-14. Presumably this is due to exchange with the unlabeled aqueous media during the polyketide assembly process.

Stereochemistry of Dehydrocurvularin (1) and Oleic Acid (2) Formation by *A. cinerariae*. Configurations of the lone acetate-derived hydrogens at C-1 and C-7 of **1** are of key interest. Stereochemistry at C-7 should be determined by the polyketide enoyl thiol ester reductase reaction whereas that at C-1 is important because two rather than one acetate hydrogen would be expected at that site.[1] Hence, the dehydrocurvularin sample obtained after incorporation of sodium [2-^{13}C,$^{2}H_3$]acetate was examined by deuterium-decoupled $^{1}H,^{13}C$ shift correlation NMR spectroscopy.[1,16] The spectrum of the methylene group at C-1 clearly shows CHD correlation signals for both the upfield and downfield hydrogens of the AB system (Figure 4), thereby demonstrating that labeling by the lone acetate deuterium at that site is nonstereospecific. In contrast, the corresponding spectrum of the C-7 group suggests that hydrogen in the CHD species occupies only the position which resonates upfield. However, the small separation of the *pro-R* and *pro-S* hydrogens (<0.1 ppm) together with their location on a large ring makes definitive stereochemical assignment difficult.

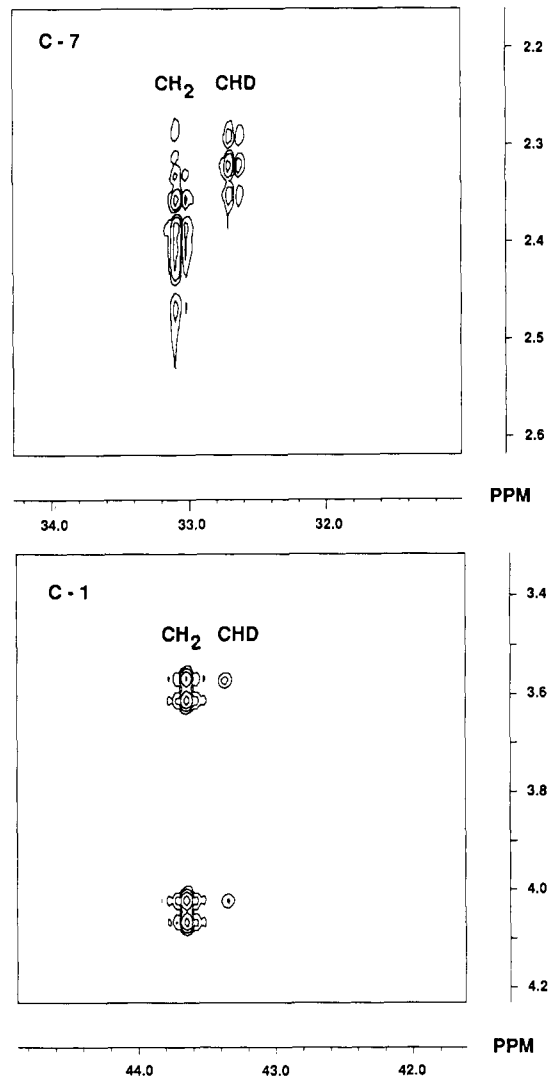

Figure 4. ^{2}H-decoupled $^{1}H,^{13}C$ shift correlation NMR spectra of dehydrocurvularin (**1**) derived from sodium [2-^{13}C,$^{2}H_3$]acetate. Upfield CHD correlations show that C-7 is stereospecifically labeled but C-1 is not. Spectra were obtained on ca. 0.15 M solutions of **1** in [$^{2}H_6$]acetone on a Bruker WH400 instrument. See ref 33.

To circumvent this problem, the dehydrocurvularin (**1**) was converted to its dimethyl ether **6** which was degraded and derivatized as shown in Figure 5. Oxidation of **6** with a mixture of potassium permanganate and sodium periodate[17] gives modest yields of diacids **7** (28%) and **8** (25%). Hydrolysis of **7** according to the Gassman procedure[18] affords the known[19] chiral δ-caprolactone **9** in 39% yield, thereby confirming the absolute stereochemistry at C-4 of **1** from *A. cinerariae* as *S*. The next objective was esterification of the aliphatic carboxyl group with methyl (*S*)-(+)-mandelate because it is known that the NMR signal of the *pro-R* hydrogen of the adjacent methylene group (corresponding to C-7 of **1**) generally appears downfield of the *pro-S* resonance in such esters.[1,20] Reaction of diacid **7** with dicyclohexylcarbodiimide and methyl mandelate in the presence of (dimethylamino)pyridine produces the lactone ester **10** in 39% yield. Replacement of the alcohol in this reaction with methanol generates the corresponding methyl ester **11** in similar yield. In order to briefly examine the scope of this reaction, the homo-

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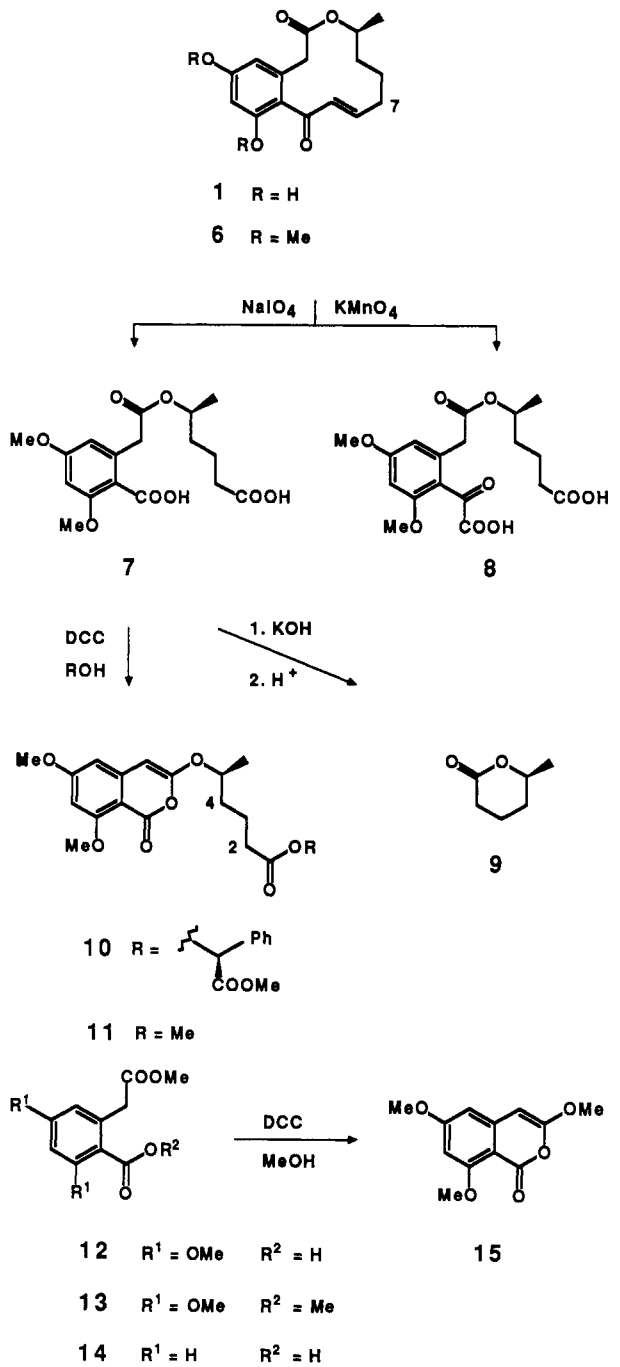


Figure 5. Degradation of dehydrocurvularin (1).

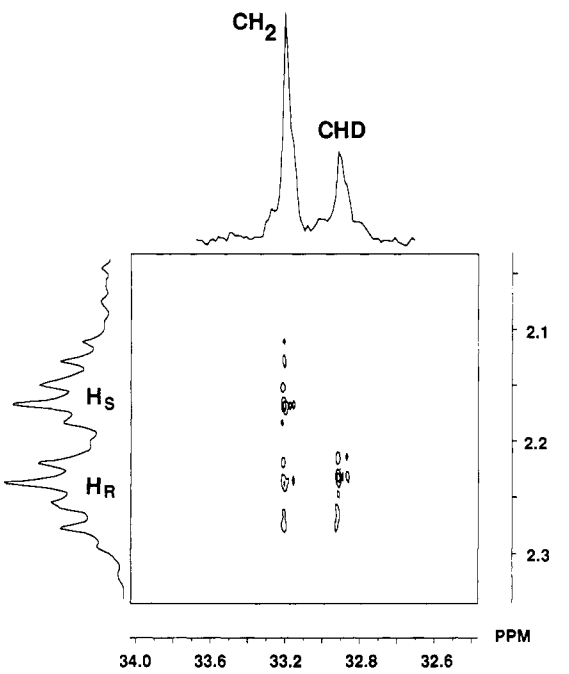
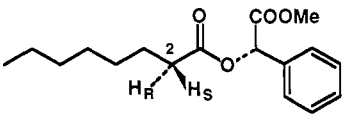


Figure 6. ^{2}H-decoupled ^{1}H,^{13}C shift correlation NMR spectrum of the C-2 methylene group of **10** obtained by degradation of dehydrocurvularin (**1**) derived from sodium [2-^{13}C,^{2}H$_3$]acetate. The ^{2}H,^{1}H-decoupled ^{13}C NMR spectrum and ^{1}H NMR spectrum are shown on the horizontal and vertical axes, respectively. Spectra were obtained on ca. 0.05 M solutions of **10** in C_6D_6. See ref 33.

phthalic esters **12**, **13**, and **14** were subjected to the same conditions with methanol as the alcohol partner. Only the dimethoxy acid **12** cyclizes to the analogous lactone **15** in significant amounts (51%). The two-dimensional deuterium-decoupled ^{1}H,^{13}C shift correlation NMR spectrum[1,16] of the key methylene carbon (C-2) of labeled **10** shows that the hydrogen of the CHD group occupies only the downfield *pro-R* position (Figure 6). Thus acetate-derived deuterium at C-7 of dehydrocurvularin (**1**) obtained after incorporation of [2-^{13}C,^{2}H$_3$]acetate is in the *S position on the growing chain* as a result of the polyketide enoyl thiol ester reductase reaction. This configuration and the *S* stereochemistry of the acetate-derived carbon–oxygen bond at C-4 of **1** correspond to those observed for the polyketide cladosporin.[1]

In order to determine the stereochemistry of the fatty acid enoyl thiol ester reductase in *A. cinerariae*, the fats from the fermentation with sodium [2-^{13}C,^{2}H$_3$]acetate were hydrolyzed, methylated, and separated as described previously.[1] Methyl oleate obtained in this way was degraded to octanoic acid and monomethyl nonanedioate, which were then individually esterified with methyl (*S*)-(+)-mandelate to **16** and **17**; these compounds were



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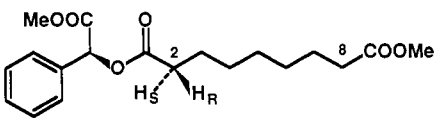

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analyzed by the deuterium-decoupled ^{1}H,^{13}C shift correlation NMR method exactly as before.[1] The spectra are essentially identical with those obtained with corresponding samples from *Cladosporium cladosporioides*[1] and show that oleic acid (**2**) from *A. cinerariae* bears acetate deuterium in the *S* position at C-8 and the *R* position at C-12. No deuterium is present at C-10. These results demonstrate that acetate-derived hydrogens reside at the *pro-R sites on the growing chain* during saturated fatty acid biosynthesis. Hence, the stereochemical outcome of this enoyl thiol ester reductase reaction is opposite to that which generates the C-7 methylene of dehydrocurvularin (**1**).

Formation of Antibiotic A26771B (3) and Oleic Acid (2) by *P. turbatum*. Assignment of the ^{1}H and ^{13}C NMR spectra of A26771B (**3**) employed the same approach as used for dehydrocurvularin (**1**). Combination of the two-dimensional INADEQUATE spectra of samples of **3** derived from sodium [1,2-^{13}C$_2$]acetate (intraunit coupling) and a 1:1 mixture of sodium [1-^{13}C]- and [2-^{13}C]acetates (interunit coupling) gives the complete carbon connectivity of the lactone ring except for the C-8 to C-9, C-9 to C-10, and C-11 to C-12 bonds. In these cases the chemical shifts of the carbon pairs are so similar that the INADEQUATE experiments fail to show the connections. However, C-8 and C-12 can be assigned by their observable interaction with C-7 and C-13, respectively, and the C-10 to C-11 bond can be seen in the interunit spectrum. The resonances of the three-carbon fragment (C-9,

Table II. ^{13}C Chemical Shifts of A26771B (**3**) and Isotope Shifts in ^{13}C NMR Spectra[a]

carbon	^{13}C δ	isotope shifts, ppb	
		^{2}H[b]	^{18}O
16	19.54	280, 560, 840	
7	22.08		
13	23.33		
9	26.75		
10	26.91	400	
8	27.05	410	
12	27.66	410	
11	27.77		
2′[c]	28.11		
3′[c]	28.41		
6	28.72	340	
14	34.34	450, 900	
15	72.57		41[d]
5	77.32		29[e]
2	132.78	310	
3	135.59		
1	165.28		36[d]
1′	171.85		37,[d] 13[e]
4′	177.03		
4	195.95		

[a] Obtained in $CDCl_3$ at 100.6 MHz on a Bruker WH400 spectrometer. [b] Sample of **3** derived from sodium [2-^{13}C,$^{2}H_3$]acetate; α shifts in ^{1}H,^{2}H-decoupled spectra. [c] These two signals may be interchanged. [d] Sample of **3** derived from sodium [1-^{13}C,$^{18}O_2$]acetate. [e] Sample of **3** derived from $^{18}O_2$ fermentation.

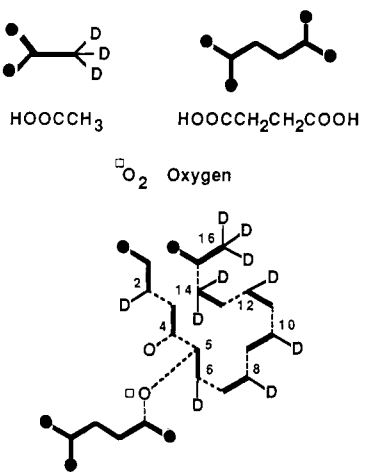

Figure 7. Distribution of biosynthetic units in antibiotic A26771B (**3**) showing intact acetate bonds.

C-10, and C-11) were assigned by making the reasonable assumption that the normal head to tail arrangement of intact acetate units observed in the rest of the lactone ring is maintained. The assignments for the carbons are given in Table II.

To determine the biosynthetic origin of atoms in antibiotic A26771B (**3**), individual fermentations of *P. turbatum* were done with sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [2-^{13}C,$^{2}H_3$]-, and [1-^{13}C,$^{18}O_2$]acetates as well as with oxygen-18 gas[15] and [1,4-$^{13}C_2$]succinic acid. Application of the same NMR techniques[2a,c] described above for analysis of labeled dehydrocurvularin gives the arrangement of biosynthetic units and the bonds derived intact from acetate in **3** (Figure 7). This proposal does contain the reasonable assumption that the C-9 to C-10 and C-11 to C-12 bonds remain intact from acetate because resonance overlap precludes direct observation of this by INADEQUATE NMR or by analysis of coupling patterns. In experiments with labeled acetates, some ^{13}C enrichment can also be seen in the succinyl residue due to operation of the citric acid cycle,[2a] but the signal enhancements are much smaller. It is interesting that the carbon–oxygen bond at C-15 of **3**, which has *R* configuration, originates intact from acetate just like the C–O bond at C-4 of dehydrocurvularin (**1**) which possesses *S* stereochemistry. The oxygen at C-4 of A26771B (**3**) is not labeled by either oxygen gas or acetate and presumably originates from the aqueous media. Examination of **3** derived from [2-^{13}C,$^{2}H_3$]acetate for α-isotope shifts in ^{2}H,^{1}H-decoupled ^{13}C NMR spectra[2a] shows that the number of deuteriums at any site corresponds to that expected from the oxidation state achieved by the previous acetate unit during polyketide biosynthesis.[1]

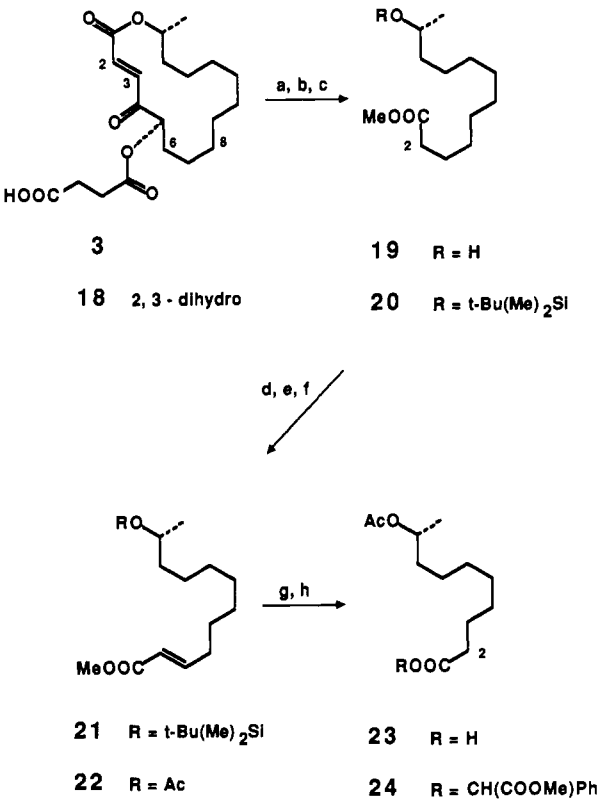

Figure 8. Degradation of A26771B (**3**): (a) H_2, Rh/C; (b) NaOH, O_2 then HCl, CH_2N_2; (c) *t*-Bu(Me)$_2$SiCl, imidazole; (d) LDA, PhSeBr; (e) H_2O_2; (f) Ac_2O, $FeCl_2$; (g) $NaIO_4$, $RuCl_3$·$3H_2O$; (h) methyl (*S*)-(+)-mandelate, DCC, DMAP.

In order to determine the stereochemistry of the polyketide thiol ester reductase involved in biosynthesis of A26771B (**3**), the sample derived from [2-^{13}C,$^{2}H_3$]acetate was degraded (Figure 8). The original objective was cleavage of four carbons to form the parent acid of **20** which could be esterified with methyl (*S*)-(+)-mandelate for stereochemical analysis of its C-2 position (corresponding to C-6 of **3**). Unfortunately, deuterium loss from that site by exchange could not be avoided during the basic degradation. Therefore, two additional carbons were removed to allow generation of ester **24** whose C-2 methylene group (corresponding to C-8 of **3**) retains deuterium and can be analyzed by NMR spectroscopy. Hydrogenation of **3** to its dihydro derivative **18** (97% yield) followed by basic oxidative degradation and esterification with diazomethane gives the hydroxy ester **19** (66% yield). Protection of the alcohol affords **20** into which a double bond is introduced via selenoxide fragmentation to form **21** (62% yield). Exchange of the protecting group to generate the acetate **22** (77% yield) proved necessary for satisfactory oxidative cleavage to produce the desired acid **23** (65% yield). Esterification as before with methyl (*S*)-(+)-mandelate generates **24** in 95% yield. Two-dimensional deuterium-decoupled ^{1}H,^{13}C shift correlation NMR spectra[16] of the C-2 methylene of **24** show that the CHD signal bears hydrogen exclusively in the downfield *pro-R* position (Figure 9). The stereochemical assignment is based on extensive precedent on the chemical shifts of both functionalized (e.g. **17**)[1] and unfunctionalized[20] mandelate esters of fatty acids. Hence, the results show that the acetate-derived deuterium at C-8 of **3** has *S configuration on the growing chain* during polyketide biosynthesis. This is the same stereochemistry as that at C-11 of cladosporin[1] and at C-7 of dehydrocurvularin (**1**).

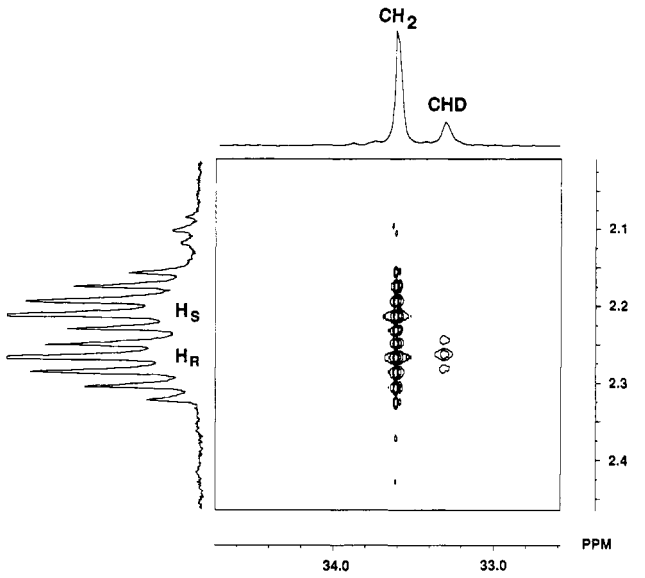

Figure 9. ^{2}H-decoupled ^{1}H,^{13}C shift correlation NMR spectrum of the C-2 methylene of **24** obtained from **3** derived from sodium [2-^{13}C,^{2}H$_3$]acetate. Spectra were obtained on ca. 0.5 M solutions of **24** in C_6D_6.

The stereochemistry of fatty acid enoyl thiol ester reduction in *P. turbatum* was checked by the same method used for *A. cinerariae* (see above) and *C. cladosporioides* (which produces cladosporin).[1] Methyl oleate derived from the sodium [2-^{13}C,^{2}H$_3$]acetate fermentation used to produce labeled A26771B (**3**) was degraded to **17** as before.[1] Analysis by the same 2D NMR technique gave results essentially identical with those seen with the other fungal systems. All *fungal* fatty acid enoyl thiol ester reductases examined to date leave the lone acetate-derived hydrogen in the *pro-R* site on the growing saturated chain.[1,4,5]

Discussion

Full NMR assignment of macrocyclic lactones like dehydrocurvularin (**1**) and antibiotic A26771B (**3**) presents a considerable challenge because of extensive overlap of both the ^{1}H and ^{13}C signals of the methylene groups. For example, eight carbon resonances of **3** fall within a 2-ppm range. Fortunately, 2D INADEQUATE NMR spectra[12] of small (ca. 0.05 mmol) samples that are biosynthetically ^{13}C enriched to give intraunit or interunit couplings allow elucidation of the carbon connectivity pattern (Figure 2).[3d] Since independent identification of a single carbon resonance then gives the complete carbon assignment, a routine ^{1}H,^{13}C shift correlation experiment[12] yields the proton assignments. However, direct stereochemical designation of the methylene AB patterns as *pro-R* or *pro-S* hydrogen is often not possible in such lactones without isotopic labeling and degradation.

The eight intact acetate units in dehydrocurvularin (**1**) (Figure 3) and A26771B (**3**) (Figure 7) have the expected[7a,8h] head to tail connection typical of polyketide biogenesis.[2,3] In examining the carbon–oxygen and carbon–hydrogen bonds derived intact from acetate, it is interesting to compare the results to those of other partially reduced fungal polyketides which have been examined. Among these are heptaketides like palitantin (**25**)[21] and monocerin (**26**),[2b,22] octaketides like cladosporin[1] and brefeldin A (**27**),[4a,b] the nonaketide mevinolin (**28**),[23] and the "decaketide" aflatoxin precursor[24] averufin (**29**)[4d,6] (Figure 10). Usually the number of acetate-derived hydrogens (deuteriums) at any 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 akin to fatty acid biosynthesis. In certain instances where two acetate deuteriums are expected because the preceding unit bears an intact acetate carbon–oxygen bond, only one deuterium is observed, for example, C-1 of dehydrocurvularin (**1**) and C-14 of brefeldin A (**27**).[4b] Generally such sites are nonstereospecifically labeled and result from extensive exchange of the precursor hydrogens (probably at the malonate or β-keto thiol ester stage) with unlabeled aqueous media prior to incorporation. This process also accounts for the invariably *nonstereospecifically* labeled CHD groups at locations which do have substantial amounts of CD_2 species. Typical examples include C-13 of cladosporin,[1] C-5 of dehydrocurvularin (**1**), and C-14 of A26771B (**3**).

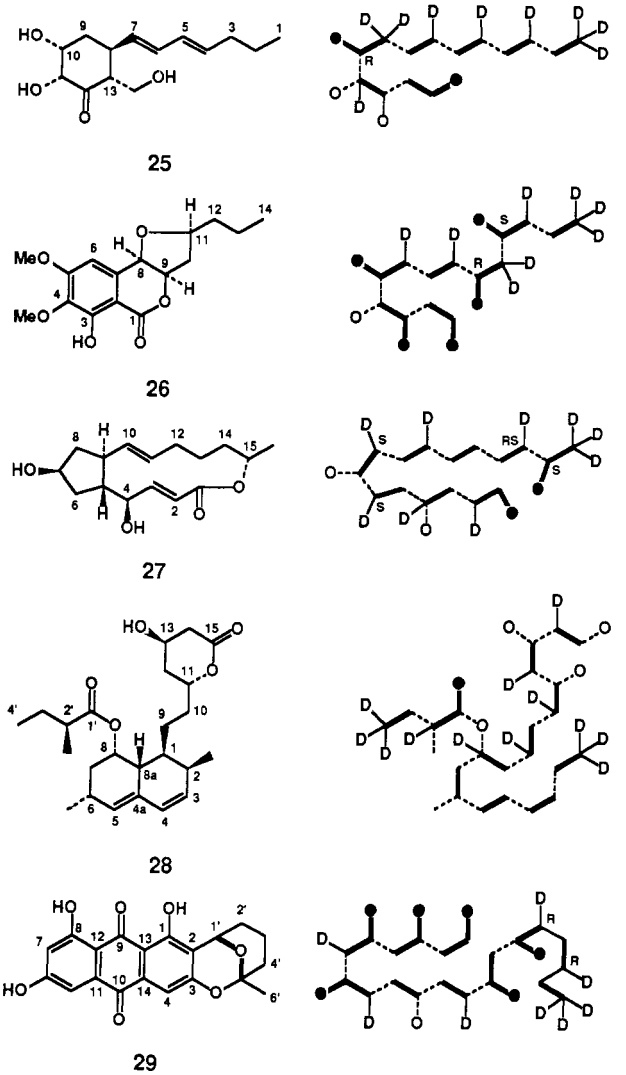

Figure 10. Distribution of C–C, C–O, and carbon–hydrogen (C–D) bonds derived intact from acetate in the polyketides palitantin (**25**), monocerin (**26**), brefeldin A (**27**), mevinolin (**28**), and averufin (**29**). Where known, the stereochemistry relative to the growing polyketide chain is indicated as *R* or *S*.

It is very likely that the stereochemistry of polyketide β-keto thiol ester reductases which generate many of the carbon–oxygen single bonds is variable, in contrast to the corresponding enzyme in fatty acid biosynthesis which in all cases examined forms an *R* alcohol.[5] For example, the octaketide lactones **1** and **3** have intact acetate C–O bonds of opposite stereochemistry in the first acetate unit (i.e., C-4 and C-15, respectively). Similarly, monocerin (**26**) has opposite configurations (relative to the growing polyketide chain) at C-9 and C-11. The possibility that this variation arises from post-assembly reactions catalyzed by oxidation/reduction enzymes (cf. patulin biosynthesis[25]) cannot be

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excluded in many cases at present. However, intact incorporation of diastereomeric *propionate-derived* di- and triketide precursors into macrolides[3a,b,e] suggests that the assembly-linked β-keto thiol ester reduction for *acetate-derived* polyketides can also be stereochemically variable.

Although the stereochemistry of fatty acid enoyl thiol ester reductase is the same during each cycle of two-carbon condensation, it is different for various types of organisms.[4,5] In accord with results with other fungi,[1,4a,d,5f] the operation of this enzyme in *A. cinerariae* and *P. turbatum* places acetate-derived hydrogens in the *pro-R* position on growing fatty acid chains. In contrast, the corresponding polyketide enzyme in the same fungi leaves such hydrogens in the *pro-S* position on the growing chains of brefeldin A (**27**) at C-8 and C-6,[4a,b] cladosporin at C-11,[1] dehydrocurvularin (**1**) at C-7, and antibiotic A26771B (**3**) at C-8. So far the only reported case of such hydrogens being in the *pro-R* position relative to the growing polyketide chain is averufin (**29**).[4d] However, these sites (C-2′ and C-4′) are located on a portion of the molecule which may be a hexanoic acid starter unit initially formed by fatty acid biosynthesis.[6] Overall, the admittedly small number of cases examined suggests that the stereochemistry of enoyl thiol ester reductases involved in fatty acid biosynthesis and polyketide formation in fungi may be opposite. If so, the configuration of acetate-derived hydrogens may provide a useful tool for detection of fatty acid fragments acting as chain starters[6] or terminators[3d] in polyketides. Alternatively, knowledge of the cryptic stereochemistry may generate a unified stereochemical hypothesis for acetate-derived fungal polyketides analogous to that proposed for macrolides from Actinomycetes.[26]

In summary, the present work elucidates the biosynthetic patterns of acetate-derived bonds in dehydrocurvularin (**1**) and antibiotic A26771B (**3**). The use of combined 2D INADEQUATE spectra of intraunit and interunit biosynthetically ^{13}C-enriched samples further illustrates the power of this technique[3d] for assignment of NMR spectra. Incorporation of doubly labeled $^{2}H,^{13}C$ precursors and product analysis by two-dimensional deuterium-decoupled $^{1}H,^{13}C$ shift correlation NMR spectroscopy allow rapid determination of deuterium stereochemistry in fatty acid and polyketides.[1,4c,d] Further applications of this approach should determine whether the enzymes responsible for assembly of acetate-derived polyketides have predictable stereochemical behavior.

Experimental Section

General methods and instrumentation have been described previously.[1,3d,16b] Preparative thin-layer chromatography employed Merck (Darmstadt) silica gel plates (20 cm × 20 cm × 2 mm) unless otherwise stated.

Fermentation of *A. cinerariae* To Produce Dehydrocurvularin (1) and Incorporation Experiments. *A. cinerariae* ATCC 11784 was grown essentially as described by Robeson and Strobel.[8g] The cultures were maintained on slants composed of Difco potato dextrose agar (39 g/L). Spore suspensions from these were used to inoculate media containing per liter the following: sucrose (100 g); Difco Bacto-casitone (2 g); $NaNO_3$ (1.5 g); K_2HPO_4 (1.0 g); KCl (0.5 g); $MgSO_4$ (0.5 g); $FeSO_4 \cdot 7H_2O$ (0.01 g); HCl or NaOH to adjust pH to 6.0. This was incubated in three to five shake flasks (100 mL of media/500-mL flask) at 25 °C on a rotary shaker (165 rpm) for 14 days. For incorporation experiments, labeled acetate (isotopic purity >98%) was added in equal portions to each flask every 24 h for 3 days beginning at 3 days after inoculation (total 200 mg/100 mL of medium). The cultures were filtered, and the wet mycelium was extracted with chloroform/methanol (1/1; 200 mL/12 g) and then chloroform (3 × 200 mL) to give, after concentration in vacuo, a fat fraction. The filtrate was extracted with ethyl acetate (2 × 100 mL/100 mL of filtrate). The combined filtrate extracts were dried (Na_2SO_4), concentrated in vacuo, and chromatographed on preparative thin-layer plates with ethyl acetate/chloroform (1/9) to give crude dehydrocurvularin. This was recrystallized from acetone/hexane to give pure dehydrocurvularin (**1**) (yield 40–70 mg/L): mp 223–224 °C (lit.[8a] mp 223.5–224 °C); $[\alpha]_D$ −82.5° (*c* 2.14, EtOH) [lit.[8d] $[\alpha]_D$ −82.4 (*c* 0.9, EtOH)]; IR (KBr) 3428, 3312, 1712, 1636 cm^{-1}; ^{1}H NMR and ^{13}C NMR data given in Table I; exact mass 290.1158 (290.1157 calcd for $C_{16}H_{18}O_5$).

Fermentation of *P. turbatum* To Produce Antibiotic A26771B (3) and Incorporation Experiments. *P. turbatum* ATCC 28797 was grown as previously described[10] with the following modifications. Cultures grown on slants (Difco malt agar, 46 g/L) for 3–4 weeks were used to inoculate media containing per liter the following: sucrose (20 g); Difco peptone (5 g); $CaCO_3$ (2 g); glucose (10 g). This was incubated in three to ten flasks (100 mL of media/500-mL flask) at 26 °C for 3 days on a rotary shaker (165 rpm). Each flask was injected with labeled precursor (sodium acetate or succinic acid, isotopic purity >98%) in equal portions every 24 h for 3 days (total 50–100 mg/100 mL of medium). After a further 3 days of incubation, the mycelium was filtered, dried, and extracted with ethyl acetate (3 × 100 mL/100 mL of media). The combined organic extracts were dried (Na_2SO_4), concentrated in vacuo, and chromatographed on preparative thin-layer plates with ethyl acetate/chloroform (2/1) to give a fat fraction and crude antibiotic A26771B. The latter was recrystallized from ethyl acetate/hexane to give pure A26771B (**3**) (yield 60–150 mg/L): mp 121–123 °C (lit.[10] mp 124–125 °C); $[\alpha]_D$ −13.1° (*c* 0.65, MeOH) [lit.[10] $[\alpha]_D$ −14° (*c* 0.13, MeOH)]; IR (KBr) 3420, 1748, 1713, 1701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13–1.60 (m, 15 H), 1.29 (d, 3 H, 6.5 Hz), 1.68 (m, 1 H), 1.89 (m, 2 H), 2.72 (m, 4 H), 5.13 (m, 1 H), 5.31 (br t, 1 H, 5.5 Hz), 6.73 (d, 1 H, 15.5 Hz), 7.22 (d, 1 H, 15.5 Hz); ^{13}C NMR data given in Table II; MS (CI, NH_3) *m/z* 400 ($M \cdot NH_4^+$), 381 ($M - H^+$).

For incorporation of [^{18}O]oxygen gas, the cultures (300 mL total) were grown under a normal atmosphere for 3 days as before. The flasks were connected to a closed-system fermentation apparatus,[15,23] the gas was circulated above the media at 4.0 mL/min, and consumed oxygen [130 mL day^{-1} (100 mL of medium)$^{-1}$] was replaced with $^{18}O_2$. After 3 days of $^{18}O_2$ addition, the fermentation was continued another 3 days with addition of unlabeled oxygen to the closed system. Extraction and purification as described above gave 31 mg of ^{18}O-labeled A26771B (**3**).

Curvularin (4).[8a] Dehydrocurvularin (**1**) (50 mg, 0.172 mmol) in ethyl acetate (10 mL) was stirred with platinum oxide (1 mg) and hydrogen gas (1 atm) at 20 °C for 3 h. The mixture was filtered, concentrated in vacuo, and recrystallized from acetone/hexane to give pure curvularin (**4**) (49 mg, 96%). The physical and spectral properties agreed with those reported in the literature:[9a] CD (MeOH) $\Delta\epsilon_{MAX} = -2.77$ (326 nm, *c* 1.86 × 10^{-3} mol/L) [lit.[9a] $\Delta\epsilon_{MAX} = -2.4$ (320 nm)].

Dehydrocurvularin Diacetate (5). Dehydrocurvularin (**1**) (50 mg, 0.172 mmol) was dissolved in pyridine (1 mL), and acetic anhydride (1 mL) was added. The mixture was stirred at 20 °C overnight, poured into water, and extracted with chloroform. The dried (Na_2SO_4) extracts were concentrated in vacuo, and the resulting residue was purified by preparative thin-layer chromatography to give dehydrocurvularin diacetate (**5**) (56 mg, 88%). For unlabeled material: IR ($CHCl_3$ cast) 1775, 1729, 1657, 1199 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (d, 3 H, 6.4 Hz); 1.34–1.52 (m, 2 H); 1.64–1.94 (m, 2 H), 2.14 (s, 3 H), 2.26 (s, 3 H), 2.08–2.36 (m, 2 H), 3.41 (s, 2 H), 4.88 (m, 1 H), 6.22 (d, 1 H, 16 Hz), 6.44 (m, 1 H), 6.94 (d, 1 H, 2 Hz), 7.03 (d, 1 H, 2 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.41 (Me at C-4), 20.52 (Me of Ac), 20.88 (Me of Ac), 24.97 (C-6), 34.57 (C-7), 34.79 (C-5), 39.65 (C-1), 73.8 (C-4), 117.27 (C-12), 122.96 (C-14), 133.22 (C-9), 134.89 (C-15), 159.07 (C-8), 149.30 (C-13), 152.51 (C-11), 170.72 (C-2), 196.42 (C-10); exact mass 374.1374 (374.1372 calcd for $C_{20}H_{22}O_7$).

Dimethyldehydrocurvularin (6). The procedure employed by Musgrave for methylation of curvularin (**4**)[27] was adapted. Dehydrocurvularin (**1**) (100 mg, 0.345 mmol) in acetone (3 mL) was treated with potassium carbonate (200 mg) and dimethyl sulfate (0.2 mL). The mixture was heated to reflux for 1.5 h, cooled, filtered, and concentrated in vacuo. The resulting oil was purified by preparative thin-layer chromatography with ethyl acetate/chloroform (1/9) as eluent to give dimethyldehydrocurvularin (**6**) (98 mg, 89%). For unlabeled material: IR ($CHCl_3$ cast) 1724, 1652, 1604, 1312, 1202, 1159 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (d, 3 H, 6.4 Hz), 1.35–1.50 (m, 2 H), 1.70–1.93 (m, 2 H), 2.10–2.35 (m, 2 H), 3.34 (s, 2 H), 3.72 (s, 3 H), 3.79 (s, 3 H), 4.85 (m, 1 H), 6.24 (d, 1 H, 16 Hz), 6.38 (d, 1 H, 2 Hz), 6.42 (m, 1 H), 6.47 (d, 1 H, 2 Hz); exact mass 318.1464 (318.1465 calcd for $C_{18}H_{22}O_5$).

Oxidative Cleavage of 6 to Diacids 7 and 8. Dimethyldehydrocurvularin (**6**) (98 mg, 0.308 mmol) in *tert*-butyl alcohol (40 mL) was treated with a solution of potassium carbonate (35 mg, 0.253 mmol) in water (80 mL). A solution of oxidizing reagent[17] (40 mL) was added which consisted of sodium periodate (2.09 g, 9.77 mmol), potassium permanganate (40 mg, 0.253 mmol), and water (100 mL). The mixture was stirred at 20 °C for 5 days, excess sodium bisulfite (1.5 g) was added, and the solution was concentrated in vacuo. The residue was dissolved in water, acidified with HCl, and extracted with ethyl acetate (3 × equal

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volume). The dried (Na_2SO_4) extracts were concentrated in vacuo and separated by preparative thin-layer chromatography (chloroform/ethyl acetate/acetic acid, 16/3/1) to give diacid **7** (31 mg, 28%, upper band) and diacid **8** (30 mg, 25%, lower band). For unlabeled **7**: IR ($CHCl_3$ cast) 3000 (br), 1730, 1705, 1605, 1321, 1205, 1165 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (d, 3 H, 6.2 Hz), 1.48–1.72 (m, 4 H), 2.31 (m, 2 H), 3.82 (AB, 1 H, 16.4 Hz), 3.83 (s, 3 H), 3.92 (s, 3 H), 3.93 (AB, 1 H, 16.4 Hz), 4.90 (m, 1 H), 6.44 (AB, 2 H, 2 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.84, 20.51, 33.72, 34.96, 41.73, 55.53, 56.65, 70.89, 98.07, 110.35, 111.94, 140.12, 160.15, 162.76, 168.10, 170.92, 178.90; exact mass 354.1333 (354.1329 calcd for $C_{17}H_{22}O_8$).

For unlabeled **8**: IR ($CHCl_3$ cast) 3000 (br), 1712, 1602, 1157 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (d, 3 H, 6.4 Hz), 1.48–1.63 (m, 4 H), 2.33 (m, 2 H), 3.76 (d, 1 H, 12 Hz), 3.83 (s, 3 H), 3.87 (s, 3 H), 3.88 (d, 1 H, 12 Hz), 4.94 (m, 1 H), 6.44 (m, 2 H), 10.18 (br s, 2 H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.73, 20.25, 33.54, 34.87, 40.37, 55.59, 56.10, 71.11, 97.52, 110.85, 115.46, 139.85, 163.23, 164.84, 167.81, 170.54, 179.43, 186.44; MS(EI) m/z 354 (M^+ – CO). For dimethyl ester of **8** obtained by diazomethane treatment: exact mass 410.1607 (410.1577 calcd for $C_{20}H_{26}O_9$).

Hydrolysis of 7 to (*S*)-(−)-δ-Caprolactone (9). To a solution of potassium *tert*-butoxide (400 mg, 3.6 mmol) in dry THF (20 mL) at 0 °C was added water (65 μL, 3.6 mmol).[18] This was stirred for 5 min at 0 °C, a solution of diacid **7** (150 mg, 0.42 mmol) in THF (10 mL) was added, and the mixture was stirred at 20 °C overnight. The mixture was concentrated in vacuo, water (30 mL) was added to dissolve the residue, the solution was acidified with HCl, and this was extracted with ethyl ether (3 × 30 mL). The combined organic phases were dried (Na_2SO_4), solvent was removed carefully, and the residual oil was distilled on a kugelrohr apparatus to give (*S*)-(−)-δ-caprolactone (**9**) (20 mg, 39%): $[\alpha]_D$ −30.2° (*c* 1.3, EtOH) [lit.[19a] $[\alpha]_D$ −51° (*c* 1, EtOH); lit.[19b] $[\alpha]_D$ −30.4° (*c* 1.6, EtOH)]; IR ($CHCl_3$ cast) 1736, 1245 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (d, 3 H, 7 Hz), 1.40–1.54 (m, 1 H), 1.75–1.92 (m, 3 H), 2.28–2.58 (m, 2 H), 4.40 (m, 1 H); exact mass 114.0679 (114.0680 calcd for $C_6H_{10}O_2$).

Conversion of Diacid 7 to Mandelate Ester 10. A solution of diacid **7** (20 mg, 0.056 mmol) in dichloromethane (2 mL) at 0 °C was treated with methyl (*S*)-(+)-mandelate (20 mg, 0.120 mmol), dicyclohexylcarbodiimide (25 mg, 0.121 mmol), and (dimethylamino)pyridine (1 mg). The mixture was warmed to room temperature and was stirred 16 h. The resulting mixture was filtered, the filtrate was concentrated in vacuo, and the residue was purified by preparative thin-layer chromatography (chloroform/ethyl acetate, 3/1). The partially purified product was separated again by preparative thin-layer chromatography (hexane/acetone, 8/2) to give ester **10** (11 mg, 39%). For unlabeled material: IR ($CHCl_3$ cast) 1740, 1643, 1602, 1564, 1220, 1161 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 0.92 (d, 3 H, 6.4 Hz), 1.20–1.62 (m, 4 H), 1.98–2.22 (m, 2 H), 3.12 (s, 3 H), 3.20 (s, 3 H), 3.24 (s, 3 H), 4.32 (m, 1 H), 5.25 (s, 1 H), 5.96 (s, 1 H), 6.04 (AB, 1 H, ~2 Hz), 6.05 (AB, 1 H, ~2 Hz), 6.96–7.10 (m, 3 H), 7.40–7.46 (m, 2 H); ^{13}C NMR (100.6 MHz, C_6D_6) δ 19.58, 20.70, 33.53, 35.33, 51.91, 54.88, 55.35, 74.86, 75.55, 82.25, 96.30, 98.97, 101.47, 128.04, 128.91 (2 C), 129.24 (2 C), 134.62, 145.72, 156.42, 159.08, 164.18, 165.61, 169.33, 172.39; MS (CI, NH_3) m/z 502 ($M{\cdot}NH_4^+$), 485 ($M{\cdot}H^+$); exact mass 484.1757 (484.1733 calcd for $C_{26}H_{28}O_9$).

Conversion of Diacid 7 to Methyl Ester 11. Substitution of methanol for methyl (*S*)-(+)-mandelate in the procedure described above to prepare **10** gave the methyl ester **11** in similar yields: ^1H NMR (200 MHz, $CDCl_3$) δ 1.37 (d, 3 H, 3.5 Hz), 1.55–1.90 (m, 4 H), 2.30–2.44 (m, 2 H), 3.68 (s, 3 H), 3.88 (s, 3 H), 3.95 (s, 3 H), 4.65 (m, 1 H), 5.46 (s, 1 H), 6.27 (m, 2 H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.59, 20.60, 33.62, 35.37, 51.48, 55.47, 56.10, 75.77, 82.14, 96.23, 98.73, 100.50, 145.26, 157.65, 158.29, 163.55, 165.58, 173.61; exact mass 350.1376 (350.1365 calcd for $C_{18}H_{22}O_7$).

Dimethyl 3,5-Dimethoxyhomophthalate (13). A mixture of dimethyl 3,5-dihydroxyhomophthalate[28] (500 mg, 2.08 mmol), acetone (6 mL), potassium carbonate (1.5 g), and dimethyl sulfate (1.5 mL) was heated to reflux for 1.5 h. The cooled mixture was concentrated in vacuo and separated by preparative thin-layer chromatography (benzene) to give the known[29] dimethyl 3,5-dimethoxyhomophthalate (**13**) (230 mg, 41%): IR ($CHCl_3$ cast) 1732, 1605 cm^{-1}; ^1H NMR (200 MHz, [$^2H_6$]acetone) δ 3.63 (s, 3 H), 3.67 (s, 2 H), 3.77 (s, 3 H), 3.83 (s, 3 H), 3.85 (s, 3 H), 6.52 (AB, 1 H, ~2.2 Hz), 6.58 (AB, 1 H, ~2.2 Hz); exact mass 268.0945 (268.0946 calcd for $C_{13}H_{16}O_6$).

3,5-Dimethoxyhomophthalic Acid.[29] A solution of the dimethyl ester **13** (229 mg, 0.854 mmol) in ethanol (6 mL) and 1 N aqueous KOH (3.5 mL) was heated to reflux for 30 min. The cooled solution was concentrated in vacuo, redissolved in water (10 mL), and acidified with HCl. This was extracted with ethyl acetate (3 × 10 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (chloroform/ethyl acetate/acetic acid, 5/4/0.5) to give 3,5-dimethoxyhomophthalic acid[29] (95 mg, 46%): IR (KBr) 2950 (br), 1709, 1656, 1610, 1577, 1438, 1308, 1284 cm^{-1}; ^1H NMR (200 MHz, [$^2H_6$]acetone) δ 3.84 (s, 3 H), 3.86 (s, 3 H), 3.88 (s, 2 H), 6.42 (m, 2 H).

Methyl 3,5-Dimethoxyhomophthalate (12). The 3,5-dimethoxyhomophthalic acid (100 mg, 0.417 mmol) was selectively esterified with methanol and HCl by the literature procedure to give monoester **12**.[30] Recrystallization from ethyl acetate gave 81 mg (76%): mp 127 °C (lit.[30] mp 127 °C); IR (KBr) 3440 (br), 1723, 1678, 1600 cm^{-1}; ^1H NMR (200 MHz, [$^2H_6$]acetone) δ 3.58 (s, 3 H), 3.76 (s, 2 H), 3.82 (s, 3 H), 3.86 (s, 3 H), 6.53 (AB, 1 H, ~2.2 Hz), 6.59 (AB, 1 H, ~2.2 Hz); exact mass 254.0789 (254.0790 calcd for $C_{12}H_{14}O_6$).

Cyclization of 12 to Ester 15. The procedure described above to convert **7** to **10** was employed. Thus methyl 3,5-dimethoxyhomophthalate (**12**) (10 mg, 0.039 mmol) in dichloromethane (1 mL) was treated with dicyclohexylcarbodiimide (8.0 mg, 0.039 mmol) and (dimethylamino)pyridine (~1 mg) as before to give, after purification by preparative thin-layer chromatography ($CHCl_3$), the ester **15** (5.0 mg, 51%): IR (KBr) 1729, 1649, 1594, 1565, 1215, 1160 cm^{-1}; ^1H NMR (200 MHz, [$^2H_6$]acetone) δ 3.90 (s, 6 H), 3.91 (s, 3 H), 5.65 (s, 1 H), 6.39 (AB, 1 H, 1.2 Hz), 6.50 (AB, 1 H, 1.2 Hz); exact mass 236.0689 (236.0685 calcd for $C_{12}H_{12}O_5$).

Methyl (*S*)-(+)-Mandelate Esters 16 and 17. The fat fractions isolated during fermentation of *A. cinerariae* and *P. turbatum* (described above) with sodium [2-^{13}C,$^2H_3$]acetate were hydrolyzed, methylated, and separated to give labeled methyl oleates exactly as described previously.[1] The *A. cinerariae* fat fraction (430 mg) afforded 49 mg of methyl oleate and 60 mg of methyl linoleate. This methyl oleate eventually yielded 36 mg of **16** and an identical amount of **17**. The *P. turbatum* fat fraction (310 mg) gave 80 mg of methyl oleate and 110 mg of methyl linoleate. Degradation of the former produced 55 mg of **17**. The physical, chromatographic, and spectral characteristics of **16**, **17**, and the degradation intermediates were in complete accord with those observed earlier.[1]

Dihydro Antibiotic A26771B (18). Antibiotic A26771B (**3**) (200 mg, 0.524 mmol) was dissolved in ethyl acetate (15 mL) and hydrogenated at 1 atm over 5% Rh/C (50 mg) for 2 h. The mixture was filtered, the catalyst was washed with ethyl acetate (15 mL), and the combined organic phases were concentrated in vacuo. The residue was recrystallized from ethyl acetate/hexane to give pure dihydro compound **18** (195 mg, 97%): IR ($CHCl_3$ cast) 3280 (br), 2929, 2858, 1742, 1723 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (d, 3 H, 6.4 Hz), 1.05–1.60 (m, 16 H), 1.76 (m, 2 H), 2.34 (m, 1 H), 2.60 (m, 6 H), 2.88 (m, 1 H), 4.87 (m, 1 H), 5.04 (t, 1 H, 5.5 Hz); exact mass 385.2222 (385.2226 calcd for $C_{20}H_{33}O_7$, $M{\cdot}H^+$), 284.1988 [284.1987 calcd for $C_{16}H_{28}O_4$, ($M - C_4H_4O_3$)$^+$].

Methyl (*R*)-11-Hydroxydodecanoate (19). The dihydro derivative **18** (400 mg, 1.04 mmol) was dissolved in methanol (5 mL) and treated with 0.5 N aqueous sodium hydroxide (15 mL). The mixture was stirred under an oxygen atmosphere at 20 °C for 24 h, then acidified with hydrochloric acid, and extracted with ether (3 × 20 mL). The combined dried (Na_2SO_4) extracts were concentrated in vacuo, and the resulting residue (310 mg) was dissolved in ether. This solution was treated with excess diazomethane in ether for 15 min and then concentrated in vacuo. Preparative thin-layer chromatography (benzene/acetone, 5/1) gave methyl 11-hydroxydodecanoate (**19**) (158 mg, 66%). For unlabeled material: IR ($CHCl_3$ cast) 3420 (br), 2928, 2855, 1740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.17 (d, 3 H, 6.2 Hz), 1.2–1.5 (m, 14 H), 1.60 (m, 3 H), 2.29 (t, 2 H, 7 Hz), 3.68 (s, 3 H), 3.78 (m, 1 H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 23.24, 24.70, 25.49, 28.89, 28.97, 29.11, 29.27, 29.36, 33.90, 39.20, 51.27, 68.13, 174.76; exact mass 212.1778 [212.1776 calcd for $C_{13}H_{24}O_2$, ($M - H_2O$)$^+$].

Methyl (*R*)-11-(*tert*-Butyldimethylsiloxy)dodecanoate (20). The procedure of Makita et al.[31] was adapted. The hydroxy ester **19** (130 mg, 0.565 mmol) was dissolved in dimethylformamide (2 mL), and *tert*-butyldimethylsilyl chloride (110 mg, 0.73 mmol) and imidazole (110 mg, 1.6 mmol) were added. The mixture was stirred 12 h at 20 °C, water was added, and the resulting mixture was extracted with ethyl acetate. The extract was dried (Na_2SO_4) and concentrated in vacuo to give a residue which was separated by preparative thin-layer chromatography (hexane/ethyl acetate, 10/1). A total of 170 mg (87%) of **20** was obtained. For unlabeled material: IR ($CHCl_3$ cast) 2929, 2856, 1744 cm^{-1};

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^{1}H NMR (200 MHz, $CDCl_3$) δ 0.04 (s, 6 H), 0.88 (s, 9 H), 1.20 (d, 3 H, 6.4 Hz), 1.20–1.50 (m, 13 H), 1.54–1.70 (m, 3 H), 2.30 (t, 2 H, 7.2 Hz), 3.66 (s, 3 H), 3.75 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.76, −4.45, 18.11, 23.77, 24.91, 25.72, 25.87, 29.11, 29.20, 29.36, 29.52, 29.63, 34.06, 39.70, 51.34, 68.60, 174.23; exact mass 329.2506 [329.2512 calcd for $C_{18}H_{37}O_3Si$, $(M - CH_3)^+$].

Methyl (*R*)-11-(*tert*-Butyldimethylsiloxy)-2-dodecenoate (21). The procedure of Makita et al.[31] was adapted. Lithium diisopropylamide (1.2 equiv) was prepared from diisopropylamine (0.12 mL) and butyllithium (0.61 mL, 1.4 M in hexane) in THF (10 mL) and was cooled to −78 °C. After 30 min, a solution of ester **20** (240 mg, 0.696 mmol) in THF (2 mL) was added dropwise. Phenylselenyl bromide was prepared by adding Br_2 (1 equiv) in dry CCl_4 to a solution of diphenyl diselenide in the same solvent; after 30 min the solvent is removed to leave the crystalline bromide. A solution of phenylselenyl bromide (201 mg, 0.85 mmol) in THF (2 mL) was added dropwise to the ester enolate solution. The mixture was stirred at −78 °C for 1 h and then at −40 to −45 °C for 2 h. The temperature was raised to 0 °C, and a solution of water (0.6 mL), acetic acid (0.12 mL), and 30% hydrogen peroxide (0.5 mL) was added to it. After 1 h the mixture was neutralized with 0.1% aqueous $NaHCO_3$ and extracted with dichloromethane (3 × 10 mL). The extracts were washed successively with equal volumes of water, 0.1 N HCl, and water and were then dried (Na_2SO_4). The organic phases were concentrated in vacuo, and the resulting residue was purified by preparative thin-layer chromatography (ethyl acetate/hexane, 1/10) to give the dehydro derivative **21** (148 mg, 62%). For unlabeled material: IR ($CHCl_3$ cast) 2929, 2856, 1729 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05 (s, 6 H), 0.90 (s, 9 H), 1.11 (d, 3 H, 6 Hz), 1.2–1.5 (m, 12 H), 2.20 (m, 2 H), 3.73 (s, 3 H), 3.76 (m, 1 H), 5.80 (d, 1 H, 16 Hz), 6.94 (dt, 1 H, 16 and 6.4 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 23.77, 25.65, 25.89, 27.98, 29.05, 29.32, 29.53, 32.17, 39.69, 51.28, 68.59, 120.83, 149.68, 173.29; exact mass 341.2515 [341.2512 calcd for $C_{19}H_{37}O_3Si$, $(M - H)^+$], 327.2357 [327.2355 calcd for $C_{18}H_{35}O_3Si$, $(M - CH_3)^+$].

Methyl (*R*)-11-Acetoxy-2-dodecenoate (22). The procedure of Makita et al.[31] was adapted. The unsaturated ester **21** (100 mg, 0.292 mmol) was dissolved in acetic anhydride (1 mL) under inert atmosphere (Ar) at 0 °C. Anhydrous ferrous chloride (10 mg) was added, and the mixture was stirred for 30 min. Water was added, and the mixture was extracted with ethyl ether. The dried (Na_2SO_4) extracts were concentrated in vacuo, and the resulting residue was purified by preparative thin-layer chromatography (ethyl acetate/hexane, 1/10) to give **22** (61 mg, 77%). For unlabeled material: IR ($CHCl_3$ cast) 2951, 1730, 1658 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.18 (d, 3 H, 6.2 Hz), 1.20–1.60 (m, 12 H), 2.01 (s, 3 H), 2.17 (m, 2 H), 3.71 (s, 3 H), 4.85 (m, 1 H), 5.79 (br d, 1 H, 16 Hz), 6.94 (dt, 1 H, 16 and 6.8 Hz); exact mass 255.1593 [255.1596 calcd for $C_{14}H_{23}O_4$, $(M - CH_3)^+$].

(*R*)-9-Acetoxydecanoic Acid (23). The ester **22** (98 mg, 0.363 mmol) was dissolved in a mixture of acetonitrile (1 mL), carbon tetrachloride (1 mL), water (1.5 mL), and sodium periodate (302 mg, 1.41 mmol). To this was added ruthenium(III) chloride trihydrate (10 mg, 0.032 mmol), and the mixture was stirred vigorously at 20 °C for 2 h. The mixture was diluted with an equal volume of dichloromethane, and the aqueous phase was further extracted with dichloromethane (3 × 5 mL). The combined organic phases were dried (Na_2SO_4), concentrated in vacuo, and purified by preparative thin-layer chromatography (chloroform) to give 9-acetoxydecanoic acid (**23**)[32] (54 mg, 65%). For unlabeled material: IR ($CHCl_3$ cast) 3000, 2951, 2857, 1738, 1710, 1240 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (d, 3 H, 6.5 Hz), 1.20–1.70 (m, 12 H), 2.08 (s, 3 H), 2.33 (t, 2 H, 6 Hz), 4.85 (m, 1 H); exact mass 187.1334 [187.1334 calcd for $C_{10}H_{19}O_3$, $(M - C_2H_3O)^+$].

(*S*)-(+)-(Methoxycarbonyl)benzyl (*R*)-9-Acetoxydecanoate (24). A solution of 9-acetoxydecanoic acid (**23**) (53 mg, 0.230 mmol) and (dimethylamino)pyridine (2 mg) in dichloromethane (2 mL) at 0 °C was treated with methyl (*S*)-(+)-mandelate (44 mg, 0.265 mmol) and dicyclohexylcarbodiimide (55 mg, 0.267 mmol). The mixture was stirred overnight at 20 °C, filtered, and concentrated in vacuo. Purification of the residue by preparative thin-layer chromatography gave the ester **24** (84 mg, 95%). For unlabeled material: IR ($CHCl_3$ cast) 1738, 1245 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6) δ 1.07 (d, 3 H, 6.8 Hz), 1.10–1.42 (m, 9 H), 1.44 (m, 1 H), 1.46 (m, 2 H), 1.73 (s, 3 H), 2.24 (m, 2 H), 3.22 (s, 3 H), 4.92 (m, 1 H), 6.04 (s, 1 H), 7.02–7.16 (m, 3 H), 7.42–7.46 (m, 2 H); ^{13}C NMR (100.6 MHz, C_6D_6) δ 19.5, 20.3, 24.6, 25.2, 28.8, 28.9, 29.1, 33.6, 35.8, 51.6, 70.4, 74.5, 128.1 (2 C), 128.9 (2 C), 129.2, 135.0, 169.6, 169.9, 172.9; MS (CI, NH_3) *m/z* 396 ($M{\cdot}NH_4^+$), 379 ($M{\cdot}H^+$). Labeled material derived from sodium [2-^{13}C,^{2}H$_3$]acetate showed identical chromatographic properties and displayed the expected ^{13}C enhancements and ^{2}H-induced isotope shifts in ^{13}C NMR spectra.

Acknowledgment. We are indebted to Dr. David J. Robeson (ARCO Plant Cell Research Institute, Dublin, CA) for a sample of dehydrocurvularin and to Dr. Ronald F. Vesonder (Northern Regional Center, U.S. Department of Agriculture, Peoria, IL) for a sample of curvularin. Helpful discussions and technical assistance from Dr. Paul B. Reese are gratefully acknowledged. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada and by the Alberta Heritage Foundation for Medical Research.

Registry No. 1, 21178-57-4; **2**, 112-80-1; **3**, 56448-20-5; **4**, 10140-70-2; **5**, 60821-05-8; **6**, 100905-80-4; **7**, 119680-49-8; **8**, 119680-50-1; **8** (dimethyl ester), 119680-55-6; **9**, 16320-13-1; **10**, 119680-51-2; **11**, 119680-52-3; **12**, 83375-20-6; **13**, 6512-26-1; **14**, 14736-50-6; **15**, 119680-53-4; **16**, 111975-72-5; **17**, 111975-73-6; **18**, 119694-03-0; **19**, 88785-25-5; **20**, 119719-55-0; **21**, 119785-24-9; **22**, 119719-56-1; **23**, 119719-57-2; **24**, 119680-54-5; methyl (*S*)-(+)-mandelate, 21210-43-5; dimethyl 3,5-dihdyroxyhomophthalate, 6110-30-1; 3,5-dimethoxyhomophthalic acid, 4778-99-8; methyl oleate, 112-62-9; methyl linoleate, 112-63-0; polyketide reductase, 119793-82-7; fatty acid enoyl thiol ester reductase, 119793-81-6; succinic acid, 127-09-3, 110-15-6.

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(33) Careful examination of the correlation spectra of C-7 of labeled dehydrocurvularin (**1**) (Figure 4) and of C-2 of its degradation product **10** (Figure 6) reveals additional sets of signals for both the CH_2 and CHD groups slightly upfield (ca. 0.05 ppm) on the ^{13}C axis. These are due to molecules bearing ^{13}C and ^{2}H two methylene groups away (i.e., at C-5 of **1** or C-4 of **10**).