with a thymidine unit was used as support. Aftr removal of the DMTr group in the usual manner, a mixture of compound **1d** (10 equiv) and iodine (50 equiv) in CH_2Cl_2/lutidine/NEt_3 (5:4:1, v/v/v) was added to the polymer support. After being gently shaken for 10 min, the mixture was quenched with lutidine containing a small amount of water. Subsequent filtration followed by deprotection of the DMTr group gave the dimer loaded on the resin. The same cycle was repeated 3 times. The average coupling yield was 94% based on assay of the DMTr cation. After the usual deprotection, tetrathymidylate $(Tp)_3T$ was obtained and analyzed with snake venom phosphodiesterase. The enzyme assay showed complete degradation to T and pT in a 1:3 ratio.

In conclusion, compounds **1b–d** are easily prepared and stable under normal laboratory conditions. They are readily activated by iodine or silver acetate. It is noteworthy that the former reagent does not require the oxidation step, because this could be simultaneously performed during condensation. The application of the present phosphorothioite method is now in progress.

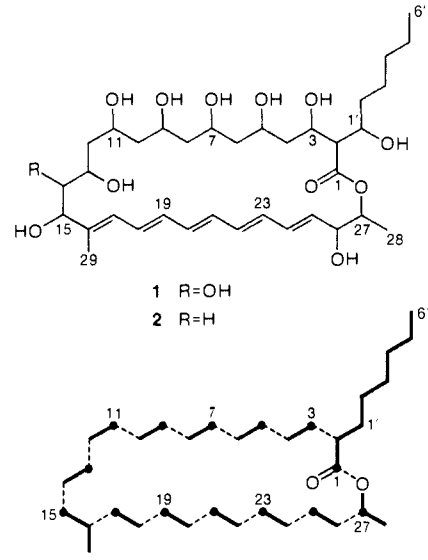

Figure 1.

Biosynthesis of Polyene Antibiotics: Intact Incorporation of ^{13}C-Labeled Octanoate into Fungichromin by *Streptomyces cellulosae*

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Fungichromin (**1**)[1] and filipin (**2**)[2] from *Streptomyces cellulosae* are typical members of the family of polyene antibiotics,[3] some of which (e.g., amphotericin B) are widely used in treatment of fungal infections.[4] Such steroid-binding antibiotics[5] usually possess a macrocyclic lactone ring which consists of a conjugated polyene chain of three to eight double bonds and has a complementary alkyl chain adorned with hydroxyl groups at alternating carbons.[3] A number of biosynthetic studies of this family have employed incorporations of radioactive precursors and subsequent chemical degradations to locate labeled sites.[6] These experiments suggest that the macrolide rings are formed from acetate and propionate by a polyketide pathway similar to fatty acid biogenesis. On this basis Martin proposed[6b] an expected[7] arrangement of biosynthetic units for filipin (**2**) with the exception of an implied introduction of a preformed eight-carbon fatty acid chain (Figure 1). Since intact incorporation of carboxylic acids longer than four carbons into microbial polyketides is extremely rare,[8] current interest in polyketide biosynthesis[7,9] led us to examine this hypothesis. The present work describes the use of ^{13}C-labeled precursors to determine the origin of the carbon skeleton of fungichromin (**1**). The results show that an unusual intact incorporation of [^{13}C]octanoate occurs.

Addition of the sodium salts of [1-^{13}C]acetate, [2-^{13}C]acetate, or [1-^{13}C]propionate to cultures of *S. cellulosae* ATCC 12625 produced samples of fungichromin (**1**)[10] whose ^{13}C NMR spectra[11]

Table I

carbon	δ	precursor	enhancement[b]
1	172.98	*c*	2.9
16	138.55		
19	135.36	*d*	2.7
21	134.81	*d*	2.4
25	134.28	*d*	2.9
23	134.21	*d*	2.5
20	134.13	*e*	2.2
22	133.66	*e*	2.5
24	131.97	*e*	2.6
17	129.91	*d*	2.5
18	129.06	*e*	2.2
15	80.43	*f*	5.6
14	78.31	*e*	2.8
27	75.25	*d*	2.5
h	74.20	*d*	2.5
h	74.08	*d*	2.6
h	73.92	*d*	2.7
3	73.41	*d*	2.3
26	73.25	*e*	2.7
1′	72.59	*g*	2.5
h	71.45	*d*	2.3
13	70.34	*d*	2.5
2	60.35		
i	45.33	*e*	2.9
i	45.17	*e*	2.8
i	44.34	*e*	2.5
4	41.38	*e*	2.9
i	39.58	*e*	2.5
2′	36.22		
4′	32.88		
3′	26.01		
5′	23.65		
28	17.96	*e*	3.0
6′	14.38		
29	11.74		

[a] 100.6-MHz ^{13}C NMR spectra in methanol-d_4 with solvent reference at 49.00 ppm. [b] Ratio of carbon signal intensities for enriched and natural abundance sample measured under identical conditions. [c] Sodium [1-^{13}C]octanoate. [d] Sodium [1-^{13}C]acetate. [e] Sodium [2-^{13}C]acetate. [f] Sodium [1-^{13}C]propionate. [g] Sodium [3-^{13}C]octanoate. [h] C-5, C-7, C-9, or C-11; see ref 17. [i] C-6, C-8, C-10, or C-12; see ref 17.

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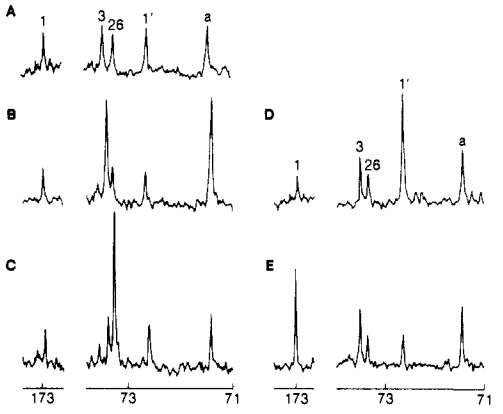

Figure 2. Portions of ^{1}H-decoupled 100.6-MHz ^{13}C NMR spectra of **1** in methanol-d_4 after incorporation of (A) no labeled precursor, (B) [1-^{13}C]acetate, (C) [2-^{13}C]acetate, (D) [3-^{13}C]octanoate, and (E) [1-^{13}C]octanoate. Peak a is due to C-5, C-7, C-9, or C-11; see ref 17.

display enhancements of specific resonances which are listed in Table I. As expected for polyketide biosynthesis,[7-9] carbon atoms derived from the carboxyl and methyl of acetate alternate around the lactone ring of **1**. Specific enrichment of C-15 by [1-^{13}C]-propionate indicates that positions 15, 16, and 29 originate from one propionate unit. However, there was no significant labeling of C-1, C-2, or the saturated side chain (C-1′ to C-6′) of fungichromin (**1**) in any of these three experiments (Figure 2A–C). Incorporation of sodium [1,2-$^{13}C_2$]acetate followed by detection of coupled resonances in **1** by double quantum coherence NMR spectroscopy (2D INADEQUATE)[9] demonstrated that the macrolide portion contains two groups of six intact acetate units connected in head to tail fashion (Figure 1). In the normal ^{13}C NMR spectrum of this sample the presence of small coupled satellites flanking the natural abundance C-6′ singlet showed that a low level of acetate incorporation does occur in the saturated side chain.[13]

To determine the origin of the eight-carbon fragment (C-1, C-2, and C-1′ to C-6′), sodium [1-^{13}C]octanoate and [3-^{13}C]octanoate[14] were fed[10] in separate experiments to *S. cellulosae*. The resulting fungichromin (**1**) samples exhibit large specific enhancements (Table I) at C-1 and C-1′, respectively (Figure 2D,E). Although [1-^{13}C]octanoate causes very slight labeling of positions derived directly from [1-^{13}C]acetate (25% enhancement), [3-^{13}C]octanoate gives no detectable enrichment at those sites. This suggests that a small amount of β-oxidation[15] of [1-^{13}C]octanoate to [1-^{13}C]-acetate and hexanoate occurs. In a separate experiment, no incorporation of [1-^{13}C]hexanoate[14] could be observed. Clearly octanoate is the preferred specific precursor for the eight-carbon unit that terminates the polyketide chain in fungichromin (**1**) (Figure 1). This contrasts the usual tendency of microorganisms to degrade longer chain fatty acids to acetate before incorporation.[8,16] Degradation of fats[15] present in the medium (e.g., Span 85)[12] probably accounts for octanoate formation under normal circumstances; this may explain the requirement for fats (especially oleic acid esters) to obtain good fungichromin (**1**) production.[12] Additional studies on details of the biosynthesis of polyene antibiotics are in progress.

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Registry No. 1, 6834-98-6; $Me(CH_2)_6CO_2H$, 124-07-2.

(10) Week-old cultures of *Streptomyces cellulosae* ATCC 12625 grown on Bacto yeast/malt extract agar were used to inoculate 100 mL of sterile liquid media (per liter: 5.0 g of bactopeptone; 2.5 g of yeast extract; 4 g of NaCl; 10 g of glucose; 10 mL of Span 85;[12] $NaHCO_3$ to adjust pH to 7.0). After a 48-h incubation at 26 °C in the dark on a rotary shaker (165 rpm), 2 mL of the resulting suspension was transferred to each of 10 500-mL flasks containing the same medium (100 mL/flask). These were incubated (same conditions) for 7–8 days. Labeled precursors (≥98% isotopic purity) (6–8 mg/flask/day, except for acetates: 10–20 mg/flask/day) were added in H_2O after 3, 4, 5, and 6 days growth. The mycelium and filtrate were extracted separately (2:1 hexane/benzene, then hot ethyl acetate). The combined ethyl acetate extracts were concentrated in vacuo and chromatographed on Sephadex LH-60 in methanol. Chromatography of the polyene antibiotic fractions on a Merck Lobar RP-8 column (65:35 methanol/water) gave 10–30 mg of pure fungichromin (**1**).

(11) The ^{1}H and ^{13}C NMR spectra were assigned by using a variety of techniques; these include homonuclear decoupling, COSY, spin echo, and heteronuclear shift correlation on unlabeled fungichromin (**1**) as well as INADEQUATE on **1** enriched by [1,2-$^{13}C_2$]acetate. The full details will be reported later. For reviews of modern methods of NMR assignment, see: (a) Benn, R.; Günther, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 350–380. (b) Shoolery, J. N. *J. Nat. Prod.* **1984**, *47*, 226–259.

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Geometry of Formal Nucleophilic Substitution at First-Row Heteroatoms: The Transfer of Oxygen from Nitrogen to Phosphorus

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The geometry of reaction at nonstereogenic atoms can, in principle, be determined by studies of systems in which the atom of interest and its reaction partners are joined by a small number of intervening atoms. When the ring required to bring the reactants together is geometrically well-defined, limits are placed on the bond angles allowed for intramolecular reaction, particularly for endocyclic displacements. If the bond angle required for reaction cannot be met in a cyclic mode, the process is expected to be intermolecular. Although the same product may result from intra- and intermolecular pathways, experimental distinction can be made and limits assigned to the reaction trajectory. The general approach has been used to investigate displacement at carbon,[1] to define the possibilities for facile ring formation,[2] to investigate radical substitution at sulfur,[3] and to provide a mechanistic distinction for a formal displacement at anionic nitrogen.[4] We now

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