redox[12] potential (−540 mV vs Ag wire at −20 °C in CH_2Cl_2 under argon) is considerably lower than those of blue copper proteins, the present experimental result clearly proves that the striking spectroscopic characteristics of blue copper proteins (a strong absorption band at ca. 600 nm (ϵ, 1500–5000) and an unusually small hyperfine constant ($A_\parallel \leq 70$ G)) can be mimicked by a simple synthetic model, a tetrahedral thiolato copper(II) complex in the absence of the coordination of a thioether.[13]

Acknowledgment. We thank Dr. R. Makino and Prof. Y. Ishimura of Keio University for low-temperature measurements of electronic spectra. Support of this work by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (62430018 and 01607003) and by Kawakami Memorial Foundation is gratefully acknowledged.

Registry No. 1, 125782-21-0; **2**, 124688-39-7; **3**, 125782-22-1; **4**, 123676-54-0; **5**, 125782-23-2.

Supplementary Material Available: Tables S-I–S-V of the summary of X-ray analyses, atomic coordinates, anisotropic thermal parameters, and bond distances and angles for **1** and **3** (19 pages); Table S-VI listing observed and calculated structure factors for **1** and **3** (19 pages). Ordering information is given on any current masthead page.

(11) Other absorption bands of **5** are observed at 349 nm (ϵ, ~11 000) and ≳900 nm (~500). Because of the very high solubility, complex **5** has not been isolated as a crystalline solid so far. However, the quantitative measurement of the EPR signal led us to the conclusion that the reaction of **4** and tBuSH proceeds quantitatively (since the binuclear hydroxo complex **4** is EPR silent, the measurement is accurate). The details of the properties and structure of **5** will be described elsewhere.

(12) The completely reversible redox couple of the thiolato complex is indicative of considerable stability of the reduced state. We infer that the structure is identical with that of a tetrahedral thiolato copper(I) complex reported by Marks et al.[9]

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Intact Incorporation of Acetate-Derived Di- and Tetraketides during Biosynthesis of Dehydrocurvularin, a Macrolide Phytotoxin from *Alternaria cinerariae*

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Extensive studies with simple precursors (e.g., acetate, propionate) labeled with stable isotopes support the hypothesis that polyketide biosynthesis resembles fatty acid formation except that particular reductive steps are absent during assembly of the carbon chain.[1,2] This results in incorporation of keto, hydroxy, or olefinic functionality in the growing enzyme-bound polyketide that can lead to further transformations (e.g., cyclization) or provide sites for post-assembly processing (e.g., oxidation, alkylation) by other enzymes.[3,4] Key support for this proposal is provided by recent experiments in which functionalized propionate-derived di- or

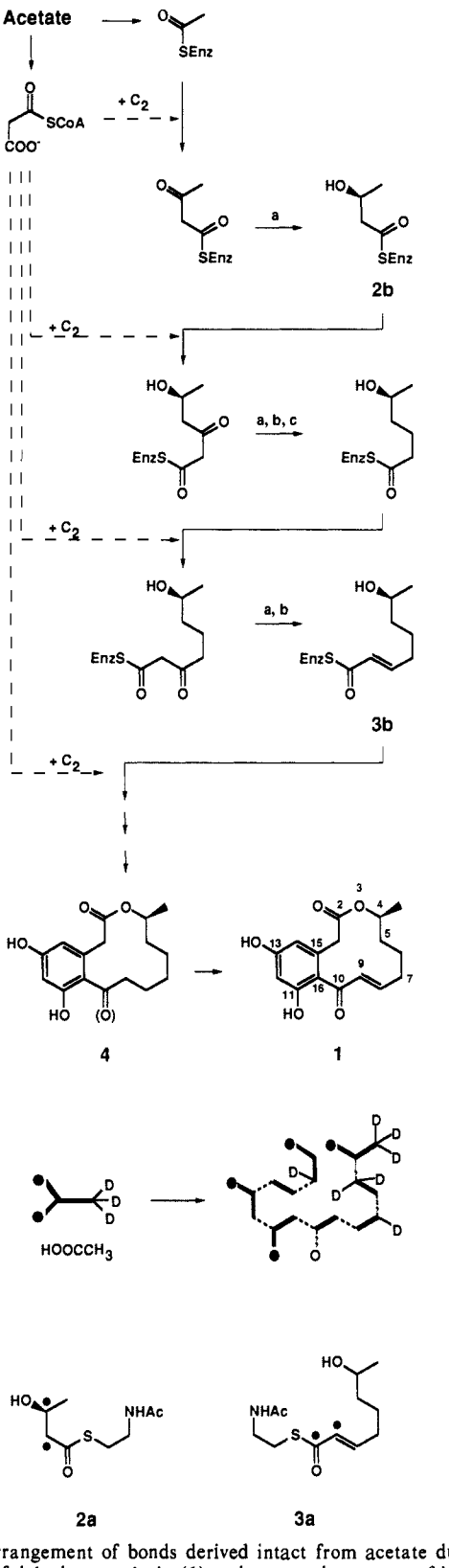

Figure 1. Arrangement of bonds derived intact from acetate during biosynthesis of dehydrocurvularin (**1**) and proposed sequence of its assembly by a polyketide synthase. In between each addition of two carbons (+C_2) from malonyl-CoA (with CO_2 loss), functionality changes can occur: (a) reduction of β-hydroxy thiol ester; (b) dehydration to α,β-unsaturated thiol ester; (c) reduction to saturated thiol ester.

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(1) (a) Vederas, J. C. *Nat. Prod. Rep.* **1987**, *4*, 277–337. (b) Simpson, T. J. *Chem. Soc. Rev.* **1987**, *16*, 123–160. (c) Simpson, T. J. *Nat. Prod. Rep.* **1987**, *4*, 339–376. (d) O'Hagan, D. *Nat. Prod. Rep.* **1989**, *6*, 205–219.

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(3) Addition of oxygenase inhibitors to polyketide fermentations can yield deoxy compounds that presumably resemble the product initially produced by the synthase enzyme complex: Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1988**, 600–602.

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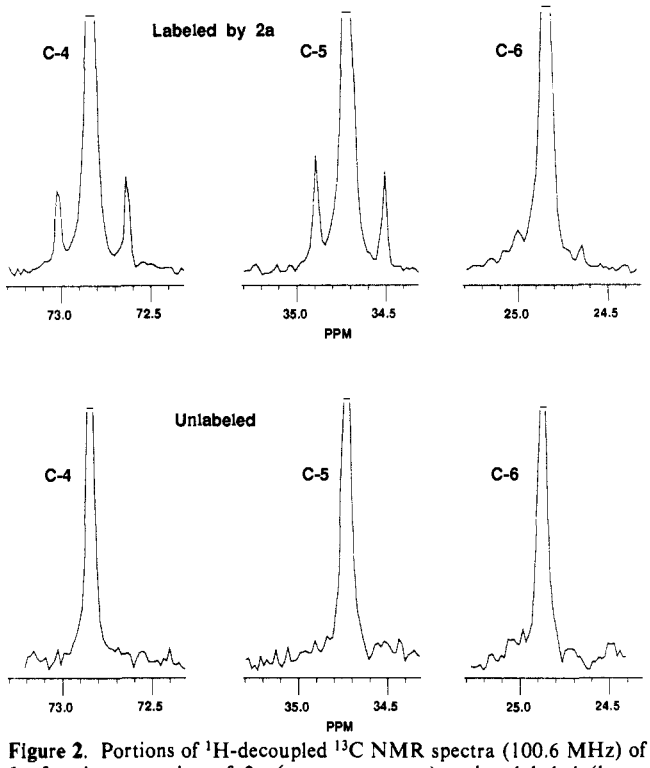

Figure 2. Portions of ^{1}H-decoupled ^{13}C NMR spectra (100.6 MHz) of **1** after incorporation of **2a** (upper spectrum) and unlabeled (lower spectrum). The highly ^{13}C enriched C-6 resonance in the upper spectrum has small satellites due to coincidental incorporation of [1-^{13}C]- and [2-^{13}C]acetates (from β-oxidation of **2a**) in adjacent units.

triketide precursors with the correct stereochemistry were incorporated intact into tylactone,[5] erythromycin A,[6] nargenicin,[7] and nonactin.[8] Although saturated fatty acids can act as chain starters or terminators in special cases,[9] intact utilization of functionalized *acetate-derived* polyketides has not been previously reported because of their efficient degradation by β-oxidation.[10] Herein we describe the intact utilization of the *N*-acetylcysteamine thiol esters of (*S*)-[2,3-$^{13}C_2$]-3-hydroxybutyrate (**2a**) and [1,2-$^{13}C_2$]-7-hydroxy-2-octenoate (**3a**) in the biosynthesis of dehydrocurvularin (**1**)[2b] by mutants of the plant pathogen *Alternaria cinerariae*.

Previous studies show that dehydrocurvularin (**1**) is a typical polyketide with a head-to-tail arrangement of eight acetate units and that it contains a number of carbon–oxygen and carbon–hydrogen bonds derived intact from acetate (Figure 1).[2b] This suggests that its assembly proceeds via the enzyme-bound intermediates (e.g., **2b** and **3b**) to produce a less oxidized precursor, for example, curvularin (**4**),[11] which is released and subsequently transformed by oxidative enzymes to **1**. To test this, doubly labeled **2a** (99% isotopic purity) was synthesized (see supplementary material) because *N*-acetylcysteamine thiol ester (NAC) derivatives are more likely to be substrates for a polyketide synthase than corresponding carboxylic acids.[5-8] Since in vivo cleavage by β-oxidation would generate singly labeled acetates,[10] which would be unlikely to recouple to a doubly labeled four-carbon fragment because of dilution by unlabeled species from the fermentation medium, the extent of intact incorporation can be estimated by observation of ^{13}C-coupled signals.[1a,b] Initial experiments in which **2a** was added to wild-type *A. cinerariae* ATCC 11784 under various conditions gave dehydrocurvularin (**1**) whose ^{13}C NMR spectra indicated complete degradation of the precursor to acetate prior to incorporation (i.e., enhanced singlets at every carbon). Intact utilization of a portion (12%) of **2a** could only be achieved by the combined use of high glucose replacement media,[12,13] a UV mutant deficient in the ability to grow on fatty acids,[14] and the addition of 4-pentynoic acid as a potential β-oxidation inhibitor.[15] Under these conditions, carbon-coupled signals could be seen at C-4 and C-5 of **1**, thereby indicating that the four-carbon chain of **2a** had been incorporated without cleavage of its C-2 to C-3 bond (Figure 2). Nevertheless, even in this experiment, a majority of the precursor was degraded by β-oxidation.

To test whether longer precursors could be loaded into the polyketide synthase machinery, NAC [1,2-$^{13}C_2$]-7-hydroxy-2-octenoate (**3a**) was prepared (see supplementary material) and administered as a mixture of isomers to *A. cinerariae* under the above conditions[12] except that 4-pentynoic acid was not added. Enhancement of the coupled ^{13}C NMR signals for C-9 and C-10 in the resulting dehydrocurvularin (**1**) clearly indicates that some intact incorporation (ca. 2%) occurs.[16] These results support the hypothesis that **2b** and **3b** are enzyme-bound intermediates during the assembly of **1**.[2b] Studies are in progress to improve incorporation of advanced intermediates into polyketides and to determine which isomer of **3a** is utilized in **1**.

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Supplementary Material Available: Description of the synthesis of **2a** and **3a** (5 pages). Ordering information is given on any current masthead page.

(4) Mutants of *Micromonospora griseorubida* produce shorter polyketides that resemble proposed intermediates in the biosynthesis of protomycenolide IV: (a) Kinoshita, K.; Takenaka, S. Hayashi, M. *J. Chem. Soc., Chem. Commun.* **1988**, 943–945. (b) Takano, S.; Sekiguchi, Y.; Shimazaki, Y.; Ogasawara, K. *Tetrahedron Lett.* **1989**, *30*, 4001–4002.

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(9) (a) Townsend, C. A.; Christensen, S. B.; Trautwein, K. *J. Am. Chem. Soc.* **1984**, *106*, 3868–3869. (b) Noguchi, H.; Harrison, P. H.; Arai, K.; Nakashima, T. T.; Trimble, L. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 2938–2945.

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(11) Dehydrocurvularin (**1**), curvularin (**4**), and 8-hydroxycurvularin occur together in various fungi: (a) Hyeon, S. B.; Ozaki, A.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1976**, *40*, 1663–1664. (b) Robeson, D. J.; Strobel, G. A. *Z. Naturforsch., C: Biosci.* **1981**, *36C*, 1081–1083. (c) Robeson, D. J.; Strobel, G. A.; Strange, R. N. *J. Nat. Prod.* **1985**, *48*, 139–141. (d) Kobayashi, A.; Yata, S.; Hino, T.; Kawazu, K. *Agric. Biol. Chem.* **1987**, *51*, 2857–2860.

(12) Fermentations employed previously described conditions[2b] except that after 96 h the mycelium (ca. 10 g) was filtered and washed with a replacement medium consisting of glucose (100 g), Na_2HPO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), and $FeSO_4 \cdot 7H_2O$ (0.01 g) (per liter). The mycelium was transferred to a 500-mL Erlenmeyer flask containing 125 mL of replacement medium. Labeled precursor (e.g., 50 mg of **2a**) and 4-pentynoic acid (15 mg) were added in 0.5 mL of 98% ethanol. Incubation on a rotary shaker (160 rpm) at 25 °C for 96 h gave **1** (42.6 mg).[2b]

(13) Since dehydrocurvularin (**1**) is excreted from cells, media replacement removes unlabeled **1** formed prior to feeding of labeled precursor. Glucose may suppress induction of β-oxidation caused by fatty acid derivatives.[10b]

(14) Mutants of *A. cinerariae* were generated by 300-s exposure to 254-nm UV light and selected for inability to grow well on agar media having fatty acids as a primary carbon source.

(15) Although 4-pentynoic acid has not been proven to inhibit β-oxidation enzymes, it may be degraded in vivo to a propiolic acid derivative. The 2-alkynoic acids are potent inactivators of acyl-CoA dehydrogenase, the first enzyme in the β-oxidation pathway.[10c]

(16) Since β-oxidation of **3a** produces a unit of doubly labeled [1,2-$^{13}C_2$]acetate,[10] incorporation after degradation generates coupled resonances in the ^{13}C NMR spectrum for all carbons of **1**. Nevertheless, the intensities of the coupled resonances for C-9 and C-10 are significantly higher than at any other sites.