the S-Me group in **4a** was comparable with reported values for similar compounds.[8] With the bulkier Ph_3PCl_2[13] (in place of PCl_5) in $CHCl_3$, **1a** yielded only **4a** at 10-15 °C. Further, even monosilyl sulfonamides **2** were found to react cleanly with Ph_3PCl_2 near 0 °C, in the presence of Et_3N, to produce only **4**. Upon standing at room temperature, the sulfonimidoyl chlorides slowly decomposed, but they were stable in solution for several hours at 0 °C.

The sulfonimidoyl chlorides **4** were then allowed to react in situ at 0 °C with a mixture of alcohol and triethylamine to yield the corresponding sulfonimidates **5** as distillable liquids.[14] The 2,2,2-trifluoroethyl sulfonimidates exhibited diastereotopic CH_2CF_3 protons in the ^1H NMR spectra, thereby aiding their identification by confirming the chirality at sulfur.

When heated in evacuated Pyrex ampules between 120 and 160 °C, the sulfonimidates **5** condensed over 3-6 days, producing silyl ether, the solid homopolymers **6a** and **6b**, and copolymer **7**. While some irreproducibility was observed in the polymerization behavior of the 2,2,2-trifluoroethyl sulfonimidates, the phenyl sulfonimidates always cleanly produced polymer and silyl ether. Polymer **6a** was purified by precipitation from DMF solution into toluene, while **6b** and **7** were precipitated into hexanes from dichloromethane solution and chloroform solution, respectively.

The polymeric nature of **6a**, **6b**, and **7** was determined by gel permeation chromatography (GPC), which showed relatively high molecular weights for the polymers derived from the phenyl sulfonimidates, but lower molecular weights for those derived from the 2,2,2-trifluoroethyl sulfonimidates (Table I). Additional characterization was obtained by elemental analysis, by ^1H and ^{13}C NMR[15] spectroscopy for **6a**, and by differential scanning calorimetry (DSC) (Table I). The striking feature in the DSC of **6a** is a T_g in the range 55-65 °C, which contrasts sharply with the corresponding -46 °C of the analogous poly(dimethylphosphazene).[12] Polymer **6a** is soluble in DMF, DMSO, and nitromethane, but insoluble in hydrocarbons, ethers, nitriles, and chlorinated hydrocarbons.

Further work on the novel conversion of silyl sulfonamides to sulfonimidoyl halides and the synthesis of poly(oxothiazenes) from sulfonimidates is in progress in our laboratories, and details on these will appear in future publications.

Acknowledgment. A.K.R. thanks Dow Corning Corporation for financial support of this work.

(13) Appel, R.; Schöler, H. *Chem. Ber.* **1977**, *110*, 2382.

(14) Conversion of **2** to **5** was carried out by addition of Et_3N to Ph_3PCl_2 at 0 °C, followed by addition of **2** at -78 °C, warming to 0 °C till the mixture became clear, and then addition of a mixture of alcohol and Et_3N at 0 °C. For sulfonimidate **5a**: yield 73%; bp 77-78 °C/7.7 mm; ^1H NMR (in benzene) δ 0.28 (s, Me_3Si), 2.35 (s, Me-S, 2.98 in $CDCl_3$), 3.92 (m, CH_2CF_3, diastereotopic protons); ^{13}C NMR (in $CDCl_3$) δ 1.8 (s, Me_3Si), 43.2 (s, Me-S), 63.7 (q, CH_2CF_3, $^2J_{FC}$ = 36.9 Hz), 122.9 (q, CH_2CF_3, $^1J_{FC}$ = 278.1 Hz). Anal. Calcd: C, 29.14; H, 5.66; N, 5.62. Found: C, 29.01; H, 5.47; N, 5.65. For **5b**: yield 40-50%; bp 83-85 °C/0.25 mm; ^1H NMR (in CH_2Cl_2) δ 0.03 (s, Me_3Si), 3.05 (s, Me-S), 7.1-7.6 (m, OC_6H_5); ^{13}C NMR (in $CDCl_3$) δ 1.7 (Me_3Si), 42.6 (Me-S), 150.2 (ipso-C), 122.9 (*o*-C), 129.6 (*m*-C), 126.4 (*p*-C). Anal. Calcd: C, 49.35; H, 7.04; N, 5.75. Found: C, 49.52; H, 7.06; N, 5.80. For **5c**: yield 27%; bp 84-86 °C/0.7 mm; ^1H NMR (in benzene) δ 0.37 (s, Me_3Si), 3.87 (m, CH_2CF_3, diastereotopic protons), 7.4-8.0 (m, C_6H_5, in CH_2Cl_2); ^{13}C NMR (in $CDCl_3$) δ 1.8 (s, Me_3Si), 64.2 (q, CH_2CF_3, $^2J_{FC}$ = 36.9 Hz), 122.5 (q, CH_2CF_3 $^1J_{FC}$ = 277.9 Hz), 139.5 (ipso-C), 127.6 (*o*-C), 129.1 (*m*-C), 133.1 (*p*-C). Anal. Calcd: C, 42.43; H, 5.18; N, 4.50. Found: C, 41.90; H, 5.16; N, 4.59. For **5d**: yield 18%; bp 113-120 °C/0.06 mm: ^1H NMR (in $CDCl_3$) δ 0.29 (s, Me_3Si), 6.9-7.9 (m, S-C_6H_5 and O-C_6H_5); ^{13}C NMR (in $CDCl_3$) δ 2.0 (Me_3Si), [S-C_6H_5; 140.1 (ipso-C), 127.8 (*o*-C), 128.5 (*m*-C), 132.6 (*p*-C)], [O-C_6H_5; 150.7 (ipso-C), 122.9 (*o*-C), 129.1 (*m*-C), 126.1 (*p*-C)]. Anal. Calcd: C, 58.98; H, 6.27; N, 4.59. Found: C, 59.49; H, 6.32; N, 4.39. Slight condensation, producing the relatively high boiling Me_3SiOPh, always occurred during distillation of phenyl sulfonimidates. All NMR chemical shifts are relative to the solvents shown in parentheses.

(15) For **6a**: ^1H NMR (in d_6-DMSO) δ 3.40-3.56 (br, S-Me); ^{13}C NMR (in d_6-DMSO) δ 46.4 (S-Me). Anal. Calcd: C, 15.58; H, 3.92; N, 18.17. Found: C, 16.07; H, 3.83; N, 18.32. Once dissolved in DMF, the polymer retained 2-3% of the solvent, which was extremely difficult to remove even after precipitation and repeated vacuum drying at 100-135 °C. Reprecipitation from $MeNO_2$ into toluene was finally used to obtain a sample for microanalysis. For **6b**: Anal. Calcd: C, 51.78; H, 3.62; N, 10.06. Found: C, 51.97; H, 3.77; N, 9.99. For **7**: Anal. Calcd (for 1:1 copolymer): C, 38.87; H, 3.73; N, 12.95. Found: C, 39.89; H, 4.03; N, 12.55.

Biosynthetic Incorporation of Labeled Tetraketide Intermediates into Dehydrocurvularin, a Phytotoxin from *Alternaria cinerariae*, with Assistance of β-Oxidation Inhibitors

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Microorganisms produce a host of commercially important natural products by the polyketide biosynthetic pathway.[1] Isotopic labeling studies,[2] genetic investigations,[3] and experiments with mutants[4] and enzyme inhibitors[5] support the current view that polyketide formation occurs with complete construction of a functionalized carbon skeleton from short fatty acids by an organized enzyme complex. In some cases, further localized transformations (e.g., oxidation, alkylation) involving separate enzymes follow this construction of the parent molecule. The assembly process is similar to fatty acid biosynthesis, but reductive steps are bypassed in particular cycles to lead to incorporation of keto, hydroxy, or olefinic functionality in the growing polyketide chain.[3c] With the exception of polyketide synthases that form simple aromatic compounds (e.g., 6-methylsalicylic acid),[6] the cell-free production of complex polyketides or isolation of their assembly enzymes has not been reported. Intact incorporations of correctly functionalized di- and triketides as their *N*-acetylcysteamine (NAC) thiolesters into *propionate*-derived metabolites such as erythromycin,[7] tylactone,[8] nargenicin,[7b,9] and nonactin[10] provide key support for the proposed biosynthetic pathways and structures of enzyme-bound intermediates. Unfortunately, such experiments are generally plagued by rapid degradation of the labeled precursors to acetate (or propionate) by efficient β-oxi-

(1) *Macrolide Antibiotics, Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic Press: Orlando, FL, 1984.

(2) (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.

(3) (a) Cortes, J.; Haydock, S. F.; Roberts, G. A.; Bevitt, D. J.; Leadley, P. F. *Nature* **1990**, *348*, 176-178. (b) Hopwood, D. A.; Sherman, D. H. *Annu. Rev. Genet.* **1990**, *24*, 37-66. (c) Donadio, S.; Staver, M. J.; McAlpine, J. B.; Swanson, S. J.; Katz, L. *Science* **1991**, *252*, 675-679. (d) Caffrey, P.; Green, B.; Packman, L. C.; Rawlings, B. J.; Staunton, J.; Leadley, P. F. *Eur. J. Biochem.* **1991**, *195*, 823-830. (e) Sherman, D. H.; Bibb, M. J.; Simpson, T. J.; Johnson, D.; Malpartida, F.; Fernandez-Moreno, M.; Martinez, E.; Hutchinson, C. R.; Hopwood, D. A. *Tetrahedron* **1991**, *47*, 6029-6043. (f) Floss, H. G.; Strohl, W. R. *Tetrahedron* **1991**, *47*, 6045-6058. (g) Kakinuma, S.; Ikeda, H.; Omura, S. *Tetrahedron* **1991**, *47*, 6059-6068. (h) Strohl, W. R.; Bartel, P. L.; Li, Y.; Connors, N. C.; Woodman, R. H. *J. Ind. Microbiol.* **1991**, *7*, 163-174.

(4) (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. (c) Huber, M. L. B.; Paschal, J. W.; Leeds, J. P.; Kirst, H. A.; Wind, J. A.; Miller, F. D.; Turner, J. R. *Antimicrob. Agents Chemother.* **1990**, *34*, 1535-1541. (d) Suzuki, H.; Takenaka, S.; Kinoshita, K.; Morohoshi, T. *J. Antibiot.* **1990**, *43*, 1508-1511. (e) Weber, J. M.; Leung, J. O.; Swanson, S. J.; Idler, K. B.; McAlpine, J. B. *Science* **1991**, *252*, 114-117. (f) Kinoshita, K.; Takenaka, S.; Hayashi, M. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2547-2553.

(5) (a) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1988**, 600-602. (b) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1990**, 908-909. (c) Oikawa, H.; Murakami, Y.; Ichihara, A. *Tetrahedron Lett.* **1991**, *32*, 4533-4536.

(6) For leading references, see: (a) 6-Methylsalicylic acid synthase: Lam, K. S.; Neway, J. O.; Gaucher, G. M. *Can. J. Microbiol.* **1988**, *34*, 30-37. (b) Orsellinate synthase: Woo, E. R.; Fujii, I.; Ebizuka, Y.; Sankawa, U.; Kawaguchi, A.; Huang, S.; Beale, J. M.; Shibuya, M.; Mocek, U.; Floss, H. G. *J. Am. Chem. Soc.* **1989**, *111*, 5498-5500. (c) Chalcone synthases: Schroeder, J.; Schroeder, G. *Z. Naturforsch., C: Biosci.* **1990**, *45*, 1-8.

(7) (a) Cane, D. E.; Yang, C. C. *J. Am. Chem. Soc.* **1987**, *109*, 1255-1257. (b) Cane, D. E.; Prabhakaran, P. C.; Tan, W.; Ott, W. R. *Tetrahedron Lett.* **1991**, *32*, 5457-5460.

(8) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253-1255.

(9) Cane, D. E.; Ott, W. R. *J. Am. Chem. Soc.* **1988**, *110*, 4840-4841.

(10) Spavold, Z. M.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 4-6.

0002-7863/92/1514-1531$03.00/0

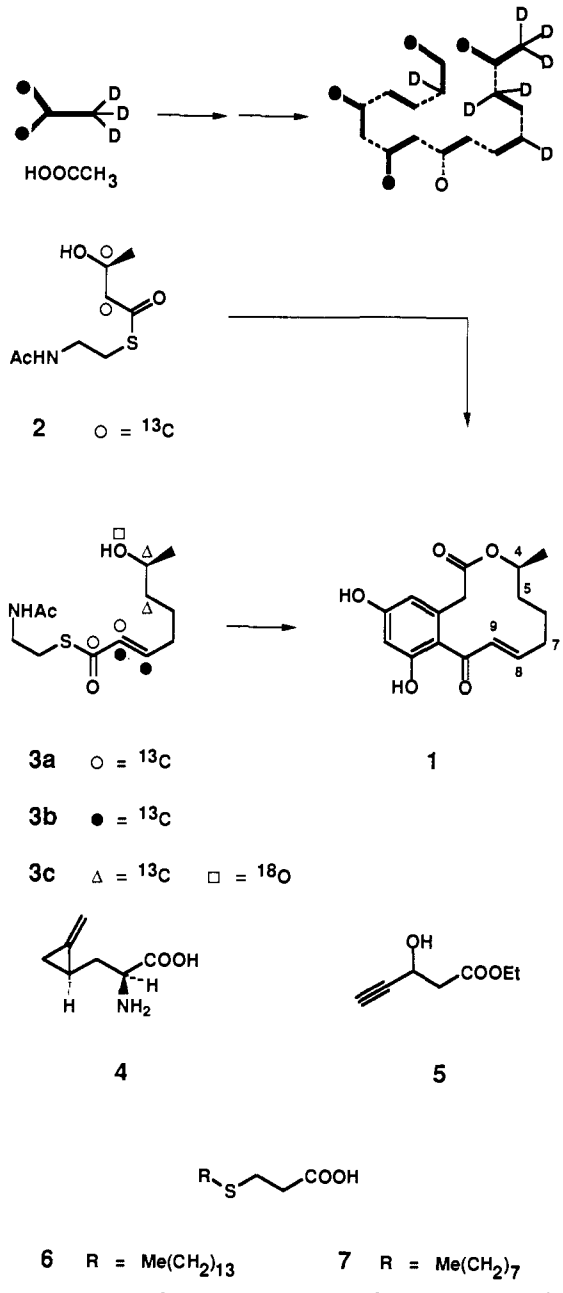


Figure 1. Pattern of bonds derived intact from acetate in dehydrocurvularin (**1**) and incorporation experiments with labeled **2** and **3**.

dation.[11,12] Analogous experiments with intact utilization of *acetate*-derived di- and tetraketide precursors (e.g., **2** and **3a**) in dehydrocurvularin (**1**), an antibiotic phytotoxin from the fungus *Alternaria cinerariae*, required UV mutants deficient in their ability to utilize fatty acids (Figure 1).[13,14] In the present work we describe the use of several potential β-oxidation inhibitors to suppress precursor breakdown and enhance intact incorporation

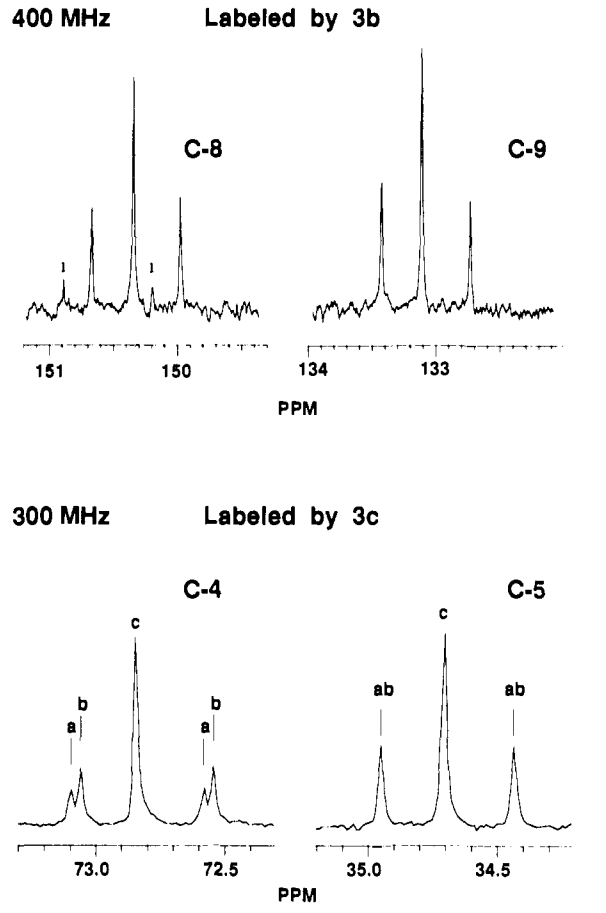


Figure 2. Expansions of ^{1}H-decoupled ^{13}C NMR spectra of dehydrocurvularin (**1**) after incorporation of **3b** (upper spectrum, 400 MHz, C-8 and C-9) and **3c** (lower spectrum, 300 MHz, C-4 and C-5) with addition of **6** as β-oxidation inhibitor. All other resonances show no detectable level of one-bond couplings. Signals i due to slight contamination by **3b** in the upper spectrum for C-8 are absent in other incorporation experiments with this precursor. Signals **a** are due to ^{13}C–^{13}C–^{16}O, **b** are due to ^{13}C–^{13}C–^{18}O, and **c** are due to ^{12}C–^{13}C–^{16}O (natural abundance) species.[2a]

of the functionalized tetraketide **3** into dehydrocurvularin (**1**) by wild-type *A. cinerariae* ATCC 11784.

Since β-oxidation of **3** (or the corresponding carboxylic acid) produces acetate by sequential removal of C_2 units from the carboxyl end,[11] placement of two ^{13}C labels at either end of a cleavable bond allows determination of the extent of intact incorporation by observation of ^{13}C-coupled signals in **1**.[2a,15] The required precursor, NAC (7*S*)-[2,3-$^{13}C_2$]-7-hydroxy-2-octenoate (**3b**) (isotopic purity 99% $^{13}C_2$; ee ≥ 70%), was prepared (see Supplementary Material) and administered in multiple pulse feedings to wild-type *A. cinerariae* cells in a high glucose replacement medium.[16] In order to enhance intact incorporation and suppress precursor degradation to singly labeled acetates, we added a variety of potential β-oxidation inhibitors together with **3b** (Table I). Examination of the ^{13}C NMR spectra of the resulting dehydrocurvularin (**1**) samples shows that the resonances for C-8 and C-9 each consist of a doublet (due to intact incorporation of **3b**) flanking a singlet which arises from natural-abundance ^{13}C combined with enrichment by singly labeled

(11) For discussions of β-oxidation, see: (a) Miyakoshi, S.; Uchiyama, H.; Someya, T.; Satoh, T.; Tabuchi, T. *Agric. Biol. Chem.* **1987**, *51*, 2381–2387. (b) Kunau, W. H.; Buhne, S.; De La Graza, M.; Kionka, C.; Mateblowski, M.; Schultz-Borchard, U.; Thieringer, R. *Biochem. Soc. Trans.* **1988**, *16*, 418–420. (c) Thorpe, C. *Trends Biochem. Sci.* **1989**, *14*, 148–151. (d) Schulz, H. *Biochim. Biophys. Acta* **1991**, *1081*, 109–120. (e) Thieringer, R.; Kunau, W.-H. *J. Biol. Chem.* **1991**, *266*, 13110–13117.

(12) Holmes, D. S.; Sherringham, J. A.; Dyer, U. C.; Russell, S. T.; Robinson, J. A. *Helv. Chim. Acta* **1990**, *73*, 239–259.

(13) Yoshizawa, Y.; Li, Z.; Reese, P. B.; Vederas, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 3212–3213.

(14) For discussion of dehydrocurvularin biosynthesis, see: Arai, K.; Rawlings, B. J.; Yoshizawa, Y.; Vederas, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 3391–3399.

(15) Cleavage by β-oxidation generates singly ^{13}C labeled species that are unlikely to recouple during polyketide formation because of dilution by unlabeled material from the medium.[13]

(16) Conditions for incorporation experiments are analogous to those reported previously,[13,14] except that it was advantageous to add precursor and inhibitor together in four pulse feedings (equal amounts in 0.4 mL of 98% EtOH) at 8-h intervals to a high glucose replacement culture in a single 125-mL shake flask.[13] In these experiments as well as others, timing of advanced precursor addition was found to be critical.

Table I. Effect of β-Oxidation Inhibitors on Intact Incorporation of **3b** into Dehydrocurvularin (**1**)

inhibitor	amoung (mg)[a]	intact incorporation (%)[b]
none		7[c]
4	36	7[d]
5	36	16
6	15	70
7	15	7

[a] Total amount added. [b] Minimum value determined by integration of ^{13}C NMR spectra of coupled resonances (intact incorporation) and singlets (natural abundance + incorporation of degraded precursor) for C-8 and C-9 of **1**. Absolute (total) incorporation rate of precursor was 1–3%. [c] Substitution of NAC ester by Me ester gave no intact incorporation. Shorter precursors (e.g., **2**) are completely degraded without β-oxidation inhibitors. [d] Negligible effect in this case, but **4** enhances incorporation of **2**.

acetates derived from β-oxidation of **3b**. The most effective inhibitors of precursor degradation are hypoglycin (**4**),[17] ethyl 3-hydroxypentynoate (**5**),[18] 3-(tetradecylthio)propanoic acid (**6**),[19] and 3-(octylthio)propanoic acid (**7**).[19] Integration of the coupled and uncoupled signals in **1** indicates that addition of **6** allows at least 70% of incorporated **3b** to be utilized intact. An exceptional recovery of unchanged precursor when this inhibitor is used suggests that there is actually little if any breakdown of the labeled tetraketide by β-oxidation.

In order to eliminate the possibility of partial breakdown of tetraketide **3** at the "starter end" and to confirm that the hydroxyl oxygen is incorporated into **1**, triply labeled NAC (7*S*)-[6,7-$^{13}C_2$,hydroxy-^{18}O]-7-hydroxy-2-octenoate (**3c**) (isotopic purity 99% $^{13}C_2$, 66% ^{18}O; ee ≥ 85%) was synthesized (Supplementary Material) and administered as before together with **6** to wild-type *A. cinerariae* cells. The ^1H-decoupled ^{13}C NMR spectra of the resulting dehydrocurvularin clearly show that (Figure 2) no oxygen label is lost from the intact tetraketide precursor. It is highly likely that there is no significant degradation of **3c**, but if any cleavage does occur between the adjacent carbon labels, virtually all of the attached oxygen label must also be lost before reincorporation of the resulting singly ^{13}C labeled acetate because there is no significant ^{18}O isotope shifted singlet visible.

The results further support the intermediacy of an enzyme-bound tetraketide precursor resembling **3** during the biosynthesis of **1** and demonstrate that use of appropriate β-oxidation inhibitors can assist incorporation of larger precursors into polyketides. Further studies with other precursors and polyketide natural products are in progress.[20]

Acknowledgment. We thank Drs. Paul B. Reese (University of the West Indies, Kingston, Jamaica), Yuko Yoshizawa (Research and Development Corporation of Japan, Eniwashi, Japan), Thomas Henkel, and T. Mark Zabriskie for valuable advice and assistance. Prof. E. A. Kean (University of the West Indies, Kingston, Jamaica) is gratefully acknowledged for a gift of authentic hypoglycin. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada.

Supplementary Material Available: Schemes for the synthesis of **3b**, **3c**, and **5** and comparison spectrum (7 pages). Ordering information is given on any current masthead page.

(17) (a) Kean, E. A. *Biochim. Biophys. Acta* **1976**, *422*, 8–14. (b) Wenz, A.; Thorpe, C.; Ghisla, S. *J. Biol. Chem.* **1981**, *256*, 9809–9812. (c) Baldwin, J. E.; Ostrander, R. L.; Simon, C. D.; Widdison, W. C. *J. Am. Chem. Soc.* **1990**, *112*, 2021–2022. (d) Ikeda, Y.; Tanaka, K. *Biochim. Biophys. Acta* **1990**, *1038*, 216–221. (e) Lai, M. T.; Liu, H.-W. *J. Am. Chem. Soc.* **1990**, *112*, 4034–4035. (f) Melde, K.; Jackson, S.; Bartlett, K.; Sherratt, H. S. A.; Ghisla, S. *Biochem. J.* **1991**, *274*, 395–400.

(18) Although **5** has not been directly demonstrated to be an inhibitor of β-oxidation enzymes, other alkynoic acids (e.g., 4-pentynoic acid) are effective at inhibiting precursor degradation.[7b,13]

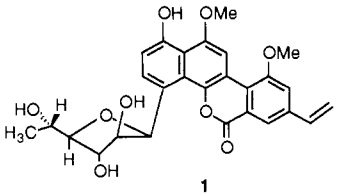
(19) (a) Spydevold, Ø.; Bremer, J. *Biochim. Biophys. Acta* **1989**, *1003*, 72–79. (b) Skorve, J.; Ruyter, B.; Rustan, A. C.; Christiansen, E. N.; Drevon, C. A.; Berge, R. K. *Biochem. Pharmacol.* **1990**, *40*, 2005–2012. (c) Hovik, R.; Osmundsen, H.; Berge, R.; Aarsland, A.; Bergseth, S.; Bremmer, J. *Biochem. J.* **1990**, *270*, 167–173. (d) Aarsland, A.; Berge, R. K. *Biochem. Pharmacol.* **1991**, *41*, 53–61.

(20) Elegant experiments on incorporation of advanced intermediates into aspyrone have been published since submission of this manuscript: Staunton, J.; Sutkowski, A. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1108–1110 and accompanying papers.

Additions and Corrections

Reductive Aromatization of Quinol Ketals: A New Synthesis of *C*-Aryl Glycosides [*J. Am. Chem. Soc.* **1991**, *113*, 8516].
KATHLYN A. PARKER* and CRAIG A. COBURN

Page 8516: Structure **1** should be



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Page 8517, left column, line 4: This sentence should read as follows:

Among the natural products with this substitution pattern are the ravidomycin members of the gilvocarcin class of antitumor antibiotics.