impressive example of 1,5-asymmetric induction. Additional studies employing both cationic iridium and rhodium catalysts are continuing in this laboratory.[14]

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Supplementary Material Available: Detailed procedure for the synthesis of rhodium catalyst **2** along with precise experimental conditions for the hydrogenation process (2 pages). Ordering information is given on any current masthead page.

(14) After completion of this study we made the observation that the reaction diastereoselectivity in the iridium-catalyzed reductions is dependent upon the *amount* of catalyst employed. At lower catalyst/substrate ratios (2.5%) improved levels of directivity may be achieved; however, under even optimal conditions the rhodium catalysts appear to be superior in the reduction of acyclic systems. This data will be reported elsewhere.

Hexanoate as a Starter Unit in Polyketide Biosynthesis

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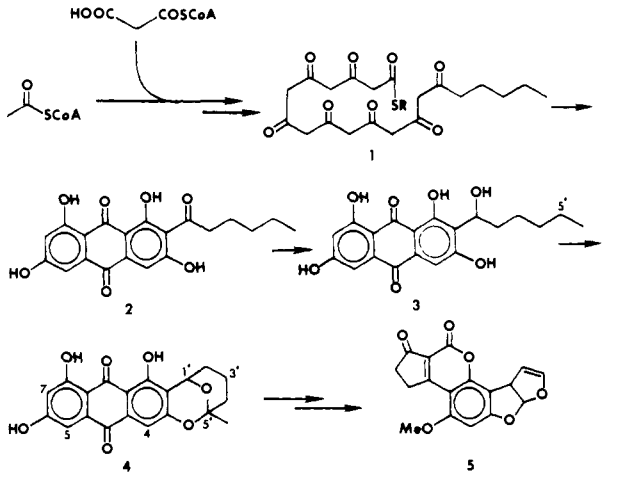
Almost without exception polyketide-derived aromatic natural products utilize acetate as the primer in their biosynthesis.[1,2] In plants benzenoid starters having their origins in shikimate are observed, most notably *p*-coumaric acid in flavone/isoflavone formation. Low molecular weight acids as propionic[3] and branched acids as isobutyric[4,5] and 2-methylbutyric[5] from catabolism of valine and isoleucine, respectively, have been demonstrated to serve as initiators in a few instances.[6] Prior to the early 1970s, when ^{13}C NMR rapidly became the predominant tool of polyketide biosynthetic investigation, observations had been made that the levels of specific radioactivity from incorporation of [^{14}C]acetate in polyketide-derived metabolites occasionally differed in saturated hydrocarbon side chains (usually lower by 5-10%) with respect to aromatic nuclei to which they were bound.[11] Attempts to test intact incorporation of the corresponding C_4 or greater acid, however, fell victim to rapid catabolism by β-oxidation, and only incorporation of radiolabel as acetate/malonate was experimentally observed.[12] In the absence of experimental proof, therefore, the important fundamental point that linear primers C_4 or larger may function in polyketide biosynthesis has remained moot. We provide in this paper the first demonstration for the case of hexanoate in averufin (**4**) biosynthesis (Scheme I).

Scheme I



The development of *Aspergillus parasiticus* mutants blocked in the anthraquinone portion of the aflatoxin B_1 (**5**) biosynthetic pathway was key to progress in this field[13] beyond the now classic [^{14}C]acetate incorporation experiments of Büchi.[14] It emerged from studies using these mutants[15] (Scheme I) that norsolorinic acid (**2**) is the first-formed anthraquinone precursor of the potent mycotoxin **5** followed linearly by averantin (**3**) and averufin (**4**).[16] The latter is apparently generated by oxidation at C-5′—the center derived from the carboxyl of the acetate starter. This realization was surprising in view of a generalization, which may be formulated from an admittedly limited number of [1-^{13}C,$^{18}O_2$]acetate incorporation studies reported recently,[17] that oxygen bound to a carboxyl-derived carbon typically has its origins in the progenitor polyketide.

[1-^{13}C]Hexanoic acid, therefore, was examined for its intact incorporation into averufin under two distinct feeding protocols.

† Research Fellow of the Alfred P. Sloan Foundation 1982-1986; Camille and Henry Dreyfus Teacher-Scholar 1983-1988.

(1) For reviews of polyketide biosynthesis, see: Bu'Lock, J. C. In "Comprehensive Organic Chemistry"; Barton D., Ollis, W. D., Eds.; Pergamon: Oxford, 1979; Vol. 5, pp 927-987. Weiss, U.; Edwards, J. M. "The Biosynthesis of Aromatic Compounds"; Wiley-Interscience: New York, 1980; pp 326-459. Turner, W. B. "Fungal Metabolites"; Academic Press: London, 1971; pp 26-61.

(2) Tetracyclines appear to be unique in the use of a malonate starter unit: Thomas, R.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1983**, 677-679.

(3) See, for example: Ollis, W. D.; Sutherland, I. O.; Codner, R. C.; Gordon, J. J.; Miller, G. A. *Proc. Chem. Soc.* **1960**, 347-349. Mosbach, K. *Acta Chem. Scand.* **1964**, *18*, 1591-1595. Paulick, R. C.; Casey, M. L.; Whitlock, H. W. *J. Am. Chem. Soc.* **1976**, *98*, 3370-3371.

(4) Zylber, J.; Zissman, E.; Polonsky, J.; Lederer, E.; Merrien, A. M. *Eur. J. Biochem.* **1969**, *10*, 278-283.

(5) Drawert, F.; Beier, J. *Phytochemistry* **1976**, *15*, 1695-1696.

(6) In fatty acid biosynthesis the analogous roles for valine, isoleucine, and leucine have been established.[7] Similarly, aleprolic acid [(2-cyclopentenyl)-carboxylic acid], derived from (2-cyclopentenyl)glycine,[8] and cyclohexane-carboxylic acid, derived from shikimate,[9,10] have been shown to serve as starters in specialized instances of fatty acid biosynthesis.

(7) For an early review, see: Lennarz, W. J. *Adv. Lipid Res.* **1966**, *4*, 175-225.

(8) Mangold, H. K.; Spener, F. In "The Biochemistry of Plants"; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: New York, 1980; Vol. 4, pp 647-663.

(9) DeRosa, M.; Gambacorta, A.; Minale, L.; Bu'Lock, J. D. *Biochem. J.* **1972**, *128*, 751-754. Oshima, M.; Ariga, T. *J. Biol. Chem.* **1975**, *250*, 6963-6968.

(10) DeRosa, M.; Gambacorta, A.; Bu'Lock, J. D. *Phytochemistry* **1974**, *13*, 905-910.

(11) See, for example: Hadfield, J. R.; Holker, J. S. E.; Stanway, D. N. *J. Chem. Soc. C* **1967**, 751-755. Tannenbaum, S. W.; Nakajima, S. *Biochemistry* **1969**, *8*, 4626-4631. Gellerman, J. L.; Anderson, W. H.; Schlenk, H. *Lipids* **1974**, *9*, 722-725.

(12) Birch, A. J. *Proc. Chem. Soc.* **1962**, 3-13. Birkenshaw, J. M.; Gowland, A. *Biochem. J.* **1962**, *84*, 342-347. Tannenbaum, S. W.; Nakajima, S. *Biochemistry* **1969**, *8*, 4626-4631. Grove, J. F. *J. Chem. Soc. C* **1970**, 1860-1863. Cross, B. E.; Hendley, P. *J. Chem. Soc., Chem. Commun.* **1975**, 124-125. Vederas, J. C.; Graf, W.; David, L.; Tamm, Ch. *Helv. Chim. Acta* **1975**, *58*, 1886-1898.

(13) Bennett, J. W.; Christensen, S. B. *Adv. Appl. Microbiol.* **1983**, *29*, 53-92.

(14) Biollaz, M.; Büchi, G.; Milne, G. *J. Am. Chem. Soc.* **1968**, *90*, 5017-5019, 5019-5020; **1976**, *92*, 1035-1043.

(15) Bennett, J. W.; Lee, L. S.; Shoss, S. M.; Boudreaux, G. H. *Appl. Environ. Microbiol.* **1980**, *39*, 835-839. Hsieh, D. P. H.; Lin, M. T.; Yao, R. C.; Singh, R. *J. Agric. Food Chem.* **1976**, *24*, 1170-1174.

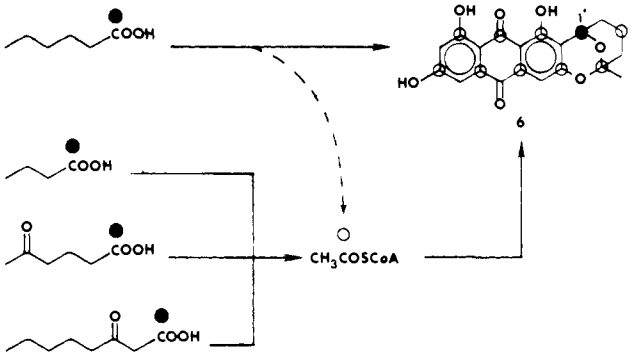
(16) The central role of averufin in aflatoxin biosynthesis has been the focus of recent efforts: Townsend, C. A.; Christensen, S. B.; Davis, S. G. *J. Am. Chem. Soc.* **1982**, *104*, 6152-6153, 6154-6155. Simpson, T. J.; deJesus, A. E.; Steyn, P. S.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1982**, 631-632. Townsend, C. A.; Davis, S. G. *Ibid.* **1983**, 1420-1422.

(17) Lane, M. P.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1982**, *104*, 913-915 and references compiled therein. In reduced polyketides the possibility exists for reduction/dehydration to occur to afford olefin/polyene intermediates that could be rehydrated or epoxidized to lead on to final products. The latter appears to play a significant role in polyether biosynthesis: Hutchinson, C. R. *Acc. Chem. Res.* **1983**, *16*, 7-14. Cane, D. E.; Celmer, W. D.; Westley, J. W. *J. Am. Chem. Soc.* **1983**, *105*, 3594-3600.

0002-7863/84/1506-3868$01.50/0

In the first of these (expt 1), 12 g of 48-h old mycelial pellets of the averufin-accumulating mutant ATCC 24551 were suspended in a low-sugar replacement medium[18] (100 mL) in each of 12 500-mL Erlenmeyer flasks. Labeled acid (12 mg) was administered to each flask under sterile conditions, and they were shaken at 28 °C for 24 h. The averufin produced was isolated by extraction of the filtered pellets and purified by chromatography on silica gel. In the second experiment (expt 2), a 100-mL 48-h-old standing culture of the same mutant grown on a low-salts medium[19] was treated with [1-^{13}C]hexanoic acid (25 mg) and again at 72 and 96 h. At 120 h the fermentation was terminated and the averufin was isolated.

When compared to a $^{13}C\{^1H\}$ NMR spectrum of averufin at natural abundance (normalization to C-5, an unlabeled center derived from C-2 of acetate), the corresponding spectra of the ^{13}C-enriched averufin from both expt 1 and 2 showed the resonance for C-1′ at δ 66.2 to be enhanced more than 3 times the natural abundance (solid circle in **6**). Some degradation of the labeled



hexanoate to [1-^{13}C]acetate and secondary incorporation (open circles in **6**) were observed, being about 0.5%/site in expt 1 and 1%/site in expt 2.

The implications of these findings were further examined by testing the incorporation of *equimolar* amounts of [1-^{13}C]acetate, [1-^{13}C]butyrate, [1-^{13}C]-5-oxohexanoate,[20] and [1-^{13}C]-3-oxooctanoate[21] under the suspended-cell conditions above. For each of these substrates the pattern of ^{13}C enrichment in averufin, while varying somewhat in overall magnitude from experiment to experiment, was the same as that for the uniform incorporation of [1-^{13}C]acetate (about 0.5%/site for the latter). Therefore, all of these experiments to a first approximation label the acetate pool to roughly the same extent as reflected in the incorporation of C-1 throughout averufin (**6**) but [1-^{13}C]hexanoate alone shows a significant level of intact incorporation.

These data suggest an intact hexanoate starter unit arising from a separate synthetase or from β-oxidation. Alternatively, a single polyketide synthetase may produce the initially reduced segment which is able to exchange with free hexanoyl CoA at the C_6 stage. This process would have to be quite efficient given the 3% specific incorporation of label from hexanoate compared with about 0.5% from acetate. Butyrate and 3-oxooctanoate, which might be expected to show analogous exchange, fail detectably to do so or at best with significantly reduced efficiency. Lastly, it is possible that acylation of a preformed anthraquinone may take place. 1,3,6,8-Tetrahydroxyanthraquinone has been isolated from *A. versicolor*,[22] and while structurally symmetrical, its labeling pattern from [1,2-$^{13}C_2$]acetate is not. Such a process would require acylation to occur in the enzyme-bound state. Parallel formation of averufin from 5-oxohexanoate is not supported experimentally.

Of these interpretations we prefer, but cannot strictly prove, the first for its simplicity and direct relation to precedents noted at the outset. It is of interest at this point to recall the observation of Holker and Mulheirn[23] that the bisfuran carbons of sterigmatocystin, from *A. versicolor*, bore about 10% lower specific activity from [1-^{14}C]acetate than did nuclear carbons. In contrast Büchi's extensive degradations of aflatoxin B_1 (**5**) from the corresponding incorporation experiment in *A. flavus* revealed no differential labeling.[14] In the event, it is now clear that the seemingly elaborate formation of averufin (**4**) is necessary given the constraint of an hexanoate primer (Scheme I) and equally necessary one might assume to ultimately construct the singular bisfuran that characterizes this family of mycotoxins.[24,25]

Acknowledgment. Professor J. M. Schwab (Catholic University of America) is thanked for recording the [1-^{13}C]hexanoate incorporation spectra. We are pleased to note helpful conversations with Dr. U. Weiss (NIH) and Professor J. C. Vederas (University of Alberta). Financial support of the National Institutes of Health (ES 01670) and the Alfred P. Sloan Foundation is gratefully acknowledged.

Registry No. 4, 14016-29-6; hexanoic acid, 142-62-1.

(18) Lin, M. T.; Hsieh, D. P. H.; Yao, R. C.; Donkersloot, J. A. *Biochemistry* **1973**, *12*, 5167–5171.
(19) Reddy, T. V.; Viswanathan, L.; Venkitasubramanian, T. A. *Appl. Microbiol.* **1971**, *22*, 393–396.
(20) Cf.: Fitzell, D. L.; Hsieh, D. P. H.; Reece, C. A.; Seiber, J. N. *J. Labelled Compd.* **1975**, *11*, 135–139 and references cited.
(21) Prepared by the reaction of *tert*-butyldimethylsilyl [1-^{13}C]acetate in the presence of lithium diisopropylamide (2.4 equiv) in tetrahydrofuran at −78 °C with hexanoyl chloride. Locquin, R. *Bull. Soc. Chem. Fr.* **1904**, *108*, 595–599.
(22) Berger, Y. *Phytochemistry* **1980**, *19*, 2779–2780.
(23) Holker, J. S. E.; Mulheirn, L. J. *J. Chem. Soc., Chem. Commun.* **1968**, 1576–1577.
(24) Townsend, C. A.; Christensen, S. B. *Tetrahedron* **1983**, *39*, 3357–3582.
(25) See for a perhaps fortuitous comparison the effects of, inter alia, added unlabeled butyrate, hexanoate, and octanoate on natural fatty acid production in *Baccillus acidocaldaria*.[10]

Total Synthesis of (±)-Poitediol and (±)-4-Epipoitediol

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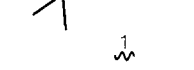
Poitediol (**1**) is an unusual sesquiterpene diol isolated from the red seaweed *Laurencia Poitei*.[1] It is one of the less complex



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members of a growing family of cyclooctane-containing natural products, many of which have interesting biological activities. We wish to report the total synthesis of racemic poitediol and 4-epipoitediol.[2]

Our earlier work[3] had shown that the anionic oxy-Cope rearrangement of dialkenyl cyclobutoxides was an efficient method for the synthesis of substituted cyclooctenones, and our strategy for the synthesis of poitediol is based upon this approach. In order to construct the bicyclo[6.3.0]undecane system of poitediol, we required the bicyclo[3.2.0]heptanone **9** whose synthesis is outlined in Scheme I.[4]

(1) Fenical, W.; Schulte, G. R.; Finer, J.; Clardy, J. *J. Org. Chem.* **1978**, *43*, 3628.
(2) Other total and partial synthesis of cyclooctanoid natural products: (a) (pleuromutilin) Gibbons, E. G. *J. Am. Chem. Soc.* **1982**, *104*, 1767. (b) (epiprecapnelladiene) Birch, A. M.; Pattenden, G. *J. Chem. Soc., Chem. Commun.* **1980**, 1195; *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1913. (c) (ophiobolins) Paquette, L. A.; Andres, D. R.; Springer, J. P. *J. Org. Chem.* **1983**, *48*, 1147 and references cited therein.
(3) Gadwood, R. C.; Lett, R. M. *J. Org. Chem.* **1982**, *47*, 2268.

0002-7863/84/1506-3869$01.50/0