1:4 trans/cis gives the same product with high preference for the trans isomer (i.e., axial bond formation). Synthetically, the lack of dependence of stereochemistry of the product on that of the starting material and the bias for axial C–C bond formation are advantages of this approach. Clearly steric effects and not charge distribution determine regioselectivity. The compatibility with esters, silyl ethers, and hydroxyl groups and the diastereoselectivity in the latter instance enhances the utility of this methodology. At the moment, the reaction requires a somewhat stabilized carbon nucleophile such as an alkynyl or vinyl system. The high selectivity for transfer of such "stabilized" carbanions in preference to saturated alkyl groups is highlighted in the reaction of **10** where, in spite of the many methyl and ethylaluminum bonds, only alkynyl transfer occurs. Alternative Lewis acid type alkylating agents may provide the opportunity for alkyl transfer. Indeed, these results suggest that organosulfones are a new general class of substrates as electrophilic conjunctive reagents in the presence of Lewis acids as mild as alkylaluminum halides.

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Synthesis of (*R*)- and (*S*)-[1-$^{13}C_1$,2-$^{2}H_1$]Malonate and Its Stereochemical Analysis by NMR Spectroscopy

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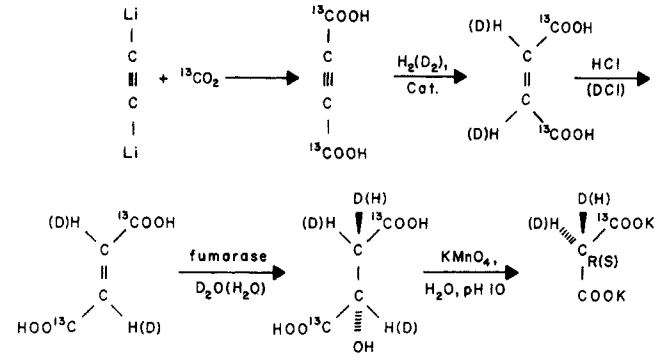
A wide variety of natural products are formed from malonylcoenzyme A by the so-called "polyketide pathway",[1-3] a biosynthetic route sharing many features with the biosynthesis of fatty acids.[4] The formation of the polyketides or acetogenins poses many stereochemical questions which have so far not been elucidated. The stereochemistry of fatty acid biosynthesis has been unraveled in the elegant studies of Cornforth and Sedgwick[5,6] using stereospecifically tritiated malonylcoenzyme A as the chiral substrate. This approach, however, is limited to work with isolated enzymes. Most polyketide biosyntheses have not yet been achieved in cell-free systems and must therefore be studied in in vivo fermentations. We therefore synthesized a chiral version of malonate, a pro-prochiral molecule of the Caabb type,[7] as a substrate for studies on the steric course of polyketide biosynthesis.

A major problem in the preparation and use of chiral malonate is the propensity of the molecule to undergo proton exchange and racemization in aqueous solutions[5] ($t_{1/2}$ for ^{1}H exchange at 30 °C 112 min at pH 8, 216 min at pH 9).[8] Clearly the compound must be generated and used at a pH above 8 and all operations must be carried out on a time scale of minutes, ruling out operations like chromatography or similar purification steps. We decided to prepare (*R*)- and (*S*)-[1-$^{13}C_1$, 2-$^{2}H_1$]malonate from configurationally stable precursors, (2*S*,3*R*)-[1,4-$^{13}C_2$, 3-$^{2}H_1$]-malate and (2*S*,3*S*)-[1,4-$^{13}C_2$-2,3-$^{2}H_2$]malate, which were synthesized as shown in Scheme I. The malate samples contained

Scheme I. Synthesis of Potassium (*R*)- and (*S*)-[1-$^{13}C_1$,2-$^{2}H_1$]Malonate



Scheme II. Derivatization of Chiral Malonate for NMR Analysis

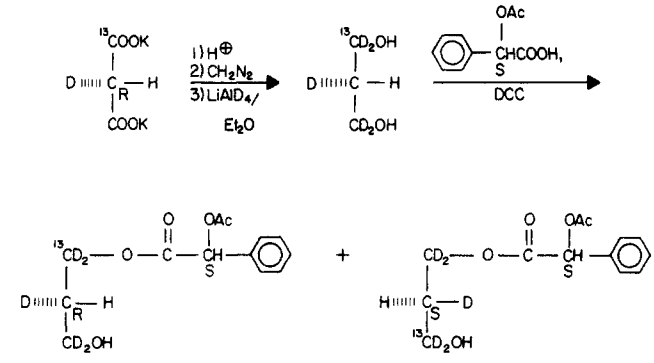
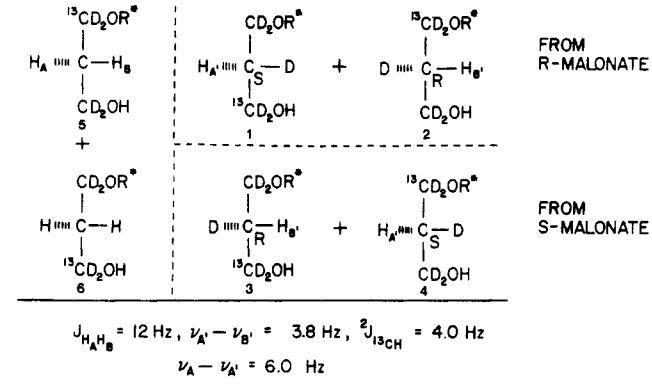


Chart I



$J_{H_AH_B}$ = 12 Hz, $\nu_{A'} - \nu_{B'}$ = 3.8 Hz, $^{2}J_{^{13}CH}$ = 4.0 Hz
$\nu_A - \nu_{A'}$ = 6.0 Hz

99% ^{13}C and 90% ^{2}H per labeled position. Their configurations follow from the known[9,10] steric course of the fumarase reaction. Oxidation of the malate (134 mg, 1 mmol) with $KMnO_4$ (300 mg, 1.1 mmol) in aqueous solution (1.9 mL) adjusted to pH 10.0 for 5 min in an ice bath, followed by immediate filtration to remove MnO_2, gave a solution of the potassium salts of malic acid (less than 10%), malonic acid (20%), and oxalic acid (70%) as determined by HPLC. If desired, the oxalic acid could be removed by addition of a cold solution of $CaCl_2$ immediately before filtration. Proton NMR analysis of the oxidation product from [3-$^{2}H_2$]malic acid in H_2O and unlabeled malic acid in D_2O showed less than 10% exchange under these conditions.

To demonstrate that the samples of chiral malonate so generated did indeed contain an excess of one enantiomer, each product was converted to dimethyl malonate by rapid acidification and lyophilization followed by treatment with ethereal diazomethane. The ether solutions were then immediately reduced with $LiAl^{2}H_4$ and the 1,3-propanediol was converted to the (*S*)-(+)-*O*-acetyl-D-mandelate monoester[11] (Scheme II). The latter (25% yield based on malonate) was purified by HPLC (Hamilton PRP-1 column,

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(1) Packter, N. M. "The Biosynthesis of Acetate-Derived Compounds"; Wiley: London, 1973.
(2) Manitto, P. "Biosynthesis of Natural Products"; Wiley: New York, 1981.
(3) Herbert, R. B. "The Biosynthesis of Secondary Metabolites"; Chapman and Hall: London, 1981.
(4) Lynen, F. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1961**, *20*, 941.
(5) Sedgwick, B.; Cornforth, F. W.; French, S. F.; Gray, R. T.; Kelstrup, E.; Willadsen, P. *Eur. J. Biochem.* **1977**, *75*, 481.
(6) Sedgwick, B.; Morris, C.; French, S. J. *J. Chem. Soc., Chem. Commun.* **1978**, 193.
(7) Floss, H. G.; Tsai, M. D.; Woodard, R. W. *Top. Stereochem.* **1984**, *15*, 253.
(8) Huang, S. Ph.D. Thesis, Purdue University, West Lafayette, IN, 1984.
(9) Gawron, O.; Fondy, T. P. *J. Am. Chem. Soc.* **1959**, *81*, 6333.
(10) Anet, F. A. L. *J. Am. Chem. Soc.* **1960**, *82*, 994.
(11) Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1983**, 83.

0002-7863/86/1508-1100$01.50/0

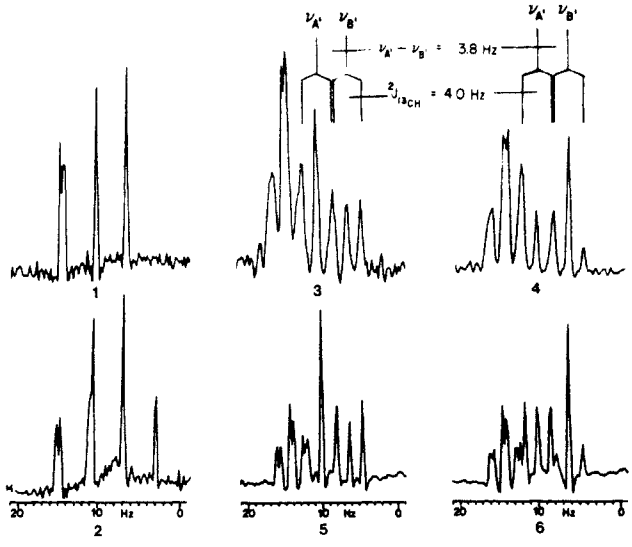

Figure 1. ^1H NMR signal for the C-2 protons of [1,1,2,3,3-$^2H_5$]-propane-1,3-diol mono-*O*-acetyl-D-mandelate observed with broad-band deuterium decoupling. (1) Product from (*R*)- or (*S*)-malonate with broad-band ^{13}C decoupling; (2) product from (*R*)- or (*S*)-malonate without ^{13}C decoupling; (3) product from (*S*)-malonate with selective ^{13}C decoupling of upfield (57.96 ppm) resonance; (4) product from (*S*)-malonate with selective ^{13}C decoupling of downfield (62.05 ppm) resonance; (5) product from (*R*)-malonate with selective ^{13}C decoupling of downfield resonance; (6) product from (*R*)-malonate with selective ^{13}C decoupling of upfield resonance.

CH_3CN/H_2O 3:7) and subjected to proton NMR analysis (solvent benzene-d_6, 7.1 T, Bruker WM 300). The different species present in the sample and the relevant NMR parameters are shown in Chart I. Since the esterification does not discriminate between the two hydroxymethyl groups of 1,3-propanediol, each pure enantiomer of malonate will give two species of the monester; i.e., (*R*)-malonate gives **1** + **2** and (*S*)-malonate **3** + **4**. Exchange of the malonate will also produce some diprotio species, **5** + **6** by C-D bond cleavage, and some monoprotio species of opposite configuration, **3** + **4** from (*R*)-malonate and **1** + **2** from (*S*)-malonate, by C-H bond cleavage. Figure 1 displays the ^1H NMR signal for the C-2 protons of the propanediol moiety. Upon broad-band decoupling of both ^{13}C and deuterium, species **5** + **6** show the center part of an AB system[12] and species **2** + **3** and **1** + **4** give single resonances 3.8 Hz apart which are deuterium-shifted 6.0-Hz upfield from the resonance frequencies of the diprotio species. Upon removal of the ^{13}C decoupling, each of these resonances splits into two due to the 4.0-Hz ^{13}C-^1H two-bond coupling. Sets **1** + **2** and **3** + **4** can be distinguished by ^2H broad-band decoupling, with simultaneous single-frequency ^{13}C decoupling, of either the upfield or the downfield ^{13}C-enriched carbon. Irradiation of C-1 (downfield) leaves H_A' of **1** and H_B' of **3** as doublets but collapses H_B' of **2** and H_A' of **4** into singlets. The intensity ratio of these two singlets gives the ratio of species **1** + **2** to **3** + **4** in the sample and hence the minimum ratio of *R*/*S* isomers in the original malonate. The opposite pattern was observed when the C-3 (upfield) ^{13}C resonance was selectively irradiated.

Analysis of the spectra showed that the sample from (*R*)-malonate contained **1** + **2** and **3** + **4** in a 2:1 ratio, indicating at least 34% ee *R* isomer in the original malonate. The ratio for the (*S*)-malonate-derived material was 1:2 of **1** + **2** and **3** + **4**, corresponding to 34% ee *S* isomer. These are minimum values, because the derivatization procedure involves considerable exchange and racemization, as evidenced by the presence of 24% diprotio species in the propanediol monoester. From the measured exchange in the oxidation of unlabeled malate in D_2O, less than 10%,[8] and the deuterium enrichment of the starting malate, it follows that the chiral purity of the original dipotassium malonate samples is greater than 80% ee.

The work reported here demonstrates the feasibility of generating chiral versions of malonic acid of good enantiomeric purity. It sets the stage for stereochemical studies on the biosynthesis of polyketides, provided conditions can be worked out for the incorporation of these substrates into products without complete obliteration of the stereochemical information by exchange. Work is under way to try to implement such applications.

Acknowledgment. We are indebted to the Purdue University Biochemical Magnetic Resonance Laboratory (supported by NIH Grant RR01077) and The Ohio State University Chemical Instrument Center for NMR and mass spectral measurements, to the Los Alamos Stable Isotope Resource (supported by NIH Grant RR02231) for labeled compounds, and to the National Science Foundation (Grant CHE-8408412) for financial support. We thank the National Institutes of Health for a postdoctoral fellowship (GM 10207 to J.B.). This project was initiated while the senior author was a visitor in the laboratory of Professor H. Simon at the Technical University of Munich under a Humboldt Senior Scientist Award. He thanks Prof. Simon for his hospitality and stimulating discussions and the Humboldt Foundation for financial support.

(12) The ^2H-broad-band decoupled ^1H NMR spectrum of the (*S*)-(+)-*O*-acetyl-D-mandelate monoester of [1,1,3,3-$^2H_4$]propane-1,3-diol displays a 0.6-Hz doublet for the methylene protons. These are the center two lines of an AB quartet; the outer two lines were too weak to be observed. The separation between the center lines is predicted to be $|((\nu_A - \nu_B)^2 + J^2)^{1/2} - J|$ (Becker, E. D. "High Resolution NMR"; Academic Press: New York, 1969; pp 135-138). Since $(\nu_A - \nu_B)$ was measured with the chiral samples, $^2J_{HH}$ could be calculated to be 12 Hz.

Flavin-Catalyzed Reductive Dioxygen Activation with *N*-Methyldihydronicotinamide

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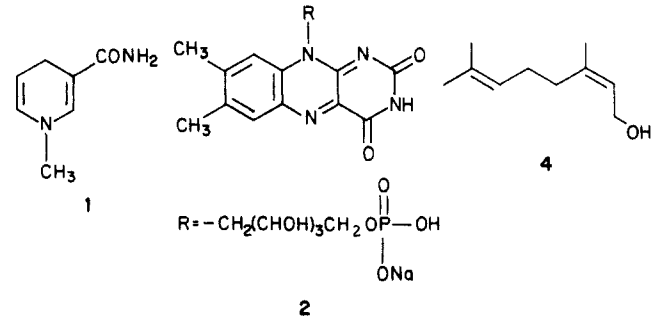
Mechanistic studies[1] and synthetic application of the P-450 type reaction[2] are currently attracting the interest of chemists. Surprisingly, however, components used for artificial O_2 activation systems have little structure similarity to those in the native enzyme systems: native, protoporphyrin-Fe/NADH/FAD·FMN/cys-S$^-$, O_2 (lipoic acid); artificial, TPPS·Mn/H_2/colloidal Pt/N-MeIm, O_2 (benzoic anhydride).

We now wish to report that MeNAH (**1**), a NADH analogue,



efficiently activates O_2 in the presence of FMN (flavin mononucleotide) (**2**) and TPPS·Mn (tetraphenylporphyrintetra-

(1) (a) White, R. R.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315-356. (b) Tabushi, I.; Yazaki, A. *J. Am. Chem. Soc.* **1981**, *103*, 7371-7373. (c) Tabushi, I.; Kodera, M. *J. Am. Chem. Soc.* **1985**, *107*, 4466-4473. (d) Groves, J. T.; Watanabe, Y.; McMurry, T. T. *J. Am. Chem. Soc.* **1983**, *105*, 4489-4490. Groves, J. T.; Nemo, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 5786-5791. (e) Powell, M. F.; Pai, E. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1984**, *106*, 3277-3285. (f) Collman, J. P.; Brauman, J. I.; Meunier, B.; Hayashi, T.; Kodadek, T.; Raybuck, S. A. *J. Am. Chem. Soc.* **1985**, *107*, 2000-2005.

(2) (a) Tabushi, I.; Morimitsu, K. *J. Am. Chem. Soc.* **1984**, *106*, 6871-6872. (b) Groves, J. T.; Myers, R. S. *J. Am. Chem. Soc.* **1983**, *105*, 5791-5796.

0002-7863/86/1508-1101$01.50/0