be contrasted with the value of $2 \times 10^{-2}$ $M^{-1}$ $s^{-1}$ obtained from studies using the Marcus cross-relationship.[3] Apparently the direct self-exchange reaction occurs by a pathway that is more efficient than the outer-sphere mechanism implicit in Marcus theory. Presumably the transition state has substantial bonding between $NO_{2(aq)}$ and $NO_2^-$. As noted in our study of the reaction between $ClO_2$ and $NO_2^-$, strong overlap in the transition state may be a general property of main-group electron-transfer reactions.[24]

**Acknowledgment.** This research was supported by the NSF, Grant CHE-8716929. Dr R. Smith is thanked for his contributions with the uric acid experiments.

(24) Stanbury, D. M.; Martinez, R.; Tseng, E.; Miller, C. E. *Inorg. Chem.* **1988**, *27*, 4277-4280.

## Nonstereospecific Proton Removal in the Enzymatic Formation of Orsellinic Acid from Chiral Malonate

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The biosynthesis of orsellinic acid (**1**, Scheme I) involves the assembly of one molecule of acetyl-CoA and three molecules of malonyl-CoA into a tetraketide which then cyclizes, connecting carbons 2 and 7, and enolizes to the final structure. The enzyme catalyzing this process has been isolated from *Penicillium madriti* and some of its properties have been reported.[1]

Following the elucidation of the steric course of fatty acid formation by Cornforth and his colleagues,[2] various aspects of the stereochemistry of polyketide biosynthesis have been studied in different systems.[3] However, except in a few cases, e.g., 6-methylsalicylic acid and rubrofusarin formation,[4] no information has been obtained on the stereospecificity of hydrogen removal from the polyketide precursor, by enolization or dehydration, during the transformation into aromatic products. The recent synthesis of the two enantiomers of chirally labeled malonate[5] and the availability of orsellinic acid synthase[6] enabled us to examine this question.

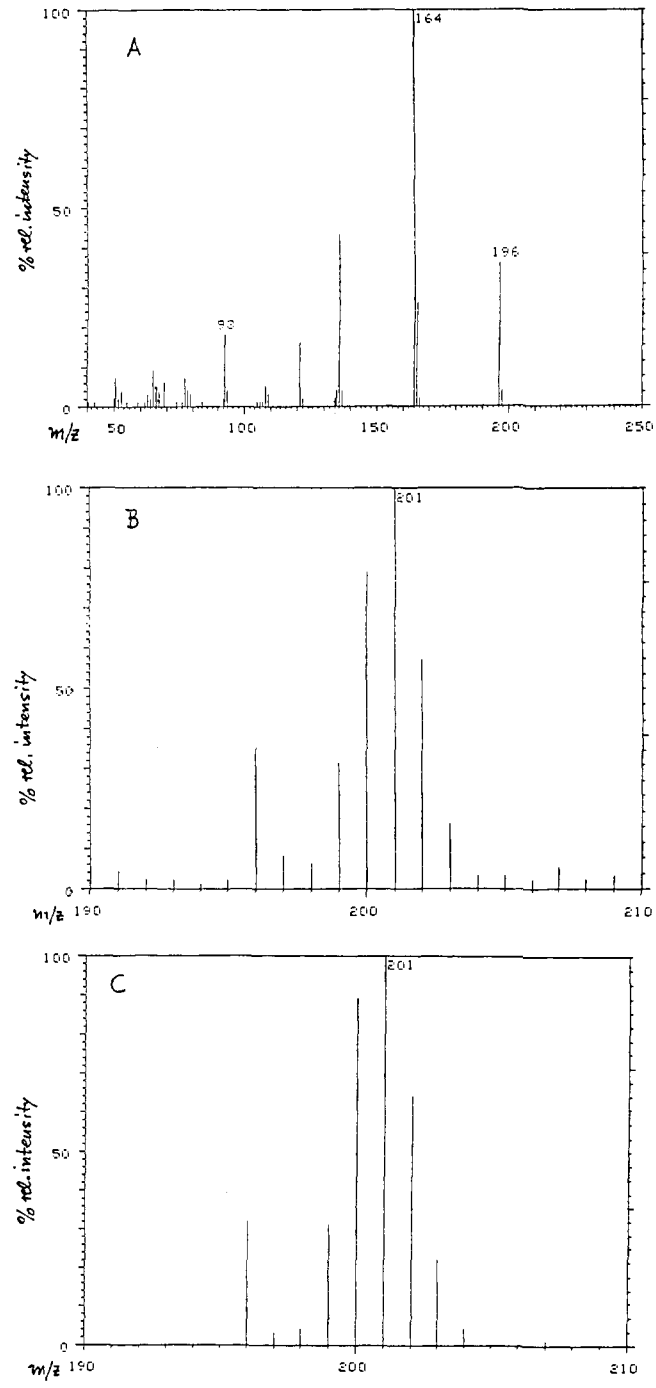


**Figure 1.** A: GC-mass spectrum of unlabeled dimethyl orsellinate; B, C: molecular ion region of the mass spectra of dimethyl orsellinate from (A) *R*-[1,2-$^{13}C_2$,$^2H_1$]malonate and (B) *S*-[1,2-$^{13}C_2$,$^2H_1$]malonate.

*S*- and *R*-[1,2-$^{13}C_2$,2-$^2H_1$]malonate were prepared from (2*S*,3*R*)-[2,3-$^{13}C_2$,3-$^2H_1$]malate and (2*S*,3*S*)-[2,3-$^{13}C_2$,2,3-$^2H_2$]malate, respectively, by oxidation with an equimolar amount of $KMnO_4$ at pH 9.5 for 15 or 40 min,[5a] respectively, at room temperature. Without isolation, these samples were used directly in enzyme incubations containing 6 milliunits of orsellinic acid synthase from *Penicillium cyclopium*[6] and 3.75 units[7] of succi-

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[∥] University of Washington.

(1) Gaucher, G. M.; Shepherd, M. G. *Biochem. Biophys. Res. Commun.* **1968**, *32*, 664.
(2) (a) Sedgwick, B.; Cornforth, J. W. *Eur. J. Biochem.* **1977**, *75*, 465. (b) Sedgwick, B.; Cornforth, J. W.; French, S. J.; Gray, R. T.; Kelstrup, E.; Willadsen, P. *Eur. J. Biochem.* **1977**, *75*, 481. (c) Sedgwick, B.; Morris, D.; French, S. J. *J. Chem. Soc., Chem. Commun.* **1978**, 193.
(3) For example: (a) Abell, C.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1981**, 856. (b) Hutchinson, C. R.; Kurobane, I.; Cane, D. E.; Hasler, H.; McInnes, A. G. *J. Am. Chem. Soc.* **1981**, *103*, 2477. (c) Hutchinson, C. R.; Sherman, M. M.; McInnes, A. G.; Walter, J. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 5956. (d) Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakeshima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694. (e) Cane, D. E.; Liang, T.-C.; Taylor, P. B.; Chang, C.; Yang, C.-C. *J. Am. Chem. Soc.* **1986**, *108*, 4957.
(4) (a) Abell, C.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1984**, 1005. (b) Leeper, F. J.; Staunton, J. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2919.
(5) (a) Huang, S.; Beale, J. M.; Keller, P. J.; Floss, H. G. *J. Am. Chem. Soc.* **1986**, *108*, 1100. (b) Jordan, P. M.; Spencer, J. B.; Corina, D. L. *J. Chem. Soc., Chem. Commun.* **1986**, 911.
(6) Woo, E.-R.; Fujii, I.; Ebizuka, Y.; Sankawa, U., unpublished work.
(7) As defined with succinate and acetoacetyl-CoA as substrates; the activity of the enzyme with malonate as substrate is 42 times lower.

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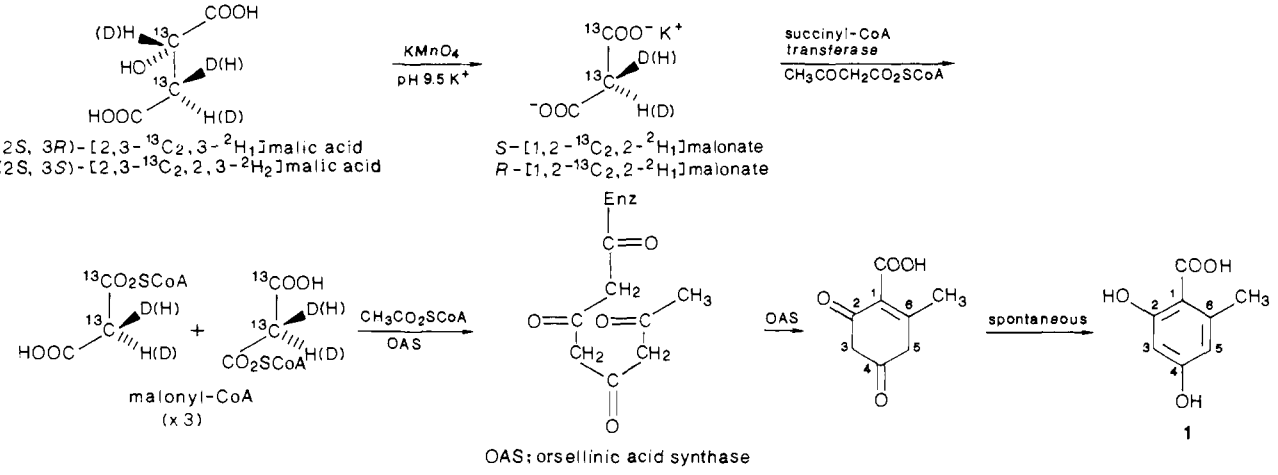
Scheme I




nyl-CoA:keto acid CoA-transferase in addition to acetyl-CoA, dithiothreitol, EDTA, bovine serum albumin, and Tris buffer, pH 7.8. The reactions were initiated with acetoacetyl-CoA as a source of CoA to generate malonyl-CoA and terminated 10 min later. Orsellinic acid was extracted with ether, methylated with trimethylsilyldiazomethane, and analyzed by GC-MS in a Hitachi M-80 instrument with a Thermon 3000 GC column (0.53 mm × 25 m, helium carrier gas at 10 mL/min).

Each of the three malonate-derived chain extension units in orsellinic acid is expected to contain 100% of a $^{13}C$ atom from C-2 and 50% of the $^{13}C$ from C-1 of malonate. In addition, barring isotope effects, the two central units will each contain 50% of the deuterium from C-2 of malonate. Following the rationale delineated previously,[8] stereospecific incorporation of one enantiomer of malonate will give orsellinic acid in which the deuterium at C-3 and C-5 is present strictly in molecules which also retain $^{13}C$ from C-1 of the same malonate unit. The other enantiomer of malonate will give orsellinic acid in which deuterium from C-2 and $^{13}C$ from C-1 of malonate are present strictly in separate units or molecules. These two modes of incorporation will produce distinctly different mass spectral patterns for the molecular ion and for diagnostic fragment ions. On the other hand, nonstereospecific proton removal in the enolization to form the aromatic ring will produce identical mass spectral patterns from both enantiomers of the precursor. As can be seen in Figure 1 (parts B and C), the labeled samples show the expected upfield displacement of the molecular ion cluster, relative to the natural abundance spectrum (Figure 1A), but the mass spectral patterns of the material derived from *R*- and *S*-malonate are virtually identical.[9] The same was observed for the fragment ion cluster at $m/z$ 164 (data not shown). These results indicate that either the proton abstraction (or, less likely, the condensation step) is not stereospecific or, alternatively, that the stereochemical information was lost due to exchange of malonate prior to or during its incorporation into orsellinic acid. The following two lines of additional evidence favor the first interpretation.

To exclude the possibility that the substrate racemized prior to or during incorporation, e.g., by hydrogen exchange at the malonyl-coA stage, the chirally labeled malonic acids were enzymatically converted into palmitic acid under conditions similar to those for the synthesis of orsellinic acid. The incubation mixtures contained 3.75 units of succinyl-CoA transferase and 176 milliunits of purified yeast fatty acid synthase. The resulting palmitic acid samples were methylated and subjected to GC-MS analysis. The spectra (Figure 2 (parts A and B)) showed the

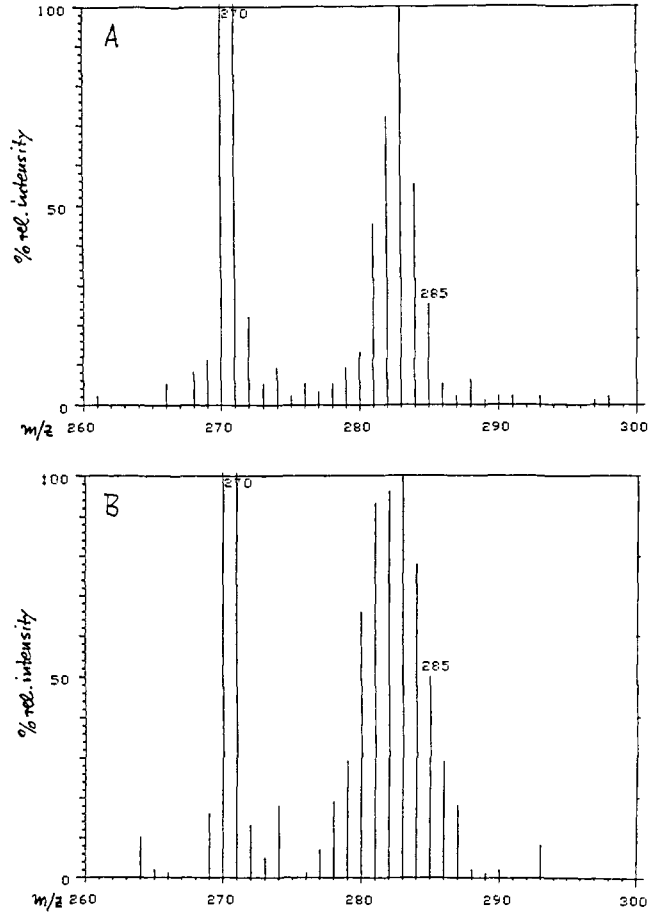



**Figure 2.** Molecular ion region of the mass spectra of methyl palmitate from (A) *R*-[1,2-$^{13}C_2$,$^{2}H_1$]malonate and (B) *S*-[1,2-$^{13}C_2$,$^{2}H_1$]malonate.

patterns expected[5b,8] for stereospecific loss of the *pro-R* hydrogen of malonyl-CoA,[2] although the discrimination between the two enantiomers of malonate was not as pronounced as in the experiments reported by Jordan et al.[5b] Thus, despite some hydrogen exchange, and consequently racemization, under the reaction conditions the experiment does clearly report stereospecific hydrogen loss. The deuterium retention in orsellinic acid formation was somewhat lower than in palmitic acid formation (av D-enrichment per labeled site: from *R*-malonate 14% vs 29%, from *S*-malonate 24% vs 27%) but not enough to obliterate completely the stereochemical information in the orsellinic acid experiments.

A second line of evidence in favor of nonstereospecific proton removal in orsellinic acid formation comes from a comparison of the degree of deuterium retention from [2-$^{2}H_1$]- and [2-

(8) Floss, H. G.; Tsai, M.-D.; Woodard, R. W. *Topics Stereochem.* **1984**, *15*, 253.

(9) A very small difference in the patterns is consistently observed, but it is not certain that this difference is significant. If it is, it might suggest that the proton removal, although not enzyme-mediated, does take place in the chiral environment of an enzyme and therefore shows a slight preference for elimination of the original *pro-S* hydrogen of malonyl-CoA.

$^{13}C,^{2}H_2$]malonate in this process, an experiment patterned after the earlier work of Abell and Staunton with mono- and trideuterated acetic acid as polyketide precursors.[4a] Stereospecific proton removal will result in nearly equal degrees of deuterium loss from a CHD and a $CD_2$ methylene group in the polyketide, because there is no intramolecular competition between H and D. However, if the proton removal is nonstereospecific, a substantially higher deuterium retention from the CHD than from the $CD_2$ methylene group is expected because of the high primary kinetic deuterium isotope effect ($k_H/k_D \sim 4-7$)[2a] associated with enolization reactions. [2-$^{2}H_1$]Malonate (87% $D_1$)[10] and [2-$^{13}C,^{2}H_2$]malonate (99% $^{13}C$, 98% $D_2$) were converted into orsellinic acid in the same coupled enzyme system used with the chiral malonate samples. GC-MS analysis of the products showed 51% deuterium retention per labeled site from the monodeuterated precursor and 26% from the dideuterated precursor.[11]

The results of the above experiments strongly suggest that the proton abstractions from C-3 and C-5 of the polyketide precursor in the formation of orsellinic acid are not stereospecific and therefore not enzyme-mediated (Scheme I), although they may occur while the molecule still resides in the chiral environment of the protein. This contrasts with the biosynthesis of 6-methylsalicylic acid and of rubrofusarin, for which Staunton and co-workers[4] proposed stereospecific proton removal based on comparisons of the incorporation of [2-$^{2}H_1$]- and [2-$^{2}H_3$]acetic acid.

**Acknowledgment.** This work was supported by grants from the National Science Foundation and from the National Institutes of Health (GM 32333). We are grateful to T. Kasama and Y. Seyama, Faculty of Medicine, The University of Tokyo, for GC-MS measurements.

(10) Prepared by generating the trianion of malonic acid with sodium hydride and quenching with $D_2O$.

(11) Theoretical values, in the absence of any exchange and isotope discrimination, in both cases are 50%. The data indicate extensive (~50%) exchange and a sizeable primary kinetic isotope effect ($k_H/k_D > 2$).

## Ohioensin-A: A Novel Benzonaphthoxanthenone from *Polytrichum ohioense*

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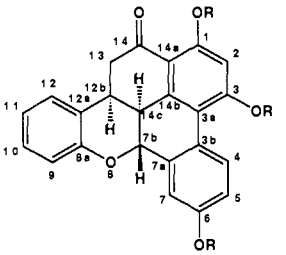
The National Cancer Institute has conducted an initial screening program to discover novel biologically active compounds from the Bryophyta.[1] As part of this program to isolate antineoplastic agents from mosses, *Polytrichum ohioense* (*Polytrichaceae*) has been studied. Although a number of mosses have been examined phytochemically, there were no published examinations of this species prior to our investigations. In this communication we report the structure of ohioensin-A (**1**) which contains a novel polycyclic skeleton and exhibits cytotoxicity against PS and MCF-7 tumor cells in culture at $ED_{50}$ 1.0 and 9.0 $\mu$g/mL, respectively.[2]



**1** R = H

**2** R = Ac

Ohioensin-A (**1**, 1.5 × $10^{-4}$% yield) was isolated from the ethanol extract of *P. ohioense* following solvent partitioning and silica gel ($CHCl_3$–MeOH, 97:3) chromatography. Ohioensin-A (**1**) ($C_{23}H_{16}O_5$), yellow needles from $CHCl_3$–MeOH (1:1), mp 274–275 °C dec, $[\alpha]_D^{27}$ +37° (*c* 0.1, MeOH), had IR bands at 3500–2500 (OH, br), 1620 (C=O), 1600, and 1570 (aromatic ring) $cm^{-1}$, indicating the presence of intramolecularly hydrogen bonded hydroxyl and conjugated carbonyl functions. The UV bands at 272.5 and 361.0 nm, which exhibited a bathochromic shift upon adding $AlCl_3$, suggested the presence of a phenolic hydroxyl group in the vicinity of a keto function. EIMS showed a significant fragment at *m/z* 354 by a loss of water from the stable molecular ion. This ion may originate by a rearrangement involving the transfer of H-13 to the C-14 carbonyl oxygen to give the enol form.[3] On the basis of this, the carbonyl carbon was linked to an aliphatic carbon with at least one hydrogen attached. Analysis of the $^{13}C$ NMR spectrum established the presence of one carbonyl, one methylene, 11 methine (eight aromatic), and ten aromatic quaternary carbons, which supported the presence of a polycyclic skeleton with highly aromatic character. The $^{1}H$ NMR spectrum of **1** indicated three exchangeable singlets at $\delta$ 7.43, 8.81, and 12.13 assigned to three phenolic hydroxyls, one of which was hydrogen bonded. This was confirmed by the formation of a triacetate (**2**). One uncoupled proton in the high field aromatic region (6.53, 1 H, s) could be assigned to H-2. This signal showed NOE enhancements of 9% on irradiation of the hydroxy group at $\delta$ 12.13 of **1** and of 16% on irradiation of the C-3 acetoxyl methyl protons at $\delta$ 2.36 of **2**. Upon acetylation, the H-2 signal exhibited an unusual downfield shift of $\delta$ 0.63 which confirmed its location between two phenolic hydroxyl groups. The $^{1}H$ NMR spectrum of **1** also indicated two groups of aromatic proton signals based on homonuclear $^{1}H$–$^{1}H$ decoupling experiments. The downfield shifts of H-7($\Delta\delta$ 0.32) and H-5($\Delta\delta$ 0.47) in the $^{1}H$ NMR of **2** established that a hydroxyl group was substituted at the C-6 position. The remaining interrelated aliphatic proton signals were also unambiguously assigned by selective decoupling experiments.

A computer-generated perspective drawing of the final X-ray model of **1** is given in the Supplementary Material.[4] The X-ray analysis did not define the absolute but only the relative stereochemistry; the enantiomer shown was arbitrarily chosen. The asymmetric unit of crystalline **1** consists of two independent molecules with identical structures and only minor conformational differences. The $sp^3$ centers at C7b, C14c, C12b, and C13 give the essentially planar molecule some slight three-dimensionality. For example, the twist about the C3a–C3b biphenyl bond is −17°. With the stereochemistry being relative, **1** was named

(1) Spjut, R. W.; Suffness, M.; Cragg, G. M.; Morris, D. H. *Economic Botany* **1986**, *40*, 310.

(2) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1.

(3) Chmielenska, K.; Prajer-Janczewska, L. *Polish J. Chem.* **1985**, *59*, 139.

(4) Two molecules of composition $C_{23}H_{16}O_5 \cdot CH_3OH$ formed the asymmetric unit. The structure was solved and refined with the SHELXTL PLUS package of programs. The final model using anisotropic heavy atoms and fixed isotropic hydrogens has refined in a full-matrix least-squares to a conventional *R*-factor of 0.041 for the observed reflections. Both molecules comprising the asymmetric unit had identical stereostructures. See the paragraph entitled Supplementary Material Available for additional crystallographic details.

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