obtain an authentic sample of stenine, the ^1H NMR, IR, and MS of racemic stenine were in agreement with values reported for the natural product and the ^{13}C NMR spectrum was consistent with the assigned structure.[29]

In summary, a synthesis of stenine (1) has been accomplished in 25 steps and 5% overall yield from tetraene 8. Application of this strategy to the synthesis of tuberostemonine is underway.

(29) We thank Professor M. Haruna (Meijo University, Nagoya, Japan) for supplying a 400-MHz ^1H NMR spectrum of (-)-1 for the purpose of comparison with our 300-MHz ^1H NMR spectrum of *dl*-1.

Acknowledgment. We thank the National Institutes of Health (GM-27647) for their generous support, Dr. Charles Cottrell and Mr. Richard Weisenberger for assistance in recording NMR and Mass Spectra at The Ohio State University Chemical Instrumentation Center, and Mr. Carl Engleman for recording NMR spectra at The Ohio State University NMR Facility.

An Unexpected Reversal of Fluorine Substituent Effects in the Biomethylenation of Two Positional Isomers: A Serendipitous Discovery

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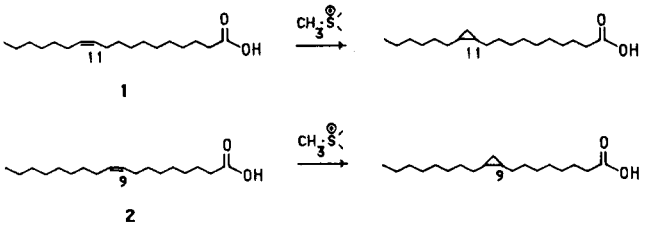
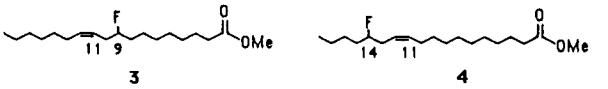
Summary: The mechanism of biological cyclopropyl fatty acid biosynthesis as it occurs in *Lactobacillus plantarum* has been probed using fluorine substituent effects. It has been shown that the pattern of rate retardations induced by homoallylic fluorine substitution is numerically the same but opposite in sense for two series of olefinic fatty acid substrates bearing the double bond at either the 9- or the 11-position.

The bacterium, *Lactobacillus plantarum*, is capable of methylenating both the *cis*-11-octadecenoic (*cis*-vaccenic) acid (1) produced by its own biosynthetic machinery as well as the *cis*-9-octadecenoic (oleic) acid (2) it meets in its natural environment.[1] The cyclopropyl products are dead-end metabolites and accumulate to such an extent that by the end of the growth cycle of this microorganism, some 80% of the cellular olefinic fatty acids have been methylenated. While considerable information has been gained on the cyclopropane synthase[2] the mechanistic details of this intriguing transformation are obscure and controversial. The current working hypothesis views this reaction as a genetic variation on a theme of olefin methylation/proton elimination which has been extensively elaborated on the sidechains of sterols[3] (See Scheme I). We have sought evidence for the existence of the putative carbocationic intermediate with the help of fluorine substituent effects—an approach which has been successful in the study of isoprenoid bioalkylations.[4] Thus we have shown that fluorine substitution at the 12-position of oleic acid has a substantially greater rate-retarding effect on biomethylenation than fluorine substitution at the 7-position.[5] It was while attempting to reproduce this result with the corresponding *cis*-fluorovaccenate series, that we stumbled upon a surprising result—namely that the pattern of fluorine-induced rate retardations is reversed as we now document.

Methyl *cis*-9-fluorovaccenate (3) and methyl *cis*-14-fluorovaccenate (4) were synthesized in racemic form using



precisely the same methodolgy as was used for the preparation of the fluorooleates referred to above.[5] All analytical data for the two homoallylically fluorinated vaccenates was satisfactory, and the compounds were judged to be pure by several chromatographic critera including capillary GC, GC/MS, and reverse-phase HPLC.



The two racemic[6] fluorinated substrates along with the parent compound were administered separately at a concentration of 40 mg/L to growing cultures of *L. plantarum* ATCC 8014. Growth of the microorganism proceeded to a similar extent in each set of experiments, and the degree of methylenation of each olefinic substrate was determined by analyzing the extracted fatty acid fraction via capillary GC as previously reported.[5]

When the results of the *cis*-vaccenate feedings are compared with the results of the oleate feedings,[5] an unexpected picture emerges as graphically illustrated in Figure 1. It is immediately obvious that the pattern of fluorine substituent effects is reversed when the double bond of the substrate is at the 11-position rather than at the 9-position. What is striking is that the numerical values obtained yield a near perfect "mirror image" pattern of rate retardations.

The simplest explanation for this phenomenon is that both substrates are visiting the same active site but are presenting *opposite* faces of the double bond to SAM—the

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(2) Law, J. H. *Acc. Chem. Res.* **1971**, *4*, 199.
(3) Lederer, E. *Q. Rev.* **1969**, *23*, 453.
(4) Poulter, C. D.; Rilling, H. C. *Acc. Chem. Res.* **1978**, *11*, 306.
(5) Buist, P. H.; Findlay, J. M.; Leger, G.; Pon, R. A. *Tetrahedron Lett.* **1987**, *28*, 3891.

(6) We were able to show by capillary GC analysis[5] that *L. plantarum* does not discriminate between the two enantiomers of methyl 7-fluorooleate. Unfortunately our capillary GC system was unable to resolve any diastereomeric fluorocyclopropanes which were in all likelihood also present in the fluorovaccenate series.

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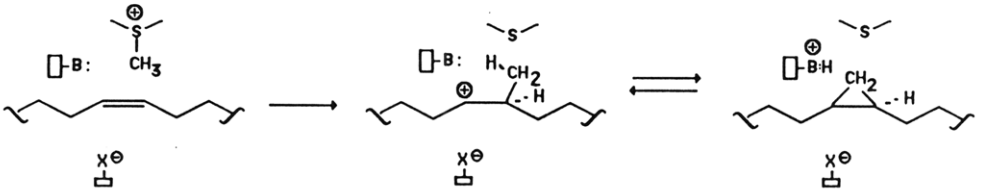
Scheme I



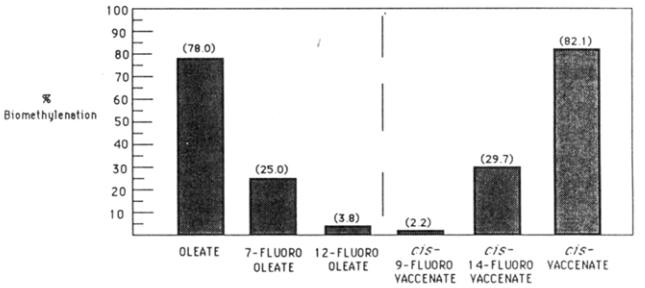


Figure 1. Percent biomethylenation of fluorinated olefinic fatty acids as a function of double bond position. The cyclopropyl product was identified in each case by comparison of its GC capillary retention time and MS (obtained by GC/MS) with authentic synthetic standards as previously described.[5]

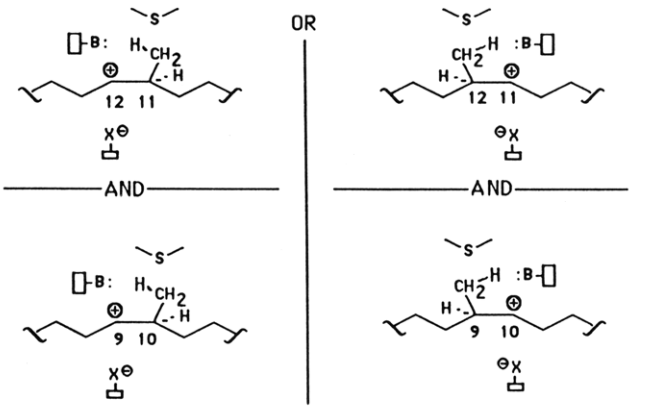


Figure 2. Relationship between the possible carbocationic intermediates derived from methylation of *cis*-9-octadecenoic acid and *cis*-11-octadecenoic acid.

methylating agent as shown in Figure 2.[7] Thus if the carbocation is generated at carbon 12 during methylation of vaccenic acid, then the carbocation must be formed at carbon 9 during methylation of oleic acid. Alternatively, if the carbocation is generated at carbon 11 during methylation of vaccenic acid, then it must be formed at carbon 10 during methylation of oleic acid. At this time we cannot distinguish between these two alternatives since we do not know precisely at what point(s) in the catalytic cycle the fluorine substituent effect is acting. The situation is complicated by the fact that we and others have produced evidence that the deprotonation step may be reversible.[8,9]

Our probe for the regiochemistry of carbocation formation has borne an important stereochemical consequence: namely that the absolute configurations of the cyclopropyl products derived from our fluorinated *cis*-vaccenates and oleates should turn out to be opposite in sense. We are currently preparing these fluorinated compounds in enantiomerically pure form in order to address this intriguing question. An important clue has already been obtained in collaboration with D. Arigoni and coworkers who have determined that the absolute configuration of the cyclopropyl product produced by *L. plantarum* from **1** is 11*R*,12*S*, while the absolute configuration of the cyclopropyl product biosynthesized from **2** by the same bacterium is 9*S*,10*R*.[10] These findings rule out any mode of binding in which the same olefinic face of the two positional isomers is engaged.

In conclusion, we would like to point out that what began as a mechanistic study has serendipitously led us to raise an entirely unexpected issue: namely can a single enzyme or enzymic active site handle both positional isomers or are two isozymes involved? We anticipate being able to solve this problem using appropriate competition experiments with our fluorinated substrates. Should it turn out that a single active site is involved, important questions of fatty acid conformation will have been raised.

Acknowledgment. The financial support of NSERC and Carleton University is gratefully acknowledged. Gabe Leger and Judy Findlay were of invaluable assistance. We wish to thank Professor D. Arigoni (ETH, Zürich) for catalytic discussions.

(7) We wish to thank Professor D. Arigoni (ETH, Zürich) for helpful discussions on this point.
(8) Buist, P. H.; Maclean D. B. *Can. J. Chem.* **1982**, *60*, 371.
(9) Arigoni, D. *Chimia* **1987**, *41*, 9.
(10) Manuscript in preparation.

Enantioselective Route to γ-Butyrolactones: Chiral Auxiliary Mediated Amide Alkylation and Iodolactonization

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Summary: A number of chiral, γ,δ-unsaturated amides (**1** and **2**) have been prepared and their subsequent alkylation and iodolactonization to γ-butyrolactones (**3**) investigated.

Earlier work in our laboratories has established that resident stereochemistry can profoundly influence the course of electrophilic cyclization reactions. For example, *d,l*-3,5-dimethylhepta-1,6-diene-4-carboxylic acid cyclizes[2]

(1) Recipient of an Alfred P. Sloan Research Fellowship, 1987–1991, and an NIH Research Career Development Award (ES00182), 1989–1994.
(2) Kurth, M. J.; Brown, E. G. *J. Am. Chem. Soc.* **1987**, *109*, 6844.

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