wavelengths between 350 and 725 nm, but no transient species were observed in this wavelength range.[15]

Acknowledgment. We thank Dr. S. Shimokawa and Mr. M. Kitaichi, Faculty of Engineering, Hokkaido University, for the NMR and pulse radiolysis experiments.

Registry No. MNP, 917-95-3.

(15) Because of the strong light absorption due to the MNP dimer in the range of wavelengths between 200 and 350 nm, aqueous solutions containing low concentrations of MNP must be used for pulse radiolysis experiments at wavelengths below 350 nm. Nevertheless, an absorbance spectrum could be observed in the range of wavelengths between 250 and 300 nm with a maximum absorbance at the wavelength around 275 nm after electron pulse irradiation and was regarded as the OH-induced transients of the MNP dimer. No information concerning the OH-induced transients of the monomer was obtained because preparation of an N_2O-saturated solution containing only the monomer was impossible. When the competition method using CNS^- as a reference solute was carried out to determine the rate constants between MNP and OH radicals, it was observed that the MNP monomer reacted more rapidly with OH radicals than the MNP dimer did in the solution that both monomer and dimer coexisted, and the rate constant between the MNP monomer and OH radicals was roughly estimated to be 6×10^9 mol^{-1} s^{-1}.

Reevaluation of the Stereochemical Courses of the Allylic Rearrangement and the Double-Bond Reduction Catalyzed by *Brevibacterium ammoniagenes* Fatty Acid Synthase

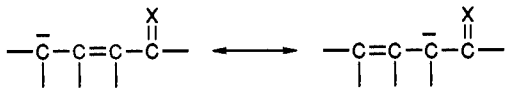
Mary C. O'Sullivan and John M. Schwab*

Department of Medicinal Chemistry and Pharmacognosy
School of Pharmacy, Purdue University
West Lafayette, Indiana 47907

T. Mark Zabriskie, Gregory L. Helms, and John C. Vederas

Department of Chemistry, University of Alberta
Edmonton, Alberta, Canada T6G 2G2
Received November 14, 1990

Substrates for enzymatic allylic rearrangements[1] belong to two general groups: (a) those with isolated allylic systems and (b) those from which resonance-stabilized carbanions could in principle be formed (cf. **1**).[2] Studies of net reaction stereochemistry have revealed striking mechanistic uniformity among the allylic rearrangements of the second group:[1,3] With one reported exception,[4] all of these reactions proceed suprafacially, suggesting a single active site acid/base.[5,6]



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The exception is the fatty acid synthase (FAS) of *Brevibacterium ammoniagenes*, a multienzyme complex[7] producing both saturated and monounsaturated[8] fatty acids. The critical reaction in the O_2-independent pathway to unsaturated fatty acids in *B. ammoniagenes* is the allylic rearrangement of enzyme-bound (*E*)-2-dodecenoyl thiol ester **3** to (*Z*)-3-dodecenoyl thiol ester **4**. Compound **3** can be reduced and elongated to saturated fatty acids,

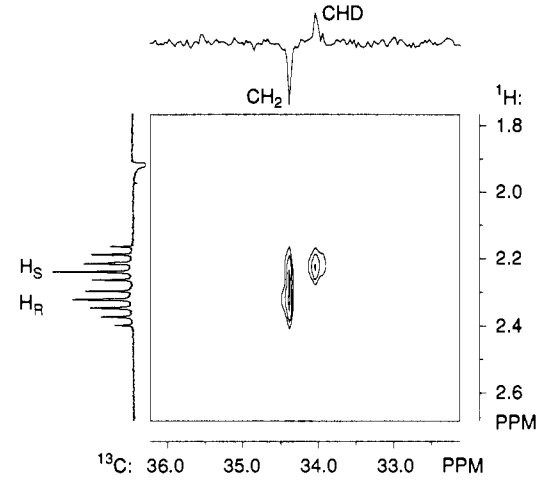
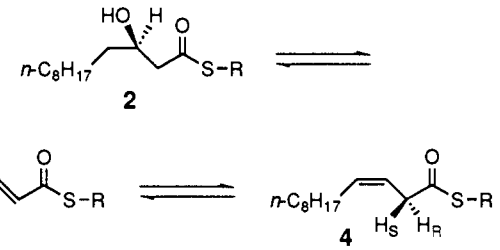


Figure 1. ^2H-decoupled HETCOR spectrum of the C-2 CH_2 group of **5** from the degradation of oleic acid derived from sodium [2-^{13}C,$^2H_3$]-acetate. The ^{13}C spectrum shown in the horizontal dimension was obtained by using a DEPT pulse sequence.

while direct elongation of **4** gives monounsaturated fatty acids including oleic (9-octadecenoic) acid.



It has been suggested that the *B. ammoniagenes* FAS interconverts **3** and **4** via an antarafacial rearrangement.[4] We now show that this process is suprafacial and that the overall steric course of the enoyl reductase step of fatty acid biosynthesis by the *B. ammoniagenes* FAS is anti, rather than syn as proposed previously.[9]

Following the strategy of Vederas and co-workers,[10-16] *B. ammoniagenes* was grown on sodium [2-^{13}C,$^2H_3$]acetate, and methyl oleate was isolated.[17] The ^1H-decoupled ^2H NMR spectrum of

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(17) Nutrient broth (2 L) containing sodium [2-^{13}C,$^2H_3$]acetate (1 g) and unlabeled sodium acetate (1 g) was inoculated with *B. ammoniagenes* ATCC 6871 (the strain used by Kawaguchi and co-workers for studies of *B. ammoniagenes* FAS[8]) and incubated at 30 °C with shaking (220 rpm) for 27 h. The cells were collected by centrifugation and resuspended along with silica gel (230–400 mesh; 50 g) in deionized water (100 mL). The mixture was homogenized in a Waring blender for 20 min, 1:1 $CHCl_3$/MeOH (600 mL) added, and the mixture stirred overnight. The suspension was filtered and the crude fatty acid extract saponified with potassium *tert*-butoxide (3.0 g) and water (0.486 mL) in tetrahydrofuran (70 mL) at room temperature overnight. The solvent was removed, 5% HCl was added, and the aqueous phase was extracted with CH_2Cl_2. The residue from removal of the solvent was purified by flash chromatography (2–4%, MeOH in CH_2Cl_2) to give a mixture of fatty acids (48 mg), which was treated with excess ethereal CH_2N_2 to give a mixture of fatty acid methyl esters (54 mg). Preparative TLC in the dark (silica gel impregnated with 20% $AgNO_3$ (Analtech); 1% MeOH in CH_2Cl_2) gave methyl oleate (34 mg) and a mixture of methyl esters of saturated fatty acids.

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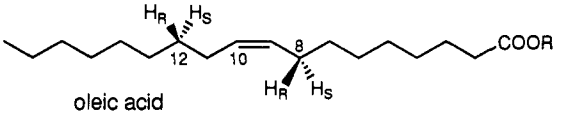
the labeled ester showed a broad *singlet* at 5.4 ppm, assigned to ^2H in natural abundance at the olefinic positions (C-9 and C-10). Thus, there were no intact ^2H–^{13}C units at C-10. Confirmation came from the ^1H-decoupled ^{13}C NMR spectrum of the methyl oleate, since the multiplicity of the 130 ppm C-10 signal was unaffected by ^2H decoupling. Following dilution with unlabeled material, labeled methyl oleate was degraded[14] to octanoic acid (from C-11 through C-18 of oleic acid) and to monomethyl nonanedioate (from C-1 through C-9 of oleic acid, the esterified carboxyl of the derivative corresponding to C-1 of oleic acid). The degradation products were converted to methyl mandelate ester derivatives (**5** and **6**), which were analyzed by ^2H-decoupled HETCOR spectroscopy.



oleic acid

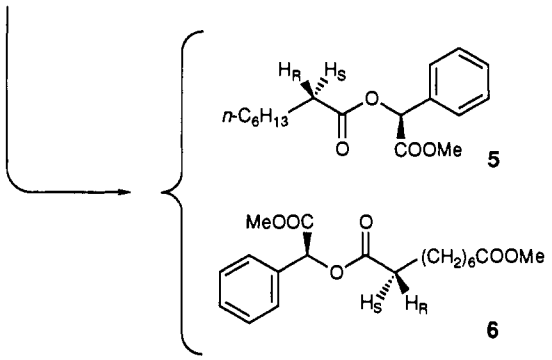
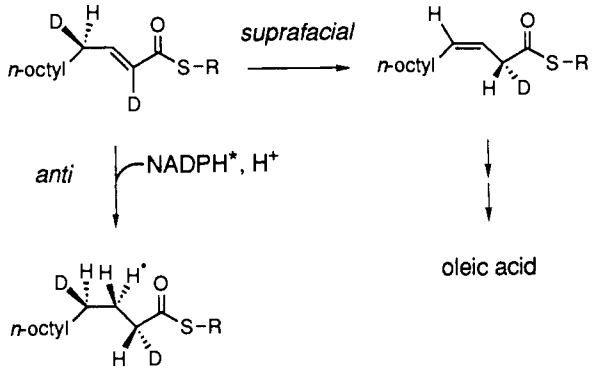


Figure 1 shows the C-2 region of the ^2H-decoupled HETCOR spectrum of labeled **5**. The proton of the CHD group (readily identified by the positive DEPT signal in the ^{13}C dimension) at C-2 is clearly at higher field, corresponding to the *pro*-2*S* position of **5**.[14,18,19] This indicates that ^2H had been incorporated by the *B. ammoniagenes* FAS into the *pro*-12*R* position of oleic acid and therefore into the *pro*-*R* position at all even-numbered carbons on the growing saturated chain.[21] Because C-10 of methyl oleate lacks ^2H, the conversion of **3** to **4** must involve loss of the *pro*-4*R* hydrogen.

To confirm the configuration of acetate-derived ^2H at C-8 of methyl oleate, the ^2H-decoupled HETCOR spectrum of **6** was examined. The proton cross peak of the CHD group at C-2 of **6** was found at lower field (ca. 2.45 ppm vs 2.41 ppm), indicating that ^1H and ^2H are in the *pro*-*R* and *pro*-*S* positions, respectively. Thus, ^2H had been in the *pro*-8*S* position of the labeled oleic acid and in the *pro*-2*R* position of biosynthetic intermediate **4**.

From these experiments, it is clear that the *B. ammoniagenes* FAS-mediated allylic rearrangement is *suprafacial*, suggesting a *stepwise* mechanism involving a single active site acid/base.[5,6] This reaction therefore *conforms* to the stereochemical (hence, mechanistic) trends noted at the beginning of the paper. The basis of the earlier report of antarafacial rearrangement[4] is the assignment of the configuration at C-12 of deuterated oleic acid (from incorporation of [$^2H_2$]malonyl-CoA) opposite[9] to that found in the present work.[24]

The configuration at C-12 of oleic acid indicates the stereochemical course of the NADPH-dependent enoyl reductase reaction. It is now clear that the substrate is protonated at C-2 on the *si* face of the double bond and that the overall stereochemical course of the reduction must be anti.[25] It is interesting that both syn and anti reductions by FAS enoyl reductases are known.[22,26] Further studies are in progress.



Acknowledgment. We thank the National Institutes of Health (Grant GM36286 to J.M.S.) and the Natural Sciences and Engineering Research Council of Canada for generous support. NATO and the Killam Foundation are gratefully acknowledged for fellowships (to T.M.Z.). We also thank Mr. Jerome F. Baker for suggestions pertaining to cell lysis.

Supplementary Material Available: ^1H-decoupled ^2H and ^{13}C NMR spectra of methyl oleate derived from feeding of sodium [2-^{13}C,$^2H_3$]acetate to *B. ammoniagenes* as well as the C-2 region of the ^2H-decoupled HETCOR spectrum of **6** from the degradation of biosynthetically deuterated oleic acid (3 pages). Ordering information is given on any current masthead page.

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Synthesis, Characterization, Crystal Structures, and CO and O_2 Binding Properties of Novel Four-Atom-Linked Capped Porphyrins

Martin R. Johnson, Won K. Seok, and James A. Ibers*

Department of Chemistry, Northwestern University Evanston, Illinois 60208-3113

Received November 14, 1990

Frequent reports on the active-site properties of oxygen-carrying hemoproteins[1] and synthetic models[2] continue. Of major interest

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