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Configurational Analysis of Cyclopropyl Fatty Acids Isolated from *Escherichia coli*

Laura J. Stuart, James P. Buck, Amy E. Tremblay, and Peter H. Buist*

Department of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6

pbuist@ccs.carleton.ca

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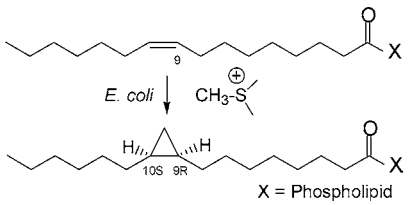
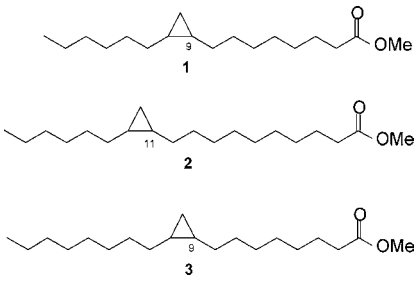
ABSTRACT



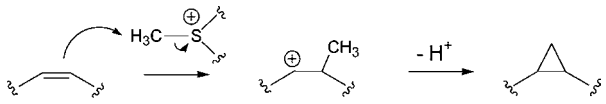
The absolute configuration of methyl lactobacillate and its 9,10 homologue, both isolated from *Escherichia coli* B–ATCC 11303, was found to be 11*R*,12*S* and 9*R*,10*S*, respectively.

Lipids containing cyclopropyl fatty acids such as **1**−**3** occur widely in microorganisms[1,2] and in the seed oils of various subtropical plants.[3] Interest in these natural products has grown with the discovery that the pathogenicity of *Mycobacterium tuberculosis* is highly dependent on the presence of cyclopropyl moieties in their membrane lipids.[4] Thus,



mycobacterial cyclopropane synthases constitute promising targets for mechanism-based inhibitors, and an X-ray crystallographic study has been published recently.[5] Several in vitro studies on a related *Escherichia coli* enzyme utilizing simpler olefinic substrates have also been undertaken.[6−11] Earlier hypotheses[12−14] featuring rate-limiting methyl transfer from *S*-adenosyl-L-methionine (SAM) to olefin followed by rapid proton loss have gained support (Scheme 1). A metal-assisted, sulfonium ylid−carbenoid-type process[15] has been effectively ruled out on the basis of observed fluorine

Scheme 1. Proposed Mechanism for Biochemical Cyclopropane Ring Formation via Methylation of the Corresponding Unsaturated Substrate



* To whom correspondence should be addressed. Phone: 613-520-2600 ext 3643. Fax: 613-520-3749.

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substituent effects,[6] SAM analogue[7] and KIE studies,[8] inductively coupled plasma-atomic emission spectrometry analysis (ICP-AES),[9] and X-ray crystallographic data.[5] Despite this progress, the facial selectivity of cyclopropanation (methylenation) as it occurs in *E. coli* has not been elucidated. Because cyclopropanation is catalyzed by a single gene product in this organism, the enantioselectivity of initial methyl transfer can be probed by determining the absolute configuration of the two major cyclopropane fatty acids found in *E. coli* lipids. Herein, we report on the results of our stereochemical analysis.

The lipid fraction (1 g) of *E. coli* B−ATCC 11303 (Avanti Polar Lipids, Inc., Alabaster, Alabama) was hydrolyzed (refluxing 2 N KOH, 50% ethanol), and the free fatty acids were isolated and methylated (BF_3/MeOH) essentially as previously described.[16] The fatty acid methyl ester fraction (FAME, 729 mg) was analyzed by GC-MS; the presence of two cyclopropyl fatty acids, methyl 9,10-methanohexadecanoate **1** (20%) and its C-19 homologue commonly known as methyl lactobacillate **2** (12%), was detected. The remaining FAMEs were identified as methyl tetradecanoate (1%), methyl hexadecanoate (36%), methyl octadecanoate (1%), methyl (*Z*)-11-octadecenoate (28%), and methyl (*Z*)-9-hexadecenoate (2%). This profile is typical of *E. coli* FAME.[17] The identity of each analyte was initially confirmed through a comparison of retention time and mass spectral characteristics of authentic standards. (Synthetic cyclopropyl fatty acid methyl esters **1** and **2** were prepared from the corresponding, commercially available, olefinic precursors by a modified Simmons−Smith reaction.[18]) To isolate each individual biosynthetic cyclopropyl fatty acid, the *E. coli* lipid extract was chromatographed using reversed-phase HPLC (Whatman Partisil Magnum 9 10/50 ODS-2 column, 25% EtOAc/ACN), and fractions enriched in **1** (238 mg) and **2** (112 mg) were obtained from a total of 70 chromatographic runs. Crude **1** was treated with *meta*-chloroperbenzoic acid (55% pure, 165 mg, 0.5 mmol) to convert coeluting olefinic fatty acids to the more polar epoxides which were subsequently removed by flash chromatography (SiO_2, 10% EtOAc/hexanes). In this manner, 72 mg of purified biosynthetic **1** was obtained as a colorless oil; the GC-MS, ^{1}H NMR, and ^{13}C NMR data of this material correlated well with those of a synthetic reference standard (see Supporting Information). Crude **2** was not purified further to remove methyl hexadecanoate because the presence of this saturated fatty acid methyl ester did not affect the subsequent stereochemical analysis. The diagnostic GC-MS, ^{1}H NMR, and ^{13}C NMR data of biosynthetic **2** matched those of an authentic standard in all respects (see Supporting Information).

Quasisymmetrical cyclopropyl fatty acids such as **1** and **2** are only weakly optically active, which renders comparison with chiral reference standards[19−21] problematic. However, long-chain cyclopropyl fatty acids are readily oxidized to a pair of separable, regioisomeric, keto derivatives and these compounds can be easily correlated with the appropriate reference compounds on the basis of their distinctive chiroptical properties.[22] Thus, mild CrO_3 oxidation of **1** (72 mg) yielded ketones **4** (7.9 mg, R_f = 0.08, [SiO_2, Hexane/Et_2O (10:1)]) and **5** (6.2 mg, R_f = 0.11); in a similar manner, **6** (8.0 mg, R_f = 0.11) and **7** (4.4 mg, R_f = 0.13) were obtained from **2** (112 mg) (see Figure 1). The keto derivatives

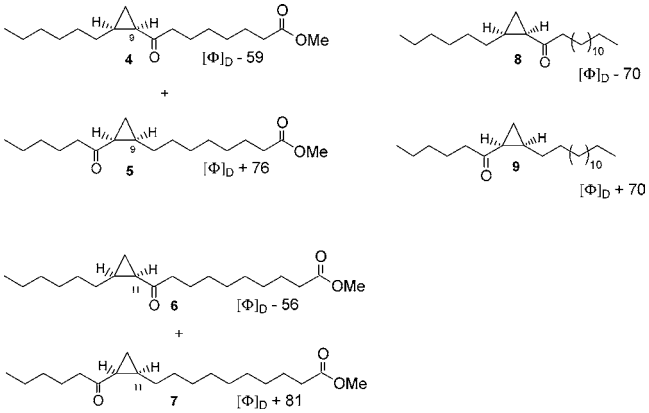

Figure 1. Comparison of $[\Phi]_D$ values obtained for ketones **4**−**7** derived from biosynthetic **1** and **2** with synthetic standards **8** and **9**.[22]

were separated by flash chromatography (SiO_2, Hexane/Et_2O [10:1]) and identified on the basis of diagnostic mass spectral fragmentation patterns which are typical for this class of compounds.[22] All analytical data (R_f values and MS, ^{1}H NMR, and ^{13}C NMR data) matched those for authentic standards obtained upon oxidation of synthetic **1** and **2** (see Supporting Information). The optical rotation of each ketone was obtained (**4**, $[\alpha]_D^{21} = -20.1$ (*c* 0.70, Et_2O); **5** $[\alpha]_D^{21} = +25.7$ (*c* 0.62, Et_2O); **6**, $[\alpha]_D^{21} = -17.4$ (*c* 0.62, Et_2O); **7** $[\alpha]_D^{21} = +24.9$ (*c* 0.44, Et_2O)), and the corresponding molecular rotations $[\Phi]_D$ were compared to the values obtained by Tocanne[22] for related compounds **8** and **9**, as displayed in Figure 1.

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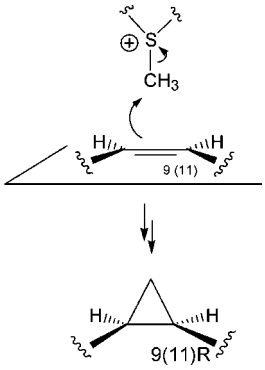
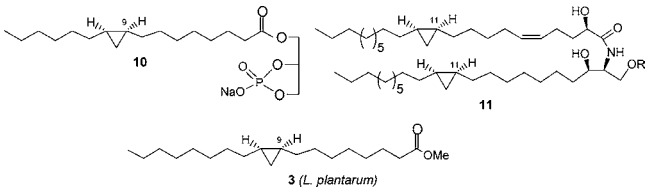
On the basis of these considerations, it is clear that **1** and **2** isolated from *E. coli* B−ATCC 11303 bear the (*R*) configuration at the cyclopropyl methine carbon closest to the carboxyl group (C-9 for **1** and C-11 for **2**) and the (*S*) configuration at the cyclopropyl methine carbon proximal to the methyl terminus (C-10 for **1** and C-12 for **2**). This implies that (*Z*)-9-hexadecenoate and (*Z*)-11-octadecenoate are attacked by the methylating agent, as shown in Scheme 2.[23] These results match those obtained for methyl lactobacillate **2** isolated from two other microorganisms, *Brucella milletensis*[24] and *Lactobacillus plantarum*[25], as well as those for methyl dihydrosterculate **3** obtained from a phytochemical source, *Litchi chinensis*.[26] However, there have been several reports of cases where the absolute configuration of long-chain cyclopropyl fatty acid derivatives is reversed. These include **10** (PHYLPA) isolated from the slime mold, *Physarum polycephalum*,[19] **11** (plakoside A)[27] found in the Caribbean sponge, *Plakortis simplex*, and methyl dihydrosterculate **3** isolated from *L. plantarum*.[25] That **2** and **3** are produced as quasienantiomers in *L. plantarum* is of particular interest and raises intriguing questions regarding binding of regioisomeric substrates to cyclopropane synthases.[25,28] These issues are relevant to the case of *E. coli* cyclopropane synthase in that this enzyme also methylenates (*Z*)-9-octadecenoate in addition to (*Z*)-11-octadecenoate.[29] Interestingly, other (*Z*)-C-18 monoene positional isomers are relatively poor substrates for this enzyme.[29] It would be of interest to compare the facial selectivity of (*Z*)-9-octadecenoate methylenation by *E. coli* cyclopropane synthase with that found in the present work for the (*Z*)-11 isomer. In this manner, one might gain new insights into the topology of the active site of the *E. coli* enzyme, the details of which could be correlated with new protein structural information as this becomes available. Experiments designed to address this issue are being planned.

Scheme 2. Enantioselectivity of Methyl Transfer to (*Z*)-9-Hexadecenoyl and (*Z*)-11-Octadecenoyl Substrates in *E. coli*





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Supporting Information Available: Experimental procedures and characterization data for racemic **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) *E. coli*, typically cyclopropanates, preexisting (*Z*)-9-C-16 and (*Z*)-11-C-18 olefinic fatty acyl chains at late exponential or early stationary phase of growth.[2] Both olefinic fatty acid derivatives are known to be substrates of *E. coli* cyclopropane synthase.[2] It is considered less likely that **2** is a chain elongation product derived from **1** or that **1** is a β-oxidation product of **2**.

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