

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

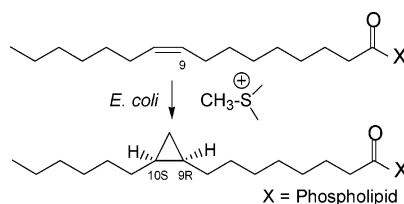
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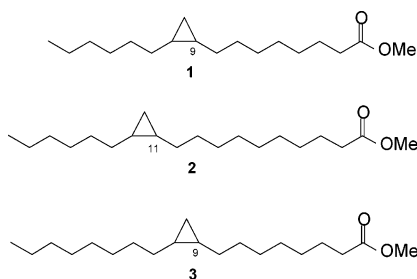
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## ABSTRACT



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.

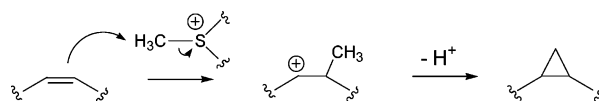
Lipids containing cyclopropyl fatty acids such as **1–3** occur widely in microorganisms<sup>1,2</sup> and in the seed oils of various subtropical plants.<sup>3</sup> Interest in these natural products has grown with the discovery that the pathogenicity of *Mycobacterium tuberculosis* is highly dependent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



mycobacterial cyclopropane synthases constitute promising

targets for mechanism-based inhibitors, and an X-ray crystallographic study has been published recently.<sup>5</sup> Several in vitro studies on a related *Escherichia coli* enzyme utilizing simpler olefinic substrates have also been undertaken.<sup>6–11</sup> Earlier hypotheses<sup>12–14</sup> 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-

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



assisted, sulfonium ylide–carbenoid-type process<sup>15</sup> has been effectively ruled out on the basis of observed fluorine

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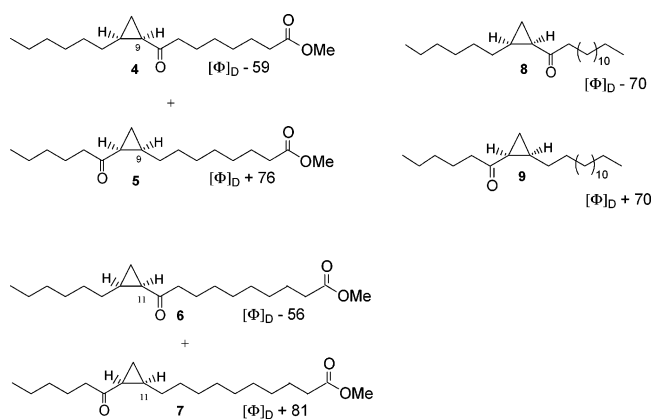
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substituent effects,<sup>6</sup> SAM analogue<sup>7</sup> and KIE studies,<sup>8</sup> inductively coupled plasma-atomic emission spectrometry analysis (ICP-AES),<sup>9</sup> and X-ray crystallographic data.<sup>5</sup> Despite this progress, the facial 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<sub>3</sub>/MeOH) essentially as previously described.<sup>16</sup> 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.<sup>17</sup> 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.<sup>18</sup>) 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<sub>2</sub>, 10% EtOAc/hexanes). In this manner, 72 mg of purified biosynthetic **1** was obtained as a colorless oil; the GC-MS, <sup>1</sup>H NMR, and <sup>13</sup>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, <sup>1</sup>H NMR, and <sup>13</sup>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<sup>19–21</sup> 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.<sup>22</sup> Thus, mild CrO<sub>3</sub> oxidation of **1** (72 mg) yielded ketones **4** (7.9 mg, *R*<sub>f</sub> = 0.08, [SiO<sub>2</sub>, Hexane/Et<sub>2</sub>O (10:1)]) and **5** (6.2 mg, *R*<sub>f</sub> = 0.11); in a similar manner, **6** (8.0 mg, *R*<sub>f</sub> = 0.11) and **7** (4.4 mg, *R*<sub>f</sub> = 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**.<sup>22</sup>

were separated by flash chromatography (SiO<sub>2</sub>, Hexane/Et<sub>2</sub>O [10:1]) and identified on the basis of diagnostic mass spectral fragmentation patterns which are typical for this class of compounds.<sup>22</sup> All analytical data (*R*<sub>f</sub> values and MS, <sup>1</sup>H NMR, and <sup>13</sup>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<sub>2</sub>O); **5**,  $[\alpha]_D^{21} = +25.7$  (*c* 0.62, Et<sub>2</sub>O); **6**,  $[\alpha]_D^{21} = -17.4$  (*c* 0.62, Et<sub>2</sub>O); **7**,  $[\alpha]_D^{21} = +24.9$  (*c* 0.44, Et<sub>2</sub>O)), and the corresponding molecular rotations  $[\Phi]_D$  were compared to the values obtained by Tocanne<sup>22</sup> for related compounds **8** and **9**, as displayed in Figure 1.

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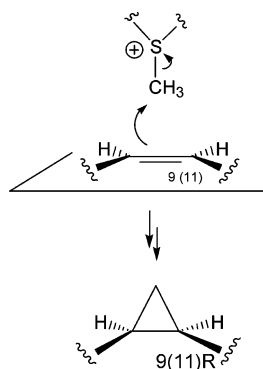
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On the basis of these considerations, it is clear that **1** and **2** isolated from *E. coli* B-ATCC 11303 bear the (*R*) configuration at the cyclopropyl methine carbon closest to the carboxyl group (C-9 for **1** and C-11 for **2**) and the (*S*) 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.<sup>23</sup> These results match those obtained for methyl lactoba-

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



cillate **2** isolated from two other microorganisms, *Brucella melitensis*<sup>24</sup> and *Lactobacillus plantarum*<sup>25</sup>, as well as those for methyl dihydrosterulate **3** obtained from a phytochemical source, *Litchi chinensis*.<sup>26</sup> However, there have been several reports of cases where the absolute configuration of long-chain cyclopropyl fatty acid derivatives is reversed. These

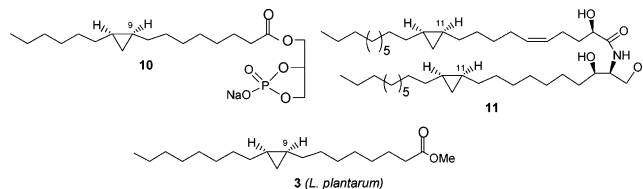
(23) *E. coli*, typically cyclopropanates, preexisting (*Z*)-9-C-16 and (*Z*)-11-C-18 olefinic fatty acyl chains at late exponential or early stationary phase of growth.<sup>2</sup> Both olefinic fatty acid derivatives are known to be substrates of *E. coli* cyclopropane synthase.<sup>2</sup> It is considered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

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include **10** (PHYLPA) isolated from the slime mold, *Physarum polycephalum*,<sup>19</sup> **11** (plakoside A)<sup>27</sup> found in the Caribbean sponge, *Plakortis simplex*, and methyl dihydrosterulate **3** isolated from *L. plantarum*.<sup>25</sup> 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.<sup>25,28</sup> 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.<sup>29</sup> Interestingly, other (*Z*)-C-18 monoene positional isomers are relatively poor substrates for this enzyme.<sup>29</sup> 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.

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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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