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The absolute configuration of methyl dihydrosterculate: an unusual phytofatty acid isolated from the seed oil of *Litchi chinensis*

Laura J. Stuart and Peter H. Buist*

Department of Chemistry, Carleton University, Ottawa, ON, Canada K1S 5B6

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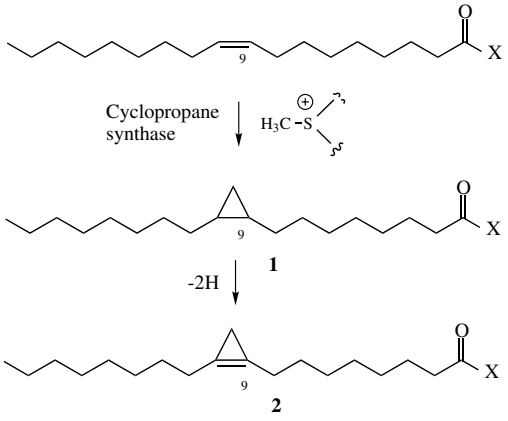
Abstract—The absolute configuration of methyl dihydrosterculate isolated from the seed oil of *Litchi chinensis* was determined to be (9*R*,10*S*) by correlation of the corresponding keto derivatives.

1. Introduction

The occurrence of various cyclopropyl fatty acids (CPA-FA) in microorganisms is relatively common and constitutes an important lipidic signature.[1–3] Fatty acids of this type are also found in some subtropical plants and their role in the biosynthetic pathway leading to sterculic acid **2** has recently been examined.[4,5] It is thought that **2** is formed by the desaturation[6] of the dihydrosterculate intermediate **1** (Scheme 1). The mechanism of cyclopropane formation is postulated to involve methyl transfer followed by 1,3-deprotonation of the resultant carbocationic intermediate.[7–10] The cyclopropane fatty acid synthase found in *Sterculia foetida* has been characterized with the C-terminal half of this enzyme shown to bear a significant sequence similarity to the *E. coli* CPA-FA synthase gene.[4] A more detailed comparison of microbial and plant enzymes would benefit from a stereochemical analysis of the cyclopropyl products. Efforts in this direction have recently been reported for some bacterial systems.[11,12] Herein, we report the first configurational determination of a plant cyclopropyl fatty acid.



Scheme 1. Proposed biosynthetic pathway for sterculate **2** formation in plants (X = thio or phospholipid ester).

2. Results and discussion

The oil of *Litchi chinensis* (*Lychee*) seeds is known to contain dihydrosterculate **1** as the major component[13] and the ready availability of this fruit provides a convenient entry into this project. GC–MS analysis of a transmethylated $CHCl_3$/MeOH (2:1) extract[14] of homogenized *Lychee* seeds (supplied locally by Pattenaude Produce, Perth, ON; imported from Thailand) revealed the presence of methyl dihydrosterculate **1** (39%) along with its C17 homologue (4%) as well as methyl palmitate (11%), methyl stearate (4%), methyl oleate (25%) and methyl linoleate (17%) in good agreement with the literature.[13] The extract (1.0 g) was treated with *meta*-chloroperbenzoic acid (MCPBA) (0.55 g, 3.2 mmol) to convert the olefinic fraction to the more polar epoxides. This step facilitated the subsequent isolation of crude methyl dihydrosterculate **1** (277 mg) by flash chromatography [SiO_2, hexane/Et_2O (10:1)]. The spectroscopic properties (IR, ^1H and ^{13}C NMR, MS)[15] of **1** matched those previously reported for this material.[8,16]

* Corresponding author. Tel.: +1-613-520-2600x3643; fax: +1-613-520-3749; e-mail: pbuist@ccs.carleton.ca

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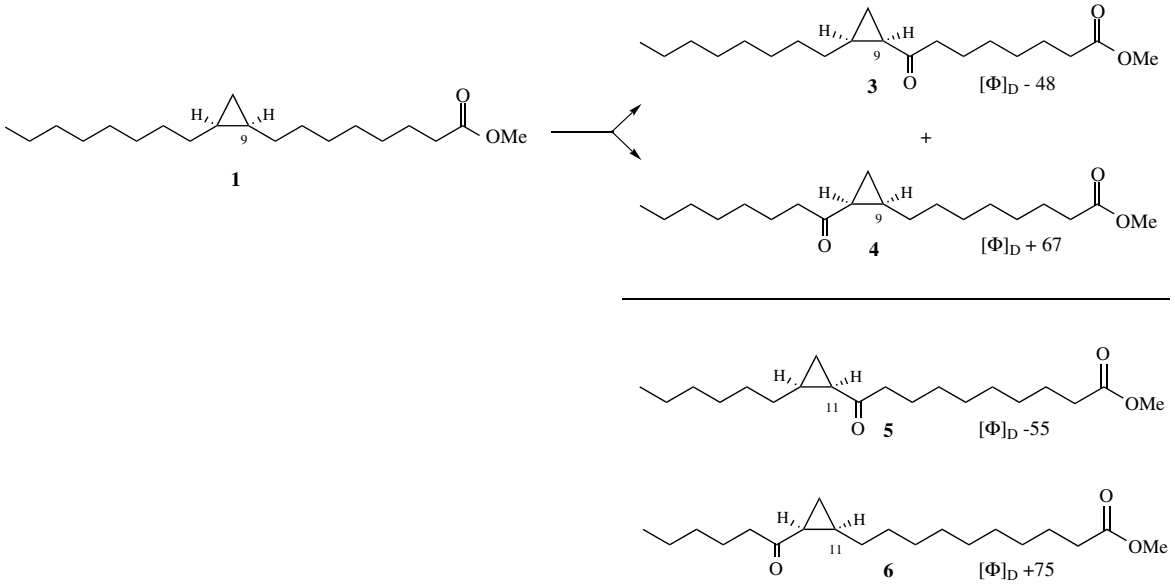

Scheme 2. Comparison of $[\Phi]_D$ values obtained for ketones derived from dihydrosterculate **1** and its 11,12-positional isomer, lactobacillic acid (Ref. 17).

Methodology for the stereochemical analysis of long chain cyclopropyl fatty acids has previously been established by Tocanne[17] and involves comparing the chiroptical properties of α-keto derivatives with stereochemically defined, synthetic standards.[18] Thus **1** was submitted to mild oxidation procedures as described by Tocanne[17] † and the product mixture chromatographed [SiO_2, Hexane/Et_2O (10:1)] to give recovered starting material (27 mg) and each ketone **3** (10 mg) and **4** (11 mg). The analytical data (IR, ^1H and ^{13}C NMR, MS)[19,20] of **3** and **4** were in accord with previous structural assignments.[16] The location of the keto group follows clearly from the mass spectral fragmentation patterns as reported initially by Thiele et al.[21] and these assignments have been confirmed through degradation experiments.[16] The molar rotation of each ketone was evaluated and compared to the corresponding value obtained by Tocanne[17] for the related compounds **5** and **6** (Scheme 2). The validity of Tocanne's configurational assignments for **5** and **6** has recently been confirmed through the total synthesis of the enantiomers of the parent cyclopropyl fatty acid.[12] Based on these considerations, it is clear that **1** isolated from *Litchi* bears the (*R*)-configuration at C-9 and (*S*)-configuration at C-10. Interestingly, this material is the enantiomer of dihydrosterculate, previously isolated from *Lactobacillus plantarum* cultures grown in a medium containing oleate.[16] To our knowledge, the cyclopropane synthase in this micoorganism has not been characterized but such an undertaking is clearly now warranted. In addition, the availability of both enantiomers of dihydrosterculate will allow us to determine the stereospecificity of the unusual desaturation reaction[6] that produces the corresponding, highly strained, cyclopropenyl fatty acid **2** (Scheme 1).

† Methyl dihydrosterculate (102 mg) in CCl_4 (1 mL) was treated with a solution of CrO_3 (220 mg, 2.2 mmol) in glacial acetic acid (5.5 mL) containing 3% water for 66 h with stirring. The excess oxidant was quenched with methanol (250 μL) and the reaction mixture diluted with hexanes (25 mL), washed with ice-cold water, satd sodium bicarbonate and satd NaCl, dried over Na_2SO_4 and evaporated to give 50 mg of crude products.

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15. Methyl dihydrosterculate (methyl (*Z*)-8-(2-octylcyclopropane-1-yl)-octanoate) **1**: colourless oil. R_f 0.40 (Et_2O/hexane 1:10); IR (film) 2925, 2854, 1774, 1465, 1436, 1360, 1246, 1197, 1170, 1113, 1020 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.67 (3H, s), 2.31 (2H, t, $J = 7.6$ Hz), 1.62 (2H, m), 1.1–1.4 (24H, m), 0.88 (3H, t, $J = 6.9$ Hz), 0.64 (2H, m), 0.56 (1H, m), −0.33 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.38, 51.44, 34.14, 31.94, 30.23, 30.14, 29.66, 29.61, 29.47, 29.38, 29.27, 29.17, 28.74, 28.68, 24.98, 22.71, 15.78, 15.74, 14.13, 10.92; EI MS m/z 310 (M^+), 278 ($[M-32]^+$).
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19. Methyl 8-ketodihydrosterculate (methyl (*Z*)-8-(2-octylcyclopropane-1-yl)-8-oxo-octanoate) **3**: colourless oil. R_f 0.15 (Et_2O/hexane 1:10); IR (film) 2926, 2855, 1741, 1458, 1394, 1258, 1196, 1166, 1079, 1021 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.66 (3H, s), 2.54 (2H, t, $J = 7.4$ Hz), 2.30 (2H, t, $J = 7.4$ Hz), 2.00 (1H, m), 1.61 (4H, m), 1.15–1.50 (19H, m), 1.06 (1H, m), 0.94 (1H, m), 0.87 (3H, t, $J = 6.9$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.31, 174.23, 51.47, 44.71, 34.03, 31.88, 29.97, 29.60, 29.36, 29.30, 28.97, 28.94, 26.23, 25.74, 25.52, 24.80, 23.89, 22.69, 14.32, 14.12; EI MS m/z 324 (M^+), 293 ($[M-31]^+$), 171 ($[CO(CH_2)_6CO_2CH_3]^+$); HRMS (EI): m/z calcd for $C_{20}H_{36}O_3$: 324.2665, found: 324.2675; $[\alpha]_D^{21} = -14.8$ (*c* 0.9, Et_2O).
20. Methyl 11-ketodihydrosterculate (methyl (*Z*)-8-(2-(1-oxo)-octylcyclopropane-1-yl)-octanoate) **4** colourless oil. R_f 0.18 (Et_2O/hexane 1:10); IR, ^1H NMR similar to **3**; ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.58, 174.34, 51.45, 44.90, 34.11, 31.73, 29.87, 29.31, 29.22, 29.14, 29.14, 29.12, 26.19, 25.68, 25.41, 24.94, 24.16, 22.64, 14.30, 14.09; EI MS m/z 324 (M^+), 293 ($[M-31]^+$), 127 ($[CH_3(CH_2)_6CO]^+$); HRMS (EI): m/z calcd for $C_{20}H_{36}O_3$: 324.2665, found: 324.2681; $[\alpha]_D^{21} = +20.7$ (*c* 1, Et_2O).
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