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The synthesis of methyl 4-(2-octadecylcyclopropen-1-yl)butanoate: a possible inhibitor in mycolic acid biosynthesis

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Abstract

The high molecular weight 2-alkyl-3-hydroxy mycolic acids are key structural components of the cell envelope of pathogenic mycobacteria such as *Mycobacterium tuberculosis*. It has been shown recently that (*Z*)-tetracos-5-enoic acid is a key initial intermediate in mycolic acid biosynthesis in extracts of *Mycobacterium smegmatis*. This acid is presumably formed by desaturation of tetracosanoate, and previous studies on oleic acid biosynthesis suggest that a cyclopropene analogue may be a potential inhibitor. This communication describes the synthesis of methyl 4-(2-octadecylcyclopropen-1-yl)butanoate, which is shown elsewhere to be an inhibitor of the initial stages of mycolic acid synthesis.

Key words: Mycobacteria; Mycolic acids; Cyclopropene fatty acid; Biosynthetic inhibitor

1. Introduction

The integrity of the mycobacterial cell envelope depends on the presence of large amounts of covalently bound mycolic acids associated with different types of characteristic free lipids [1,2]. The mycolic acids occur as mixture of different types, consisting of the so-called α-mycolates, which have no oxygen functions other than the 3-hydroxy acid unit, and a characteristic selection of oxygenated types (Table 1). The high degree of complexity in the structure of these mycolic acids suggests that many enzyme systems must be involved in the biosynthesis of these lipids. The identification of intermediates in mycolic acid biosynthesis would allow key enzymes to be identified as possible drug targets [3].

A detailed investigation into mycobacterial mycolic acid biosynthesis was carried out by Takayama and co-workers for *Mycobacterium*

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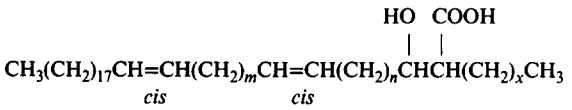
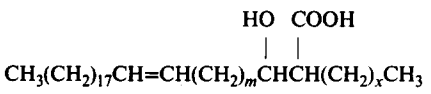
Table 1
Essential structures of representative mycolic acids

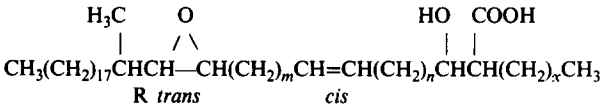
α-Mycolates[a]



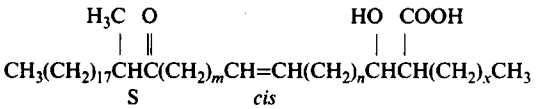
α′-Mycolates



Epoxymycolates[a]



Ketomycolates[a]



[a]In addition to the *cis* double bonds shown, other structures with *cis* cyclopropane rings or *trans* double bonds with adjacent methyl branches are also encountered.

tuberculosis H37Ra [4], and several intermediates for mycolic acid biosynthesis were suggested. One proposed key intermediate was (*Z*)-tetracos-5-enoic acid, formed by desaturation of tetracosanoate. In recent studies this acid was synthesized [5], and its role was confirmed by its specific stimulation of the incorporation of radioactive label from [1-^{14}C]acetate into mycolic acids in *Mycobacterium smegmatis* [6]. Structural analogues of this intermediate may serve as potential inhibitors of mycolic acid biosynthesis. It is established that the desaturation of stearate to produce oleate is inhibited by the cyclopropenyl analogue of the latter [7,8]. This present report describes the synthesis of methyl 4-(2-octadecylcyclopropen-1-yl)butanoate, the cyclopropene analogue of (*Z*)-tetracos-5-enoate.

2. Experimental procedures

Melting points (uncorrected) were obtained on a Kofler hot stage apparatus. Elemental analyses were performed on a Carlo-Erba Instrumentazione model 1106 CHN analyser. Infrared spectra (cm^{-1}) were recorded on a Nicolet 20 SXB or a Nicolet 20 PC Fourier Transform spectrometer; peaks are labelled 'br' (broad) 's' (strong), 'm' (medium) and 'w' (weak). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectra (δ values, ppm) were obtained using solutions in deuteriochloroform with tetramethylsilane as internal standard on a Bruker WP 200 instrument. ^{1}H signals are labelled 's' (singlet), 'd' (doublet), 't' (triplet), 'q' (quadruplet) and 'm' (multiplet). ^{13}C signals were assigned according to previous

studies [9,10]; tentative assignments are indicated. Electron-impact (EI) mass spectra were recorded on AEI MS9 and Kratos MS 80RF spectrometers. Starting materials and chemical reagents were purchased from Aldrich or Lancaster Synthesis. Column chromatography was carried out at medium pressure using Merck 7736 grade silica gel. Fluka 60738 silica gel 60 was used for flash chromatography. Thin-layer chromatography (tlc) used Merck 5554 silica gel aluminium-backed sheets.

2.1. *Eicos-1-yne* (2)

Lithium acetylide-ethylenediamine complex (13.5 g, 150 mmol) was added to dimethylsulphoxide (100 ml) at 0°C and stirred under nitrogen for 15 min. 1-Bromooctadecane [1] (33.3 g, 100 mmol) in dimethyl sulphoxide (50 ml) was added slowly and the resulting reaction mixture allowed to reach ambient temperature and stirred at room temperature overnight, followed by quenching with 40% hydrochloric acid (40 ml). The reaction was extracted with diethyl ether (3 × 150 ml), and the combined organic extracts were washed with water (2 × 100 ml) and dried over anhydrous magnesium sulphate. Evaporation of the solvent gave an off-white solid that was further purified by flash column chromatography using petroleum ether (b.p. 60–80°C) as eluent to afford eicos-1-yne (2) as a white solid (18.1 g, 65%). NMR and mass spectral analyses were identical to an authentic sample [11]. M.p. 36–38°C. IR (film) 3314br m, 2117w, 2920s, 2851s, 1464m, 712m; ^{1}H-NMR (200 MHz) 0.89 (3H, t, *J* 6.9 Hz, CH_3), 1.26–1.79 (32H, complex multiplet, aliphatic), 1.94 (1H, t, *J* 2.6 Hz, $(CH_2)_{17}C{=}CH$), 2.19 (2H, dt, *J* 2.6 and 4.3 Hz, $CH_2CH_2C{=}CH$); ^{13}C-NMR (50 MHz) 84.80 (C2), 68.03 (C1), 31.97 (C18), 29.15–29.72 (C6–C17), 28.81 (C5), 28.55 (C4), 22.73 (C19), 18.44 (C3), 14.14 (C20); *m/z* (EI) 278 (M^+, 18%). Found: C, 86.5; H, 13.7. $C_{20}H_{38}$ requires C, 86.3; H, 13.7%.

2.2. *2-Bromoeicos-1-ene* (3)

Hydrogen bromide gas was bubbled through a solution of tetraethylammonium bromide (14.5 g, 69 mmol) in dichloromethane (150 ml) at 0°C for 35 min. The reaction mixture was purged with nitrogen, eicos-1-yne (2) (17.5 g, 63 mmol) was added and the reaction was stirred overnight at room temperature. Diethyl ether (300 ml) was added, and the resulting white precipitate was removed by filtration. The precipitate was washed with diethyl ether (100 ml). The combined organic extracts were evaporated to yield a white precipitate in a yellow oil. This was then purified by flash column chromatography using petroleum ether (b.p. 60–80°C) as eluent to afford 2-bromoeicos-1-ene (3) as a colourless oil (19.6 g, 87%). IR (film) 2924s, 2853s, 1630m, 1466m, 1377m, 721w; ^{1}H-NMR (200 MHz) 0.88 (3H, t, *J* 6.7 Hz, CH_3), 1.26–1.58 (32H, complex multiplet, aliphatic), 2.41 (2H, t, *J* 6.9 Hz, CH_2CH_2 $CBr{=}CH_2$), 5.38 (1H, s, $CH_2CBr{=}CH_2$), 5.55 (1H, s, $CH_2CBr{=}CH_2$); ^{13}C-NMR (50 MHz) 135.00 (C2), 116.18 (C1), 41.47 (C3), 31.97 (C18), 29.38–29.73 (C6–C17), 28.46 and 27.94 (C4 and C5?), 22.73 (C19), 14.15 (C20); *m/z* (EI) 358 (M^+, 63%), 279 (M^+—Br). Found: C, 66.9; H, 10.9. $C_{20}H_{39}Br$ requires C, 66.9; H, 10.5%.

2.3. *1,1,2-Tribromo-2-octadecylcyclopropane* (4)

To a rapidly stirred solution of 2-bromoeicos-1-ene (3) (19.6 g, 54.75 mmol) in bromoform (47.9 g, 191.63 mmol) and cetrimide (0.46 g) was added, in one portion, sodium hydroxide (22.88 g, 572 mmol) in water (22.8 ml). The temperature of the reaction mixture was not allowed to exceed 60°C using a cold water bath. The reaction mixture was stirred for an 1 h, after which it was heated at 60°C for an 1 h using a water bath. The reaction mixture was poured into water (100 ml) and extracted with dichloromethane (3 × 100 ml). The combined organic extracts were washed with dilute hydrochloric acid (100 ml) and sodium bicarbonate (100 ml) and finally with water (100 ml). The organic layer was then dried over anhydrous magnesium sulphate and the solvent removed in vacuo. Diethyl ether (150 ml) was added and the precipitated catalyst was removed by filtration. The solvent was again evaporated and excess bromoform was removed under reduced pressure (oil pump) to afford the product. The product was purified using flash column chromatography using

petroleum ether (b.p. 60–80°C) as eluent to give the title compound (**4**) as a white solid (1.1 g, 65%). M.p. 43–45°C; IR (film) 2918s, 2849s, 1464s, 1415s, 1377m, 1016m, 694s; ^{1}H-NMR (200 MHz) 0.86 (3H, t, *J* 6.6 Hz, CH_3), 1.23–1.77 (32H, complex multiplet, aliphatic), 1.82–2.15 (4H, complex multiplet, C (2)BrCH_2CH_2 and cyclopropane CH_2); ^{13}C-NMR (50 MHz) 45.94 (C1?), 41.83 (cyclopropane CH_2?), 38.18 (C3?), 33.27 (C2?), 32.01 (C18), 29.05–29.77 (C5–C17), 27.78 (C4?), 22.78 (C19), 14.22 (C20); *m/z* (EI) 529 (MH^+, 14%), 450 (MH^+—Br), 371 (MH^+—Br_2). Found: C, 47.5; H, 7.4. $C_{21}H_{39}Br_3$ requires C, 47.5; H, 7.5%.

2.4. *1-Iodo-3-(2-octadecylcyclopropen-1-yl)-propane* (**5**)

n-Butyl lithium (44 ml, 44 mmol, in hexane) was added over 5 min to a stirred solution of 1,1,2-tribromo-2-octadecylcyclopropane [**4**] (10.56 g, 20 mmol) in dry diethyl ether (250 ml) under nitrogen at −40°C. The reaction was allowed to reach ambient temperature and stirred for an additional 30 min, then hexamethylphosphorus triamide (5.90 g, 33 mmol) was added followed by 1,3-diiodopropane (11.84 g, 40 mmol). The reaction mixture was stirred overnight at room temperature. Water (40 ml) was added followed by diethyl ether (100 ml) and the two layers separated. The organic layer was washed with more water (3 × 50 ml) and dried and the solvent removed under reduced pressure. The product was purified by flash column chromatography using petroleum ether (b.p. 60–80°C) as eluent to yield (**5**) as a white solid (2.1 g, 23%), which was used immediately.

2.5. *1-Cyano-3-(2-octadecylcyclopropen-1-yl)-propane* (**6**)

Sodium cyanide (0.042 g, 0.84 mmol) was slurried in dry dimethylsulphoxide (4 ml) and heated at 90°C with stirring for 15 min. The reaction was allowed to cool to 60°C, the 1-iodo-3-(2-octadecylcyclopropen-1-yl)propane (**5**) (0.35 g, 0.76 mmol) was added and stirring continued for a further 2 h. Water (5 ml) was added and the solution was extracted with ether (3 × 10 ml). The organic extracts were combined, dried and evaporated in vacuo to yield an off-white solid, which was further purified by flash column chromatography using petroleum ether (b.p. 60–80°C) and ethyl acetate (95:5) as eluent to afford pure 1-cyano-3-(2-octadecyl-cyclopropen-1-yl)propane (**6**) as a white solid (0.21 g, 77%). M.p. 33–35°C; IR (film) 3434br w, 2976s, 2853s, 2247m, 1714m, 1682m, 1628w, 1466m, 1350m, 1120s, 721w; ^{1}H-NMR (200 MHz) 0.79 (2H, s, cyclopropene CH_2), 0.86 (3H, t, *J* 6.6 Hz, CH_3), 1.23–1.98 (34H, complex multiplet, aliphatic), 2.24–2.56 (6H, complex multiplet); *m/z* (EI) 358 (M^+—H, 75%). Found: M^+, 359.3534. $C_{25}H_{45}N$ requires M, 359.3552.

2.6. *Methyl 4-(2-octadecylcyclopropen-1-yl)-butanoate* (**7**)

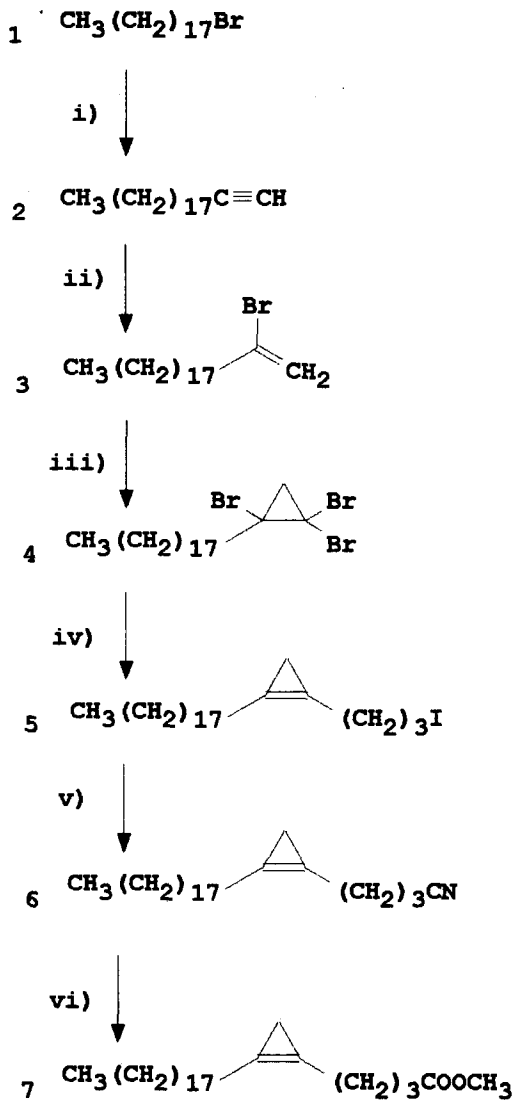
1-Cyano-3-(2-octadecylcyclopropropen-1-yl) propane (**6**) (0.25 g, 0.7 mmol) was added to a solution of sodium hydroxide (0.22 g, 5.5 mmol) in ethanol (2.4 ml) and water (0.3 ml) and the solution refluxed for 7.5 h. Water (10 ml) was added, followed by methanol (10 ml), and the reaction mixture was cooled to 0°C, acidified with dilute hydrochloric acid (10 ml) and extracted with petroleum ether (b.p. 60–80°C) and diethyl ether (1:1), (3 × 20 ml). The organic extracts were combined, washed with water (2 × 50 ml) and dried and the solvent removed in vacuo at 0°C. Aqueous tetrabutyl ammonium hydroxide (5%, 15 ml), dichloromethane (15 ml) and iodomethane (1 g, 7 mmol) were added to the residue and the reaction mixture stirred overnight at ambient temperature. The organic layer was separated, dried and concentrated in vacuo and the product purified by flash column chromatography using petroleum ether (b.p. 60–80°C) and ethyl acetate (95:5) as eluent to yield the title compound (**7**) as a colourless oil (0.21 g, 77%). IR (film) 2924s, 2855s, 1745s, 1367m, 1167m, 1008m; ^{1}H-NMR (200 MHz) 0.77 (2H, s, cyclopropene CH_2), 0.86 (3H, t, *J* 6.6 Hz, CH_3), 1.23–1.94 (34H, complex multiplet, aliphatic), 2.29–2.45 (6H, complex multiplet), 3.65 (3H, s, $COOCH_3$); ^{13}C-NMR (50 MHz) 173.88 (C1), 110.65 (C6), 108.17 (C5), 51.45 (methyl ester), 33.56 (C2), 31.97 (C22),

29.42–29.73 (C9–C21), 27.38 (C4?), 26.02 (C3), 25.40 (C7?), 22.82 (C8?), 22.71 (C23), 14.12 (C24), 7.39 (cyclopropene CH_2); *m/z* (EI) 392 (M^+, 36%), 361 (M^+—OCH_3). Found: M^+ 392.3804. $C_{26}H_{48}O_2$ requires M, 392.3654.

3. Results and discussion

The synthesis of methyl 4-(2-octadecyl-cyclopropen-1-yl)-butanoate (**7**) follows the strategy developed previously [12]. The first step (Scheme 1) involves the preparation of eicos-1-yne (**2**) by reaction of lithium acetylide-ethylenediamine complex with 1-bromooctadecane [13]. The terminal acetylene (**2**) is treated with dry hydrogen bromide, using tetraethylammonium bromide to direct the addition in a Markownikov manner [14], to afford 2-bromoeicos-1-ene (**3**). The ^{1}H-NMR of (**6**) contained two broad singlets at δ 5.38 and 5.55 for the two alkene protons; however, no signals associated with anti-Markownikov addition were visible, as these would have resulted in a more complex spectrum in the alkene region due to allylic coupling. 2-Bromoeicos-1-ene (**3**) was converted to a dibromocyclopropane with dibromocarbene, using a phase transfer catalysis method [15], to give 1,1,2-tribromo-2-octadecyl-cyclopropane (**4**). The procedure requires the use of bromoform, both as a solvent and a source of dibromocarbene, sodium hydroxide as base and cetyltrimethylammonium bromide (cetrimide) as a phase transfer catalyst [15].

The tribromocyclopropane (**4**) was then treated with two equivalents of *n*-butyl lithium at −40°C. This resulted in a lithium-bromine exchange followed by elimination of lithium bromide [16]. As the reaction mixture warmed up to 0°C, a second lithium-bromine exchange occurred, giving rise to 1-lithio-2-octadecylcyclopropene. This species was coupled with two equivalents of 1,3-diiodopropane [12], providing a modest yield (23%) of 1-iodo-3-(2-octadecyl-cyclopropen-1-yl)-propane (**6**). The iodocyclopropene (**6**) was converted immediately to 1-cyano-3-(2-octadecylcyclopropen-1-yl)-propane (**7**), using a well documented procedure [17]. The infrared spectrum showed a signal for the nitrile at 2247 cm^{-1}, a singlet in the



Scheme 1. Synthesis of methyl 3-(2-octadecylcyclopropen-1-yl)butanoate. (i) Lithium acetylide-ethylenediamine complex, dimethyl sulphoxide; (ii) hydrogen bromide gas, tetraethylammonium bromide, dichloromethane; (iii) bromoform, cetrimide, sodium hydroxide; (iv) *n*-butyl lithium, diethyl ether, hexamethylphosphorus triamide, 1,3-diiodopropane; (v) sodium cyanide, dimethyl sulphoxide; (vi) aqueous ethanolic sodium hydroxide followed by tetrabutylammonium hydroxide, iodomethane, dichloromethane.

^{1}H NMR spectrum at δ 0.79 characteristic of a cyclopropene methylene group and also a triplet at δ 2.54 (*J* 6.9 Hz) for the methylene protons α to the nitrile group. Mild base hydrolysis yielded the

cyclopropene fatty acid which was immediately esterified, using a phase transfer catalysis method [18], to afford the target compound, methyl 4-(2-octadecylcyclopropen-1-yl)butanoate (**8**). The ^{1}H-NMR spectrum showed a singlet at δ 0.77 (cyclopropene) and a sharp singlet at δ 3.65 (methyl ester). The infrared spectrum showed a carbonyl stretch at 1745 cm^{-1} along with the cyclopropene signal at 1008 cm^{-1}.

Methyl 4-(2-octadecylcyclopropen-1-yl)butanoate (**8**), synthesized in this study, has been shown to significantly inhibit mycolic acid biosynthesis in extracts of *M. smegmatis* [19]. Presumably the cyclopropene ester inhibits the essential desaturase. This is the first example of a custom-designed inhibitor of a key stage of mycolic acid biosynthesis. This opens up the possibility of identifying at least one enzyme as a potential target for antimycobacterial drugs. The hexane-water assay system used to study the incorporation of long-chain compounds into mycobacterial extracts [6,19] has the convenient capability of being able to utilise methyl esters. Presumably the mycobacterial preparation includes a lipase which liberates the free acid from the methyl ester. This is extremely important, as cyclopropene acids are prone to polymerisation.

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