ELSEVIER SCIENTIFIC PUBLISHERS IRELAND

Chemistry and Physics of Lipids 66 (1993) 23–34

Chemistry and Physics of LIPIDS

Synthesis of methyl (*Z*)-tetracos-5-enoate and both enantiomers of ethyl (*E*)-6-methyltetracos-4-enoate: possible intermediates in the biosynthesis of mycolic acids in mycobacteria

Gurdyal S. Besra[a], David E. Minnikin*[a], Paul R. Wheeler[b], Colin Ratledge[b]

[a]*Department of Chemistry, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK*
[b]*Department of Applied Biology, University of Hull, Hull HU6 7RX, UK*

(Received 4 November 1992; revision received 12 February 1993; accepted 12 February 1993)

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*. A prime target for drug action would be the initial stages where the biosynthetic pathways diverge from those of ordinary fatty acids. It has been postulated that the pathway for the α-mycolates, without oxygen functions in addition to the hydroxy-acid unit, appears to diverge from (*Z*)-tetracos-5-enoic acid. The biosynthesis of oxygenated mycolic acids is considered to possibly diverge from (*E*)-6-(*R*)-methyltetracos-4-enoic and (*E*)-6-(*S*)-methyltetracos-4-enoic acids. This communication describes the synthesis of esters of these acids in order to test their potential role in the biosynthesis of mycolic acids.

Key words: Mycolic acids; Biosynthetic intermediates; Tetracos-5-enoic acid; 6-Methyltetracos-4-enoic acid; Fatty acid synthesis

1. Introduction

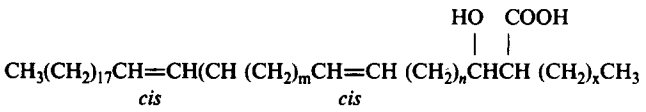
The integrity of the cell envelopes of *Mycobacterium tuberculosis* and other mycobacteria depends on the presence of large amounts of covalently bound mycolic acids associated with different types of characteristic free lipids [1,2]. The high molecular weight long-chain mycolic acids occur as a mixture of different types (Table 1), consisting of the so-called α-mycolates, which have no oxygen functions in addition to the 3-hydroxy acid unit, and a characteristic selection of oxygenated types. A detailed investigation into mycobacterial mycolic acid biosynthesis was carried out by Takayama and co-workers for *M. tuberculosis* H37Ra [3]. The suggested pathway for the biosynthesis of the α-mycolic acids in *M. tuberculosis* H37Ra involved a key desaturation of tetracosanoate to give (*Z*)-tetracos-5-enoic acid. This reaction was considered to be a step that might be sensitive to the anti-tubercular drug isoniazid [3,4], but a detailed mechanism for the

* Corresponding author.

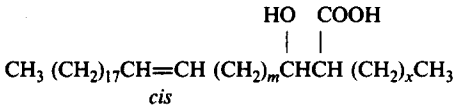
0009-3084/93/$06.00
SSDI 0009-3084(93)02164-M

Table 1
Essential structures of representative mycolic acids.

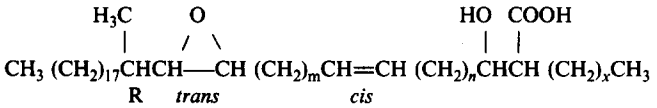
α-Mycolates[a]



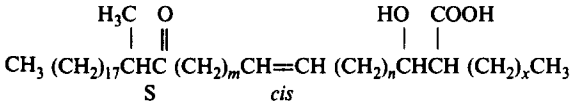
α'-Mycolates



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.

inhibition and the resistance of certain mycobacterial pathogens to this drug has not been proposed.

Many mycolic acids contain other very characteristic units (Table 1) whose biosynthetic pathways may provide potential sites for drug attack. In a number of different mycolic acids *cis*-double bonds are apparently transformed into *trans*-double bonds with an adjacent methyl branch as well as the alternative conversion to a *cis*-cyclopropane, as discussed by Minnikin [1,5]. Studies conducted by Etémadi and Gasche [6] indicated that the methyl branch next to the oxygen in wax-ester mycolates was derived from the methyl group of (*S*)-adenosyl methionine [1,5]. Detailed studies relating to the epoxymycolates [7,8] have resulted in a possible biosynthetic route [5] for oxygenated mycolates. The *trans* epoxide with an adjacent methyl branch [1,5] could be formed directly from a methyl branched *trans* alkene. Recent studies [9], however, have indicated that at least two parallel pathways must operate to give methyl branches next to oxygen functions in mycolic acids. The methyl branch adjacent to the epoxide group in epoxymycolates has an (*R*)-absolute configuration in contrast to the (*S*)-methyl group next to the keto, methoxy and wax-ester functions. This rules out a common epoxide intermediate in the biosynthetic pathway leading to all oxygenated mycolates. The pathways for α-mycolates and oxygenated mycolates appear to diverge from probable intermediates, such as (*Z*)-tetracos-5-enoic, (*E*)-6-(*R*)-methyltetracos-4-enoic and (*E*)-6-(*S*)-methyltetra-cos-4-enoic acids,

perhaps with different chain lengths. The present paper describes the synthesis of the three postulated intermediates in the mycolic acid biosynthetic pathway. Experiments on the incorporation of these long-chain compounds into extracts of *Mycobacterium smegmatis* are described elsewhere [10].

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 20PC Fourier Transform spectrometer; peaks are labelled 'br' (broad), 's' (strong), 'm' (medium) and 'w' (weak). Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance spectra (NMR) (δ values, ppm) were obtained using solutions in deuteriochloroform with tetramethylsilane as internal standard on a Bruker WP 200 instrument; ^1H signals are labelled 's' (singlet), 'd' (doublet), 't' (triplet), 'q' (quadruplet) and 'm' (multiplet). ^{13}C-NMR spectra were assigned according to previous guidelines [11,12]; tentative assignments are indicated. Electron-impact (EI) mass spectra were recorded on AEI MS9 and Kratos MS 80RF spectrometers. Optical rotations were recorded on an NPL automatic polarimeter type 143D. 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. *1-Tetrahydropyranoloxyhex-5-yne* (2)

5-Hexyne-1-ol (Aldrich) (**1**) (5 g, 51 mmol) and pyridinium-*p*-toluenesulphonate (0.98 g, 5.1 mmol) were dissolved in dry dichloromethane (100 ml). To this solution was added 3,4-dihydro-2*H*-pyran (5.14 g, 61 mmol), and the mixture was stirred at ambient temperature overnight under a nitrogen atmosphere. The reaction mixture was washed with saturated sodium bicarbonate (3 × 50 ml) and dried over anhydrous magnesium sulphate, filtered and concentrated in vacuo to give a pale yellow oil. The product was purified by flash column chromatography, using petroleum ether (b.p. 60–80°C) and acetone (95:5) as eluent, to afford the title compound (**2**) as a colourless oil (7.50 g, 81%). IR (film) 3298 br m, 2943 s, 2870 s, 1454 m, 1365 m, 1261 m, 1186 s, 1120 s, 1022 s, 974 m, 870 m; ^1H-NMR (200 MHz) 1.51–1.85 (10H, complex multiplet, aliphatic and THP-C(3)H_2, C(4)H_2, C(5)H_2), 1.95 (1H, t, *J* 2.7 Hz, $HC{=}C(CH_2)_4$), 2.24 (2H, dt, *J* 2.6 Hz, $HC{=}CCH_2CH_2$), 3.45 (2H, m, CH_2CH_2OTHP), 3.82 (2H, m, THP-C(6)H_2), 4.58 (1H, m, THP-C(2)*H*); ^{13}C-NMR (50 MHz) 98.98 (THP C2), 84.52 (C5), 68.43 (C1), 67.06 (C6), 62.42 (THP C6), 30.92 (THP C3), 29.01 (THP C5), 25.69 (C2?), 25.56 (C3?), 19.77 (THP C4), 18.42 (C4); *m/z* (EI) 183 (MH^+, 24%), 101 ($M{-}(CH_2)_4C{=}CH$), 85 ($M{-}O(CH_2)_4C{=}CH$). Found: C, 72.55; H, 9.86. $C_{11}H_{18}O_2$ requires C, 72.52; H, 9.89%. NMR and mass spectroscopic data were identical to those reported [11].

2.2. *1-Tetrahydropyranoloxytetracos-5-yne* (3)

A solution of 1-tetrahydropyranoloxyhex-5-yne (**2**) (6.02 g, 33.1 mmol) in dry diethyl ether (100 ml) and dry hexamethylphosphorus triamide (11.85 g, 66.2 mmol) was cooled to 5°C under a nitrogen atmosphere. To this solution was added *n*-butyl lithium (25 ml, 36.4 mmol, in hexane) dropwise, and the reaction mixture was stirred at 0°C for 1 h under nitrogen. The solution was cooled down to −78°C and 1-bromooctadecane (Aldrich) (12.1 g, 36.4 mmol) added. The reaction mixture was then allowed to reach ambient temperature, and stirring continued overnight. The reaction was quenched by the addition of saturated ammonium chloride solution (50 ml) and the product extracted with diethyl ether (3 × 100 ml). The combined organic extracts were washed with saturated sodium chloride (75 ml) and dried over anhydrous magnesium sulphate and concentrated in vacuo. The product was purified by flash column chromatography using petroleum ether (b.p. 60–80°C) and acetone (98:2) as eluent to afford (**3**) as a colourless oil (8.02 g, 58%). IR (film) 2926 s, 2855 s, 1732 m,

1441 m, 1352 m, 1273 w, 1138 w, 1076 m, 1022 m, 985 m; ^{1}H-NMR (200 MHz) 0.87 (3H, t, *J* 6.8 Hz, CH_3), 1.25–1.76 (42 H, complex multiplet, aliphatic and THP-C(3)H_2, C(4)H_2, C(5)H_2), 2.09–2.21 (4H, m, $CH_2C{=}CCH_2$), 3.42 (2H, m, CH_2CH_2OTHP), 3.75 (2H, m, THP-C(6)H_2), 4.57 (1H, m, THP-C(2)H,); ^{13}C-NMR (50 MHz) 98.96 (THP C2), 80.68 (C6), 79.98 (C5), 67.25 (C1), 62.41 (THP C6), 32.09 (C22), 30.95 (THP C3), 29.34–29.84 (C9–C21), 29.15 (C8?), 29.06 (THP C5), 26.17 and 25.73 (C2 and C3?), 22.82 (C23), 19.81 (THP C4), 18.93 (C7), 18.79 (C4), 14.19 (C24); *m/z* (EI) 434 (M^+, 32%). Found: M^+, 434.4224. $C_{29}H_{54}O_2$ requires M, 434.4124.

2.3. *(Z)-1-Tetrahydropycanoloxytetracos-5-ene* (4)

A solution of 1-tetrahydropyranoloxytetracos-5-yne (**3**) (8.02 g, 19.19 mmol) in dry diethyl ether (100 ml) was hydrogenated (1 atm) over Lindlar catalyst (0.45 g) with vigorous stirring until hydrogen gas (430 ml, 19.19 mmol) was taken up. The catalyst was filtered off, and the filtrate was concentrated in vacuo. Purification by flash column chromatography using petroleum ether (b.p. 60–80°C) and acetone (98:2) as eluent afforded the title compound (**4**) as a colourless oil (7.02 g, 87%). IR (film) 3005 w, 2926 s, 2853 s, 1653 w, 1466 m, 1259 w, 1201 m, 1035 m, 989 w, 758 m cm^{-1}; ^{1}H-NMR (200 MHz) 0.88 (3H, t, *J* 6.8 Hz, CH_3), 1.25–1.85 (42 H, complex multiplet, aliphatic and THP-C(3)H_2, C(4)H_2, C(5)H_2), 1.96–2.11 (4H m, $CH_2CH{=}CHCH_2$), 3.45 (2H, m, CH_2CH_2OTHP), 3.81 (2H, m, C(6)H_2), 4.59 (1H, m, C(2)H), 5.36 (2H, t, *J* 5.9 Hz, $CH_2CH{=}CHCH_2$); ^{13}C-NMR (50 MHz) 130.41 (C6), 129.67 (C5), 98.98 (THP C2), 67.67 (C1), 62.40 (THP C6), 32.09 (C22), 30.99 (THP C3), 29.59–29.85 (C8—C21), 29.52 (THP C5), 27.43 (C7), 27.20, 26.62 and 25.74 (C2—C4?), 22.83 (C23), 19.82 (THP C4), 14.20 (C24); *m/z* (EI) 436 (M^+, 11%), 334 (M-HOTHP). Found: M^+, 436.4286. $C_{29}H_{56}O_2$ requires M, 436.4280.

2.4. *(Z)-Tetracos-5-en-1-ol* (5)

To a solution of (*Z*)-1-tetrahydropyranoloxy-tetra-cos-5-ene (**4**) (3.5 g, 8 mmol) in methanol and toluene (2:1) (150 ml) was added pyridinium-*p*-toluenesulphonate (0.16 g, 0.8 mmol), and the reaction mixture was left stirring at ambient temperature for 3 days under a nitrogen atmosphere. The solvent was removed under reduced pressure. The crude reaction mixture was taken up in diethyl ether (100 ml) and washed with saturated sodium bicarbonate (50 ml) and saturated sodium chloride (50 ml) and dried over anhydrous magnesium sulphate. The solution was filtered and the filtrate concentrated in vacuo. Purification by flash column chromatography using petroleum ether (b.p. 60–80°C) and acetone (95:5) as eluent afforded (*Z*)-tetracos-5-en-1-ol (**5**) as a white solid (2.35 g, 83%). M.p. 45–46°C; IR (film) 3367 br w, 2926 s, 2855 s, 1466 w, 1265 m, 1062 w, 742 s, 706 m; ^{1}H-NMR (200 MHz) 0.87 (3H, t, *J* 6.7 Hz, CH_3), 1.25–1.65 (37 H, m, aliphatic and CH_2OH), 1.75–2.11 (4H, m, $CH_2CH{=}CHCH_2$), 3.64 (2H, t, *J* 6.5 Hz, CH_2CH_2OH), 5.36 (2H, m, $CH_2CH{=}CHCH_2$); ^{13}C-NMR (50 MHz) 130.59 (C6), 129.46 (C5), 63.08 (C1), 32.56 (C2), 32.08 (C22), 29.52–29.85 (C8—C21), 27.42 (C7), 27.08 and 26.06 (C3 and C4?), 22.83 (C23), 14.22 (C24); *m/z* (EI) 353 (MH^+, 32%), 334 ($M-H_2O$). Found: M^+ 352.3771. $C_{24}H_{48}O$ requires M, 352.3705.

2.5. *Methyl (Z)-tetracos-5-enoate* (6)

(*Z*)-Tetracos-5-en-1-ol (5) (2.35 g, 6.7 mmol) in dry dichloromethane (25 ml) was added to a stirred solution of pyridinium dichromate (30 g, 79.8 mmol) in dry dimethylformamide (100 ml) and dry dichloromethane (75 ml) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was poured into water (200 ml) and extracted with diethyl ether (3 × 100 ml). The combined diethyl ether extracts were washed with saturated sodium chloride (100 ml) and dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure. The crude product was repeatedly recrystallised from methanol to give (*Z*)-tetracos-5-enoic acid (2.12 g, 83%). M.p. 42–44°C; IR (film) 3003 br w, 2922 s, 2851 s, 1689 s, 1464 s, 1265 s, 1128 w, 922 m, 740 s; ^{1}H-NMR (200 MHz) 0.88 (3H, t, *J* 6.8 Hz, CH_3), 1.25–1.62 (32 H, complex multiplet, aliphatic), 1.69 (2H, m,

CH_2CH_2COOH), 2.08 (4H, m, $CH_2CH{=}CHCH_2$), 2.36 (2H, t, *J* 7.6 Hz, CH_2CH_2COOH), 5.37 (2H, m, $CH_2CH{=}CHCH_2$); ^{13}C (50 MHz) 180.06 (C1), 131.55 (C6), 128.47 (C5), 33.60 (C2), 32.09 (C22), 29.51–29.87 (C8–C21), 27.42 (C7), 26.65 (C4), 24.81 (C3), 22.83 (C23), 14.23 (C24); *m/z* (EI) 366 (M^+, 3%), 348 ($M-H_2O$). Found: C, 78.7; H, 13.0. $C_{24}H_{46}O_2$ requires C, 78.7; H, 12.6%.

(*Z*)-Tetracos-5-enoic acid (2.00 g, 5.5 mmol) was dissolved in dichloromethane (50 ml) and 0.1 M tetrabutylammonium hydrogen sulphate in 0.2 M aqueous sodium hydroxide (50 ml) followed by iodomethane (0.93 g, 6.6 mmol). The reaction mixture was stirred for 1 h. The upper aqueous layer was discarded and the lower layer concentrated in vacuo. The title compound (**6**) was purified by recrystallisation from acetone (1.61 g, 77%). M.p. 32–34°C; IR (film) 2924 s, 2855 s, 1743 s, 1458 m, 1365 w, 1313 w, 1213 w, 1167 w, 740 w; ^1H-NMR (200 MHz) 0.87 (3H, t, *J* 6.8 Hz, CH_3), 1.25–1.60 (32 H, complex multiplet, aliphatic), 1.68 (2H, m, $CH_2CH_2COOCH_3$), 2.03 (4H, m, $CH_2CH{=}CHCH_2$), 2.31 (2H, t, *J* 7.4 Hz, $CH_2CH_2COOCH_3$), 3.66 (3H, s, CH_2COOCH_3), 5.35 (2H, m, $CH_2CH{=}CHCH_2$); ^{13}C-NMR (50 MHz) 174.18 (C1), 131.35 (C6), 128.47 (C5), 51.43 (methyl ester), 33.66 (C2), 32.09 (C22), 29.48–29.85 (C8–C21), 27.39 (C7), 26.72 (C4), 25.11 (C3), 22.83 (C23), 14.19 (C24); *m/z* (EI) 380 (M^+, 8%), 348 ($M-CH_3OH$). Found: M^+, 380.3604. $C_{25}H_{48}O_2$ requires M, 380.3654.

2.6. *1-Octadecanal* (**8**)

A solution of 1-octadecanol (**7**) (100 g, 370 mmol) in dry dichloromethane (300 ml) was added to a rapidly stirred suspension of pyridinium chlorochromate (120 g, 558 mmol) in dry dichloromethane (100 ml) under nitrogen at room temperature. After stirring for 1.5 h, dry diethyl ether (150 ml) was added and the supernatant liquid decanted. The insoluble residue was washed with more diethyl ether (5 × 100 ml). The combined organic solution was passed through a pad of Florisil™ and the solvent evaporated. NMR and TLC evidence showed that the reaction had gone to completion, and the crude product (**8**), obtained in 92% yield, was not purified further.

2.7. *Ethyl (E)-2-methyleicos-2-enoate* (**9**)

1-Carboethoxyethylidene triphenylphosphorane (Lancaster) (136.5 g, 376 mmol) in dry dichloromethane (300 ml) was charged to a three-necked 250 ml round-bottom flask fitted with a reflux condenser and drying tube, a dropping funnel, dry nitrogen inlet and magnetic stirrer. All apparatus was dried and flushed with nitrogen before use. 1-Octadecanal (**8**) (84 g, 313.4 mmol) in dry dichloromethane (100 ml) was added slowly with stirring via the dropping funnel. The mixture was then stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the residue taken up in diethyl ether (300 ml) and passed through a pad of silica gel which was washed with more diethyl ether (5 × 100 ml). The combined diethyl ether fractions were evaporated under reduced pressure to give an off-white solid which was further purified by flash column chromatography using petroleum ether (b.p. 60–80°C) and acetone (98:2) as eluent to afford ethyl (*E*)-2-methyleicos-2-enoate (**9**) as a white solid (92.3 g, 84%). M.p. 29–31°C; IR (film) 2924 s, 2855 s, 1713 s, 1651 s, 1466 m, 1367 m, 1271 m, 1136 m, 1037 m; ^1H-NMR (200 MHz) 0.88 (3H, t, *J* 6.8 Hz, CH_3), 1.26–1.58 (36H, m, aliphatic, $CH{=}C(CH_3)$ CO, $COOCH_2CH_3$), 1.83 (2H, s, $CH_2CH_2CH{=}C$), 4.22 (2H, q, *J* 7.1 Hz, $COOCH_2CH_3$), 6.76 (1H, t, *J* 7.5 Hz, $CH_2CH{=}C(CH_3)CO$); ^{13}C (50 MHz) 168.38 (C1), 142.51 (C3), 127.65 (C2), 60.38 (ethyl ester CH_2), 31.94 (C18), 29.39–29.69 (C6–C17), 28.70 and 28.60 (C4 and C5?), 22.71 (C19), 14.30 (ethyl ester CH_3), 14.15 (C20), 12.35 (C2 methyl branch); *m/z* (EI) 352 (M^+, 9%), 307 ($M^+-OCH_2CH_3$). Found: C, 78.6; H, 13.2. $C_{23}H_{44}O_2$ requires C, 78.4; 12.5%.

2.8. *Ethyl 2-methyleicosanoate* (**10**)

Ethyl (*E*)-2-methyleicos-2-enoate (**9**) (19.24 g, 54.66 mmol) in dry diethyl ether (100 ml) and palladium, 10% on carbon (2 g) was stirred vigorously while connected to the Parr hydrogenator with hydrogen gas (1232 mls, 55 mmol) being taken up. The reaction mixture was filtered to remove the catalyst, and the solvent was removed in vacuo. Flash column chromatography using

petroleum ether (b.p. 60–80°C) and acetone (95:5) as eluent yielded the title compound (**10**) as a white solid (17.94 g, 93%). M.p. 30–32°C; IR (film) 2922 s, 2853 s, 1736 s, 1468 s, 1350 m, 1180 m, 721 m; ^{1}H-NMR (200 MHz) 0.81 (3H, t, *J* 6.8 Hz, CH_3), 1.06 (3H, d, *J* 7 Hz, $CH_2CH(CH_3)COOCH_2CH_3$), 1.18–1.55 (37H, complex multiplet, aliphatic and $COOCH_2CH_3$), 2.31 (1H, m, $CH_2CH(CH_3)COOCH_2CH_3$), 4.05 (2H, q, *J* 7.1 Hz, $COOCH_2CH_3$); ^{13}C-NMR (50 MHz) 177.06 (C1), 60.09 (ethyl ester CH_2), 39.63 (C2?), 33.89 (C3?), 32.01 (C18), 29.44–29.76 (C5–C17), 27.30 (C4?), 22.75 (C19), 17.13 (C2 methyl branch), 14.33 (ethyl ester CH_3), 14.16 (C20); *m/z* (EI) 354 (M^+, 58%), 309 (M^+—OCH_2CH_3). Found: M^+, 354.3537. $C_{23}H_{46}O_2$ requires M, 354.3498.

2.9. 2-Methyleicosanoic acid (11)

Ethyl 2-methyleicosanoate (10) (17.94 g, 50.68 mmol) was heated at 75°C overnight in a 5% solution of potassium hydroxide (15 g, 268.9 mmol) in methanol/toluene/water (1:1:1). The reaction mixture was acidified with dilute hydrochloric acid (150 ml) and the product extracted with diethyl ether (5 × 100 ml). The combined organic extracts were washed with water (100 ml) and brine (100 ml) and dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure. NMR and TLC evidence showed that the reaction had gone to completion and the crude product 2-methyleicosanoic acid (**11**) was obtained in 90% yield and was not purified further.

2.10. General procedure for the preparation of amides

The starting material containing the carboxylic acid function (1 eq) was heated under reflux with oxalyl chloride (10 eq) for 1 h using a drying tube for protection. Excess reagent was removed under reduced pressure. Dry dichloromethane (20 ml) and 4-dimethylaminopyridine (1.2 eq) were added to the crude acid chloride followed by the amine (1.1 eq). The reaction mixture was stirred at ambient temperature overnight. The reaction mixture was washed with dilute hydrochloric acid (3 × 15 ml) and water (2 × 10 ml), and the organic layer was dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure to afford the crude product, which was purified by medium pressure chromatography using an appropriate solvent system as eluent.

2.11. Diastereoisomers of N-[1-hydroxy-2-(R)-phenyl]ethyl-2-methyl-eicosanamide (12, 13)

The diastereoisomers, prepared by reaction of 2-methyleicosanoic acid (**11**) with (R)-(-)-2-phenylglycinol, were separated by medium pressure chromatography.

2.12. N-[1-hydroxy-2-(R)-phenyl]ethyl-2-(R)-methyl-eicosanamide (12)

(5.1 g, 34%). R_f 0.54 (toluene/acetone 70:30); M.p. 126–128°C; $[\alpha]_D$ −32.1°C (c 1.0% in $CHCl_3$, 21°C); IR (film) 3302 br m, 2981 s, 2849 s, 1630 m, 1541 m, 1496 m, 1234 m, 1059 w, 752; ^{1}H-NMR (200 MHz) 0.86 (3H, t, *J* 6.7 Hz, CH_3), 1.13 (3H, d, *J* 6.9, $CH_2CH(CH_3)CO$), 1.23–1.58 (34H, complex multiplet, aliphatic), 2.21 (1H, m, $CH_2CH(CH_3)CO$), 2.83 (1H, br, OH), 3.88 (2H, br, CH_2OH), 5.04 (1H, m, $NHCHCH_2OH$), 6.05 (1H, br d, NH), 7.29 (5H, m, C_6H_5); ^{13}C-NMR (50 MHz) 177.51 (C1), 139.49 (phenyl C1), 129.08 (phenyl C2,C6?), 128.08 (phenyl C4), 126.75 (phenyl C3,C5), 67.23 (NH-CH-Ph?), 56.17 (CH_2OH?), 41.79 (C2?), 34.47 (C3), 32.01 (C18), 29.45–29.79 (C5–C17), 27.60 (C4?), 22.79 (C19), 17.93 (C2 methyl branch), 14.22 (C20); *m/z* (EI) 446 (MH^+, 16%), 427 ($M—H_2O$). Found: M^+, 445.3904. $C_{29}H_{51}NO_2$ requires M, 445.3919.

2.13. N-[1-Hydroxy-2-(R)-phenyl]ethyl-2-(S)-methyl-eicosanamide (13)

(6.5 g, 43%). R_f 0.32 (toluene/acetone 70:30); M.p. 111–113°C; $[\alpha]_D$ −22.4°C (c 1.0% in $CHCl_3$, 19°C); IR (film) 3296 br m, 2918 s, 2851 s, 1645 m, 1549 m, 1473 m, 1251 w, 1039 w, 719 w; ^{1}H-NMR (200 MHz) 0.86 (3H, t, *J* 6.6 Hz, CH_3), 1.15 (3H, d, *J* 6.6 Hz, $CH_2CH(CH_3)CO$), 1.25–1.59 (34H, complex multiplet, aliphatic), 2.23 (1H, m, $CH_2CH(CH_3)CO$), 2.97 (1H, br, OH), 3.85 (2H, br, CH_2OH), 5.06 (1H, m, $NHCHCH_2OH$), 6.02

(1H, br d, N*H*), 7.30 (5H, m, C_6H_5); ^{13}C-NMR (50 MHz) 177.48 (C1), 139.22 (phenyl C1), 128.97 (phenyl C2,C6?), 127.94 (phenyl C4), 126.76 (phenyl C3,C5?), 66.89 (NH-CH-Ph?), 55.91 (CH_2OH), 41.74 (C2?), 34.55 (C3), 32.05 (C18), 29.42–29.82 (C5–C17), 27.62 (C4?), 22.83 (C19), 18.02 (C2 methyl branch), 14.26 (C20); *m/z* (EI) 446 (MH^+, 18%), 427 (M—H_2O). Found: M^+, 445.3938. $C_{29}H_{51}NO_2$ requires M, 445.3919.

2.14. General procedure for the hydrolysis of amides

The pure amide was refluxed in 3N sulphuric acid in dioxan and water (1:1) (100 ml) overnight and then diluted with water (100 ml). The liberated acid was extracted with dichloromethane (3 × 100 ml) and the solvent removed in vacuo. Solid carboxylic acids were recrystallised and liquid acids were purified by flash column chromatography.

2.15. (R)-(-)-2-Methyleicosanoic acid (14)

Hydrolysis of *N*-1-[hydroxy-2-(*R*)-phenyl]ethyl-2-(*R*)-methyleicosanamide (**12**) gave (**14**) (3.1 g, 83%). M.p. 55–57°C (recrystallised from acetone); $[\alpha]_D$ −8.8° (c 1% in $CHCl_3$, 21°C).

2.16. (S)-(+)-2-Methyleicosanoic acid (15)

Hydrolysis of *N*-[1-hydroxy-2-(*R*)-phenyl]-ethyl-2-(*S*)- methyleicosanamide (**13**) gave (**15**) (3.5 g, 74%). M.p. 55–57°C (recrystallised from acetone); $[\alpha]_D$ +8.4° (c 1% in $CHCl_3$, 21°C).

2.17. N-Methoxy-N-methyl-2-(R)-methyleicosanamide (16)

Reaction of *N,O*-dimethylhydroxylamine hydrochloride (Aldrich) and (*R*)-2-methyleicosanoic acid (**14**) gave (**16**) (3.1 g, 86%). M.p. 48–50°C; [α]D −8.0° (c 1% in $CHCl_3$, 22°C); IR (KBr) 2918 br m, 2849 s, 1657 s, 1473 m, 1385 w, 1180 w, 1113 w, 999 m, 729 m; ^{1}H-NMR (200 MHz) 0.85 (3H, t, *J* 6.6 Hz, CH_3), 1.08 (3H, d, *J* 6.9 Hz, $CH_2CH(CH_3)CO$), 1.23–1.66 (34H, complex multiplet, aliphatic), 2.84 (1H, m, $CH_2CH(CH_3)$ CO), 3.17 (3H, s, NCH_3), 3.66 (3H, s, OCH_3); ^{13}C-NMR (50 MHz) 178.59 (C1), 61.52 (OCH_3), 35.21 (C2?), 33.95 (NCH_3), 32.40 (C3?), 32.04 (C18), 29.47–29.79 (C5–C17), 27.69 (C4?), 22.78 (C19), 17.56 (C2 methyl branch), 14.22 (C20); *m/z* (EI) 370 (MH^+, 78%), 309 (M—NCH_3OCH_3), 281 (M—$CONHCH_3OCH_3$). Found: M^+, 369.3563. $C_{23}H_{47}NO_2$ requires M, 369.3607.

2.18. N-Methoxy-N-methyl-2-(S)-methyleicosanamide (17)

Reaction of *N,O*-dimethylhydroxylamine hydrochloride and (*S*)-2-methyleicosanoic acid (**15**) gave (**17**) (3.57 g, 92%). M.p. 48–50°C; $[\alpha]_D$ +8.4° (c 1% in $CHCl_3$, 21°C); IR, ^{1}H- and ^{13}C-NMR as for compound (**16**); *m/z* (EI) 369 (M^+, 19%), 309 (M—NCH_3OCH_3). Found: M^+, 369.3658. $C_{23}H_{47}NO_2$ requires M, 369.3607.

2.19. Reduction of N-methoxy-N-methylamides (16, 17) using lithium aluminium hydride

Lithium aluminium hydride (2 eq) in dry THF (20 ml) was added in one portion to the *N*-methoxy-N-methylamide (1 eq) in dry THF at −78°C under a nitrogen atmosphere. The reaction mixture was stirred at −78°C for 10 min and then quenched by the addition of ethyl acetate (20 ml) followed by water (20 ml). The reduced product was extracted with diethyl ether (3 × 30 ml). The combined organic extracts were washed with water (30 ml) and dried over anhydrous magnesium sulphate. The organic solvent was removed in vacuo to afford the crude aldehydes (**18, 19**) in approximately 90% yield (NMR and TLC evidence).

2.20. Reaction of aldehydes (18, 19) with vinyl magnesium bromide

A solution of vinyl magnesium bromide (Aldrich) (1.2 eq, 1.0 M in THF) was added to a stirred solution of an aldehyde (1 eq) in dry THF (30 ml) at 0°C. The reaction mixture was then allowed to reach ambient temperature and stirred for 1 h. The reaction mixture was quenched by the addition of saturated ammonium chloride (50 ml) and the product extracted with diethyl ether

(3 × 50 ml). The combined diethyl ether extracts were washed with water (50 ml) and saturated sodium chloride (50 ml) and dried over anhydrous magnesium sulphate. The organic solvent was removed under reduced pressure and the crude product purified by flash column chromatography using petroleum ether (b.p. 60–80°C) and diethyl ether (9:1) as eluent.

2.21. 3-Hydroxy-4-(R)-methyldocos-1-ene (**20**)

Reaction of vinyl magnesium bromide with (*R*)-2-methyleicosanal (**18**) gave (**20**) (1.52 g, 70%). M.p. 40–42°C; IR (film) 3402 br m, 2924 s, 2855 s, 1645 m, 1466 m, 1377 m, 1263 m, 993 w, 942 w; ^{1}H-NMR (200 MHz) 0.86 (6H, m, C*H*$_3$ and $CH_2CH(CH_3)CH$), 1.23–1.40 (34H, m, aliphatic), 1.45–1.60 (2H, br m, O*H* and $CH_2CH(CH_3)CH$), 3.96 (1H, m, $CH(CH_3)CH(OH)$), 5.17 (2H, m, $CHCH{=}CH_2$), 5.85 (1H, m, $CHCH{=}CH_2$); ^{13}C-NMR (50 MHz) 140.06 (C2), 139.32 (C1), 115.76 and 115.24 (C3, diastereoisomers), 77.39 and 76.91 (C4, diastereoisomers), 38.66 (C5?), 32.74 (unassigned), 32.04 (C20), 29.47–29.80 (C7–C19), 27.41 (C6?), 22.81 (C21), 15.02 and 14.43 (C4 methyl branch, diastereoisomers), 14.22 (C22); *m/z* (EI) 338 (M^+, 22%), 320 ($M-H_2O$). Found: M^+, 338.3508. $C_{23}H_{46}O$ requires M, 338.3549.

2.22. 3-Hydroxy-4-(S)-methyldocos-1-ene (**21**)

Reaction of vinyl magnesium bromide and (*S*)-2-methyleicosanal (**19**) gave (**21**) (1.72 g, 56%). M.p. 40–42°C; IR, ^{1}H- and ^{13}C-NMR as for compound (20); *m/z* (EI) 338 (M^+, 14%), 320 ($M-H_2O$). Found: M^+, 338.3584. $C_{23}H_{46}O$ requires M, 338.3549.

2.23. Preparation of R and S isomers of ethyl (E)-6-methyltetracos-4-enoate (**22**, **23**)

Isomers of 3-hydroxy-4-methyldocos-1-ene (**20**, **21**) were heated at 138°C for 1 h in triethyl orthoacetate (7 eq) and a small amount of propionic acid (0.06 eq). The products were extracted with diethyl ether (50 ml) and the diethyl ether layer washed with dilute hydrochloric acid (30 ml) followed by water (30 ml) and dried over anhydrous magnesium sulphate. The organic solvent was removed under reduced pressure and the crude product purified by flash column chromatography using petroleum ether (b.p. 60–80°C) and diethyl ether (9:1) as eluent to afford the title compounds as white solids.

2.24. Ethyl (E)-6-(R)-methyltetracos-4-enoate (**22**)

Reaction of triethyl orthoacetate and 3-hydroxy-4-(*R*)-methyldocos-1-ene (**20**) gave (**22**) (1.64 g, 89%). M.p. 25–26°C; $[\alpha]_D$ −9.9°C (c 10% in $CHCl_3$, 22°C); IR (film) 2924 s, 2855 s, 1741 s, 1466 m, 1371 w, 1346 w, 1246 w, 1174 m, 1041 m; ^{1}H-NMR (200 MHz) 0.85 (3H, t, *J* 6.5 Hz), 0.90 (3H, d, *J* 6.7 Hz, $CH_2CH(CH_3)CH{=}$), 1.22–1.60 (37H, m, aliphatic and $COOCH_2CH_3$), 2.01 (1H, br m, $CH_2CH(CH_3)CH{=}$), 2.29 (4H, br m, ${=}CH(CH_2)_4COOCH_2CH_3$), 4.10 (2H, q, *J* 7.1 Hz, $COOCH_2CH_3$), 5.30 (2H, m, $CHCH{=}CHCH_2$); ^{13}C-NMR (50 MHz) 173.30 (C1), 137.92 (C5), 126.06 (C4), 60.24 (ethyl ester CH_2), 37.12, 36.69 and 34.59 (C2, C6 and C7?), 31.98 (C22), 29.42–29.76 (C9–C21), 28.01 and 27.35 (C3 and C8?), 22.75 (C23), 20.80 (C6 methyl branch), 14.30 (ethyl ester CH3), 14.16 (C24); *m/z* (EI) 408 (M^+, 18%), 362 ($M-CH_3CH_2OH$). Found: M^+, 408.3943. $C_{27}H_{52}O_2$ requires M, 408.3967.

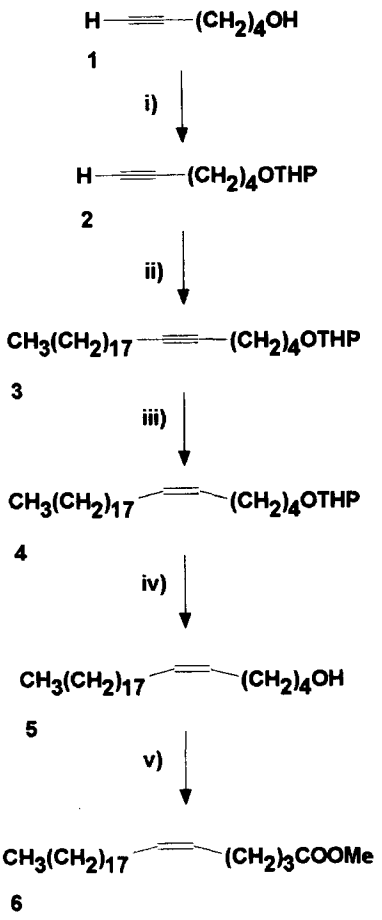
2.25. Ethyl (E)-6-(S)-methyltetracos-4-enoate (**23**)

Reaction of triethyl orthoacetate and 3-hydroxy-4-(*S*)-methyldocos-1-ene (**21**) (1.56 g, 76%). M.p. 25–26°C; $[\alpha]_D$ +9.5° (c 10% in $CHCl_3$, 21°C); IR, ^{1}H- and ^{13}C-NMR as for compound (22); *m/z* (EI) 408 (M^+, 36%), 362 ($M-CH_3CH_2OH$). Found: M^+, 408.3991. $C_{27}H_{52}O_2$ requires M, 408.3967.

3. Results and discussion

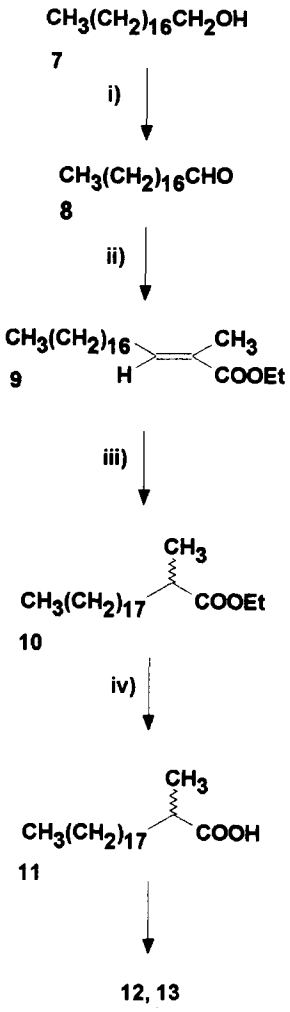
3.1. Synthesis of methyl (Z)-tetracos-5-enoate (**6**)

The synthesis of methyl (*Z*)-tetracos-5-enoate (**6**) is outlined in Scheme 1. The method is based on a synthetic strategy used by Gilman and Holland [14], who prepared 5-tetradecynoic acid from a terminal acetylene and a haloalkane. The



Scheme 1. Synthesis of methyl (*Z*)-tetracos-5-enoate. (i) 3,4-Dihydro-2*H*-pyran, pyridinium-*p*-toluenesulphonate, dichloromethane; (ii) 1-bromooctane, n-butyl lithium, hexamethylphosphorus triamide, diethyl ether; (iii) hydrogen, Lindlar catalyst, diethyl ether; (iv) pyridinium-*p*-toluenesulphonate, methanol, toluene; (v) pyridinium dichromate, dimethylformamide, dichloromethane followed by 5% aqueous tetrabutylammonium hydroxide, iodomethane, dichloromethane.

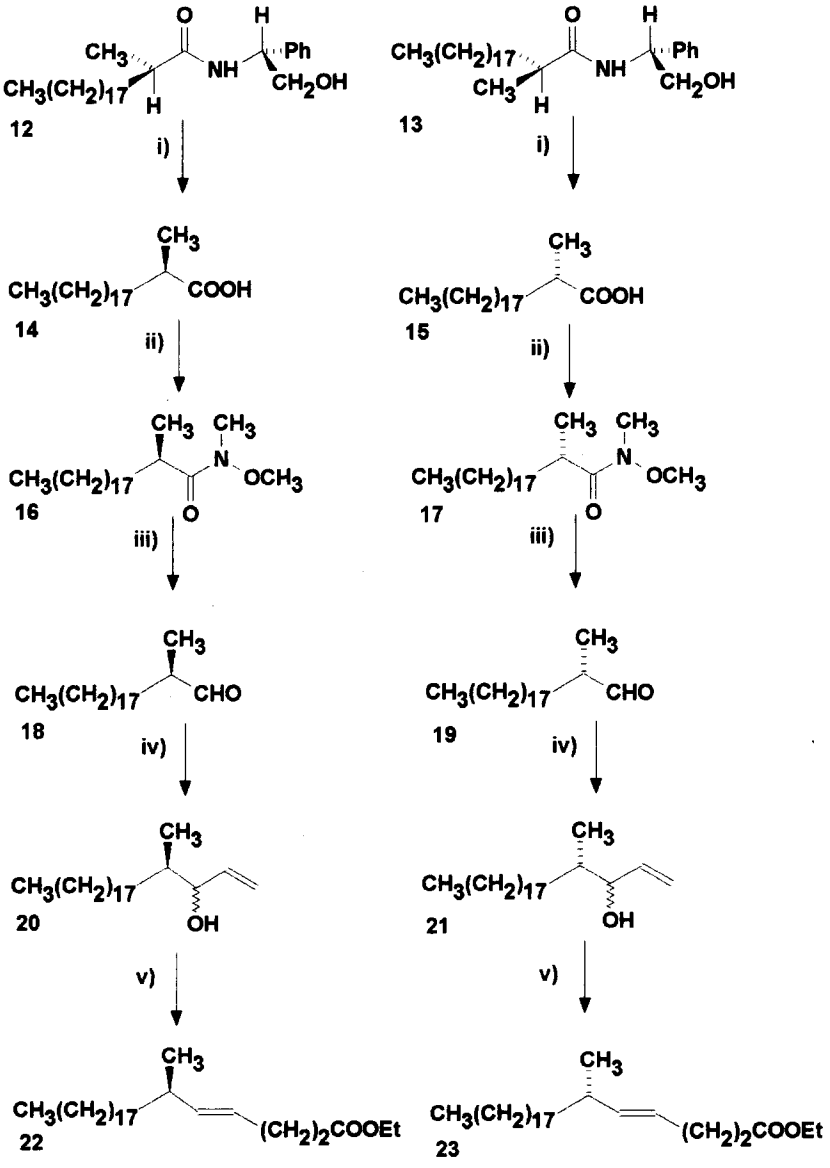
initial step involved the protection of 5-hexyn-1-ol (**1**) with 3,4-dihydro-2*H*-pyran in the presence of pyridinium-*p*-toluenesulphonate to afford 1-tetrahydropyranoloxyhex-5-yne (**2**) in high yield. The protected terminal alkyne (**2**) was then coupled with 1-bromooctadecane, according to a procedure used by Mori and Takeuchi [15]. The product, 1-tetrahydropyranoloxytetracos-5-yne (**3**), was obtained in 58% yield after column chromatography. The partial hydrogenation of (**3**) was achieved in a very high yield, using a Lindlar catalyst [16], to afford (*Z*)-1-tetrahydro-pyranoloxy-tetracos-5-ene (**4**). Deprotection of (**4**) using pyridinium-*p*-toluenesulphonate in methanol-toluene solution occurred readily to afford (*Z*)-tetracos-5-en-1-ol (**5**), which was oxidised with pyridinium dichromate [17] to give (*Z*)-tetracos-5-enoic acid. The carboxylic acid was then esterified, using phase transfer catalysis [18], to afford methyl (*Z*)-tetracos-5-enoate (**6**).



Scheme 2. Synthesis of diastereoisomers of *N*-[1-hydroxy-2-(*R*)-phenyl]ethyl-2-methyl-eicosamide. (i) Pyridinium chlorochromate, dichloromethane, dichloromethane; (ii) 1-carboethoxyethylidene triphenylphosphorane, dichloromethane; (iii) hydrogen, 10% palladium on carbon, diethyl ether; (iv) potassium hyroxide, methanol, toluene, water; (v) oxalyl chloride followed by *R*-(-)-2-phenylglycinol, 4-dimethylaminopyridine, dichloromethane.

3.2. Synthesis of ethyl (E)-6-(R)-methyltetracos-4-enoate (22) and ethyl (E)-6-(S)-methyltetracos-4-enoate (23)

The synthesis of the title compounds (**22**, **23**) is outlined in Schemes 2 and 3. The initial reaction was the oxidation of 1-octadecanol (**7**) to 1-octadecanal (**8**) with pyridinium chlorochromate [19]. The next step involved the Wittig reaction of 1-octadecanal (**8**) with 1-carboethoxyethylidene triphenylphosphorane [20] to afford ethyl (*E*)-2-methyl-eicos-2-enoate (**9**) as the major product.



Scheme 3. Synthesis of *R* and *S* isomers of ethyl (*E*)-6-methyltetracos-4-enoate. (i) Sulphuric acid, dioxane, water; (ii) oxalyl chloride followed by *N,O*-dimethylhydroxylamine, 4-dimethylaminopyridine, dichloromethane; (iii) lithium aluminium hydride, THF; (iv) vinyl magnesium bromide, THF; (v) triethyl orthoacetate, propionic acid.

The stereochemistry of (**9**) was confirmed by the chemical shift of the olefinic proton according to Matter et al. [21]. The (*E*)- to (*Z*) ratio was determined after column chromatography of the reaction mixture to be 13:1. Since the next step of the synthesis involved hydrogenation of the double bond, the (*E*) and (*Z*) products were not separated when the reaction was repeated on a large scale. Hydrogenation of the (*E*) and (*Z*) products was achieved using a palladium catalyst, 10% on carbon. The hydrogenated ester (**10**) was then saponified using mild base to afford 2-methyleicosanoic acid (**11**).

The resolution of 2-methyl-eicosanoic acid (**11**) was achieved by reaction with the chiral amino-alcohol (*R*)-(-)-2-phenylglycinol [20] and liquid adsorption chromatography of the resulting diastereomeric amides. The diastereoisomers (**12**) and (**13**) were well separated by thin layer chromatography (R_f 0.54 and 0.32, respectively), and they were obtained in 34 and 43% yield after column chromatography. The chiral identity of each diastereoisomer was assigned according to the rules of Helmchen et al. [22]. The amides (**12**) and (**13**) were then cleaved using mild acid hydrolysis [22] to afford (*R*)-2-methyleicosanoic acid (**14**) and (*S*)-2-methyleicosanoic acid (**15**), respectively. After recrystallisation from methanol, the acids (**14**) and (**15**) gave optical rotation values of −8.8° and +8.4°, respectively.

The conversion of the acids (**14**, **15**) to aldehydes was achieved by the preparation of *N*-methoxy-N-methylamide derivatives [23] (**16**, **17**), followed by lithium aluminium hydride reduction of the amides at −78°C [22] to afford (*R*)-2-methyleicosanal (**18**) and (*S*)-2-methyleicosanal (**19**), respectively. No over-reduction products of the amides were detected by TLC. The conversion is thought to proceed through a very stable metal-chelated intermediate [23], which accounts for the observed lack of over-reduction of the amide to the alcohol.

The aldehydes (**18**) and (**19**) were allowed to react with vinyl magnesium bromide to afford 3-hydroxy-4-(*R*)-methyldocos-1-ene (**20**) and 3-hydroxy-4-(*S*)-methyldocos-1-ene (**21**), respectively. The allylic alcohols were converted to the target compounds (**22**) and (**23**) via a version of the Claisen rearrangement. The method [24,25] simply involved heating the allylic alcohols (**20**) and (**21**) with an excess of triethyl orthoacetate in the presence of a trace of weak acid. Initially a mixed orthoester is formed, which loses ethanol to form a ketene acetal that rearranges to the olefinic esters (**22**) and (**23**). Model studies [24,25] established the stereoselectivity of the (*E*)-disubstituted olefinic bonds formed by this reaction as being greater than 98%. Optical rotations of (**26**) and (**27**) were recorded as −9.9° and +9.5°, respectively.

The possible role of the three fatty acids in the biosynthesis of mycolic acids has been studied using an extract from *Mycobacterium smegmatis* [10]. The only acid to stimulate the incorporation of radioactive label from [1-^{14}C]acetate into mycolic acids was (*Z*)-tetracos-5-enoic acid. This confirms that this acid is a mycolic acid precursor, but further studies will be needed to find a specific methyl-branched precursor of oxygenated mycolic acids.

4. Acknowledgements

The study was supported by a grant to D.E.M., P.R.W. and C.R. from the Medical Research Council (G89000176SB) and a British Leprosy Relief Association studentship to G.S.B. Dr. R.F.W. Jackson is thanked for invaluable assistance in devising synthetic strategies. R.M. Hall and M. Morpurgo carried out preliminary synthetic studies.

5. References

1 D.E. Minnikin (1982) in: C. Ratledge and J.L. Stanford, (Eds.) The Biology of the Mycobacteria, Vol. 1, Academic Press, London, pp. 95–184.
2 P.J. Brennan (1988) in: C. Ratledge and S.G. Wilkinson (Eds.), Microbial Lipids, Vol. 1, Academic Press, London, pp. 203–298.
3 K. Takayama and N. Qureshi (1984) in: G.P. Kubica and L.G. Wayne (Eds.), The Mycobacteria: a Sourcebook, Part A, Marcel Dekker, New York, pp. 315–344.
4 F.G. Winder (1982) in: C. Ratledge and J. Stanford (Eds.), The Biology of the Mycobacteria, Vol. 1, Academic Press, London, pp. 353–438.
5 D.E. Minnikin (1987) in: M. Hooper (Ed.), Chemotherapy of Tropical Diseases (Critical Reports on Applied Chemistry, Vol. 21), Wiley, Chichester, pp. 19–43.

6 A.H. Etémadi and J. Gasche (1965) Bull. Soc. Chim. Biol. 47, 2095–2104.
7 D.E. Minnikin, S.M. Minnikin and M. Goodfellow (1982) Biochim. Biophys. Acta 712, 616–620.
8 M. Daffé, M.A. Lanéelle, G. Puzo and C. Asselineau (1981) Tetrahedron Lett. 4515–4516.
9 C. Lacave, M.A. Lanéelle, M. Daffé, H. Montrozier, M.P. Rols and C. Asselineau (1987) Eur. J. Biochem. 163, 369–378.
10 P.R. Wheeler, G.S. Besra, D.E. Minnikin and C. Ratledge (1993), Biochim. Biophys. Acta, 1167, 182–188.
11 F.D. Gunstone, M.R. Pollard, C.M. Scrimgeour, N.W. Gilman and B.C. Holland (1976) Chem. Phys. Lipids 17, 1–13.
12 F.D. Gunstone, M.R. Pollard, C.M. Scrimgeour and H.S. Vedanayagam (1977) Chem. Phys. Lipids 18, 115–129.
13 R.W. Evans and H. Sprecher (1985) Chem. Phys. Lipids 38, 327–342.
14 N.W. Gilman and B.C. Holland (1974) Chem. Phys. Lipids 13, 239–248.
15 K. Mori and T. Takeuchi (1988) Tetrahedron 44, 333–342.
16 E.N. Marvell and T. Li (1973) Synthesis, 457–468.
17 E.J. Corey and G. Schmidt (1979) Tetrahedron Lett. 399–402.
18 O. Gyllenhaal and H. Ehrsson (1975) J. Chromatogr. 107, 327–333.
19 E.J. Corey and J.W. Suggs (1975) Tetrahedron Lett. 2647–2650.
20 I.D. Doyle, R.A. Massy-Westropp and R.F.O. Warren (1986) Synthesis, 845–847.
21 U.E. Matter, C. Pascual, E. Pretsch, A. Pross, W. Simon and S. Sternhell (1969) Tetrahedron 25, 691–697.
22 G. Helmchen, G. Nill, D. Flockerzi, W. Schühle and M.S.K. Youssef (1979) Angew. Chem., Int. Ed. Engl. 18, 62–63.
23 S. Nahm and S.N. Weinreb (1981) Tetrahedron Lett. 3815–3818.
24 W.S. Johnson, L. Werthemann, W.R. Bartlett, T.J. Brocksom, Tsung-tee Li, D.J. Faulkner and M.R. Petersen (1970) J. Am. Chem. Soc. 92, 741–743.
25 D.J. Faulkner and M.R. Petersen (1973) J. Am. Chem. Soc. 95, 553–563.