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Note

Synthetic mirror cord factors: Protected dimycolyl esters of an α,α-(1 ↔ 1)-bisheptosiduronic acid

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We have recently described[1] the synthesis of "mirror" corynocord factors, namely, dicorynomycolyl esters (**2**) of the bis-heptosiduronic acid **1**. To complement that work, we approached the synthesis of analogous dimycolyl esters (**3**). Such esters are structurally related to mycobacterial cord factors (α,α-trehalose 6,6′-dimycolates) but differ from these in possessing regioinverted ester functionalities. It should be interesting to compare bioactivities of synthetic mirror compounds with some of the multifaceted biological manifestations of exposure to natural cord factors[2].

Mycolic acids are high-molecular weight, aliphatic β-hydroxy acids substituted at the α-position with a long *n*-alkyl branch ($C_{22}H_{45}$ or $C_{24}H_{49}$). Many variants exist that differ in methylene homology and other structural features of the main chain, which may contain cyclopropane rings, a methoxy substituent, a methyl branch, a keto group, or other constitutional elements, depending on the mycobacterial species of origin[2]. The specimen of mycolic acid used in the present work originated[3] from dried bacillary residues of *Mycobacterium tuberculosis* H37Rv, and represented a mixture having two principal components which had been revealed[4] by mass spectrometry to belong to the dicyclopropano series of acids (**6a**, earlier designated[5] "alpha" or[6] Type I), and the methoxycyclopropano series (**6b**, Type II)[6a,b,7], respectively; both series are typical of mycolic acids encountered in human tubercle bacilli[2].

The mycolic acids were esterified with diazomethane, and column chromatography of the resulting mixture led to the isolation of two methyl mycolates in almost equal proportions (yields, 43 and 40%), although each contained traces of the other. From the methyl esters as well as from derivatives subsequently prepared we obtained clear NMR-spectroscopic evidence for the structures involved. The data for the slightly less polar component were as expected for the methyl ester (**7a**) of

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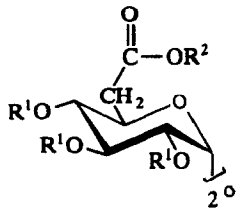
type I acids (**6a**), whereas those for the other component agreed with the formula for the ester **7b** of type II acid **6b**. Both esters were benzylated by use of benzyl trichloroacetimidate in the presence of triflinic acid[8] to give the 3-benzyl ethers **8a** and **8b**, respectively, reduction of which by lithium triethylborohydride furnished the 3-*o*-benzylmycolyl alcohols **9a** and **9b**. Mitsunobu esterification[9] of the 2,3,4,2′,3′,4′-hexabenzyl ether[1] **4** of the diacid **1** with **9a** and **9b** afforded the benzyl-protected mirror cord factors **5a** and **5b**.



1 $R^1 = R^2 = H$

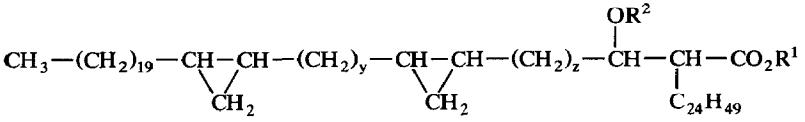
2 $R^1 = H$, R^2 = corynomycolyl

3 $R^1 = H$, R^2 = mycolyl

4 R^1 = Bn, $R^2 = H$

5a R^1 = Bn, R^2 = 3-*O*-benzylmycolyl-I

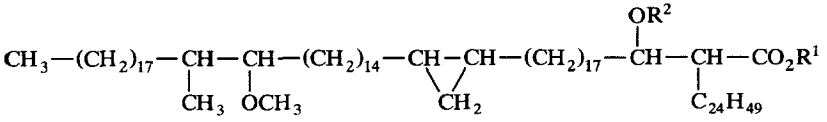
5b R^1 = Bn, R^2 = 3-*O*-benzylmycolyl-II



6a $R^1 = R^2 = H$ y = 14, 16

7a R^1 = Me, $R^2 = H$ z = 9, 11, 13

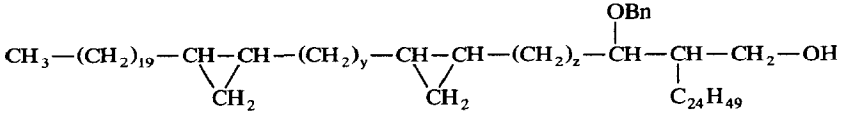
8a R^1 = Me, R^2 = Bn



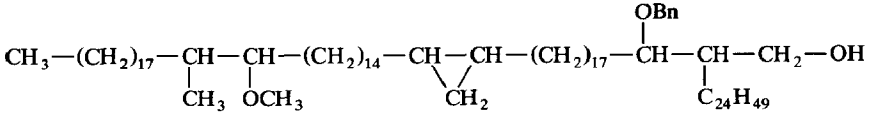
6b $R^1 = R^2 = H$

7b R^1 = Me, $R^2 = H$

8b R^1 = Me, R^2 = Bn



9a 3-*O*-Benzylmycolyl-I alcohol



9b 3-*O*-Benzylmycolyl-II alcohol

Owing to the limited quantity of mycolic acids that was available for these studies, only small amounts (~ 10 mg) of **5a** and **5b** were finally in hand, insufficient for experiments aimed at deprotection to produce free mirror cord factors (**3**). However, debenzylation by catalytic hydrogenolysis has been achieved in the course of syntheses of[1] **2** and of numerous carbohydrate mycolates (cord factor analogs)[3,4,10], and should also be applicable in the present case.

EXPERIMENTAL

General methods.—General procedures were the same as those previously employed[1]. The mycolic acid mixture was prepared[3] and kindly donated by Dr. A. Liav. Solvents for TLC and column chromatography were mixtures (v/v) of EtOAc and hexanes: 1:8 (*A*), 1:10 (*B*), 1:100 (*C*), 1:200 (*C*), 1:400 (*E*), and 1:600 (*F*). The ^{1}H NMR data were obtained with $CDCl_3$ solutions, chemical shifts being measured from the chloroform signal at δ 7.24.

Methyl mycolates I (**7a**) *and II* (**7b**).—Mixed mycolic acids[3] (120 mg) were dissolved in a freshly prepared, ethereal solution of CH_2N_2. Immediate evolution of N_2 occurred, and after 10 min TLC (solvent *A*) showed two major spots (R_f 0.2 and 0.25) for methyl esters and several faster-moving trace spots, whereas mycolic acid (R_f 0.0) was absent. The excess of CH_2N_2 was decomposed by addition of EtOAc containing 1% of AcOH, the solvent was evaporated, and the residue dissolved in hexane. The solution was washed with water, dried (Na_2SO_4), and concentrated. The crude ester (~ 130 mg) was subjected to column chromatography with pure hexane followed by solvents *F*, *D*, and *B* as eluents. After elution of small amounts of the unidentified, fast-moving components, fractions of almost pure **7a** (52 mg, 43%) and **7b** (50 mg, 40%) were obtained, although the separation was not quite complete. ^{1}H NMR data (200 MHz): for **7a**, δ 3.68 (s, 3 H, CO_2Me), 3.64 (m, 1 H, H-3), 2.41 (m, 2 H, H-2 and O*H*), 1.68–1.09 (large peak, bulk of CH_2 groups), 0.85 (2 overlapping t, 6 H, 2 terminal Me), 0.57 and −0.35 (m, 8 H, 2 *cis*-cyclopropane rings); for **7b**, δ 3.68 (s, 3 H, CO_2Me), 3.64 (m, 1 H, H-3), 3.31 (s, 3 H, CHOC*H*$_3$), 2.94 (m, 1 H, C*H*OMe), 2.41 (m, 2 H, H-2 and O*H*), 1.7–1.1 (large peak, bulk of CH_2 groups), 0.85 and 0.82 (t and d, 9 H, 2 terminal and 1 branch Me), 0.58 and −0.35 (m, 4 H, *cis*-cyclopropane ring).

Methyl 3-O-benzylmycolates I (**8a**) *and II* (**8b**).—Compound **7a** (52 mg) dissolved in CH_2Cl_2 (1 mL) and cyclohexane (10 mL) was stirred for 2 days with benzyl trichloroacetimidate (75 mg) and CF_3SO_3H (20 μL). The reaction was quenched by addition of pyridine (0.5 mL), and after successive washings with water, M HCl, water, aq $NaHCO_3$, and water, the solution was dried and evaporated. The residue showed a strong spot for **8a** (R_f 0.66), a weak spot attributable to **8b** (R_f 0.55), and traces of less-mobile impurities (TLC with solvent *A*). Column chromatography employing gradient elution (solvents $E \rightarrow D \rightarrow C \rightarrow B$) furnished nearly homogeneous **8a** (43 mg, 82%); ^{1}H NMR (200 MHz): δ 7.34 (Ph), 4.47 (AB-q, 2 H, *J* 11.6 Hz, OC*H*$_2$Ph), 3.36 (s, 3 H, CO_2Me), 3.61 (m, 1 H, H-3), 2.61 (m, 1 H, H-2),

1.7–1.1 (large peak, bulk of internal CH_2 groups), 0.86 (t, 6 H, 2 terminal Me), 0.59 and −0.35 (m, 8 H, 2 cyclopropane rings); ^{13}C NMR (50.3 MHz, ADEPT): δ 175.2 (*C*O), 138.5 (C-1 of Ph), 128.2, 127.6, and 127.4 (Ph), 80.5 (C-3), 72.0 (CH_2Ph), 51.3 (CO_2CH_3), 49.8 (C-2), 31.9, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 30.9, 30.2, 30.0, and 29.6 (highest peak), 29.5, 29.4, 29.3, 28.7, 27.8, and 27.6 (bulk of internal chain carbons), 24.4 (possibly C-1 of α-branch), 15.7 and 10.8 (*C*H and CH_2 of 2 cyclopropane rings).

Application of the same procedure of benzylation and product separation to mycolate **7b** (50 mg) furnished **8b** (38 mg, 76%); ^1H NMR (300 MHz): δ 7.30–7.24 (Ph), 4.48 (AB-q, 2 H, *J* 11.2 Hz, CH_2Ph), 3.64 (s, 3 H, CO_2Me), ~3.61 (m, 1 H, H-3), 3.32 (s, 3 H, CHOCH_3), 2.94 (m, 1 H, C*H*OMe), 2.64 (m, 1 H, H-2), 1.65–1.0 (m, and large peak at 1.24, bulk of internal CH_2 groups), 0.88–0.81 (t and d, 9 H, 2 terminal and 1 branch CH_3 groups), 0.64–0.50 (2 m, 3 H total), and −0.35 (m, 1 H) for 1 cyclopropane ring; ^{13}C NMR (75.43 MHz, ADEPT): δ 175.2 (*C*O), 138.5 (C-1 of Ph), 128.2, 127.6, and 127.4 (Ph), 85.4 (*C*HOMe), 80.5 (C-3), 72.0 (CH_2Ph), 57.6 (CHOCH_3), 51.3 (CO_2CH_3), 49.8 (C-2), 35.2 (*C*HMe), 31.9, 22.6, and 14.1 (terminal $CH_2CH_2CH_3$), 32.3–26.1 (several peaks, the highest at 29.6; bulk of internal chain carbons), 24.4 (possibly C-1 of α-branch), 14.4 (CHCH_3), 15.7 and 10.8 (*C*H and CH_2 of cyclopropane ring).

3-O-Benzylmycolyl alcohols I (**9a**) *and II* (**9b**).—To a solution of **8a** (43 mg) in oxolane (0.5 mL, freshly distilled from Na) was added M $LiBHEt_3$ in oxolane (0.5 mL), by syringe at 0°C. The mixture was kept at room temperature for 30 min, after which TLC (solvent *B*) revealed complete consumption of the ester (R_f 0.7) and showed a single spot for **9a** (R_f 0.4). Several drops of MeOH were added and the solvent was evaporated. A solution of the residue in EtOAc (10 mL) was washed successively with M HCl, water, aq $NaHCO_3$, and water, and evaporated with the addition of acetone. Column chromatography of the material with hexane as the initial eluent, followed by solvents *D*, *C*, and *B*, gave the alcohol **9a** (31 mg, 74%) as an amorphous solid. ^1H NMR (300 MHz): δ 7.33–7.24 (Ph), 4.52 (AB-q, 2 H, *J* 11.4 Hz, CH_2Ph), 3.85 (dd, $J_{1a,2}$ 2.6, $J_{1a,1b}$ 11.2 Hz, H-1a), 3.55 (dd, $J_{1b,2}$ 5.6 ($J_{1a,1b}$ 11.2 Hz, H-1b), 3.49 (m, H-3), 2.35 (br, O*H*), 1.7–1.0 (large m, highest peak at 1.24; bulk of internal CH_2 groups), 0.87 (t, 6 H, *J* 6.5 Hz, 2 terminal Me), 0.64–0.51 (2 m, 6 H) and −0.35 (m, 2 H) for 2 cyclopropane rings; ^{13}C NMR (75.43 MHz, verified by HETCOR): δ 138.3 (C-1 of Ph), 128.4, 127.75, and 127.65 (Ph), 83.4 (C-3), 72.2 (CH_2Ph), 63.3 (C-1), 42.6 (C-2), 31.9, 22.6, and 14.1 (terminal $CH_2CH_2CH_3$), 31.5–27.3 (12 peaks, the highest at 29.6, for internal CH_2 groups), 25.1 (possibly C-1 of α-branch), 15.7 (*C*H of cyclopropane, $^1J_{C,H}$ 154 Hz), and 10.9 (CH_2 of cyclopropane, $^1J_{C,H}$ 158 Hz).

Methyl ester **8b** (38 mg) was reduced as just described for **8a**, to give alcohol **9b** as an amorphous solid (29 mg, 78%) after chromatographic purification, R_f 0.35 (TLC with solvent *B*); ^1H NMR (200 mHz): δ 7.3–7.2 (Ph), 4.50 (AB-q, 2 H, *J* 11.3 Hz, CH_2Ph), 3.83 (dd $J_{1a,2}$ 1.9, $J_{1a,1b}$ 11.1 Hz, H-1a), 3.54 (dd $J_{1b,2}$ 5.5, $J_{1a,1b}$ 11.1 Hz, H-1b), 3.49 (m, H-3), 3.31 (s, 3 H, CHOCH_3), 2.94 (m, 1 H, C*H*OCH_3), 2.25

(br, O*H*), 1.7–1.1 (m, including large peak at 1.23; bulk of internal CH_2 groups), 0.88–0.80 (t and d, 9 H, 2 terminal and 1 branch CH_3), 0.68–0.52 (m, 3 H) and −0.35 (m, 1 H) for cyclopropane; ^{13}C NMR (50.3 MHz): δ 138.3 (C-1 of Ph), 128.4, 127.8, and 127.7 (Ph), 85.4 (*C*HOMe), 83.5 (C-3), 72.2 (CH_2Ph), 63.4 (C-1), 57.7 (OMe), 42.6 (C-2), 35.3 (*C*HMe), 31.9, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 32.3–26.2 (several peaks, the highest at 29.7; bulk of internal chain carbons), 25.2 (possibly C-1 of α-branch), 14.9 ($CHCH_3$), 15.8 and 10.9 (*C*H and CH_2 of cyclopropane ring).

*3-O-Benzylmycolyl-I [(3-O-benzylmycolyl-I 2,3,4-tri-O-benzyl-6-deoxy-α-*D*-gluco-heptopyranosyluronate) 2,3,4-tri-O-benzyl-6-deoxy-α-*D*-gluco-heptopyranosid]uronate* (**5a**) *and analog* **5b**.—A vessel was charged with bis-heptosiduronic acid[1] **4** (8 mg, 8.5 μmol), mycolyl alcohol **9a** (30 mg, 17.7 μmol), and PPh_3 (9 mg, 34 μmol), and the mixture was dried overnight in a high vacuum. An atmosphere of N_2 was provided and toluene (3 mL, dried over 4A molecular sieves) was added at 0°C, followed by diisopropyl azodicarboxylate (7 μL, 34 μmol). The mixture was stirred at room temperature overnight and then processed by washing it with water, drying (Na_2SO_4), and evaporation of the solvent. Preparative TLC (solvent *B*) of the residue gave diester **5a** (12 mg, 33%) and unreacted **9a** (11 mg). Compound **5a** had $[\alpha]_D$ +20.3° (*c* 0.4, $CHCl_3$); ^1H NMR (300 MHz, COSY; data refer to one substituted uronate moiety): sugar and benzyl protons, δ 7.34–7.14 (m, 2 O*H*, 4 Ph), 5.46 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.87, 4.83, 4.75, and 4.28 (centers of 4 AB-q, 8 H, *J* 11.7, 11.5, 11.8, and 11.6 Hz, 4 CH_2Ph), 4.34 (dt, 1 H, $J_{4,5} = J_{5,6b} = 9.8$, $J_{5,6a}$ 3.1 Hz, H-5), 4.13 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), 3.60 (dd, 1 H, $J_{1,2}$ 3.3, $J_{2,3}$ 9.4 Hz, H-2), 3.305 (t, 1 H, $J_{3,4} + J_{4,5} = 19.4$ Hz, H-4), 2.63 (dd, 1 H, $J_{5,6a}$ 2.7, $J_{6a,6b}$ 16.1 Hz, H-6a), 2.32 (dd, 1 H, $J_{5,6b}$ 9.6, $J_{6a,6b}$ 16.2 Hz, H-6b); mycolyl protons, δ 4.09 (dd, 1 H, *J* 5.6 and 11.2 Hz, H-1a), 4.00 (dd, 1 H, *J* 6.0 and 11.2 Hz, H-1b), 3.32 (nm, 1 H, H-3), 1.81 (m, 1 H, H-2), 1.57 (m), 1.34 (m), 1.23 (large peak), and 1.12 (m) for internal chain protons, 0.86 (t, 6 H, *J* 6.7 Hz, 2 terminal CH_3), 0.63–0.50 (2 m, 6 H), and −0.36 (m, 2 H) for 2 cyclopropane rings; ^{13}C NMR (75.43 MHz): δ 170.9 (*C*O), 138.8 and 138.4 (C-1 of Ph), 128.2–127.2 (multiple peaks, Ph), 90.4 (C-1), 81.2, 81.0, 79.9, and 79.1 (C-3 of mycolyl and C-2,3,4 of sugar), 75.4, 74.6, and 72.7 (3 PhCH_2 on sugar), 71.6 (PhCH_2 on mycolyl), 67.2 (C-5), 64.6 (C-1 of mycolyl), 40.2 (C-2 of mycolyl), 36.3 (C-6), 31.9, 22.6, and 14.1 (terminal $CH_2CH_2CH_3$), 30.5, 30.2 and 29.6 (highest peak), 29.5, 29.3, 28.7, and 27.4 (internal CH_2), 25.6 (C-1 of α-branch), 15.7 and 10.9 (*C*H and CH_2 of cyclopropane rings).

Esterification of the diacid **4** (3 mg) with alcohol **9b** (10 mg) in the presence of diisopropyl azodicarboxylate (3 μL) and PPh_3 (3 mg) as just described for **9a** gave the analogous diester **5b** (8 mg, 63%) after chromatographic purification. Its ^1H NMR spectrum (300 MHz) showed in the δ 7.3–3.5 and 2.7–1.0 regions the same pattern as that of **8a**. Significant differences were the following: the multiplet at δ 3.34–3.31 (H-3 of mycolyl and H-4 of sugar) was superposed on a 3-proton singlet (δ 3.32) for OMe; a multiplet was present at δ 2.94 for C*H*OMe; a signal at

0.88–0.81 consisted of a triplet and a doublet (total intensity, 9 H) for 2 terminal and 1 branch CH_3 groups; and the multiplets at δ 0.69–0.52 and −0.36 integrated jointly to 4 protons, indicating a single cyclopropane ring in the mycolyl chain.

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REFERENCES

1 H.H. Baer, Y. Shen, and X. Wu, *Carbohydr. Res.*, 241 (1993) 117–129.
2 (a) C. Asselineau and J. Asselineau, *Prog. Chem. Other Lipids*, 16 (1978) 59–99; (b) E. Lederer, in G. Kubica and L.G. Wayne (Eds.), *The Mycobacteria. A Sourcebook*, Marcel Dekker, New York, 1984, pp 361–378; (c) N. Rastogi and H.L. David, *Biochimie*, 70 (1988) 1101–1120; (d) M.B. Goren, in M. Kates (Ed.), *Handbook of Lipid Chemistry*, Vol. 6, Plenum Press, New York, 1990, pp 363–461.
3 A. Liav and M.B. Goren, *Carbohydr. Res.*, 125 (1984) 323–328.
4 A. Liav, M.B. Goren, Y.-M. Yang, and H.M. Fales, *Carbohydr. Res.*, 155 (1986) 223–228.
5 A.H. Etemadi, A.M. Miquel, E. Lederer, and M. Barber, *Bull. Soc. Chim. Fr.*, (1964) 3274–3276; A.H. Etemadi and E. Lederer, *ibid.*, (1965) 2640–2645; A.H. Etemadi, *J. Chem. Soc., Chem. Commun.*, (1967) 1074–1075; C. Asselineau, H. Montrozier, and J.C. Prome, *Bull. Soc. Chim. Fr.*, (1969) 592–596.
6 D.E. Minnikin and N. Polgar, (a) *Tetrahedron Lett.*, (1966) 2643–2647; (b) *J. Chem. Soc., Chem. Commun.*, (1966) 648–649; (c) *ibid.*, (1967) 313–314; (d) *ibid.*, (1967) 915–918.
7 D.E. Minnikin and N. Polgar, *J. Chem. Soc., Chem. Commun.*, (1967) 1172–1174; A. Adam, M. Senn, E. Vilkas, and E. Lederer, *Eur. J. Biochem.*, 2 (1967) 460–468; L.A. Davidson, P. Draper, and D.E. Minnikin, *J. Gen. Microbiol.*, 128 (1982) 823–828.
8 H.P. Wessel, T. Iversen, and D.R. Bundle, *J. Chem. Soc., Perkin Trans. 1*, (1985) 2247–2250.
9 O. Mitsunobu, *Synthesis*, (1981) 1–28.
10 A. Liav and M.B. Goren, *Carbohydr. Res.*, 129 (1984) 121–129; *ibid.*, 155 (1986) 229–235; A. Liav, H.M. Flowers, and M.B. Goren, *ibid.*, 133 (1984) 53–58.