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CORD-FACTOR ANALOGS: SYNTHESIS OF 6,6′-DI-*O*-MYCOLOYL- AND -CORYNOMYCOLOYL-(α-D-GALACTOPYRANOSYL α-D-GALACTOPYRANOSIDE)

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ABSTRACT

Appropriate solvolysis of 2,3,2′,3′-tetra-*O*-benzyl-4,6,4′,6′-tetra-*O*-mesyl-α,α-trehalose gave 2,3,2′,3′-tetra-*O*-benzyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**2**). Selective tosylation or mesylation of **2** respectively gave the 6,6′-ditosylate (**3**) and 6,6′-dimesylate (**4**), the structures of which were confirmed by the ^{1}H-n.m.r. spectra of the corresponding 4,4′-di-*O*-acetyl derivatives. Treatment of **3** with potassium mycolate in toluene, and subsequent hydrogenolysis, gave the 6′-mycolate 6-tosylate derivative. Treatment of **3** with potassium mycolate or potassium corynomycolate in hexamethylphosphoric triamide, followed by catalytic hydrogenolysis, yielded the respective cord-factor analogs 6,6′-di-*O*-mycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside) and 6,6′-di-*O*-corynomycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside). The same 6,6′-diesters were obtained from the 6,6′-dimesylate **4**. Putative 4,6-anhydro-6′-monomycolates are also described.

INTRODUCTION

Our laboratory has been involved in the synthesis of cord factor (6,6′-di-*O*-mycoloyl-α,α-trehalose) and cord-factor analogs for the purpose of correlating their structures with their biological activities. We have synthesized several such analogs, in which diverse linkages betwen the core disaccharide and the lipid moieties were utilized (see ref. 1, and references cited therein). More recently, we have described the synthesis of a new type of cord-factor analog in which the natural sugar (α,α-trehalose) was replaced by the corresponding D-*manno* disaccharide[2].

We now describe the synthesis, from α,α-trehalose, of a suitably blocked α-D-galactopyranosyl α-D-galactopyranoside derivative, and its conversion into

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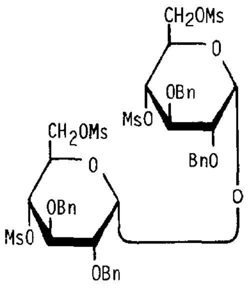
6,6′-di-*O*-mycoloyl- and -corynomycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside).

RESULTS AND DISCUSSION

Treatment of 2,3,2′,3′-tetra-*O*-benzyl-4,6,4′,6′-tetra-*O*-(methylsulfonyl)-α,α-trehalose[3] (**1**) with sodium benzoate in hexamethylphosphoric triamide, followed by catalytic deacylation, yielded 2,3,2′,3′-tetra-*O*-benzyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**2**) in good yield. Selective tosylation or mesylation of compound **2** gave the corresponding 6,6′-ditosylate (**3**) and 6,6′-dimesylate (**4**). The structures of **3** and **4** were confirmed by the 360-MHz, ^{1}H-n.m.r. spectra of the 4,4′-di-*O*-acetyl derivatives **5** and **6**. In both spectra, the H-4,4′ signal appeared at low field (δ 5.34 for compound **5**, and δ 5.50 for **6**), owing to the deshielding effect of the adjacent *O*-acetyl group. The small values of the coupling constants ($J_{4,3}$ 2.8, $J_{4,5}$ 0 Hz for compound **5**, and $J_{4,3}$ 3.2, $J_{4,5}$ 1.0 Hz for **6**) confirmed that compounds **5** and **6** had the *galacto-galacto* configurations. The structural assignment was also confirmed by double-irradiation experiments. The reaction of the 6,6′-disulfonate (either **3** or **4**) with potassium mycolate or potassium corynomycolate was studied under various conditions. In our earlier experience, the conversion of the mannopyranosyl mannopyranoside analog proceeded satifactorily, to give the corresponding 6,6′-diesters[2]. However, the reaction of the galactopyranosyl galactopyranoside derivatives was more complex. Under relatively mild conditions (in toluene, at 90°, in the presence of a crown ether) essentially insignificant conversion of the ditosylate **3** into the 6,6′-di-*O*-mycoloyl ester was achieved; instead, following catalytic hydrogenolysis, the 6′-mycolate 6-sulfonate derivative **7** was isolated as the major product.

When the 6,6′-ditosylate **3** was treated with potassium mycolate under more-forcing conditions (in hexamethylphosphoric triamide at 120°), two products were obtained in almost equal proportions: the desired 2,3,2′, 3′-tetra-*O*-benzyl-6,6′-di-*O*-mycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**8**) and a by-product that contained one mycoloyl and no sulfonyl group. Catalytic hydrogenolysis of this product afforded a homogeneous compound that differed in mobility from the monomycolate monosulfonate **7**; its elemental analysis was in agreement with that of a *monoanhydro-monomycolate*.

Formation of a 3,6-anhydro derivative from 6,6′-di-*O*-*p*-tolylsulfonyl-α,α-trehalose and potassium corynomycolate had already been observed[4]. In compound **3**, however, OH-3 is blocked by a benzyl group, so that the by-product should be a 4,6-anhydride, a structure that, to the best of our knowledge, has not previously been described for such compounds. Similarly, when the 6,6′-ditosylate **3** was treated with potassium corynomycolate, the desired 2,3,2′,3′-tetra-*O*-benzyl-6,6′-di-*O*-corynomycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**9**) was accompanied by a similar by-product, again judged to be a 4,6-anhydro-6′-corynomycolate. The same mixtures of 6,6′-diesters and 4,6-anhydrides were



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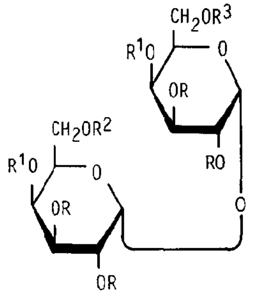
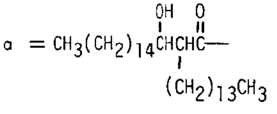


	R	R^1	R^2	R^3
2	Bn	H	H	H
3	Bn	H	Ts	Ts
4	Bn	H	Ms	Ms
5	Bn	Ac	Ts	Ts
6	Bn	Ac	Ms	Ms
7	H	H	Ts	mycoloyl
8	Bn	H	mycoloyl	mycoloyl
9	Bn	H	a	a
10	H	H	mycoloyl	mycoloyl
11	H	H	a	a

Bn = CH_2Ph

Ms = O_2SMe



$\mathrm{a} = CH_3(CH_2)_{14}\overset{OH}{\overset{|}{C}}H\underset{(CH_2)_{13}CH_3}{\underset{|}{C}}H\overset{O}{\overset{\|}{C}}—$

Ts = $O_2SC_6H_4Me$-*p*

obtained when the dimesylate **4** was treated with potassium mycolate or potassium corynomycolate under the conditions just described. Catalytic hydrogenolysis of the benzylated diesters **8** and **9** gave the respective cord-factor analogs, 6,6′-di-*O*-mycoloyl- (**10**) and -corynomycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**11**). Animal studies indicate that the *galacto-galacto* analog is less than one-tenth as toxic in mice as 6,6′-di-mycoloyl-α,α-trehalose.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are not corrected. Optical rotations were determined with a Jasco DIP-140 polarimeter. Eastman-Kodak plates were used for thin-layer chromatography. Chromatography columns were packed with silica gel (Baker, No. 3405). Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee. N.m.r. spectra were recorded at 60 MHz with a Varian EM 360A spectrometer, and at 360 MHz with an NT 360 spectrometer, with tetramethylsilane as the internal standard and $CDCl_3$ as the solvent.

*2,3,2′,3′-Tetra-*O*-benzyl-(α-D-galactopyranosyl α-D-galactopyranoside)* (**2**). — A mixture of 2,3,2′,3′-tetra-*O*-benzyl-4,6,4′,6′-tetra-*O*-(methylsulfonyl)-α,α-trehalose[3] (**1**, 2.0 g) and sodium benzoate (6.0 g) in hexamethylphosphoric triamide (40 mL) was stirred for 20 h at 130°, cooled, diluted with water, and extracted with toluene. The extract was washed 6 times with water, dried (Na_2SO_4), and evaporated. The residue was applied to a column of silica gel, and the homogeneous product was eluted with 9:1 benzene–ether; yield, 2.25 g (99%) of presumed 4,6,4′,6′-tetra-*O*-benzoyl-2,3,2′,3′-tetra-*O*-benzyl-(α-D-galactopyranosyl α-D-galactopyranoside); n.m.r. data: δ 7.30–8.25 (40 H, aryl) and 5.50 (d, 2 H, *J* 3 Hz, H-1,1′).

Catalytic deacylation of this material (1.2 g) with sodium methoxide in 1:3 chloroform–methanol, followed by extraction of the product into chloroform, washing with water, and evaporation, gave an almost homogeneous product which was purified by column chromatography. Elution with 20:1 chloroform–methanol gave pure, syrupy **2** (0.65 g, 88%); $[\alpha]_D$ +131° (*c* 1.05 chloroform); n.m.r. data: δ 7.35–7.40 (20 H, aryl) and 5.40 (d, 2 H, *J* 3 Hz, H-1,1′).

Anal. Calc. for $C_{40}H_{46}O_{11} \cdot H_2O$: C, 66.65; H, 6.60. Found: C, 66.43; H, 6.55.

*2,3,2′,3′-Tetra-*O*-benzyl-6,6′-di-*O*-p-tolylsulfonyl-(α-D-galactopyranosyl α-D-galactopyranoside)* (**3**). — To a stirred solution of **2** (0.85 g) in pyridine (20 mL), cooled to 0°, was added *p*-toluenesulfonyl chloride (0.75 g, 3 mol. equiv.). The mixture was stirred for 24 h at room temperature, diluted with cold water, and extracted with toluene. The extract was washed successively with cold, M hydrochloric acid and a cold, aqueous solution of sodium hydrogencarbonate, dried, and evaporated; the product was purified by column chromatography. Elution with chloroform yielded amorphous **3** (1.0 g, 82%); $[\alpha]_D$ +85° (*c* 0.47, chloroform).

Anal. Calc. for $C_{54}H_{58}O_{15}S_2$: C, 64.16; H, 5.78; S, 6.34. Found: C, 64.06; H, 5.99; S, 6.11.

A trace (20 mg, 2.5%) of unchanged starting-material was recovered.

*2,3,2′,3′-Tetra-*O*-benzyl-6,6′-di-*O*-(methylsulfonyl)-(α-D-galactopyranosyl α-D-galactopyranoside)* (**4**). — Mesylation of **2** (1.15 g) under conditions similar to those described for **3**, using a 20% molar excess of methanesulfonyl chloride, gave a syrup (eluted by 19:1 chloroform–methanol from a column of silica gel); yield 1.34 g (95%); $[\alpha]_D$ +84° (*c* 0.84, chloroform).

Anal. Calc. for $C_{42}H_{50}O_{15}S_2$: C, 58.73; H, 5.87; S, 7.47. Found: C, 58.59; H, 5.63; S, 7.20.

*4,4′-Di-*O*-acetyl-2,3,2′,3′-tetra-*O*-benzyl-6,6′-di-*O*-p-tolylsulfonyl-(α-D-galactopyranosyl α-D-galactopyranoside)* (**5**).— The ditosylate **3** (30 mg) was acetyllated in the usual way with acetic anhydride–pyridine. The mixture was evaporated, and the residue was chromatographed. Elution with 3:2 hexane–ethyl acetate gave pure, syrupy **5** (30 mg, 92%); $[\alpha]_D$ +113° (*c* 0.83, chloroform); ^{1}H-n.m.r. data (360 MHz): δ 7.12–7.66 (m, 28 H, aryl), 5.34 (d, 2 H, $J_{3,4}$ 2.8, $J_{4,5}$ 0 Hz, H-4,4′), 4.95 (d, 2 H, $J_{1,2}$ 3.5 Hz, H-1,1′), 4.37–4.64 (m, 8 H, 4 CH_2), 4.31 (bt, 2 H, H-5,5′), 3.91 (dd, 2 H, $J_{5,6}$7.0, $J_{6a,6b}$ 9.9 Hz, H-6a,6′a), 3.82 (dd, 2 H, $J_{2,3}$ 10.0 Hz, H-3,3′), 3.76 (dd, 2 H, $J_{5,6b}$ 5.2 Hz, H-6b,6′b), 3.64 (dd, 2 H, H-2,2′), 2.35 (s, 6 H, CH_3-Ts), and 1.92 (s, 6 H, 2 AcO).

Anal. Calc. for $C_{58}H_{62}O_{17}S_2$: C, 63.60; H, 5.70; S, 6.34. Found: C, 63.70; H, 5.84; S, 6.12.

*4,4'-Di-O-acetyl-2,3,2',3'-tetra-O-benzyl-6,6'-di-O-(methylsulfonyl)-(α-*D*-galactopyranosyl α-*D*-galactopyranoside)* (**6**). — The dimesylate **4** (50 mg) was acetylated, and the product was purified as described for **5**; yield, 37 mg (67%); $[\alpha]_D$ +103° (*c* 1.1, chloroform); ^{1}H-n.m.r. data (360 MHz); δ 7.25–7.36 (m, 20 H, 4 phenyl), 5.50 (dd, 2 H, $J_{4,3}$ 3.2, $J_{4,5}$ 1.0 Hz, H-4,4'), 5.20 (d, 2 H, $J_{1,2}$ 3.5 Hz, H-1,1'), 4.50–4.84 (m, 8 H, 4 CH_2), 4.45 (broad dt, 2 H, H-5,5'), 4.09 (dd, 2 H, $J_{6a,5}$ 6.7, $J_{6a,6b}$ 10.9 Hz, H-6a,6'a), 4.02 (dd, 2 H, $J_{6b,5}$ 5.7 Hz, H-6b,6'b), 3.98 (dd, 2 H, $J_{2,3}$ 9.8 Hz, H-3,3'), 3.83 (dd, 2 H, H-2,2'), 2.91 (s, 6 H, 2 CH_3-Ms), and 2.11 (s, 6 H, 2 AcO).

Anal. Calc. for $C_{46}H_{54}O_{17}S_2$: C, 58.58; H, 5.77; S, 6.80. Found: C, 58.25; H, 5.69: S, 6.75.

*6-O-Mycoloyl-6'-O-*p*-tolylsulfonyl-(α-*D*-galactopyranosyl α-*D*-galactopyranoside* (**7**). — The ditosylate **3** (0.45 g) was treated with potassium mycolate[5] (1.9 g) in toluene (15 mL) in the presence of a crown ether[6] (18-crown-6, 0.4 g) for 18 h at 90°. The mixture was evaporated, and the residue was partially purified by column chromatography. The benzylated product was dissolved in 1:1 ethyl acetate–ethyl alcohol (100 mL), and hydrogenolyzed at 344 kPa in the presence of 10% Pd–C catalyst for 18 h. The catalyst was filtered off, and the filtrate was evaporated. The residue was chromatographed on silica gel. Elution with 19:1 chloroform–methanol removed various impurities, including some 18-crown-6. Elution with 9:1 chloroform–methanol yielded **7**, which solidified on trituration with methanol; yield, 0.6 g (77%); m.p. 50–52°,$[\alpha]_D$ +48° (*c* 1.0, chloroform); ^{1}H-n.m.r. data: δ 7.32–8.10 (m, aryl), 2.42 (s, CH_3-Ts), and 0.72–1.30 (m, mycoloyl group).

Anal. Calc. for $C_{10}H_{202}O_{15}S$: C, 72.76; H, 11.26; S, 1.88. Found: C, 72.45; H, 11.40; S, 1.68.

*2,3,2',3'-Tetra-O-benzyl-6,6'-di-O-mycoloyl-(α-*D*-galactopyranosyl α-*D*-galactopyranoside)* (**8**). — The ditosylate **3** (67 mg) was treated with potassium mycolate (260 mg) in hexamethylphosphoric triamide (3 mL) for 65 h at 120°. Ice and water were added, the precipitate was filtered off, and washed with water, and the excess of mycolic acid was removed by dissolving the precipitate in chloroform–methanol, and treating the solution with Bio-Rad AG-MPI (OH^-) resin. The resulting mycolic acid-free mixture was then chromatographed; elution with 5:1 hexane–ethyl acetate removed fast-moving impurities. Continued elution with the same solvent system, followed by 4:1 hexane–ethyl acetate, gave compound **8**; yield, 60 mg (29%); $[\alpha]_D$ +30° (*c* 1.2, chloroform).

Anal. Calc. for $C_{208}H_{370}O_{15}$: C, 80.29; H, 11.98. Found: C, 80.02; H, 12.07.

The column was then eluted with 3:1 hexane–ethyl acetate to give the other major product, considered to be the 4,6-anhydro-6'-*O*-mycoloyl derivative (see Discussion); yield, 38 mg (31%).

*2,3,2',3'-Tetra-O-benzyl-6,6'-di-O-corynomycoloyl-(α-*D*-galactopyranosyl α-*D*-galactopyranoside)* (**9**). — The 6,6'-dimesylate **4** (73 mg) was treated with potassium corynomycolate[7] (120 mg) in hexamethylphosphoric triamide (2 mL) for 18 h at 120°. The mixture was processed as already described, and the crude product was chromatographed. Elution with 5:1 hexane–ethyl acetate, followed by 4:1 hexane–ethyl acetate, gave the 6,6′-dicorynomycolate **9** (50 mg, 36%); $[\alpha]_D$ +63.5° (*c* 1.7 chloroform).

Anal. Calc. for $C_{104}H_{170}O_{15}$: C, 75.22; H, 10.32. Found: C, 75.04; H, 10.24.

Elution with 3:1 hexane–ethyl acetate gave the other major product (4,6-anhydro-6′-monocorynomycolate?); yield, 29 mg (30%).

6,6′-Di-O-mycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**10**). — The tetra-*O*-benzyl dimycolate **8** (46 mg) was dissolved in ethyl acetate (25 mL) and ethyl alcohol (10 mL), and hydrogenolyzed in the presence of 10% Pd–C catalyst (45 mg) for 7 h at 344 kPa. The catalyst was filtered off, washed with chloroform, and the filtrate evaporated. The residue was chromatographed on silica gel, and the product was eluted with 20:1 chloroform–methanol; yield, 28 mg (70%); $[\alpha]_D$ +37° (*c* 0.5, chloroform).

Anal. Calc. for $C_{180}H_{346}O_{15}$: C, 78.66; H, 12.60. Found: C, 78.43; H, 12.80.

6,6′-Di-O-corynomycolyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**11**). — The tetra-*O*-benzyl-6,6′-di-*O*-corynomycoloyl derivative **9** (44 mg) was hydrogenolyzed, and the product was isolated as just described. The crude product was purified by chromatography. Elution with 15:1 chloroform–methanol gave pure **11**; yield, 20 mg (59%); $[\alpha]_D$ +63.8° (*c* 0.6, chloroform).

Anal. Calc. for $C_{76}H_{146}O_{15}$: C, 70.22; H, 11.32. Found: C, 70.51; H, 11.29.

ACKNOWLEDGMENTS

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