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Note

Synthesis of 6-*O*-mycoloyl and 6-*O*-corynomycoloyl-α,α-trehalose

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For some years our laboratory has been interested in the synthesis of cord factor (6,6′-di-*O*-mycoloyl-α,α-trehalose) and cord-factor analogues, and several syntheses of this class of biologically important compounds have been described[1–5], involving 2,3,4,2′,3′,4′-hexa-*O*-benzyl-α,α-trehalose (**2**) as the key intermediate[2,3]. Compound **2** was obtained by successive tritylation of anhydrous trehalose, alkali-catalyzed perbenzylation with benzyl chloride, and detritylation of the purified or crude 2,3,4,2′,3′,4′-hexa-*O*-benzyl-6,6′-dityrityl trehalose (**1**) with 80% aqueous acetic acid. The resulting hexa-*O*-benzyltrehalose (**2**) was then purified from a byproduct by chromatography on silica gel[2,3]. The i.r. spectrum of the byproduct suggested that it was probably a 6-monoacetate (**3**) of hexa-*O*-benzyl trehalose (absorption at 1370, 1745, and 3300 cm^{-1}), and we were able to eliminate it and improve the yield of **2** considerably by brief alkaline hydrolysis of the detritylation mixture. However, we were intrigued by the surprising, apparent esterification that occurred during detritylation with aqueous acetic acid. When this presumed structural assignment (**3**) was unequivocally established (as described here) we found, as anticipated, that hexa-*O*-benzyltrehalose was directly convertible, in similar modest yield (27%), into the 6-monoacetate under the same conditions used for the detritylation of compound **1**. We also have preliminary evidence that trehalose is similarly converted into a 6-monoacetate.

[This curious, but useful, transformation was in fact described by Duff nearly 30 years ago; glucose and galactose were preferentially acetylated at O-6 with 50% aqueous acetic acid for ~24 h at 100° (yields of crude products ~30%)[6]. It is evidently a little-known reaction, but because of the ease with which the products can be obtained, it may be worthy of resurrection from its present obscurity.]

Compound **3** serves as an ideal starting material for synthesis of 6-*O*-mycoloyl- and 6-*O*-corynomycoloyl-trehalose, the isolation of which from various bacterial sources has been described[7–10]. 6′-*O*-Acetyl-6-monomycolates have also

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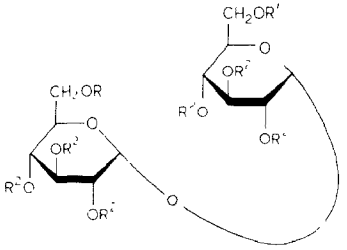
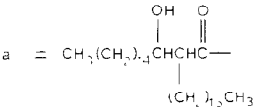


	R	R′	R″
1	Tr	Tr	Bn
2	H	H	Bn
3	H	Ac	Bn
4	Ms	Ac	Bn
5	Ms	H	Bn
6	mycoloyl	H	Bn
7	a	H	Bn
8	mycoloyl	H	H
9	a	H	H



been isolated[11]. Recent, ongoing studies in our laboratory on lipid extracts from a wide spectrum of mycobacteria suggested that what we suspect to be trehalose monomycolates may be ubiquitous in, and are evidently produced in large amount by, members of this genus. This anticipation was confirmed by the availability to us of the synthetic material. Cord factor and the presumed monomycolates are recognizable in t.l.c. of even quite complex lipid mixtures [silica gel plates are developed in 90:10:1 chloroform–methanol–water and then sprayed with 60% sulfuric acid containing a trace of orcinol (0.01%), and the plates are heated for ~5 min at 125–135°]. The monomycolates have R_F 0.06 as compared with R_F 0.45 for cord factor. The spots assume a vivid purple color that is quite characteristic and appears to be definitive[12]. We needed authentic samples of the presumed monomycolate to compare with the natural products in these extracts, and have therefore exploited the ready availability of **3** for synthesis of this material. This paper describes the synthesis of 6-*O*-mycoloyl-α,α-trehalose and the corresponding 6-*O*-corynomycoloyl-α,α-trehalose.

Hydrolysis of 2,3,4,2′,3′,4′-hexa-*O*-benzyl-6,6′-di-*O*-trityl-α,α-trehalose[2,3] (**1**) with 80% aqueous acetic acid followed by column chromatography gave 2,3,4,2′,3′, 4′-hexa-*O*-benzyl-α,α-trehalose **2** (53%), and 6-*O*-acetyl-2,3,4,2′,3′,4′-hexa-*O*-benzyl-α,α-trehalose (**3**, 24%). Alternatively, compound **2** may also be directly converted into **3** with aqueous acetic acid under the same conditions (27% yield), but with facile recovery of unaltered **2**. Treatment of **3** with methanesulfonyl chloride and subsequent *O*-deacetylation gave 2,3,4-2′,3′,4′-hexa-*O*-benzyl-6-*O*-methylsulfonyl-α,α-trehalose (**5**). Treatment of **5** with potassium mycolate[2,3] or potassium corynomycolate[13] gave the corresponding 6-monoesters **6** and **7**, respectively. Catalytic hydrogenolysis of **6** and **7** afforded 6-*O*-mycoloyl-α,α-trehalose (**8**) and 6-*O*-corynomycoloyl-α,α-trehalose (**9**), respectively.

Although n.m.r. spectra were recorded at 360 MHz, resolution of the carbohydrate protons was low because of overlap by the benzylic methylene resonances. Nevertheless, the presence monoacyl substitutents (acyloxy, mesyloxy) was clearly evident.

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. N.m.r. spectra were recorded at 360 MHz with an NT 360 spectrometer with tetramethylsilane as internal standard and $CDCl_3$ as solvent. Thin-layer chromatograms were run on Eastman Kodak silica gel plates. Chromatography columns were packed with silica gel (Baker No. 3404). Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Potassium corynomycolates and potassium mycolate (from M. tuberculosis). — Potassium corynomycolate was synthesized according to the method of Polonsky and Lederer[12], with minor modification: by sodium hydride-catalyzed Claisen condensation of methyl palmitate, reduction by sodium borohydride to the *erythro–threo* mixture of corynomycolates, and hydrolysis of the chromatographically purified esters (200 mg of esters, 1 mL of 1,4-dioxane, and 2 mL of 10% potassium hydroxide in boiling ethanol for 3 h). Pure potassium corynomycolate (140 mg) crystallized when the mixture was cooled in ice. An additional crop of 60 mg was obtained by concentrating the mother liquor.

Methyl mycolate was recovered from extensively delipidated, dried bacillary residues of *Mycobacterium tuberculosis* H37Rv. Residues (250 g) were heated for 4 h with vigorous stirring, under reflux in 700 mL of 5% methanolic potassium hydroxide. The hot mixture was filtered and the residue extensively washed with hot methanol to remove esters of lower molecular weight. Crude methyl mycolates (insoluble in boiling methanol) were recovered by prolonged Soxhlet extraction of the insoluble mass with diethyl ether; yield 30 g. Purified, mixed methyl mycolates were obtained by chromatography on silica gel, and converted into potassium mycolates by hydrolysis as already described, but with larger amounts of solvents. As useful alternative, 20 g of powdered potassium carbonate, 1.5 g of potassium hydroxide and 25 mL of methanol were stirred while 510 mg of methyl mycolates dissolved in 5 mL of ether was added dropwise. The flask was placed in a water bath and stirred while heating to evaporate the solvents and the residue was heated at 80°. The ester was essentially completely hydrolyzed in <30 min and potassium mycolate was recovered by dissolving away the inorganic materials with hot water. Traces of residual methyl mycolates were removable by chromatography if desired, but did not interfere in the ultimate conversions of mesylated carbohydrates into cord factors.

6-O-Acetyl-2,3,4,2′,3′,4′-hexa-O-benzyl-α,α-trehalose (**3**). — (*a*) 6,6′-Di-*O*-trityl-α,α-trehalose[2,3] was treated with benzyl chloride and the resulting crude hexa-*O*-benzyl derivative **1** subjected to hydrolysis with acetic acid as described before[2,3]. The mixture was evaporated and the residue was chromatographed. Elution with 1:1 ethyl acetate–hexane yielded a mixture of trityl salts. Continued elution with the same solvent system afforded 6-*O*-acetyl-2,3,4,2′,3′,4′-hexa-*O*-benzyl-α,α-trehalose (**3**, 360 mg, 24% yield based on 6,6′-di-*O*-trityl-α,α-trehalose);

$[\alpha]_D^{24}$ +88° (*c* 1.4, chloroform); n.m.r. data: δ 7.37–7.25 (6 benzylic phenyl groups), 5.15–4.55, 4.15–4.03, 3.59–3.52 (benzylic methylene protons and H-1,2,3,4,5,6), and 1.97 s, 3 H (OAc).

Anal. Calc. for $C_{56}H_{60}O_{12}$: C, 72.70; H, 6.53. Found: C, 72.47; H, 6.78.

Finally, the major product, 2,3,4,2′,3′,4′-hexa-*O*-benzyl-α,α-trehalose[2,3] (**2**) was eluted with 1:1 ethyl acetate–hexane followed by 3:2 ethyl acetate–hexane; yield: 755 mg (53%).

(*b*) 2,3,4,2′,3′,4′-Hexa-*O*-benzyl-α,α-trehalose (**2**, 210 mg) was treated with 80% aqueous acetic acid (15 mL) for 18 h at 75°. The mixture was evaporated and the residue chromatographed as already described. The monoacetate **3** was isolated in 27% yield (60 mg) and the hexa-*O*-benzyl derivative **2** was recovered in 44% yield (93 mg).

6-O-Acetyl-2,3,4,2′,3′,4′-hexa-O-benzyl-6′-O-(methylsulfonyl)-α,α-trehalose (**4**). — To a cold (ice-bath) solution of **3** (236 mg) in pyridine (2 mL) was added methanesulfonyl chloride (0.5 mL). The mixture was stirred for 30 min at 0° and for an additional 2 h at room temperature. Ice was then added and the mixture was extracted with ethyl acetate. The organic extract was successively washed with 2M hydrochloric acid, water, saturated sodium hydrogencarbonate, and water. It was dried (sodium sulfate) and evaporated to give the syrupy product **4** (238 mg, 93%) which was homogeneous in t.l.c. An analytically pure sample was obtained by chromatography. The product was eluted with 3:2 hexane–ether; $[\alpha]_D^{24}$ +86° (*c* 0.9, chloroform).

Anal. Calc. $C_{57}H_{62}O_{14}S$: C, 68.24; H, 6.22; S, 3.19. Found: C, 68.06; H, 6.22; S, 3.41.

2,3,4,2′,3′,4′-Hexa-O-benzyl-6-O-(methylsulfonyl)-α,α-trehalose (**5**). — Compound **4** (238 mg) was suspended in methanol (5 mL) and treated with M sodium methoxide solution (0.1 mL). The mixture was kept for 3 h at room temperature and made neutral with acetic acid. Evaporation of the mixture gave a syrupy product that was purified by chromatography. Elution with 1:1 ethyl acetate–hexane gave the pure product; yield 180 mg (79%), $[\alpha]_D^{24}$ +90° (*c* 0.9, chloroform); n.m.r. data: δ 7.35–7.25 (6 benzylic phenyl groups), 5.13–4.58, 4.25–3.95 and 3.61–3.51 (benzylic methylene protons and H-1,2,3,4,5,6), and 2.87 s, 3 H (Ms).

Anal. Calc. for $C_{55}H_{60}O_{13}S$: C, 68.73; H, 6.29; S, 3.33. Found: C, 68.43; H, 6.47; S, 3.14.

2,3,4,2′,3′,4′-Hexa-O-benzyl-6′-O-mycoloyl-α,α-trehalose (**6**). — Compound **5** (138 mg) was treated with potassium mycolate[2,3] (297 mg) in hexamethylphosphoric triamide (5 mL) for 4 h at 100°. Ice and water were then added and the precipitate was filtered off and washed with water. It was dried and purified by column chromatography. Elution with 3:2 hexane–ether removed minor by-products. Continued elution with the same solvent-system followed by 1:1 ether–hexane afforded compound **6**; yield 230 mg (74%), $[\alpha]_D^{24}$ +44° (*c* 1.0, chloroform); n.m.r.

data: δ 7.38–7.27 (6 benzylic phenyl group), 5.12–4.53, 4.27–4.02, 3.65–3.50 (benzylic methylene groups and H-1,2,3,4,5,6), and 1.25 s (mycoloyl residue).

Anal. Calc. for $C_{142}H_{232}O_{14}$: C, 78.83; H, 10.80. Found: C, 79.02; H , 10.94.

2,3,4,2',3',4'-Hexa-O-benzyl-6-O-corynomycoloyl-α,α-trehalose (**7**). — The monosulfonate **5** (178 mg) was treated with potassium corynomycolate[13] (250 mg) in hexamethylphosphoric triamide (5 mL) for 5 h at 100°. The product was isolated as already described as a glass (168 mg, 67%), $[\alpha]_D^{24}$ +73° (*c* 2.0, chloroform); n.m.r. data: as reported for compound **6**.

Anal. Calc. for $C_{86}H_{120}O_{13}$: C, 75.84; H, 8.88. Found: C, 75.76; H, 9.00.

6-O-Mycoloyl-α,α-trehalose (**8**). — Compound **6** (150 mg) was dissolved in 1:1 ethyl acetate–ethyl alcohol (60 mL) and hydrogenolyzed at 50 lb.in.$^{-2}$ in the presence of 110 mg of 10% palladium-on-carbon catalyst for 6 h. The catalyst was filtered off and washed with chloroform, and the filtrate evaporated to give an amorphous residue that gave one major spot in t.l.c. It was purified by chromatography. Elution with 7:1 chloroform–methanol followed by 5:1 chloroform–methanol gave the pure product; yield 81 mg (72%). It was triturated with methanol to give a crystalline sample, m.p. 170–173°, $[\alpha]_D^{24}$ +45.5° (*c* 0.66, chloroform); lit.[8] m.p. 163–167°, $[\alpha]_D^{24}$ +47 ±0.9°.

Anal. Calc. for $C_{100}H_{126}O_{14}$: C, 74.02; H, 12.17. Found: C, 73.91; H, 12.08.

6-O-Corynomycoloyl-α,α-trehalose (**9**) . — Compound **7** (100 mg) was dissolved in 1:1 ethyl acetate–ethyl alcohol (60 mL) and hydrogenolyzed as described for compound **8**. The pure product was isolated in 55% yield (33 mg) after column chromatography; $[\alpha]_D^{24}$ +76.5° (*c* 1.2, chloroform).

Anal. Calc. for $C_{44}H_{84}O_{13}$: C, 64.36; H, 10.31. Found: C, 64.09; H, 10.53.

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