Carbohydrate Research, 155 (1986) 223–228
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthesis of 4,6-anhydro-α-D-galactopyranosyl-6-*O*-mycoloyl- and -corynomycoloyl-α-D-galactopyranoside

AVRAHAM LIAV, MAYER B. GOREN*,
Department of Molecular and Cellular Biology, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206 (U.S.A.)

YI-MING YANG†, AND HENRY M. FALES
National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20205 (U.S.A.)
(Received November 12th, 1985; accepted for publication, January 7th, 1986)

A report[1] from our laboratory described the synthesis of 6,6′-di-*O*-mycoloyl- and -corynomycoloyl-(α-D-galactopyranosyl α-D-galactopyranoside) from the appropriate potassium carboxylates and 2,3,2′,3′-tetra-*O*-benzyl-6,6′-di-*O*-*p*-tolylsulfonyl-(α-D-galactopyranosyl α-D-galactopyranoside) (**2**). This synthesis also gave rise to the formation of by-products to which the tentative structures 4,6-anhydro-2,3-di-*O*-benzyl-α-D-galactopyranosyl 2,3-di-*O*-benzyl-6-*O*-mycoloyl(or corynomycoloyl)-α-D-galactopyranoside were assigned. [Our recent synthesis[2] of 4,6,4′,6′-dianhydro-(α-D-galactopyranosyl α-D-galactopyranoside) indicates that the formation of this type of anhydro sugar is indeed feasible under certain conditions]. The 6,6′-diesters described earlier[1] were readily purified by chromatography and isolated in homogeneous form, but purification of the by-products was not satisfactory. Trace amounts of the major products and other impurities were still present, even after preparative t.l.c. In order to characterize the 4′,6′-anhydro-6-*O*-mycoloyl- and -6′-*O*-corynomycoloyl derivatives, we have undertaken the direct synthesis of these compounds.

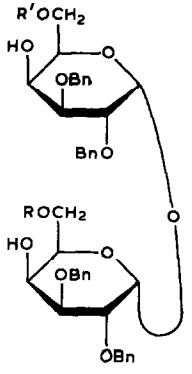
Selective tosylation of 2,3-di-*O*-benzyl-α-D-galactopyranosyl 2,3-di-*O*-benzyl-α-D-galactopyranoside[1] (**1**) gave a mixture of the 6,6′-ditosyl derivative **2** (14%), the desired monotosyl derivative **3** (41%), and unchanged starting material (24%). The three components were separated satisfactorily by column chromatography. Treatment of **3** with boiling sodium methoxide solution in methanol, followed by *p*-toluenesulfonylation gave the 4,6-anhydromonotosyl derivative **5**. (The tosyl

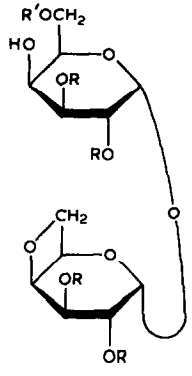
*Margaret Regan Investigator in Chemical Pathology, National Jewish Center for Immunology and Respiratory Medicine; Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, Colorado (U.S.A.).
†Visiting Scientist from Shanghai Institute of Materia Medica, Academia Sinica.

0008-6215/86/$ 03.50

group of **5** is linked to O-6 owing to the much higher reactivity of the primary OH-6 group). Treatment of **5** with potassium mycolate or potassium corynomycolate[3] in *N,N,N,N'N',N'*-hexamethylphosphoric triamide gave the corresponding 4',6'-anhydro-6-*O*-mycoloyl and 4',6'-anhydro-6-*O*-corynomycoloyl derivatives **6** and **7**, respectively. Catalytic hydrogenolysis of **6** and **7** afforded 4,6-anhydro-α-D-galacto-pyranosyl-6-*O*-mycoloyl- and -6-*O*-corynomycoloyl α-D-galactopyranoside) (**8** and **9**, respectively).

The pure glycolipids were examined by ^{252}Cf plasma desorption–sodium cationization mass spectrometry (*cf.*, for example, Puzo and Promé[4], and Shimakata *et al.*[5]). The spectrum for the corynomycolate **9** (not shown) gave a

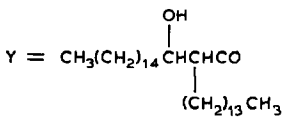


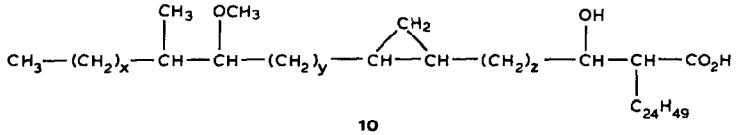


1 R = H, R' = H
2 R = Ts, R' = Ts
3 R = H, R' = Ts

4 R = Bn, R' = H
5 R = Bn, R' = Ts
6 R = Bn, R' = X
7 R = Bn, R' = Y
8 R = H, R' = Y
9 R = H, R' = Y

X = mycoloyl





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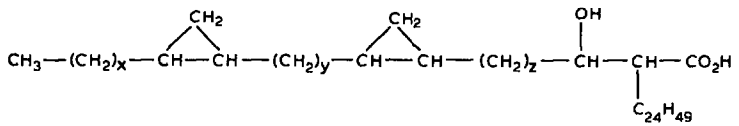

11

major peak at *m/z* 826 ($M + Na^+$) and a minor peak at *m/z* 858 ($M + 2\,Na^+ - H^+$) in accord with the expected values. Examination of the mass spectrum of **8** (Fig. 1) and of the elemental analysis showed that the major components of the mycolic acids used (derived from *Mycobacterium tuberculosis* strain H37Rv as described earlier[3]) are a series of methoxymycolic acids designated Type "IIa" (**10**), according to Davidson *et al.*[6], and a second major series comprised of dicyclopropane types that have earlier been designated Type "I" or "alpha" (**11**). (A uniform nomenclature for the more than half dozen varieties of mycolic acid structures has not yet been adopted by consensus; for reviews, see refs. 7–9).

In our crude product, both types of mycolic acids were represented as can be deduced from the mass spectrum (Fig. 1). Two principal series of monoanhydro disaccharide monomycolates may be defined by the mass spectrum. The three peaks at *m/z* 1439, 1467, and 1495 are characteristic of type I (dicyclopropane series **11**), which includes three monomycoloyl derivatives. The mass numbers define and give the sum of the methylene groups (x + y + z), which for this series is represented by 42, 44, or 46. In the principal homolog, the sum of the methylene groups is 44, but the exact distribution of the groups is not elucidated by this mass spectrum. In ordinary e.i. mass spectrometry of the methyl mycolates or of appropriate derivatives, the fragmentation patterns may be used to define the individual values of x, y, and z with greater certainty. The second series of major peaks at *m/z* 1555, 1583, 1611, and 1639 is characteristic for carbohydrate residues substituted with mycoloyl groups of type IIa (**10**), which bear the definitive methoxyl group. This group is precisely located by e.i. mass spectrometry, but not by m.s. in the plasma-desorption mode. The peaks of this second series correspond to mycolate groups in which the sum of the methylene groups (x + y + z) corresponds to 48, 50, 52, or 54, the principal homolog having the sum (x + y + z) = 50.

All of the peaks in the two series were in accord with the structures assigned. Therefore, owing to the heterogeneity of the mycoloyl groups in **6** and **8**, the elemental analysis data for these compounds represent only average values. Com-

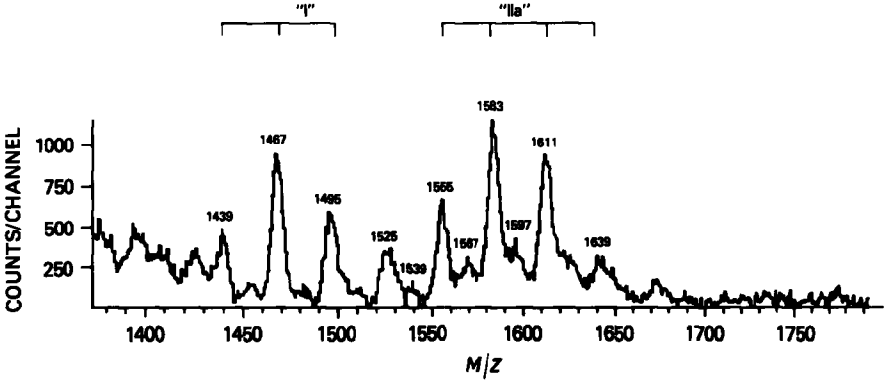

Fig. 1. Plasma-desorption mass spectrum of 4,6-anhydro-α-D-galactopyranosyl 6-*O*-mycoloyl-α-D-galactopyranoside (**8**).

parison of the mass spectrum of compound **8** with that of 6-*O*-mycoloyl-α,α-trehalose[3] (not shown) revealed that the *m/z* values for the two major sets of peaks shown in Fig. 1 are lower than the corresponding peaks in the trehalose monomycolate spectrum by 18 mass units, in agreement with the values expected in comparing the two monoanhydride series with the "parent" trehalose glycolipids.

EXPERIMENTAL

General methods. — Optical rotations were determined with a Jasco DIP-140 polarimeter. ^{1}H-N.m.r. spectra were recorded at 60 MHz with an EM360A Varian spectrometer with tetramethylsilane as the internal standard and (^{2}H)$CHCl_3$ as the solvent. ^{252}Cf Plasma-desorption mass spectra (p.d.m.s.) were obtained on an instrument, the prototype of which has been described[10] and which was built for N.I.H. by Prof. R. Macfarlane and subsequently modified in our laboratory to change samples automatically (L. Pannell, NIADDK). Solutions of the samples in polar solvents, usually methanol and chloroform, were applied to the conductive surface of an aluminized Mylar film in a sample holder either by evaporation or electrospraying[10]. The sample holder was then placed (through a vacuum lock) in front of a ^{252}Cf source (~0.4 GBq) held at 10^{-6} mm. Desorbed ions were accelerated down a 42-flight tube by a 10 kV grid and their flight times digitized and converted to mass values which were stored in a computer. Each ion was collected and added to others in the same channel to provide the mass spectrum as shown. About 1 millionth of the sample was used in the average analysis time of ~20 min and the sample could be recovered if necessary. Eastman–Kodak plates were used for t.l.c. Chromatography columns were packed with silica gel (Baker #3405). Potassium mycolate and potassium corynomycolate were prepared as described earlier[3]. Organic solutions were dried in the presence of Na_2SO_4. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

*2,3-Di-*O*-benzyl-α-*D*-galactopyranosyl 2,3-di-*O*-benzyl-6-*O*-p-tolylsulfonyl-α-*D*-galactopyranoside* (**3**). — To a solution of 2,3-di-*O*-benzyl-α-D-galactopyranosyl 2,3-di-*O*-benzyl-α-D-galactopyranoside[1] (**1**, 330 mg) in pyridine (3 mL) was added *p*-tolylsulfonyl chloride (130 mg), and the mixture was stirred at room temperature. More *p*-tolylsulfonyl chloride was added after 1.5 h (95 mg) and 4.5 h (30 mg). After 5.5 h, ethyl acetate and 2M HCl were added, and the organic layer was separated and washed with water, a saturated $NaHCO_3$ solution, and water. It was dried and evaporated, and the residue was chromatographed. Elution with chloroform yielded syrupy **2** (67 mg, 14%), R_F 0.84 (3:1 benzene–methanol). Continued elution with chloroform gave **3** (165 mg, 41%), syrup, $[\alpha]_D^{22}$ +77° (*c* 0.9, chloroform); R_F 0.72; ^{1}H-n.m.r.: δ 7.90–7.10 (24 H, arom.), and 2.42 (s, 3 H, CH_3Ts).

Anal. Calc. for $C_{47}H_{52}O_{13}S$: C, 65.87; H, 6.11; S, 3.74. Found: C, 66.23; H, 6.15; S, 3.82.

Finally, elution with 20:1 chloroform–methanol gave unchanged starting material **1** (79 mg, 24%; R_F 0.52).

*4,6-Anhydro-2,3-di-*O*-benzyl-α-*D*-galactopyranosyl 2,3-di-*O*-benzyl-α-*D*-galactopyranoside* (**4**). — The monotosyl derivative **3** (162 mg) was treated with M sodium methoxide solution (2 mL) in methanol (4 mL). The mixture was boiled under reflux for 4 h, cooled, made neutral with acetic acid, and evaporated. The residue was extracted with chloroform, and the insoluble solid material filtered off, washed with chloroform, and discarded. The filtrate was evaporated and the residue chromatographed. Elution with 1:1 ethyl acetate–hexane removed a minor by-product. Continued elution with the same solvent, followed by 3:2 ethyl acetate–hexane gave syrupy **4** (85 mg, 66%), $[\alpha]_D^{22}$ +66° (*c* 1.0, chloroform); R_F 0.20 (3:1 ethyl acetate–hexane).

*4,6-Anhydro-2,3-di-*O*-benzyl-α-*D*-galactopyranosyl 2,3-di-*O*-benzyl-6-*O*-p-tolylsulfonyl-α-*D*-galactopyranoside* (**5**). — To a solution of the monoanhydro derivative **4** (53 mg) in pyridine (0.6 mL) was added *p*-tolylsulfonyl chloride (35 mg) and the mixture was stirred at room temperature. More *p*-tolylsulfonyl chloride was added after 2 h (20 mg) and 4 h (15 mg). After 5 h, ethyl acetate and 2M HCl were added, and the mixture was processed as described for **3**. The product was purified by chromatography. Elution with 2:1 hexane–ethyl acetate gave a syrup (55 mg, 65%), $[\alpha]_D^{22}$ +120° (*c* 0.8, chloroform); R_F 0.81 (3:1 ethyl acetate–hexane); ^{1}H-n.m.r.: δ 7.90–7.10 (24 H, arom.) and 2.45 (s, 3 H, CH_3Ts).

Anal. Calc. for $C_{47}H_{50}O_{12}S$: C, 67.28; H, 6.00. Found: C, 67.16; H, 6.18.

*4,6-Anhydro-2,3-di-*O*-benzyl-α-*D*-galactopyranosyl 2,3-di-*O*-benzyl-6-*O*-mycoloyl-α-*D*-galactopyranoside* (**6**). — Compound **5** (45 mg) was treated with potassium mycolate (200 mg) in *N,N,N',N',N'',N''*-hexamethylphosphoric triamide (1.5 mL) at 115° for 17 h. Ice and 2M HCl acid were added, and the precipitate was filtered off and washed with water. The precipitate was dissolved in chloroform and the solution evaporated. The residue was treated with an AG-MPI (OH^-) anion-exchange resin in chloroform–methanol in order to remove the excess of mycolic acid and the de-ionized product was chromatographed on silica gel. Elution with 3:1 hexane–ethyl acetate removed a trace amount of impurities. Continued elution with the same solvent gave **6** as a wax (65 mg, 67%), $[\alpha]_D^{22}$ +58° (*c* 1.0, chloroform); R_F 0.50 (2:1 hexane–ethyl acetate).

Anal. Calc. for $C_{119}H_{198}O_{13}$: C, 77.81; H, 10.86. Found: C, 77.86; H, 10.66.

*4,6-Anhydro-2,3-di-*O*-benzyl-α-*D*-galactopyranosyl 2,3-di-*O*-benzyl-6-*O*-corynomycoloyl-α-*D*-galactopyranoside* (**7**). — Compound **5** (24 mg) was treated with potassium corynomycolate[3] (50 mg) in *N,N,N',N',N'',N''*-hexamethylphosphoric triamide (1 mL) as just described. The product was purified by column chromatography. Elution with 3:1 hexane–ethyl acetate gave **7** as a wax (19 mg, 58%), $[\alpha]_D^{22}$ +74° (*c* 1.0, chloroform); R_F 0.63 (3:2 hexane–ethyl acetate).

Anal. Calc. for $C_{72}H_{106}O_{12}$: C, 74.31; H, 9.18. Found C, 74.13; H, 9.35.

*4,6-Anhydro-α-*D*-galactopyranosyl 6-*O*-mycoloyl-α-*D*-galactopyranoside* (**8**). — Compound **6** (67 mg) was dissolved in ethyl acetate (25 mL) and ethyl alcohol

(20 mL), and hydrogenolyzed in the presence of 10% Pd–C catalyst (95 mg) at 350 kPa for 5 h. The catalyst was filtered off and washed with chloroform, and the filtrate evaporated. The residue was purified by column chromatography. Elution with 15:1 chloroform–methanol removed some impurities. Continued elution with 9:1 chloroform–methanol gave pure **8** (33 mg, 62%), wax, $[\alpha]_D^{22}$ +55° (*c* 0.65, chloroform); R_F 0.74 (3:1 benzene–methanol).

Anal. Calc. for $C_{91}H_{174}O_{13}$: C, 74.03; H, 11.88. Found: C, 73.96; H, 12.12.

*4,6-Anhydro-α-*D*-galactopyranosyl 6-*O*-corynomycoloyl-α-*D*-galactopyranoside* (**9**). — Compound **7** (27 mg) was dissolved in ethyl acetate (15 mL) and ethyl alcohol (15 mL), hydrogenolyzed in the presence of 10% Pd–C catalyst (55 mg), and purified by chromatography as just described (12 mg, 66%), wax, $[\alpha]_D^{22}$ +73° (*c* 0.5, chloroform); R_F 0.60 (3:1 benzene–methanol).

Anal. Calc. for $C_{44}H_{82}O_{12}$: C, 65.80; H, 10.29. Found: C, 65.88; H, 10.33.

ACKNOWLEDGMENTS

The authors thank Carol Breibart for careful preparation of the manuscript and Nadia de Stackelburg for the figure.

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