Carbohydrate Research, 84 (1980) 171–174
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

An improved synthesis of (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)uronic acid (2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)uronic acid

AVRAHAM LIAV AND MAYER B. GOREN*

*National Jewish Hospital and Research Center and *University of Colorado Health Sciences Center, Denver, Colorado 80206 (U.S.A.)*

(Received October 22nd, 1979; accepted for publication, November 20th, 1979)

INTRODUCTION

A recent report from this laboratory described the synthesis of "pseudo cord-factors"[1], in which the 6-hydroxyl groups of the trehalose core have been transformed into carboxyl groups. The "pseudo cord-factors" were synthesized by attaching lipid substituents to the carboxyl groups of the disaccharide, either by ester or amide linkages. Thus, the core carbohydrate in these pseudo cord-factors is (α-D-glucopyranosyluronic acid) (α-D-glucopyranosiduronic acid) (**1**) ("trehalose-dicarboxylic acid") and its synthesis from trehalose has been described[2]. Compound **1** was previously obtained by catalytic oxidation of trehalose, but as the reaction mixture contained several other products, the chromatographic purification of the desired acid was tedious and the yield was only modest (30%). A key intermediate in the preparation of either the diamide or diester pseudo cord-factors is the hexaacetate **2**, in which the hydroxyl groups of **1** are blocked. Compound **2** can, of course, also serve as a precursor of **1**. We previously reported[2] unsuccessful efforts at obtaining **2** by oxidation of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-α,α-trehalose (**3**).

In the present work, the oxidation of **3** to **2** in good yield was achieved by means of the Jones reagent[3] (prepared from chromic oxide and sulfuric acid), and homogeneous **2** was obtained in 60% yield after column chromatography. This compound was also characterized by the crystalline dimethyl ester **4**. Since, as we reported earlier, peracetylation of **1** was a curiously difficult operation, this new route to **2** is much more fruitful.

6,6′-Di-*O*-trityl-α,α-trehalose (**5**) was obtained from trehalose according to the published procedure of Bredereck[4], and was isolated as a homogeneous solid in 92% yield. We found that recrystallization of **5** from ethanol according to Bredereck's description[4] is beset with inordinate difficulties: **5** is very insoluble in boiling ethanol, and afterwards is recovered in meager yield from (presumably) saturated ethanolic solution. Instead, purified **5** was readily obtained by deacetylation of **6** (see later).

Obtained as described in the Experimental section, and without recrystallization, compound **5** had physical properties in good agreement with those described by Bredereck. Compound **5** was converted into the corresponding hexaacetate **6** in good yield, and it could also be prepared without isolation of **5**. 2,3,4,2′,3′,4′-Hexa-*O*-acetyl-α,α-trehalose (**3**) was prepared by a modified procedure rather than by use of the somewhat destructive, 48% hydrogen bromide in acetic acid[4]. Compound **6** was instead de-tritylated by treatment with 80% acetic acid at 75–80°, and the crude, detritylated product **3** purified by column chromatography. It was obtained crystalline in 65% yield. Treatment of **3** with Jones reagent[3] at room temperature gave one major product, which was purified by chromatography on cellulose. Compound **2** was found to be identical with the product described before[2], on the basis of optical rotation, n.m.r. spectroscopy, and t.l.c. However, the two samples differed in their melting points. This difference is probably due to the fact that compound **2** was recrystallized twice, whereas the other sample[2] was obtained by evaporation of a solution of the compound in ether.

Treatment of **2** with ethereal diazomethane gave the crystalline dimethyl ester **4**. The n.m.r. spectrum of **4**, which was well-resolved, confirmed the structure of **2**.

EXPERIMENTAL

General methods. — Melting points were measured in capillary tubes in a modified Thiele–Hershberg apparatus and are not corrected. Optical rotations were determined with a Jasco DIP-4 polarimeter. N.m.r. spectra were recorded with a Jeol FX 100 spectrometer with tetramethylsilane as internal standard and $CDCl_3$ as the solvent. Thin-layer chromatograms were run on Eastman Kodak silica gel plates. Chromatography columns were packed with silica gel (E. Merck, No. 7734), unless otherwise stated. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

*6,6′-Di-*O*-trityl-α,α-trehalose* (**5**). — Trehalose dihydrate (3.94 g) was dehydrated by dissolving it in pyridine (70 mL) and concentrating the resulting solution to ~30 mL. The concentrated solution was cooled to room temperature, and chlorotriphenylmethane (7.38 g) was added. The mixture was kept for 45 h at room temperature. Addition of ice–water (45 mL) precipitated the product as a gum, which was rendered crystalline by the addition of a small amount of ethyl alcohol. The product was filtered off, washed several times with water, and allowed to dry in air. Extraction with benzene removed triphenylmethanol, and the solid residue was triturated with ethyl alcohol (90–100 mL) to give crystalline material (7.98 g, 92%) which was found to be homogeneous in t.l.c. (4:1:1 ethyl acetate–methanol–water). Its recovery in pure form from the hexaacetate **6** is described next.

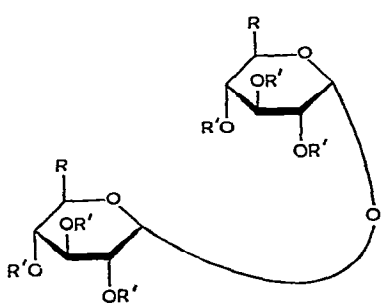
*2,3,4,2′,3′,4′-Hexa-*O*-acetyl-6,6′-di-*O*-trityl-α,α-trehalose* (**6**). — The 6,6′-ditrityl ether **5** (4.55 g) was treated with acetic anhydride (20 mL) and pyridine (45 mL). The mixture was kept overnight at room temperature and evaporated *in vacuo*. Ethyl alcohol was added to the residue, and the crystalline material recovered;

yield 4.96 g (83%); m.p. 238–241°, $[\alpha]_D^{24}$ +112.3° (*c* 1.4, chloroform) [lit.[4] m.p. 235–238°, or 245–247° after recrystallization; $[\alpha]_D$ +115.7°].

To prepare pure **5**, 0.143 g of **6** was suspended in methanol (5 mL), treated with 0.1–0.2 mL of M sodium methoxide, and stirred for 16 h at 25°. The crystalline material was filtered off and washed with methanol and then with acetone to afford 83 mg (75%) of **5**; m.p. 276–281°, $[\alpha]_D^{24}$ +64° (*c* 0.87, pyridine) [lit.[4] (after three recrystallizations from ethanol) m.p. 278–281°, $[\alpha]_D$ +62.2 — +62.8° (pyridine).

2,3,4,2',3',4'-Hexa-O-*acetyl-α,α-trehalose* (**3**). — Compound **6** (1.8 g) was suspended in 80% aqueous acetic acid (30 mL), and the suspension stirred for 1.5 h at 75°. The mixture was then evaporated and the residue chromatographed on silica gel (85 g). Elution with 3:1 ethyl acetate–hexane removed the trityl salts as well as minor by-products (probably monosaccharide derivatives). Continued elution with the same solvent system afforded the title compound, isolated as an amorphous residue (0.88 g, 88%). Crystallization from acetone–hexane gave 0.65 g (65%) of **3**, homogeneous by t.l.c., m.p. 82–86°, $[\alpha]_D^{24}$ +158° (*c* 1.05, chloroform) [lit.[4] m.p. 91–94°, $[\alpha]_D$ +158.3°].

(2,3,4-Tri-O-*acetyl*-α-D-*glucopyranosyl)uronic acid* (*2,3,4-tri*-O-*acetyl*-α-D-*glucopyranosid)uronic acid* (**2**). — A solution of the hexaacetate **3** (260 mg) in acetone (8 mL) was treated with Jones' reagent[3] (0.8 mL), and the mixture was stirred for 5 h at room temperature. Ethyl alcohol was added in order to reduce the excess of oxidizing agent, and the mixture was then made neutral with saturated sodium hydrogencarbonate solution. The insoluble material was filtered off and the filtrate applied to a column of AG-50 WX-8 (H^+) resin (12 × 150 mm). Elution with 50% aqueous methanol (60 mL), and evaporation of the effluent gave the crude dicarboxylic acid, which was then extracted into ether. The insoluble material was filtered off, and the filtrate evaporated to give an amorphous residue (250 mg), which was applied to a column (25 × 250 mm) of cellulose. The fast-moving by-products were removed by elution with 100:1 chloroform–methanol. Continued elution with the same solvent-system followed by elution with 50:1 chloroform–methanol afforded fractions found by t.l.c. (100:7:0.7 chloroform–methanol–acetic acid) to contain the homogeneous product. These fractions were pooled and evaporated to give the product (165 mg, 60%). An analytically pure sample was obtained by crystallization from ether–hexane; m.p. 143–145°, $[\alpha]_D^{24}$ +157° (*c* 1.06, chloroform) [lit.[2] m.p.: sinters at 120° and



	R	R'
1	CO_2H	H
2	CO_2H	Ac
3	CH_2OH	Ac
4	CO_2Me	Ac
5	CH_2OTr	H
6	CH_2OTr	Ac

melts at 166–170°, $[\alpha]_D$ +151.4° (*c* 0.46, chloroform)]. For analysis, the product was dried *in vacuo* for 2 h at 100°.

Anal. Calc. for $C_{24}H_{30}O_{19}$: C, 46.30; H, 4.82. Found: C, 46.17; H, 4.98.

(*Methyl 2,3,4-tri-*O*-acetyl-α-*D*-glucopyranosyluronate*)(*methyl 2,3,4-tri-*O*-acetyl-α-*D*-glucopyranosiduronate*) (**4**). — The dicarboxylic acid derivative **2** (128 mg) was treated with diazomethane (obtained from 200 mg of "Diazald") in ether for a few min. The ether was evaporated off and the product crystallized from acetone–hexane; yield 84 mg (63%). Recrystallization from the same solvent mixture gave an analytical sample, m.p. 134–135°, $[\alpha]_D^{24}$ +135° (*c* 1.0, chloroform); n.m.r. data: δ 5.53 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3 and H-3′), 5.29 (d, 2 H, $J_{1,2}$ 3.6 Hz, H-1 and H-1′), 5.20 (t, 2 H, $J_{4,5}$ 9.5 Hz, H-4 and H-4′), 5.10 (dd, 2 H, H-2 and H-2′), 4.46 (d, 2 H, H-5 and H-5′), 3.73 (s, 6 H, 2 CO_2CH_3), 2.09, 2.05 (2 s, 18 H, 6 acetoxyl groups).

Anal. Calc. for $C_{26}H_{34}O_{19}$: C, 48.00; H, 5.23. Found: C, 48.20; H, 5.47.

ACKNOWLEDGMENTS

This investigation was supported by U.S. Public Health Service Grant AI-08401 from the U.S.–Japan Cooperative Medical Science Program, administered by the National Institute of Allergy and Infectious Diseases. For n.m.r. data, we thank the Colorado State University Regional NMR Center, funded by National Science Foundation Grant CHE 78-18581. M.B.G. is the Margaret Regan Investigator in Chemical Pathology, NJHRC.

REFERENCES

1 M. B. Goren and K.-S. Jiang, *Chem. Phys. Lipids*, 25 (1979) 209–224.
2 M. B. Goren and K.-S. Jiang, *Carbohydr. Res.*, 79 (1980) 225–234.
3 K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, (1946) 39–45.
4 H. Bredereck, *Ber.*, 63 (1930) 959–965.