Carbohydrate Research, 40 (1975) 375–379

Note

Preparation and β-elimination of a 5-deoxy-5-nitromaltitol derivative: an approach to selective cleavage of the glycosidic bond at the reducing end of oligosaccharides*

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(Received April 25th, 1974; accepted October 3rd, 1974)

Selective cleavage of the glycosidic bond of oligo- and poly-saccharides by a base-catalyzed β-elimination reaction has often been found useful in structural studies[1–5]. Successful application of the cleavage reaction to all types of glycosidic linkage at the terminal residue would provide a general means for sequence-analysis by stepwise degradation, but this has not yet been accomplished. One of the major problems involved is the selection and introduction of an effective electron-withdrawing group β-related to the glycosidic bond to be cleaved[6]. Methylated oligosaccharides having a free, hemiacetalic hydroxyl group are known to be susceptible to cleavage of the glycosidic linkage to the reducing-end residue, by base-catalyzed elimination, when the linkage is β to the carbonyl group[6,7]. The technique may be extended to the scission of other types of linkage by introduction of a new electron-withdrawing group, such as a carbonyl, nitro, or sulfonyl group, at a suitable position in the molecule. This has been accomplished by opening the pyranose ring and introducing an electron-withdrawing group at C-5. In this note, the preparation of a model compound for the selective cleavage of a (1→4)-glycosidic bond at the reducing-end residue of methylated oligosaccharides is described. The compound chosen is a 5-deoxy-5-nitromaltitol derivative prepared from maltose.

The formula sequence shows the synthetic route used to obtain 1-*O*-benzoyl-5-deoxy-2,3,6-tri-*O*-methyl-5-nitro-4-*O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-D-glucitol and its C-5 epimer (compound **5**, an epimeric mixture). The acetylated glycoside **1** was obtained in 39% yield from maltose without isolation of intermediates. Methylation of **1** by the Haworth method afforded the ether **2** in 68% yield. Hydrogenolytic removal of the benzyl group from **2** gave hepta-*O*-methylmaltose in 89% yield, and subsequent reduction with sodium borohydride followed by selective

*Part of this work was presented at a meeting of the Agricultural Chemical Society of Japan, Okinawa, Japan, January 12, 1974.

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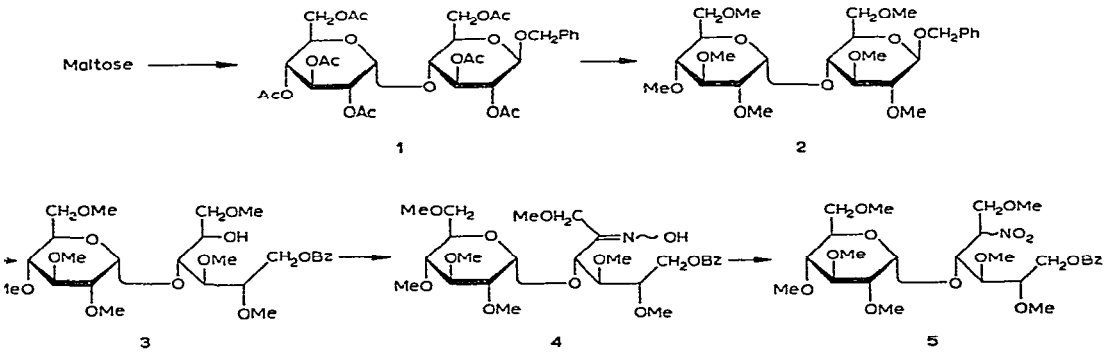
benzoylation of the resultant primary hydroxyl group gave compound **3** in an overall yield of 35%. Oxidation of compound **3** with methyl sulfoxide gave a glycosylated L-sorbose derivative that was converted into its oxime **4**. Oxidation[8,9] of the oxime with 90% hydrogen peroxide and trifluoroacetic anhydride gave the epimeric nitrohexitols **5** in 48% yield, together with 23% of a nitroalkene and an unknown product (7% yield). Column chromatography failed to separate the epimeric pair, although t.l.c. showed the presence of two components having close R_F values. P.m.r. data of the nitroalkene and the absence of 2,3,4,6-tetra-*O*-methyl-D-glucose in the reaction mixture suggested that elimination during the oxidation occurred only between H-5 and 6-OMe of compound **5**, although the exact reaction-mechanism is not clear.



The alkali-lability of glycosides having a nitro group in the β-position of the aglycon has been demonstrated by Helferich and Hase[10]. A similar observation was made with a 6-deoxy-6-nitroglucoside by Baer and Rank[11]. Treatment of **5** with 0.05M aqueous sodium hydroxide for 12 h at room temperature resulted in scission of 87% of the glycosidic bonds, as well as causing deacylation, as shown by g.l.c. and t.l.c. of the resultant 2,3,4,6-tetra-*O*-methyl-D-glucose. Under the same conditions (121 h reaction time), 15% of the tetra-*O*-methyl-D-glucose underwent β-elimination. Isolation of the unsaturated β-elimination products has not been accomplished.

Although the route presented here involves several steps and requires improvements as to yields and selection of protective groups, it provides, in principle, a means for sequential degradation of common oligosaccharides.

EXPERIMENTAL

General methods. — Melting points were observed with a Yanagimoto apparatus and are uncorrected. Specific rotations were determined with a Yanagimoto direct-reading polarimeter (Model OR-20). P.m.r. spectra were recorded at 90 MHz with a Hitachi–Perkin Elmer R-22 spectrometer and at 100 MHz with a Varian HA-100 instrument, with tetramethylsilane as the internal standard. I.r. spectra were measured on a Hitachi grating spectrophotometer (Model 215). T.l.c. was performed on Silica Gel G. The solvent systems for t.l.c. were 49:1 (v/v) benzene–methanol (solvent A),

3:1 (v/v) benzene–acetone (solvent B), 4:1 (v/v) benzene–acetone (solvent C), 17:3 (v/v) benzene–acetone (solvent D), and 9:1 (v/v) benzene–acetone (solvent E). Compounds were detected by spraying the plates with 10% sulfuric acid and then heating, or by scanning with a Shimadzu dual-wavelength t.l.c. scanner (Model CS-900). Silica gel (0.063–0.200 mm, E. Merck) was used for all column-chromatographic separations. G.l.c. was performed with a Yanagimoto gas chromatograph 550 F.

Benzyl hepta-O-acetyl-β-maltoside (**1**). — Maltose (80 g) was converted into hepta-*O*-acetylmaltosyl bromide by the method of Helferich and Speicher[12]. The syrupy halide was glycosylated[12] with benzyl alcohol (144 ml), iodine (0.8 g), silicic acid (9.6 g), and zinc oxide (4 g) for 24 h at room temperature. The mixture was filtered and the filtrate washed with 10% acetic acid, sodium hydrogen sulfite solution and water. Steam distillation of the organic layer removed benzene and benzyl alcohol to give a yellowish syrup. The syrup was crystallized from methanol–water to afford colorless, fine needles (66 g, 39%), m.p. 115–121° (lit.[12] m.p. 121–123°). $[\alpha]_D^{15}$ +31.3° (*c* 2.08, chloroform); p.m.r. (Me_2SO-d_6): δ 7.33 (5 H, aromatic), 5.29 (1 H, H-1′, d, $J_{1,2}$ 3.9 Hz), 4.77 (1 H, H-1, d, $J_{1,2}$ 9.2 Hz), and 1.91–2.04 (21 H, acetyl); t.l.c. R_F 0.84 (solvent A).

Benzyl hepta-O-methyl-β-maltoside (**2**). — To a solution of compound **1** in 50% sodium hydroxide solution (300 ml) was added dimethyl sulfate (120 ml) during 2.5 h at 75° under nitrogen. After decomposition of excess dimethyl sulfate by the addition of 30% aqueous ammonia, the mixture was neutralized with 10% sulfuric acid and extracted with chloroform. The extract was evaporated to a syrup (16 g) that contained three components (t.l.c.). The title compound (8 g, 68%) was separated by column chromatography; $[\alpha]_D^{14}$ +60.9° (*c* 1.69, chloroform); ν_{max}^{film} 1500 (phenyl) cm^{-1}, no OH absorption; n.m.r. (Me_2SO-d_6): δ 7.38 (5 H, aromatic), 5.41 (1 H, H-1, d, $J_{1',2'}$ 3.2 Hz), 4.69 (2H, q, CH_2-Ph, J_{gem} 12.0 Hz), 4.47 (1 H, d, H-1, $J_{1,2}$ 7.4 Hz), and 3.21–3.45 (21 H, OMe); t.l.c. R_F 0.55, by-products 0.27, and 0.10 (solvent A).

1-O-Benzoyl-2,3,6-tri-O-methyl-4-O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-D-glucitol (**3**). — Compound **2** (6.8 g) was hydrogenolyzed in a mixture of methanol (110 ml) and acetic acid (70 ml) at 1 atm pressure over 5% palladium-on-charcoal (6.8 g) for 5 h at room temperature to afford hepta-*O*-methyl-β-maltose (5.0 g) in 89% yield; $[\alpha]_D^{14}$ +122.4° (*c* 1.9, chloroform); ν_{max}^{film} 3400 (OH), 2940, 2840, and 1380 (Me) cm^{-1}; t.l.c. R_F 0.32 (solvent B). To a stirred solution of hepta-*O*-methyl-β-maltose (2.0 g) in a mixture of *N*,*N*-dimethylformamide (20 ml) and methanol (20 ml) was added sodium borohydride (4.0 g). Completion of the reduction was indicated after 20 h (t.l.c.). After conventional processing of the mixture, syrupy products were obtained in 85% yield. The purified product obtained in 80% yield by column chromatography had $[\alpha]_D$ +93.3° (*c* 1.2, chloroform); ν_{max}^{film} 3400 (OH) cm^{-1}, t.l.c. R_F 0.07 (solvent B). To a solution of the glucitol derivative (0.3 g) in pyridine (1 ml) was added 1 mol equiv. of benzoyl chloride with cooling, and the reaction was continued for 4 h. The mixture was poured into water and the solution extracted with chloroform. The extract was washed with sodium hydrogen carbonate solution, M

hydrochloric acid, and water, and dried (sodium sulfate). Evaporation of the mixture afforded a syrup (0.284 g) that on purification by column chromatography gave the title compound (154 mg) in 41% yield; $[\alpha]_D^{14}$ +68.8° (*c* 1.25, chloroform); ν_{max}^{film} 3480 (OH), 2980, 2930, 2830, 1720, 1600, 1580, 1450, 1270, and 1100 cm^{-1}; p.m.r. (Me_2SO-d_6): δ 8.1–7.4 (5 H, aromatic), 5.11 (1 H, H-1, d, $J_{1',2'}$ 2.9 Hz), 4.8 (1 H, d, OH, $J_{5,OH}$ 5.0 Hz), 4.46 (2 H, d, CH_2OBz), 3.47, 3.44, 3.40, 3.35, 3.27, and 3.22 (21 H, OMe); t.l.c. R_F 0.26 (solvent C).

Anal. Calc. for $C_{26}H_{42}O_{12}$: C, 57.13; H, 7.75. Found: C, 57.04; H, 7.79.

6-O-*Benzoyl-1,4,5-tri*-O-*methyl-3*-O-(*2,3,4,6-tetra*-O-*methyl-α*-D-*glucopyranosyl*)-L-*sorbose oxime* (**4**). — A mixture of compound **3** (1.42 g), dimethyl sulfoxide (13 ml), and acetic anhydride (1.10 g) was stirred overnight at room temperature. Evaporation *in vacuo* of volatile materials from the mixture gave a syrupy residue from which the corresponding ketone derivative was obtained in 55% yield by column chromatography; $[\alpha]_D^{24}$ +83.3° (*c* 1.8, chloroform); ν_{max}^{film} 1730 (C=O), 1720 (COPh), 1600, 1450, and 1100 cm^{-1}; t.l.c. R_F 0.40 (solvent C). To a solution of hydroxylamine (freshly prepared by dissolving 0.62 g of sodium hydroxide and 1.04 g of hydroxylamine hydrochloride in 50 ml of ethanol and filtering off the sodium chloride) was added the L-sorbose derivative (0.766 g). After being stirred overnight, the mixture was evaporated to a syrup that was extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and evaporated to a syrup that was purified by column chromatography on silicic acid to afford the title compound (0.600 g), $[\alpha]_D^{25}$ +80.8° (*c* 1.5, chloroform); ν_{max}^{film} 3350 (OH), 1720 (COPh), 1680, 1600, 1580, 1450, and 1200–1040 cm^{-1} (solvent C); p.m.r. (Me_2SO-d_6): δ 11.38 and 11.27 (1 H, s, N-*OH*), t.l.c. R_F 0.30.

Anal. Calc. for $C_{26}H_{41}NO_{12}\cdot 0.5H_2O$: C, 54.92; H, 7.61; N, 2.46. Found: C, 54.84; H, 7.61; N, 2.39.

1-O-*Benzoyl-5-deoxy-2,3,6-tri*-O-*methyl-5-nitro-4*-O-(*2,3,4,6-tetra*-O-*methyl*-α-D-*glucopyranosyl*)-D-*glucitol and the* -L-*iditol epimer* (**5**). — To a mixture[9] of 90% hydrogen peroxide (0.06 ml), trifluoroacetic anhydride (0.338 ml), acetonitrile (8 ml), sodium dihydrogen phosphate (690 mg), and urea (5 mg) was added a solution of the L-sorbose oxime derivative (**4**, 64.5 mg) in acetonitrile (3 ml). The mixture was kept for 2 h at room temperature with stirring, and then a small portion of water was added. The mixture was concentrated *in vacuo* and extracted with chloroform. The extract was neutralized with sodium hydrogen carbonate solution, washed with water, and dried (sodium sulfate). Evaporation of the solvent gave a syrup (63.1 mg) that was chromatographed on a column (solvent E) to afford three reaction-products: the title compound (48% yield), $[\alpha]_D^{18}$ +75.6° (*c* 2.25, chloroform); ν_{max}^{film} 2980, 2830, 1720, 1600, 1580, 1450, 1551 (sat. NO_2), 1350 (NO_2), and 1100 cm^{-1}; t.l.c. R_F 0.61 and 0.56 (solvent D); p.m.r. (100 MHz, $CDCl_3$): 8.05–7.45 (5H, aromatic), 5.1 (2H, m), 4.5 (3H, m), 4.88 (1H, d, H-1', $J_{1',2'}$ 2.5 Hz), 4.0 (2H, m), and 3.6–3.4 (m, OMe); together with a nitroalkene derivative (23% yield), $[\alpha]_D^{16}$ +55.2° (*c* 1.8, chloroform); ν_{max}^{film} 2980, 2930, 2830, 1720, 1665 (C=C), 1527 (alkenic NO_2), 1600, 1580, 1450, 1350 (NO_2), and 1100 cm^{-1}; p.m.r. ($CDCl_3$): δ 8.15–7.35 (5H, m, aromatic), 6.57

(1H, d, H-6), 5.93 (1H, d, H-6), 5.12 (1H, d, H-1′, $J_{1',2'}$ 3.5 Hz), 4.93 (1H, d, H-4, $J_{3,4}$ 6.9 Hz), 4.65 (2H, m, H_2COBz), 3.91 (1H, q, H-3, $J_{2,3}$ 3.2 Hz), 3.67, 3.60, 351, 3.48, 3.40, and 3.35 (18H, OMe); t.l.c. R_F 0.46 (solvent D); and the third component (7.5% yield), t.l.c., R_F 0.34; i.r.: no absorption for a nitro group.

β-Elimination reaction of **5** *and determination by g.l.c. of 2,3,4,6-tetra-*O*-methyl-*D*-glucose* (**6**). — To a solution of **5** (23.3 mg) and 2,3,4,6-tetra-*O*-methyl-D-glucitol[13] (**7**, 10.6 mg) in 1 ml of methanol was added 0.05M aqueous sodium hydroxide (1 ml) and the mixture was stirred at 19°. Monitoring the reaction by semi-quantitative t.l.c. with a t.l.c. scanner showed a progressive decrease in **5** with an increasing amount of **6** and a new reaction-product (R_F 0.93, solvent C). Identification of **6** in the reaction mixture was made by comparing the R_F value of the product with that of authentic 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranose {m.p. 91–96°, t.l.c. R_F 0.21 (solvent C) and 0.34 [7:3 (v/v) benzene–acetone]}. For determination of **6** by g.l.c. with **7** as an internal standard, aliquots were withdrawn from the mixture at intervals, treated with cation-exchange resin (Amberlite IR-120, H^+ form), and subjected to g.l.c. Retention times of **6** and **7** at 150° on a stainless-steel column (2 m × 4 mm) packed with 3% SE-52 on Chromosorb W were 5.2 and 7.0 min, respectively. The calibration value of 1.730 for the ratio of T_p/G_p (peak-area ratio of **6** and **7**) to T_w/G_w (weight ratio of **6** and **7**) was obtained and used for determining **6** in the mixture with a relative error of ~7%. Values for the amount of **5** reacted, calculated on the basis of the amount of **6** determined after 2, 4, and 12 h of reaction time, were 53.6, 63.8, and 87.3%, respectively, relative to the initial quantity. Under the same conditions as already mentioned, compound **6** underwent no degradation after 2 h, and 15% of **6** decomposed in 121 h with the formation of three products (R_F 0.20, 0.31, and 0.40, solvent E), whereas no degradation of **7** took place after a reaction time of 73 h. Similar treatment of 1-*O*-benzoyl-2,3,4,6-tetra-*O*-methyl-D-glucitol {$[\alpha]_D$ +1.4° (*c* 5.33, chloroform); t.l.c. R_F 0.42 (solvent C)}, which was prepared by the same method used for **3**, resulted in the formation of **7** in a few h.

ACKNOWLEDGMENT

The authors thank Miss S. Yamashita for recording the p.m.r. spectra.

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