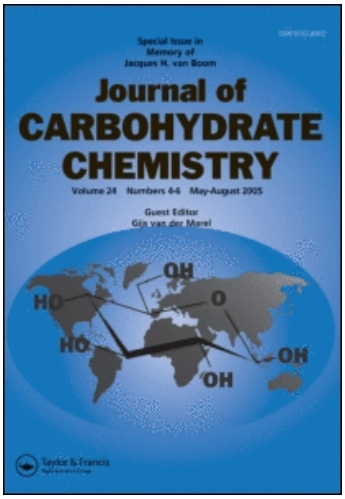

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1,4-Anhydro-2,3,6-Tri-O-Methyl-D-Glucitol Formed as an Artifact in the Reductive Cleavage of Permethylated 1,4-Linked Glucopyranosides

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1,4-ANHYDRO-2,3,6-TRI-O-METHYL-D-GLUCITOL FORMED AS AN ARTIFACT IN THE REDUCTIVE CLEAVAGE OF PERMETHYLATED 1,4-LINKED GLUCOPYRANOSIDES

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ABSTRACT

1,5-Anhydro-2,3,6-tri-O-methyl-D-glucitol (1) is formed as the major product in the reductive cleavage of permethylated 4-linked glucopyranosyl residues, but a small amount of 1,4-anhydro-2,3,6-tri-O-methyl-D-glucitol (2) is formed as an artifact when water is present. The formation of 2 can be minimized by carrying out the reductive cleavage under anhydrous conditions. The independent synthesis of 2 and its 5-O-acetyl derivative (4) is described.

INTRODUCTION

Reductive cleavage of the glycosidic carbon-oxygen bonds in permethylated polysaccharides was originally proposed[1] as a means to establish linkage positions and ring forms because of the inability of standard methylation analysis to distinguish between 4-linked aldopyranosides and 5-linked aldofuranosides. In applying this method to permethylated cellulose and cyclohexaamylose, the major product observed was indeed the expected

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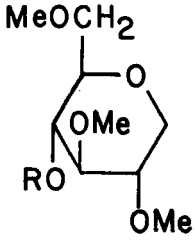
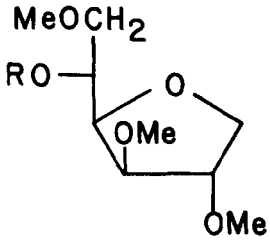
1,5-anhydroalditol (1,5-anhydro-2,3,6-tri-O-methyl-D-glucitol, 1), but a minor product was consistently observed in variable amounts that was found by chemical ionization mass spectrometry (CIMS) to be isomeric with 1.[2] The minor isomeric product has now been identified as 1,4-anhydro-2,3,6-tri-O-methyl-D-glucitol (2), the expected product of 5-linked glucofuranose residues.



1, R=H

3, R=Ac



2, R=H

4, R=Ac

Because of the absence of glucofuranose residues in these polysaccharides, an examination of the factors affecting the formation of 2 as an artifact was undertaken. Reported herein are the isolation and characterization of 2, its independent synthesis, an investigation of its route of formation, and a revised experimental protocol that minimizes its formation.

RESULTS AND DISCUSSION

The reductive cleavage of permethylated cyclohexaamylose was carried out in CH_2Cl_2 with trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst as previously reported,[2] and the products were acetylated and separated by GLC on OV225 (3.2 mm x 244 cm, 85°C-220°C, 6°C/min). Two products were observed, a major product (95%) that was shown to be identical to authentic

was dissolved in a mixture of 5 mL of H_2O and 2 mL of glacial acetic acid, and after cooling the mixture to 0°C, $NaNO_2$ (0.5 g) was slowly added. The reaction mixture was allowed to stir overnight at 4°C, and was then quenched by neutralization with $NaHCO_3$. After the addition of solid NaCl to decrease the solubility of 2, the aqueous solution was extracted with CH_2Cl_2. Drying (anhyd. Na_2SO_4) and evaporation of the CH_2Cl_2 extracts yielded crude 2 (0.11 g). Acetylation in the usual fashion and preparative GLC (6.3 mm x 91 cm SE-30, 180°C isothermal) of the major product (4, 58%) yielded pure 4 (0.007 g) as a clear colorless oil. ^1H NMR ($CDCl_3$): δ2.09 (s,3H, acetoxy), 3.361, 3.369, 3.372 (three s, 9H, methoxyl), 3.63 (dd, J = 11.22, 4.84 Hz, 1H, H-6), 3.69 (dd, J = 11.22, 2.58 Hz, 1H, H-6'), 3.70 (dd, J = 3.82, 0.80 Hz, 1H, H-3), 3.79 (dd, J = 9.80, 1.73 Hz, 1H, H-1α), 3.85 (ddd, J = 4.39, 1.73, 0.80 Hz, 1H, H-2), 4.04 (dd, J = 4.39, 9.80 Hz, 1H, H-1β), 4.15 (dd, J = 8.43, 3.82 Hz, 1H, H-4), 5.20 (ddd, J = 8.43, 4.84, 2.58 Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$) (^1H-decoupled): δ21.1 (C-2 acetoxy), 56.7, 57.6, 59.1 (methoxyl), 70.1, 71.3, 71.8, 78.2, 83.1, 83.3 (C-1,2,3,4,5,6), 169.8 (C-1 acetoxy); MS (CI, NH_3): m/e 249 ($M + H^+$), 266 ($M + NH_4^+$).

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tetramethylsilane. Reductive cleavages were carried out with TMSOTf as described previously.[2] Where water was deliberately added to the reaction, the addition was made prior to addition of the catalyst. In those reactions, a release of gas pressure was observed upon opening that appeared to correlate with the amount of water added.

Model Compounds. Permethylated cyclohexaamylose and permethylated cellulose were prepared as reported earlier.[2] Methyl 2,3,6-tri-O-methyl-4-O-(2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl)-β-D-glucopyranoside[5] and methyl 2,3,6-tri-O-methyl-4-O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-β-D-glucopyranoside[5] were prepared by Hakomori[6,7] methylation of methyl 4-O-β-D-glucopyranosyl-β-D-glucopyranoside and methyl 4-O-α-D-glucopyranosyl-β-D-glucopyranoside, respectively, and had ^{1}H NMR spectra identical to those reported. 2,3,6-Tri-O-methyl-D-glucose (5) was prepared by hydrolysis of permethylated cyclohexaamylose (2N trifluoroacetic acid, 20 h at 95°C); its ^{1}H NMR spectrum was identical to that reported by Terui, et al.[8]

1,4-Anhydro-5-O-acetyl-2,3,6-tri-O-methyl-D-glucitol (4). 2,3,6-Tri-O-methyl-D-glucose (5, 0.963 g, 4.33 mmol) and 3.34 g (43.3 mmol) of ammonium acetate were dissolved in 30 mL of methanol and 0.4 g (6.4 mmol) of freshly purified[9] $NaBH_3CN$ was added. After stirring 65 h at room temperature, 12 N HCl was added dropwise to pH 2 in a well ventilated hood (Caution: HCN evolved). Evaporation of the methanol under vacuum afforded a white salt which was dissolved in 20 mL of H_2O and extracted sequentially with three 20-mL portions of petroleum ether and three 20-mL portions of ethyl ether. The aqueous solution was subsequently made basic (pH > 10) by the addition of solid KOH and was extracted with three 25-mL portions of $CHCl_3$. After drying over anhyd. Na_2SO_4, the $CHCl_3$ solution was evaporated under vacuum to yield a clear yellow oil (0.912 g). Adsorption and elution from Dowex 50(H^+)[3] gave 6 (0.413 g) as a yellow gel. Compound 6 (0.20 g)

TABLE 1

Percentages of 1 and 2 Formed from Permethylated Cyclohexaamylose by Reductive Cleavage Under Various Reaction Conditions.

Solvent	Drying Agent (*in situ*)	Percent yield 1	2
CH_2Cl_2 (reagent grade)	None	87	13
CH_2Cl_2 (dist. from P_2O_5)	None		
CH_2Cl_2	BSTFA	0	0
CH_2Cl_2	CaH_2	98	2

the reductive cleavage of permethylated cyclohexaamylose was carried out in reagent grade CH_2Cl_2 that was not previously dried, 2 was produced in 13% yield (Fig. 1, Table 1). The amount of 2 produced was reduced to 5%, however, when the solvent was predried by distillation from P_2O_5. Recognizing the difficulty of removing traces of water from permethylated polysaccharides, however, chemical drying agents were tried. Interestingly, no reaction was observed when 0.5 equivalents of the silylating agent bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added per equivalent of acetal. The reductive cleavage reaction proceeded to completion, however, when a small amount of the insoluble drying agent CaH_2 was added, and the amount of 2 that was formed decreased to approximately 2%.

EXPERIMENTAL

General. Nuclear magnetic resonance spectra were recorded on a Nicolet NT-300 spectrometer. ^1H Spectra and ^{13}C spectra were recorded with $CDCl_3$ as solvent and are referenced to internal

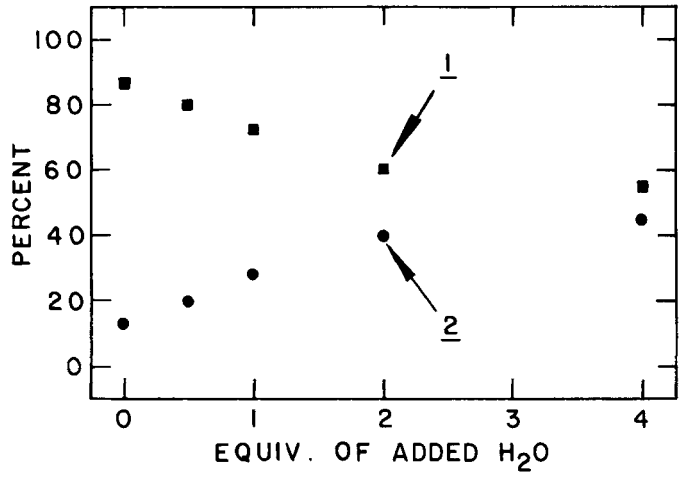
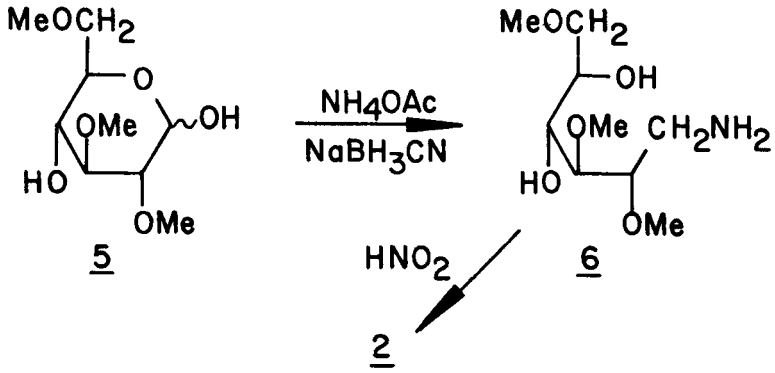


Fig. 1. Percent of 1 (■) and 2 (●) derived from permethylated cyclohexaamylose by reductive cleavage in the presence of 5 equiv. of TMSOTf (per equiv. of acetal) and the indicated amounts of added water. The maximal amount of 2 produced was 45%.

in 55% yield and 2 was formed in 45% yield (analyzed as acetyl derivatives 3 and 4 by GLC). The amount of 2 formed from 5 is therefore exactly the same as the maximal amount formed from permethylated cyclohexaamylose upon the addition of water (Fig. 1). These results suggest, but do not prove, that 2,3,6-tri-O-methyl-D-glucose (5) is an intermediate in the formation of 2 from permethylated cyclohexaamylose. It is also feasible that the reduction of both substrates proceeds *via* another common intermediate. For example, a bicyclic intermediate such as 1,4-anhydro-2,3,6-tri-O-methyl-D-glucopyranose could also be expected to undergo reductive ring opening to give both 1 and 2. Further studies will be required to establish the identity of the intermediate leading to the formation of 2.

Because of the necessity of carrying out reductive cleavage reactions under anhydrous conditions, an investigation was conducted to identify a convenient experimental protocol. When



Scheme I

Studies designed to explore the route of formation of 2 from permethylated cellulose and cyclohexaamylose quickly centered on the importance of anhydrous reaction conditions because of the observation that the amount of 2 formed was extremely variable in separate experiments. In order to explore this possibility, the reductive cleavage of cyclohexaamylose was carried out with TMSOTf as catalyst in the presence of known amounts of added water. As shown in Fig. 1, the amount of 2 formed increased with added water, reaching a maximal value of 45% when 4 equivalents of water were added per equivalent of acetal. All reactions were carried out in the presence of excess TMSOTf (5 equivalents per equivalent of acetal) in order to ensure catalysis of reductive cleavage. One possibility suggested by these results was that 2,3,6-tri-O-methyl-D-glucose (5) was formed as an intermediate in these reactions as a result of *hydrolysis* of the permethylated polysaccharide. Equilibration between the pyranose and furanose forms of 2,3,6-tri-O-methyl-D-glucose and their subsequent reduction could give rise to both 1 and 2. Indeed, when 2,3,6-tri-O-methyl-D-glucose was subjected to reductive cleavage in the presence of TMSOTf, 1 was produced

1,5-anhydro-4-O-acetyl-2,3,6-tri-O-methyl-D-glucitol (3) as previously reported[1,2] and a minor product (5%) with a slightly longer retention time (20 min vs. 19 min for 3). The same minor product was observed in varying amounts in the reductive cleavage of permethylated cellulose, methyl 2,3,6-tri-O-methyl-4-O-(2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl)-β-D-glucopyranoside and methyl 2,3,6-tri-O-methyl-4-O-(2,3,4,6-tetra-O-methyl-α-D-glucopyranosyl)-β-D-glucopyranoside. Isolation of the minor product in an amount sufficient for structural characterization was accomplished by preparative GLC of its acetate on OV225.

The identity of the minor component as 4 was readily established by 300 MHz ^{1}H NMR spectroscopy. It was known from chemical ionization mass spectral studies[2] that the minor component was isomeric with 3, i.e., that it had the composition of a mono-O-acetyl-tri-O-methyl-monoanhydrohexitol. Its ^{1}H NMR spectrum confirmed this, displaying one O-acetyl and three methoxyl resonances. In addition, the methine resonance of the ester (H-5, δ5.20), which was observed well downfield of the other resonances, was a doublet doublet of doublets (J = 2.58, 4.84 and 8.43 Hz). The multiplicity of the ester methine and the magnitude of the coupling constants can arise only in a 5-O-acetyl derivative. Decoupling experiments confirmed this and led to the identification of the C-1,2,3,4 and 6 proton resonances and their associated coupling constants.

Confirmation of the structure proposed for the artifact (2) was obtained by its independent synthesis from 2,3,6-tri-O-methyl-D-glucose (5) by the route shown in Scheme I. Direct reductive amination[3] of 5 gave 1-amino-1-deoxy-2,3,6-tri-O-methyl-D-glucitol (6) which was subjected to kinetic ring closure by treatment with nitrous acid.[4] Subsequent acetylation and preparative GLC yielded a major product (4, 58% GLC yield) which co-chromatographed with the artifact derived from reductive cleavage and had identical ^{1}H- and ^{13}C-NMR spectra.

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