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ANALYSIS OF LINKAGE POSITIONS IN 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSYL RESIDUES BY THE REDUCTIVE-CLEAVAGE METHOD*

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ABSTRACT

The fate of methylated 2-acetamido-2-deoxy-D-glucopyranosyl residues under reductive-cleavage conditions was investigated by using methyl 2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (**1**), its α anomer (**8**), and fully methylated lacto-*N*-tetraitol as test compounds. Treatment of **1** with triethylsilane and trimethylsilyl trifluoromethanesulfonate in dichloromethane gave rise to (1,2-dideoxy-3,4,6-tri-*O*-methyl-α-D-glucopyrano)-2,3-dimethyl-[2,1-*d*]-2-oxazolinium trifluoromethanesulfonate. Quenching of the reaction by addition of aqueous sodium hydrogencarbonate resulted in hydrolysis of the oxazolinium salt. Compound **8** was fully stable to reductive-cleavage conditions. Thus, participation by the 2-acetamido group is necessary for glycosidic carbon–oxygen bond-cleavage to occur. Treatment of methyl 2-deoxy-2-(ethylmethylamino)-3,4,6-tri-*O*-methyl-α,β-D-glucopyranoside under reductive-cleavage conditions resulted in some anomerization, but neither hydrolysis nor reductive cleavage of the glycosidic carbon–oxygen bond was observed, as expected. Reductive cleavage of fully methylated lacto-*N*-tetraitol gave the products predicted on the basis of these and prior model studies, including 3-*O*-acetyl-2-(acetylmethylamino)-2-deoxy-4,6-di-*O*-methyl-D-glucopyranose derived from the 3-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residue.

INTRODUCTION

In an attempt to simplify the structural characterization of complex carbohydrates, we proposed[1], for carrying out "methylation analysis", a new technique which we referred to as the Reductive-Cleavage Method. Previous studies employing several mannans[2], fructans[3], and glucans[4,5] of established structure indeed demonstrated the applicability of this new technique to the structural characteriza-

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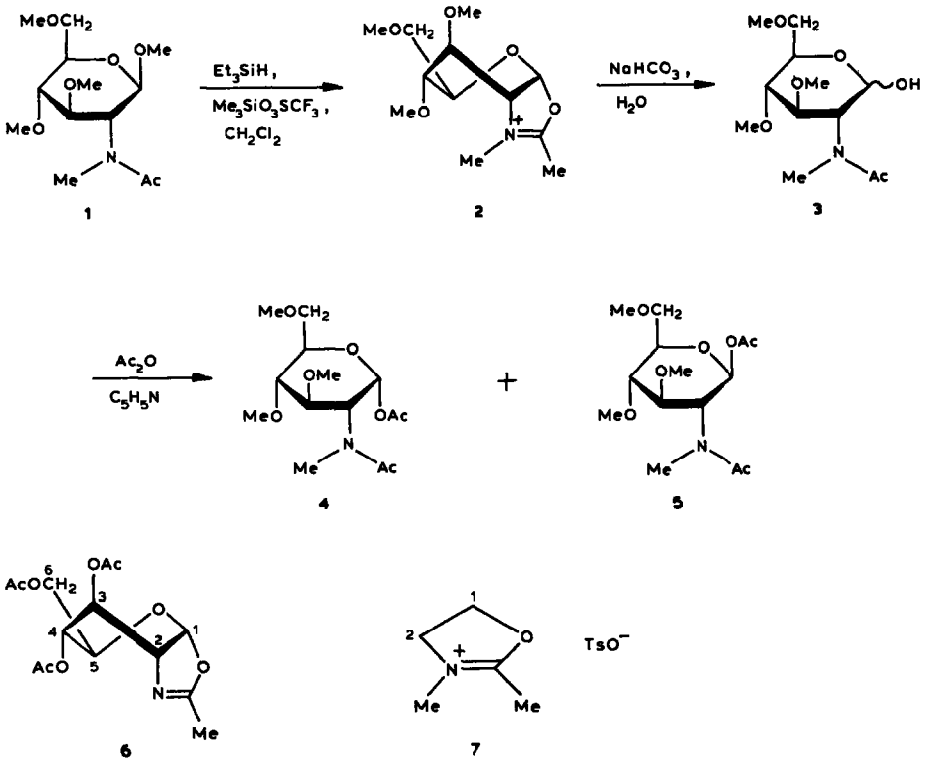
tion of polysaccharides containing these neutral monosaccharide residues, as, in all cases, the partially methylated anhydroalditol residues expected were obtained. We have since begun a program to extend this new method to the characterization of polysaccharides containing acidic and basic monosaccharide residues by examining the behavior of representative model glycosides and glycans under reductive-cleavage conditions. In this report, we describe the results obtained from a study employing methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside, as well as lacto-*N*-tetraitol, which contains a 3-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residue.

RESULTS

Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside. — Direct methylation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside by the procedure of Hakomori[6] gave methyl 2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (**1**). Treatment of **1** under the usual reductive-cleavage conditions[4], namely, with 5 equivalents each of triethylsilane (Et_3SiH) and trimethylsilyltrifluoromethanesulfonate ($Me_3SiO_3SCF_3$) in dichloromethane for 20 h at room temperature, followed by sequential, *in situ* acetylation, and extraction with aqueous sodium hydrogencarbonate, gave no detectable carbohydrate compounds in the resultant dichloromethane solution, as judged by g.l.c. (Method 1) and ^{1}H-n.m.r. spectroscopic analysis.

After three trials in which no carbohydrate products were observed, a reaction was carried out in an n.m.r. tube (compound **1** plus 5 equiv. each of Et_3SiH and $Me_3SiO_3SCF_3$ in $CDCl_3$, with Me_4Si as the internal standard), and the ^{1}H-n.m.r. spectrum of the mixture was recorded as a function of time. Four hours after mixing, the anomeric-proton signal of **1** (δ 4.32, d, *J* 8.1 Hz) was completely replaced by a signal much farther downfield (δ 6.60, d, *J* 8.1 Hz). Analysis of the entire spectral region revealed two other ring-hydrogen signals shifted downfield, namely, at δ 4.60 (broad multiplet) and at δ 3.86 (t, *J* 3.1 Hz). In addition, the *N*-methyl and *N*-acetyl signals, which were observed as two pairs of singlets for the starting material (**1**), due to rotameric equilibrium around the amide bond, were now observed as one singlet each and were shifted slightly downfield. A similar n.m.r. experiment was performed in which the reducing agent (Et_3SiH) was omitted, and identical results were obtained. Decoupling of the signals at δ 6.60, 4.60, and 3.86 in the spectrum of the product established that these resonances were attributable to H-1, H-2, and H-3, respectively. For example, decoupling at δ 4.60 collapsed the doublet at δ 6.60 to a singlet, and the triplet at δ 3.86 to a doublet.

The magnitudes of the coupling constants and the downfield shifts for H-1, H-2, H-3, and the *N*-methyl and *N*-acetyl resonances suggested that the bicyclic oxazolinium ion, (1,2-dideoxy-3,4,6-tri-*O*-methyl-α-D-glucopyrano)-2,3-dimethyl-[2,1-*d*]-2-oxazolinium trifluoromethanesulfonate (**2**) was the product formed under



reductive cleavage conditions. The structural assignment of **2** and the conformation [modified skew ($^{O}S_2$), as shown, or distorted half-chair ($^{4}H_5$)] are supported by the n.m.r. data, which agree well with the values reported for **6** and **7**, which are structurally similar (see Table I)[7–10]. Compound **6** had been reported to exist in a modified $^{O}S_2$ conformation[7,8] or distorted $^{4}H_5$ conformation[9] in solution. Compounds **2** and **6** display coupling constants of similar magnitude between H-1, H-2, and H-3, suggesting that they possess similar conformations. More importantly, the chemical shifts observed for H-1 and H-2 of **2** are those expected, taking into account the additional deshielding brought about by the presence of the positively charged, oxazolinium ring of **2**, relative to the neutral oxazoline ring of **6**.

Aided by the knowledge that **2** was formed upon treatment of **1** under reductive-cleavage conditions, another reaction was carried out in the usual way and was stopped by the addition of aqueous sodium hydrogencarbonate. Again, extraction with CH_2Cl_2 failed to yield any carbohydrate material, but examination of the remaining aqueous layer revealed the presence of 2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-D-glucopyranose (**3**), which was characterized on the basis of its 300-MHz, ^{1}H-n.m.r. spectrum, and analysis of its acetylated derivatives (**4** and **5**) by combined gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.). Evaporation of the aqueous $NaHCO_3$ solution obtained after quenching the reductive

TABLE I

A COMPARISON OF THE RELEVANT ^{1}H-N.M.R. SPECTRAL PARAMETERS FOR COMPOUNDS **2**, **6**, AND **7**

Resonance or coupling constant	*Compound*		
	2	**6**	**7**
Resonance	*Chemical shift (δ)*		
H-1	6.60[a]	5.97[b]	4.80[c]
H-2	4.60	4.14	4.06
N-CH_3	3.86	—	3.20
C-CH_3	2.50	2.09	2.30
Coupling constant	*J (Hz)*		
H-1,2	8.1	7.3	—
H-2,3	3.1	2.6	—
H-3,4	2.8	2.2	—

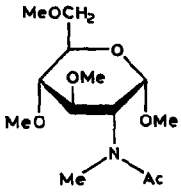
[a]Spectrum recorded at 300 MHz for a solution in $CDCl_3$. [b]Spectrum recorded at 270 MHz for a solution in $CDCl_3$ (ref. 7). [c]Spectrum recorded at 100 MHz for a solution in CD_3CN (ref. 10).

cleavage of **1**, and thorough drying, followed by acetylation (acetic anhydride–pyridine), gave two compounds, as established by g.l.c.–m.s. analysis. These two compounds were shown to be the anomeric 1-*O*-acetyl-2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-α- and -β-D-glucopyranoses (**4** and **5**, respectively) by independent synthesis. The isolated and the synthesized materials were found to co-migrate in capillary g.l.c. (Method 2). Analysis by combined gas–liquid chromatography–chemical ionization mass spectrometry (g.l.c.–c.i.m.s.), with NH_3 as the reagent gas, indicated that only compound **4** was stable enough to reveal $(M + H)^+$ and $(M + NH_4)^+$ ions, and these ions were unusually weak in intensity. However, both the isolated compounds and the synthetic standards (**4** and **5**) gave intense ions at *m/z* 260, attributable to the stable oxazolinium ion (**2**) formed *via* the facile displacement of the 1-*O*-acetyl group.

Assignment of the ^{1}H-n.m.r. spectrum of **3** (obtained for a solution in D_2O) was complicated by the presence of two anomers, each of which appeared as a pair of amide rotamers. Thus, four anomeric-proton resonances were present, two each for the axial proton of the β anomer [δ 4.92 (d, 0.37 H, *J* 7.6 Hz), and 5.01 (d, 0.43 H, *J* 7.8 Hz)] and for the equatorial proton of the α anomer [δ 5.14 (d, 0.13 H, *J* 3.4 Hz), and 5.28 (d, 0.07 H, *J* 3.3 Hz)]. Similarly, the *N*-methyl group of the two anomers gave rise to four singlets, at δ 2.93 (1.4 H), 2.99 (0.2 H), 3.07 (1.0 H), and 3.12 (0.4 H). Inspection of the integral values of these singlets led to the conclusion that the singlets at δ 2.99 and 3.12 are due to the *N*-methyl groups of the α anomer, whereas the resonances at δ 2.93 and 3.07 are due to those of the β anomer. Multiple *N*-acetyl and *O*-methyl signals were also observed, as expected.

From these results, it is apparent that the oxazolinium ion (**2**) is stable to the conditions of reductive cleavage and that, upon workup, it is hydrolyzed to produce **3**. The net result, therefore, of exposure of **1** to reductive-cleavage conditions is simple hydrolysis of its glycosidic carbon–oxygen bond.

*Methyl 2-acetamido-2-deoxy-α-*D*-glucopyranoside.* — The fate of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside after sequential methylation and reductive cleavage was examined by using a mixture of methyl 2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-α-D-glucopyranoside (**8**) and methyl 2-



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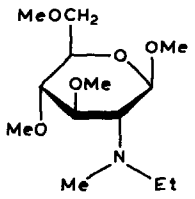
(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (**1**), prepared by direct methylation[6] of a mixture of methyl 2-acetamido-2-deoxy-α,β-D-glucopyranosides. A mixture containing **8**, **1**, and docosane (as an internal standard) was treated under reductive-cleavage conditions, using either $BF_3 \cdot Et_2O$ or $Me_3SiO_3SCF_3$ as the catalyst, and the reaction mixtures, after quenching, were analyzed by g.l.c. (Method 1). The peak areas of the starting glycosides (**1** and **8**) relative to the peak area of the internal standard (docosane) were determined both before and after reductive cleavage. Before reductive cleavage, the peak areas of **1** and **8** relative to docosane were 0.60 and 1.14, respectively. After reductive cleavage, the peak areas of **1** and **8** relative to docosane were found to be 0.00 and 1.22, respectively, when $Me_3SiO_3SCF_3$ was used as the catalyst, and 0.21 and 1.08, respectively, when $BF_3 \cdot Et_2O$ was used as the catalyst. Thus, as expected, the β-glycoside (**1**) was quantitatively hydrolyzed in the presence of $Me_3SiO_3SCF_3$ and the product (**3**) was not detected in the dichloromethane solution. When $BF_3 \cdot Et_2O$ was used as the catalyst under the same conditions[4], compound **1** was also hydrolyzed, although not as rapidly as when $Me_3SiO_3SCF_3$ was used as the catalyst. Importantly, the α-glycoside **8** was stable to reductive cleavage with either catalyst and was quantitatively recovered, within experimental error, in the dichloromethane solution.

*Methyl 2-(ethylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-β-*D*-glucopyranoside* (**9**) *and methyl 2-(ethylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-α-*D*-glucopyranoside* (**10**). — The results obtained in the attempted reductive cleavage of the anomeric glycosides **1** and **8** clearly indicated participation by the 2-acetamido group in cleavage of the glycosidic carbon–oxygen bond. These results suggested, therefore, that reduction of the acetamido group would prevent glycosidic carbon–oxygen bond-cleavage. As a test of this assumption, the starting mixture of amides **1** and **8** and docosane used previously was treated with lithium aluminum hydride, to produce a mixture containing the corresponding ethylmethylamines (**9** and **10**, respectively) and docosane. This mixture was treated under reductive-cleavage conditions with both catalysts as before, but processing of the $BF_3 \cdot Et_2O$-catalyzed

reaction was modified in order to facilitate extraction of the amine products into the organic (dichloromethane) layer. The resulting dichloromethane solutions were analyzed by g.l.c. (Method 3), and the peak areas of the starting glycosides (**9** and **10**) relative to the peak area of the internal standard (docosane) were determined.



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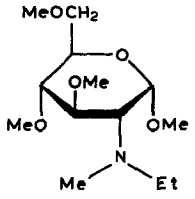

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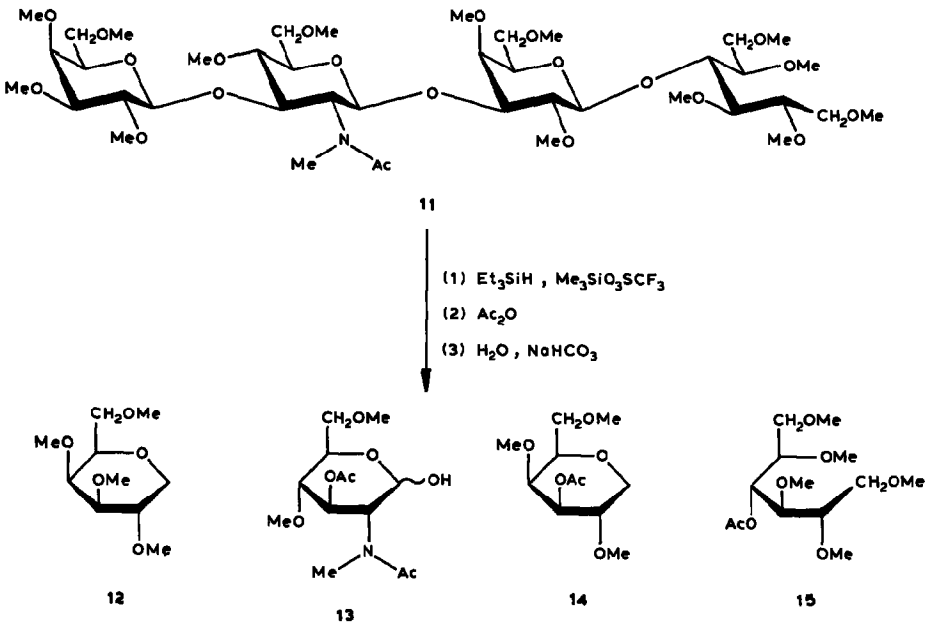
Reduction of the acetamido group with lithium aluminum hydride was accomplished in near-quantitative yield; *i.e.*, the peak areas of **9** and **10** relative to docosane (0.53 and 1.08, respectively) were virtually the same as for the starting mixture of **1** and **8** relative to docosane (0.60 and 1.14, respectively). Reductive cleavage in the presence of $Me_3SiO_3SCF_3$ led to some anomerization of **9** to **10** (peak areas of **9** and **10** relative to docosane were 0.25 and 1.56, respectively), but neither reductive cleavage nor hydrolysis was apparent. The combined-peak-area ratio of **9** and **10** after reductive cleavage in the presence of $Me_3SiO_3SCF_3$ (1.81) is, in fact, somewhat greater than before reductive cleavage (1.61), but this difference, most likely, is within experimental error. Reductive cleavage of the **9** + **10** mixture in the presence of $BF_3 \cdot Et_2O$ may possibly have led to some destruction of the β anomer **9**, as the peak areas observed for **9** and **10** relative to docosane were 0.38 and 1.07, respectively. In this case, however, overall hydrolysis is not possible, because an oxazolinium ion cannot be formed, and reductive cleavage, had it occurred, would have been noticed by the appearance of the corresponding anhydroalditol in subsequent g.l.c. analysis. Such a component was not detected, and consequently, the most likely explanation for the low peak-area ratio of **9** (0.38) is that some anomerization of **9** to **10** also occurred in the presence of $BF_3 \cdot Et_2O$ and that the recovered yield of **9** + **10** was low, due to extractive loss during workup.

*Lacto-*N*-tetraitol.* — Crystalline lacto-*N*-tetraose [β-Gal-(1→3)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc][11,12], a generous gift from Dr. Victor Ginsburg, was reduced with sodium borohydride to produce lacto-*N*-tetraitol. Hakomori methylation[6] and subsequent chromatography on silica gel[13], afforded the fully *O*- and *N*-methylated derivative (**11**). The 300-MHz, ^{1}H-n.m.r. spectrum of **11** revealed three axial anomeric hydrogen resonances, as expected, at δ 4.26 (d, J 6.9 Hz), 4.42 (d, J 7.8 Hz) and 4.62 (d, J 8.2 Hz). Given are the structures of permethylated lacto-*N*-tetraitol (**11**), as well as the expected products of sequential reductive-cleavage and *in situ* acetylation. Because the constituent amino sugar is β-linked, hydrolysis of its glycosidic linkage should occur, to give the corresponding aldose derivative (**13**).

The terminal (nonreducing) D-galactopyranosyl group and the 3-linked D-galactopyranosyl residue should give rise to **12** and **14**, respectively, whereas the remaining residue at the originally reducing end should give the (acyclic) alditol derivative **15**.

Reductive cleavage of **11** was carried out in the presence of $Me_3SiO_3SCF_3$ as the catalyst, and, after subsequent acetylation *in situ* and extraction with aqueous sodium hydrogencarbonate, the resulting aqueous and organic (dichloromethane) layers were analyzed. Analysis of the dichloromethane solution by g.l.c. (Method 2), g.l.c.–c.i.m.s., and combined g.l.c.–electron impact mass spectrometry (g.l.c.–e.i.m.s.), led to the identification of three major products, namely, 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-galactitol (**12**), 3-*O*-acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl-D-galactitol (**14**), and 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methyl-D-glucitol (**15**). Given in Table II are the g.l.c. retention-times for these components, their expected and observed molecular weights, and their relative molar ratios (normalized



to **14**). In all cases, the molecular weights observed for these components were those expected. In addition, their e.i. mass spectra were fully in accord with their assigned structures. For example, the e.i. mass spectra of compounds **12** and **14** both displayed fragment ions corresponding to $(M - CH_2OMe)^+$ at *m/z* 175 and *m/z* 203, respectively, and both spectra also displayed fragment ions corresponding to the further loss of methanol from those ions $(M - CH_2OMe - MeOH)^+$ at *m/z* 143 and *m/z* 171, respectively. The e.i. mass spectrum of **14** also displayed a major fragment-ion at *m/z* 188, corresponding to loss of acetic acid from the molecular ion $(M - AcOH)^+$ and, as expected, a fragment of this type was absent from the

TABLE II

RETENTION TIMES, MOLECULAR WEIGHTS, AND MOLE FRACTIONS OF PRODUCTS DERIVED BY REDUCTIVE CLEAVAGE OF PERMETHYLATED LACTO-*N*-TETRAITOL (**11**)[a]

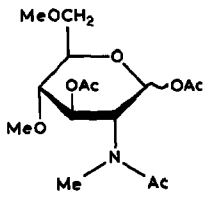
Compound	*Retention time (min.)*[b]	*Molecular weight*		*Mole fraction*[d]
		Expected	*Found*[c]	
12	9.90	220	220	0.85
14	13.04	248	248	1.00
15	14.66	294	294	0.48

[a]Dichloromethane layer. [b]G.l.c. Method 2. [c]C.i.m.s. (NH_3) gives $(M + H)^+$ and $(M + NH_4)^+$. [d]Normalized to **14**.

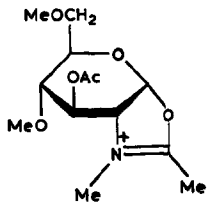
e.i. mass spectrum of **12**. The acyclic component **15** also gave the expected[14] e.i. mass spectrum. Fragment ions at *m/z* 45, 89, and 205 were particularly diagnostic.

The relative mole-fractions of these components were calculated by dividing their integral values (flame-ionization detection) by their calculated, effective-carbon-response values[2,15] (1.00 for **12**, 1.09 for **14**, and 1.29 for **15**; normalized to **12**), and normalizing to the value for compound **14**. The value, slightly lower than expected, for the relative molar ratio of **12** (0.85) is attributable to the high volatility of this compound, which is higher than that of any other tetra-*O*-methylated anhydrohexitol we have thus far observed (unpublished data). The low value obtained for the relative molar ratio of **15**, however, is a consequence of degradation of the acyclic residue during long-term, reductive-cleavage reactions. The side-products produced in this reaction are the same as were observed in the reductive cleavage of permethylated maltitol (unpublished data). Although these components have not been identified, integration established that they are present in a combined molar ratio of 0.52 (relative to **14**), thus fully accounting for all products derived from reductive cleavage of the (acyclic) D-glucitol residue.

Because the amino sugar **13** was not detected in the dichloromethane solution obtained from processing of the reductive-cleavage reaction-mixture, quantitation of this residue relative to those of the other three (**12, 14**, and **15**) could not be accomplished. Identification of the amino sugar residue was accomplished after evaporation of the aqueous sodium hydrogencarbonate solution, drying, and acetylation of the product as described for **3**. Analysis of the resulting acetates by g.l.c.–c.i.m.s. (NH_3) revealed the presence of two compounds, both of which gave intense ions at *m/z* 288. Indeed, these are the results expected due to the facile loss of acetate (59 mu) from the anomeric 1,3-di-*O*-acetyl-2-(acetylmethylamino)-2-deoxy-4,6-di-*O*-methyl-D-glucopyranoses (**16**) to produce the stable oxazolinium ion **17**. As mentioned previously, a fragment ion (**2**) arising by an identical process was present in the c.i. mass spectra of compounds **4** and **5**. Further analysis of the anomeric mixture of acetates (**16**) by 300-MHz, ^{1}H-n.m.r. spectroscopy revealed a doublet at δ 5.78 (J 8.5 Hz) attributable to H-1 of the β anomer of **16**, and *N*-methyl



16, 347 mu



17, *m/z* 288

singlets at δ 2.77 and 2.88. The chemical shift and coupling constant of the δ 5.78 resonance agree well with those observed for H-1 of **5** (δ 5.72, d, J 8.8 Hz).

DISCUSSION

The model studies reported herein suggest that the Reductive-Cleavage Method will be directly applicable to the analysis of polysaccharides containing 2-acetamido-2-deoxy-D-glucopyranosyl residues. Although in no case was reductive cleavage of the glycosidic carbon–oxygen bond of an amino sugar residue accomplished, the ability to *hydrolyze* selectively the glycosidic bond of β-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues, coupled with the stability of the glycosidic bond of α-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues under reductive-cleavage conditions, provides a direct method for the chemical determination of their anomeric configuration.

This selectivity is also likely to be useful in sequence determination where amino sugar residues are present; *i.e.*, methylated, α-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues and methylated α- or β-linked 2-(ethylmethylamino)-2-deoxy-D-glucopyranosyl residues will not be cleaved, giving rise to a disaccharide-anhydroalditol[4] or a larger fragment containing the amino sugar residue with its glycosidic linkage intact. This approach should be especially useful in combination with another method for the structural analysis of polysaccharides containing 2-acetamido-2-deoxy-D-glucopyranosyl residues, namely, cleavage by sequential *N*-deacetylation and nitrous acid deamination[16]. In the latter case, deamination leads to hydrolytic cleavage of the glycosidic carbon–oxygen bond of the amino sugar residue, to give fragments terminated ("reducing end") by a 2,5-anhydro-D-mannose residue. Further characterization of the fragments generated by these specific cleavages will therefore establish the identities of the residue to which the amino sugar is glycosidically linked, as well as the residue(s) which is glycosidically linked to the amino sugar.

It should also be mentioned that the Reductive-Cleavage Method is directly applicable to the characterization of the fragments formed by sequential *N*-deacetylation and nitrous acid deamination. Reduction ($NaBH_4$) of the terminal ("reducing") 2,5-anhydro-D-mannose residue, and sequential methylation, reductive cleavage, and acetylation will yield a partially methylated 2,5-anhydro-D-mannitol acetate where the position of the acetyl group(s) will identify the linkage posi-

tion(s). The same derivatives are produced by reductive-cleavage analysis of D-fructofuranose-containing polysaccharides, and several isomers have already been characterized[3].

EXPERIMENTAL

General. — ^{1}H-N.m.r. spectra were recorded with a Nicolet NT-300 n.m.r. spectrometer. Spectra recorded with $CDCl_3$ or C_6D_6 as the solvent were referenced to internal tetramethylsilane, and those recorded with D_2O as the solvent to internal sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. Analytical g.l.c. was performed in a Hewlett–Packard Model 5890A gas–liquid chromatograph equipped with a Hewlett–Packard Model 3392A integrator and a flame-ionization detector. The following columns and conditions were used. *Method 1.* OV-101 glass capillary column (0.32 mm × 25 m), isothermal at 150° for 4 min, and then programmed to 240° at 5°/min. *Method 2.* Cross-linked methyl silicone–glass capillary column (0.2 mm × 25 m), isothermal at 110° for 2 min, and then programmed to 300° at 6°/min. *Method 3.* Same column as in *Method 1*, except that the temperature was held at 130° for 2 min, programmed to 175° at 5°/min, held at 175° for 1 min, and then programmed to 240° at 10°/min.

G.l.c.-m.s. analyses were performed in a Finnigan 4000 mass spectrometer equipped with a VG Multispec data system. Column effluents were analyzed by chemical-ionization mass spectrometry, with ammonia as the reagent gas, whereby characteristic $(M + 1)^+$ and $(M + NH_4)^+$ ions were detected, and by electron-impact mass spectrometry in order to verify that eluted components had mass spectra identical to those of independently synthesized standards.

Reductive cleavages were performed as previously described[4]. In the $BF_3 \cdot Et_2O$-catalyzed reductive cleavage of the amine mixture (**9** + **10**), processing of the reaction mixture was altered slightly, in order to facilitate extraction of the products into the organic layer. The reaction was quenched by the addition of methanol (0.5 mL) and solid Na_2CO_3, and the mixture was stirred for 1.5 h, during which time, mild evolution of gas occurred. Solid NaOH (one pellet) was added, and stirring was continued for 1 h, after which, dichloromethane (5 mL) and water (10 mL) were added. The dichloromethane solution was removed, and filtered through anhydrous Na_2SO_4 prior to g.l.c. analysis (Method 3).

*Methyl 2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-β-*D*-glucopyranoside* (**1**). — Direct methylation[6] of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[17] (0.2 g) gave **1** (0.15 g) as a yellow oil. G.l.c. analysis (Method 1) revealed a single component having a retention time of 12.3 min; ^{1}H-n.m.r. ($CDCl_3$): δ 2.10 (s, 0.75 H, AcN, rotamer 1), 2.13 (s, 2.25 H, AcN, rotamer 2), 2.86 (s, 2.25 H, MeN, rotamer 2), 3.06 (broad s, 0.75 H, MeN, rotamer 1), 3.41, 3.45, 3.50, 3.53 (4 s, 0.75 H each, MeO, rotamer 1), 3.43, 3.47, 3.50, 3.56 (4 s, 2.25 H each, MeO, rotamer 2), and 4.32 (d, 1 H, *J* 8.1 Hz, H-1); lit.[18] δ 2.02, 2.06 (AcN), 2.89, 3.09 (MeN), 3.43–3.68 (MeO), and 4.38 (d, *J* 8 Hz, H-1).

*2-(Acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-*D*-glucopyranose* (**3**). — Compound **1** (12.5 mg) was subjected to reductive cleavage with $Me_3SiO_3SCF_3$ as the catalyst in CH_2Cl_2 as the solvent, in the usual manner[4], and the reaction mixture was extracted twice, with stirring, with 1-mL portions of saturated, aqueous $NaHCO_3$. The aqueous layers were combined, and evaporated to dryness, leaving a white, powdery residue. The residue was extracted with ethyl acetate (10 mL), the extract filtered through glass wool to remove carbonate salts, and the filtrate evaporated to dryness, yielding 17 mg of a sticky gum (some salt present). For **3**: ^{1}H-n.m.r. (D_2O): δ 2.17–2.29 (complex, 3 H, AcN), 2.93 (s, 1.4 H, MeN, β anomer, rotamer 1), 2.99 (s, 0.2 H, MeN, α anomer, rotamer 1), 3.07 (broad s, 1.0 H, MeN, β anomer, rotamer 2), 3.12 (s, 0.4 H, MeN, α anomer, rotamer 2), 3.3–4.4 (complex, 15 H, H-2,3,4,5,6,6′, MeO), 4.92 (d, 0.37 H, J 7.6 Hz, H-1, β anomer, rotamer 2), 5.01 (d, 0.43 H, J 7.8 Hz, H-1, β anomer, rotamer 1), 5.14 (d, 0.13 H, J 3.4 Hz, H-1, α anomer, rotamer 2), and 5.28 (d, 0.07 H, J 3.3 Hz, H-1, α anomer, rotamer 1).

*Methyl 2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-α-*D*-glucopyranoside* (**8**). — An anomeric mixture of methyl 2-acetamido-2-deoxy-α,β-D-glucopyranosides[19] (1.4 g) was methylated[6], and the product was chromatographed on silica gel in 1:1 (v/v) hexane–acetone, to yield 1.3 g of a 9:16 mixture of **1** and **8**. Selective removal of the β anomer **1** was accomplished by treatment of the mixture (210 mg) under reductive-cleavage conditions, namely, with Et_3SiH (0.6 mL; 5 equiv.) and $Me_3SiO_3SCF_3$ (0.7 mL; 5 equiv.) in 15 mL of CH_2Cl_2 for 24 h at room temperature. The reaction was stopped by the addition of 10 mL of saturated, aqueous $NaHCO_3$, and the mixture was stirred for 0.5 h, during which time mild evolution of gas occurred. The biphasic mixture was extracted with water (50 mL), and the organic layer was retained. The aqueous layer was extracted three times with 10-mL portions of dichloromethane, and the extracts and initial organic layer were combined, and evaporated under vacuum, to yield 157 mg of a yellow oil. Passage of the product through silica gel in 1:1 (v/v) hexane–acetone provided 113 mg of **8** that was pure by g.l.c. (Method 1, retention time 14.3 min); ^{1}H-n.m.r. ($CDCl_3$): δ 2.12 (s, 1.2 H, AcN, rotamer 1), 2.13 (s, 1.8 H, AcN, rotamer 2), 2.94 (s, 1.2 H, MeN, rotamer 1), 3.00 (s, 1.8 H, MeN, rotamer 2), 3.31, 3.43, 3.49, 3.54 (4 s, 1.8 H each, MeO, rotamer 2), 3.34, 3.44, 3.52, 3.56 (4 s, 1.2 H each, MeO, rotamer 1), 3.35–3.80 (complex, 5 H, H-3,4,5,6,6′), 4.66–4.69 (complex, 1.6 H, H-1, rotamer 2, and H-2, rotamers 1 and 2), and 4.73 (d, 0.4 H, J 3.6 Hz, H-1, rotamer 1); ^{1}H-n.m.r. (C_6D_6): δ 1.74 (s, 2.1 H, AcN, rotamer 1), 1.85 (s, 0.9 H, AcN, rotamer 2), 2.64 (s, 2.1 H, MeN, rotamer 1), 2.93 (s, 0.9 H, MeN, rotamer 2), 2.99, 3.21, 3.32, 3.34 (4 s, 2.1 H each, MeO, rotamer 1), 3.04, 3.16, 3.29, 3.39 (4 s, 0.9 H each, MeO, rotamer 2), 3.22–3.85 (complex, 5 H, H-3,4,5,6,6′), 4.32 (d, 0.3 H, J 3.5 Hz, H-1, rotamer 2), 4.81 (d, 0.7 H, J 3.5 Hz, H-1, rotamer 1), and 5.12 (dd, 1 H, J 3.5, 11.6 Hz, H-2).

*1-*O*-Acetyl-2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-α-*D*-glucopyranose* (**4**) *and 1-*O*-acetyl-2-(acetylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-β-*D*-*

glucopyranose (**5**). — A mixture of compounds **1** and **8** (79 mg, prepared as described for **8**) was hydrolyzed[20], and the product was acetylated by treatment with an excess of acetic anhydride and pyridine overnight. The reaction was quenched by the addition of water at 0°, dichloromethane was added, and the organic layer was washed successively with cold M H_2SO_4, saturated aqueous $NaHCO_3$, and water, dried (Na_2SO_4), and evaporated to dryness, to yield a mixture of **4** and **5**. Analytical g.l.c. (Method 2) of the product revealed the presence of two components, having retention times of 17.8 (**5**) and 22.7 min (**4**); ^{1}H-n.m.r. ($CDCl_3$): δ 2.06, 2.08, 2.09, 2.10, 2.11, 2.12, 2.14, 2.16 (8 s, 6 H, AcN, AcO, rotamers 1 and 2, **4** and **5**), 2.83, 2.90, 2.96 (3 s, 3 H, MeN, rotamers 1 and 2, **4** and **5**), 3.39–3.85 (complex, 14.3 H, H-2,3,4,5,6,6′ of **5**, H-3,4,5,6,6′ of **4**, MeO), 4.81 (dd, 0.7 H, *J* 3.5, 11.6 Hz, H-2 of **4**), 5.72 (d, 0.29 H, *J* 8.8 Hz, H-1 of **5**), 6.09 (d, 0.44 H, *J* 3.4 Hz, H-1 of **4**, rotamer 1), and 6.10 (d, 0.27 H, *J* 3.4 Hz, H-1 of **4**, rotamer 2).

*Methyl 2-(ethylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-β-*D*-glucopyranoside* (**9**) *and methyl 2-(ethylmethylamino)-2-deoxy-3,4,6-tri-*O*-methyl-α-*D*-glucopyranoside* (**10**). — A mixture of compounds **1** and **8** (60 mg) in 10 mL of dry diethyl ether was added dropwise to a stirred slurry of $LiAlH_4$ (120 mg) in 20 mL of dry diethyl ether, and the mixture was stirred for 1.5 h under an atmosphere of dry nitrogen. The reaction was quenched by the dropwise addition of water (5 mL) and the mixture diluted with diethyl ether (10 mL) and water (100 mL). The ethereal layer was separated and retained, and the aqueous layer was extracted twice with 30-mL portions of diethyl ether. The ethereal extracts were combined, dried (Na_2SO_4), and evaporated to dryness, to yield a 33:67 mixture of **9** and **10** (g.l.c., Method 3). Analytical g.l.c. (Method 3) after the addition of docosane revealed retention times of 10.26, 10.36, and 20.04 min for **9**, **10**, and docosane, respectively. For **9**, ^{1}H-n.m.r. ($CDCl_3$): δ 1.06 (t, 3 H, *J* 7.1 Hz, ethyl CH_3), 2.41 (s, 3 H, MeN), 2.65–2.80 (complex, 2 H, ethyl CH_2), 3.1–3.8 (complex, 6 H, H-2,3,4,5,6,6′), 3.41, 3.48, 3.54, 3.57 (4 s, 3 H each, MeO), and 4.28 (d, 1 H, *J* 8.3 Hz, H-1). For **10**, ^{1}H-n.m.r. ($CDCl_3$): δ 1.05 (t, 3 H, *J* 7.2 Hz, ethyl CH_3), 2.46 (s, 3 H, MeN), 2.65–2.80 (complex, 2 H, ethyl CH_2), 3.1–3.8 (complex, 6 H, H-2,3,4,5,6,6′), 3.33, 3.42, 3.54, 3.61 (4 s, 3 H each, MeO), and 4.69 (d, 1 H, *J* 3.2 Hz, H-1).

*Permethylated lacto-*N*-tetraitol* (**11**). — Lacto-*N*-tetraose (3.1 mg) was reduced to lacto-*N*-tetraitol as described by Kobata and Ginsburg[21], and the product was methylated by the procedure of Hakomori[6]. Purification by passage through silica gel as described by Ginsburg *et al.*[13] afforded **11** (3.2 mg) as a clear oil; ^{1}H-n.m.r. ($CDCl_3$): δ 2.17, 2.20 (2 s, 3 H, AcN), 2.90, 2.94 (2 s, 3 H, MeN), 3.3–4.0 (complex, methoxyls, ring hydrogens), 4.26 (d, 1 H, *J* 6.9 Hz, anomeric H), 4.42 (d, 1 H, *J* 7.8 Hz, anomeric H), and 4.62 (d, 1 H, *J* 8.2 Hz, anomeric H).

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REFERENCES

1 D. ROLF AND G. R. GRAY, *J. Am. Chem. Soc.*, 104 (1982) 3539–3541.
2 J. U. BOWIE, P. V. TRESCONY, AND G. R. GRAY, *Carbohydr. Res.*, 125 (1984) 301–307.
3 D. ROLF AND G. R. GRAY, *Carbohydr. Res.*, 131 (1984) 17–28.
4 D. ROLF, J. A. BENNEK, AND G. R. GRAY, *Carbohydr. Res.*, 137 (1985) 183–196.
5 D. ROLF AND G. R. GRAY, *Carbohydr. Res.*, 152 (1986) 343–349.
6 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
7 M. A. NASHED, C. W. SLIFE, M. KISO, AND L. ANDERSON, *Carbohydr. Res.*, 82 (1980) 237–252.
8 C. FOCES-FOCES, F. H. COMO, M. BERNABE, S. PENADES, AND M. MARTIN-LOMAS, *Carbohydr. Res.*, 135 (1984) 1–11.
9 V. K. SRIVASTAVA, *Carbohydr. Res.*, 103 (1982) 286–292.
10 T. SAEGUSA, H. IKEDA, AND H. FUJII, *Polym. J.*, 4 (1973) 87–92.
11 A. KOBATA, *Methods Enzymol.*, 28 (1972) 262–271.
12 A. KOBATA, K. YAMASHITA, AND Y. TACHIBANA, *Methods Enzymol.*, 50 (1978) 216–220.
13 V. GINSBURG, D. A. ZOPF, K. YAMASHITA, AND A. KOBATA, *Arch. Biochem. Biophys.*, 175 (1976) 565–568.
14 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohydr. Res.*, 5 (1967) 433–440.
15 D. P. SWEET, R. H. SHAPIRO, AND P. ALBERSHEIM, *Carbohydr. Res.*, 40 (1975) 217–225.
16 J. M. WILLIAMS, *Adv. Carbohydr. Chem. Biochem.*, 31 (1975) 9–79.
17 J. CONCHIE AND G. A. LEVVY, *Methods Carbohydr. Chem.*, 2 (1963) 332–335.
18 U. KRASKA, J. R. POUGNY, AND P. SINAŸ, *Carbohydr. Res.*, 50 (1976) 181–190.
19 A. B. FOSTER, D. HORTON, N. SALIM, M. STACEY, AND J. M. WEBBER, *J. Chem. Soc.*, (1960) 2587–2596.
20 S. HASE AND E. T. RIETSCHEL, *Eur. J. Biochem.*, 63 (1976) 93–99.
21 A. KOBATA AND V. GINSBURG, *J. Biol. Chem.*, 244 (1969) 5496–5502.