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

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ABSTRACT

The positions of linkage in the D-glucans amylose, cellulose, laminaran, pullulan were established by permethylation and subsequent reductive cleavage with triethylsilane and either trimethylsilyl trifluoromethanesulfonate ($Me_3SiO_3SCF_3$) or boron trifluoride etherate ($BF_3 \cdot Et_2O$) as the catalyst. Reductive cleavages with $Me_3SiO_3SCF_3$ as the catalyst were performed by adding a stock solution of Et_3SiH and catalyst in dichloromethane to the previously dried, fully methylated polysaccharides. Subsequent acetylation was accomplished *in situ* by adding acetic anhydride, and, after extraction of the reaction mixture with aqueous sodium hydrogencarbonate, the dichloromethane solution was analyzed directly by gas–liquid chromatography–mass spectrometry. With $Me_3SiO_3SCF_3$ as the catalyst, all of these D-glucans gave the methylated 1,5-anhydro-D-glucitol derivatives expected. Reductive cleavages with $BF_3 \cdot Et_2O$ as the catalyst were carried out in two steps, with an intermediate reaction workup prior to acetylation of the product. With $BF_3 \cdot Et_2O$ as the catalyst, permethylated amylose gave the methylated 1,5-anhydro-D-glucitol derivatives expected, but permethylated cellulose and laminaran were not cleaved. Interestingly, $BF_3 \cdot Et_2O$ effectively catalyzed reductive cleavage of the α-(1→4) linkages in permethylated pullulan but not the α-(1→6) linkages, yielding a methylated disaccharide-anhydroalditol derivative containing the intact α-(1→6) linkage as a major product. The independent synthesis, and n.m.r. and mass-spectral characterization, of the methylated 1,5-anhydro-D-glucitol derivatives formed from these D-glucans by reductive cleavage are reported.

INTRODUCTION

The reductive-cleavage method for determination of structure of polysaccharides[1] is based upon methylation analysis, but departs from it significantly with regard to the types of fragments formed by cleavage of the fully methylated polysaccharide. The development of this new technique was prompted both by the

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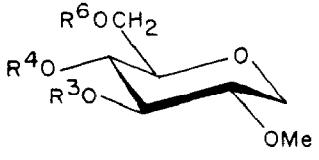
laboriousness of "standard" methylation analysis as well as its inability to distinguish between 4-linked aldopyranosyl and 5-linked aldofuranosyl residues in a polysaccharide. In the reductive-cleavage technique, the ring form and linkage positions of each monosaccharide residue are established simultaneously, and, moreover, the conversion of a fully methylated polysaccharide into derivatives suitable for g.l.c.–m.s. analysis can be accomplished in a "one-flask" reaction with only one reaction workup.

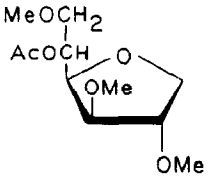
In developing this new technique, we have chosen to examine structurally well-characterized polysaccharides as models. In previous reports, several D-mannans[2] and D-fructans[3] were examined by this technique, and, in all cases, the anhydroalditol derivatives expected were obtained. We now describe the results obtained from an analysis of several D-glucans, namely, amylose, cellulose, laminaran, and pullulan. In all cases, the expected derivatives of 1,5-anhydro-D-glucitol were formed. The independent synthesis and mass spectra of these derivatives are also reported as an aid to those who may use this method in the structural characterization of other D-glucans.

RESULTS

Amylose. Amylose is a linear α-(1→4)-linked D-glucan obtained from potato starch. Reductive cleavage of per-*O*-methylated amylose and acetylation of the product was therefore expected to yield 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**), derived from nonreducing (terminal) D-glucopyranosyl groups, and 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**3**), derived from internal 4-linked D-glucopyranosyl residues. In addition, 5-*O*-acetyl-1,4-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**6**) was expected to be formed as an artifact from 4-linked D-glucopyranosyl residues if water was present in the reaction mixture during reductive cleavage[4].

In an attempt to minimize the formation of **6**, and to simplify the experimental procedure, reductive cleavages with trimethylsilyl trifluoromethanesulfonate





1 $R^3 = R^4 = R^6 = Me$

2 $R^3 = Ac, R^4 = R^6 = Me$

3 $R^4 = Ac, R^3 = R^6 = Me$

4 $R^6 = Ac, R^3 = R^4 = Me$

5 $R^3 = R^6 = Ac, R^4 = Me$

6

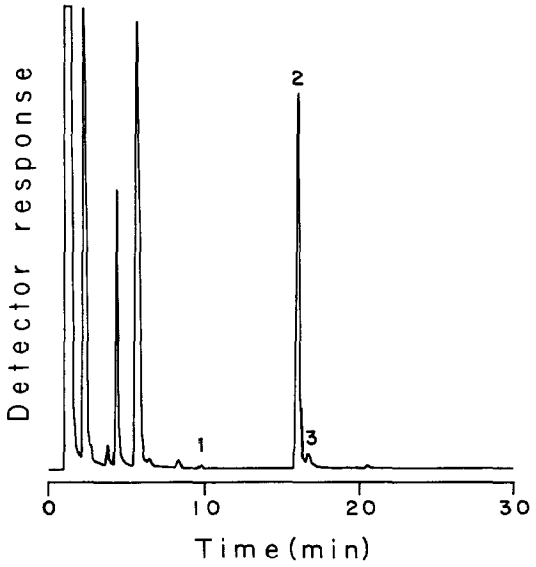


Fig. 1. Gas–liquid chromatogram, on a column (3.18 mm × 2.44 m) of SP-2401, programmed from 110 to 220° at 6°/min, of the partially methylated anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated amylose with $Me_3SiO_3SCF_3$ as the catalyst. The numbered peaks were identified as follows: (1) 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol, **1**; (2) 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol, **3**; and (3) 5-*O*-acetyl-1,4-anhydro-2,3,6-tri-*O*-methyl-D-glucitol, **6**. Peaks eluted prior to Peak 1 were present in a reagent control prepared without polysaccharide. Unnumbered peaks were not identified.

($Me_3SiO_3SCF_3$) as the catalyst were conducted by adding a suitable volume of a carefully dried dichloromethane solution of the reducing agent (Et_3SiH) and catalyst to the per-*O*-methylated polysaccharide that had been dried under high vacuum. After the mixture had been stirred for 20 h at room temperature, acetic anhydride (5 equiv./equiv. of acetal) was added, and stirring was continued overnight. The reaction mixture was then extracted twice with saturated aqueous $NaHCO_3$, and an aliquot of the remaining dichloromethane solution was analyzed directly by combined gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.).

Shown in Fig. 1 is the chromatogram obtained for per-*O*-methylamylose. The expected products were indeed obtained, as established by g.l.c.–m.s. analysis and comparison to independently synthesized standards. Integration of the peaks, and correction for molar response[2,5], gave the mole fractions listed in Table I. As expected, the major product was 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**3**), derived from 4-linked D-glucopyranosyl residues. Compound **6**, produced as an artifact from 4-linked D-glucopyranosyl residues, was also observed, but not to the extent found in earlier experiments (typically 10% or greater) where precautions to exclude water were not taken (experiments not reported). 1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**), arising from nonreducing D-glucopyranosyl groups, was also detected, but was not present in a proportion

TABLE I

MOLE FRACTIONS OF PRODUCTS (COMPOUNDS **1–7**) DERIVED BY REDUCTIVE CLEAVAGE OF PER-*O*-METHYLATED AMYLOSE, CELLULOSE, LAMINARAN, AND PULLULAN

D-*Glucan*	*Catalyst*	*Mole fraction*[a]						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Amylose	$Me_3SiO_3SCF_3$	tr	—	0 94	—	—	0 04	—
	$BF_3 \cdot Et_2O$	tr	—	0.91	—	—	0.06	—
Cellulose	$Me_3SiO_3SCF_3$	tr	—	0.89	—	—	0 04	—
	$BF_3 \cdot Et_2O$	—	—	—[b]	—	—	—	—
Laminaran	$Me_3SiO_3SCF_3$	0.11	0.77	—	0.01	0.07	—	—
	$BF_3 \cdot Et_2O$	—	—	—	—	—	—	—
Pullulan	$Me_3SiO_3SCF_3$	0.02	—	0.62	0.33	—	0.02	—
	$BF_3 \cdot Et_2O$	0.02	—	0.42	0.07	—	0.03	0 37

[a]Small amounts of unidentified products, presumably arising from incompletely methylated residues in the polysaccharides, were also observed. [b]Compound **3** was detected by g.l.c. analysis, but the "product" consisted primarily of the starting material; see text.

sufficient for accurate integration. In addition to the products expected, reductive cleavage also produced, in 2% yield, a di-*O*-acetyl-anhydro-di-*O*-methylglucitol derivative of unknown structure, presumed to be derived from incompletely methylated residues in the methylated polysaccharide.

The reductive cleavage of per-*O*-methylamylose was also conducted with $BF_3 \cdot Et_2O$ as the catalyst, and satisfactory results were again obtained (see Table I). This experiment was performed by adding a suitable volume of a carefully dried dichloromethane solution of the reducing agent and catalyst to the vacuum-dried, methylated polysaccharide. In this case, however, acetylation was accomplished in a second step after processing of the reductive-cleavage reaction mixture (see Discussion).

Cellulose. α-Cellulose, a highly purified form of cellulose, was used in this study. Reductive cleavage of the per-*O*-methylated polysaccharide, and acetylation of the product, was expected to yield the same derivatives of 1,5-anhydro-D-glucitol (*i.e.* **1** and **3**) that were obtained from per-*O*-methylamylose. With $Me_3SiO_3SCF_3$ as the catalyst, the expected products were indeed obtained (see Table I). The major product was 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**3**), derived from internal β-(1→4)-linked D-glucopyranosyl residues, and a small proportion of **6** was also observed, owing to the presence of traces of water in the reaction mixture[4]. 1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol, arising from nonreducing D-glucopyranosyl groups, was also observed by g.l.c. analysis, but it was not present in an amount sufficient for accurate integration. A significant proportion (7%) of a single di-*O*-acetyl-1,5-anhydro-di-*O*-methyl-D-glucitol derivative was also detected by g.l.c.–m.s. analysis. The latter derivative, which was not identified, is presumed to arise as a result of incomplete methylation of the polysaccharide.

Interestingly, $BF_3 \cdot Et_2O$ proved to be ineffective as a catalyst for the reduc-

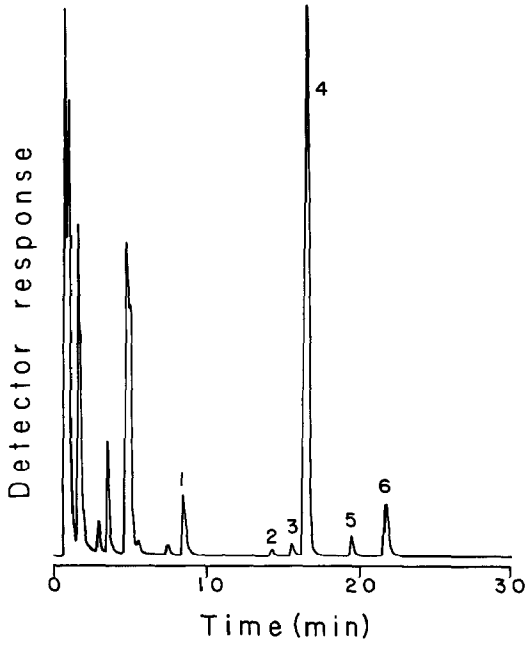


Fig. 2. Gas–liquid chromatogram, on a column (3.18 mm × 2.44 m) of SP-2401, programmed from 110 to 220° at 6°/min, of the partially methylated anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated laminaran with $Me_3SiO_3SCF_3$ as the catalyst. The numbered peaks were identified as follows: (1) 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol, **1**; (2) 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol, **4**; (3) unidentified mono-*O*-acetylmonoanhydro-tri-*O*methylhexitol; (4) 3-*O*-acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl-D-glucitol, **2**; (5) unidentified di-*O*-acetylmonoanhydro-di-*O*-methyl-D-hexitol; and (6) 3,6-di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl-D-glucitol. Peaks eluted prior to Peak 1 were present in a reagent control prepared without polysaccharide.

tive cleavage of per-*O*-methylated cellulose (see Table I); only traces of **3** were detected when the reductive cleavage of per-*O*-methylcellulose was conducted under the conditions that led to complete reductive cleavage of per-*O*-methylamylose. The reason for the failure of $BF_3 \cdot Et_2O$ to catalyze the reductive cleavage of per-*O*-methylcellulose is at present unknown. It was noted, however, that the methylated polysaccharide was not solubilized under the reaction conditions, and examination of the product by ^{1}H-n.m.r. spectroscopy revealed that starting material was present.

Laminaran. Laminaria digitata laminaran is a soluble D-glucan that is comprised of a β-(1→3)-linked D-glucopyranose backbone which is branched at some of the O-6 atoms[6]. Reductive cleavage of per-*O*-methylated laminaran and acetylation of the product was therefore expected to yield 3-*O*-acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl-D-glucitol (**2**) as the major product, along with smaller proportions of 3,6-di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl-D-glucitol (**5**), derived from branch point D-glucopyranosyl residues, and 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**), derived from nonreducing D-glucopyranosyl groups. Shown in Fig. 2 is

the gas–liquid chromatogram obtained when the reductive cleavage of the per-*O*-methylated polysaccharide was conducted with $Me_3SiO_3SCF_3$ as the catalyst. The numbered peaks were identified through comparison to independently synthesized standards by chemical-ionization (NH_3) mass spectrometry, electron-impact mass spectrometry, and g.l.c. retention-time. Integration of these peaks, and correction for molar response[2,5], gave the mole fractions listed in Table I. The major product, as expected, was 3-*O*-acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl-D-glucitol (**2**), derived from unsubstituted 3-linked D-glucopyranosyl residues of the polysaccharide backbone. Backbone residues substituted at O-6 gave rise to Peak 6, *viz.*, 3,6-di-*O*-acetyl-2,4-di-*O*-methyl-D-glucitol (**5**). Compounds **2** and **5** were produced in the ratio of 11:1, demonstrating that 1 of every 12 residues in the backbone is branched. 1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**, Peak 1), arising from nonreducing D-glucopyranosyl groups, was also observed in a significant proportion, as expected. In addition to the aforementioned expected products, traces of 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**4**, Peak 2), arising from 6-linked D-glucopyranosyl residues, and an unidentified mono-*O*-acetylanhydro-tri-*O*-methylhexitol (Peak 3) were also observed. It is not yet known whether the last two products arise from structural features present in laminaran or in a contaminating polysaccharide. Peak 5 (see Fig. 2), which was also not identified, was found to have a molecular weight corresponding to that of a di-*O*-acetylanhydro-di-*O*-methylhexitol; this component may arise from incompletely methylated residues in the polysaccharide.

When the reductive cleavage of per-*O*-methylated laminaran was performed with $BF_3 \cdot Et_2O$ as the catalyst, only traces of the expected products were observed (see Table I). In contrast to what was noted in the $BF_3 \cdot Et_2O$-catalyzed reductive cleavage of cellulose, it appeared that the polysaccharide was solubilized under the reaction conditions. The addition of trifluoroacetic acid (2 equiv./mol of acetal) to the reaction aided dissolution of the permethylated polysaccharide, and allowed reductive cleavage to occur, but unfortunately, a significant proportion of the acyclic product 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol was observed (data not included in Table I).

Pullulan. The D-glucan isolated from *Pullularia pullulans* is a linear polysaccharide comprised of a trisaccharide repeating unit of one α-(1→6)-linked and two α-(1→4)-linked D-glucopyranosyl residues. Reductive cleavage of the fully methylated polysaccharide, and acetylation of the product, was therefore expected to yield 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**4**) and 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**3**) in the molar ratio of 1:2, as well as 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**), derived from nonreducing (terminal) D-glucopyranosyl groups. Shown in Fig. 3 (upper) and Fig. 3 (lower) are the gas–liquid chromatograms obtained when the reductive cleavage of per-*O*-methylpullulan was achieved with $Me_3SiO_3SCF_3$ and with $BF_3 \cdot Et_2O$, respectively, as the catalyst. The numbered peaks were identified through comparison to independently synthesized standards, with the exception that Peak 5 (Fig. 3) was identified

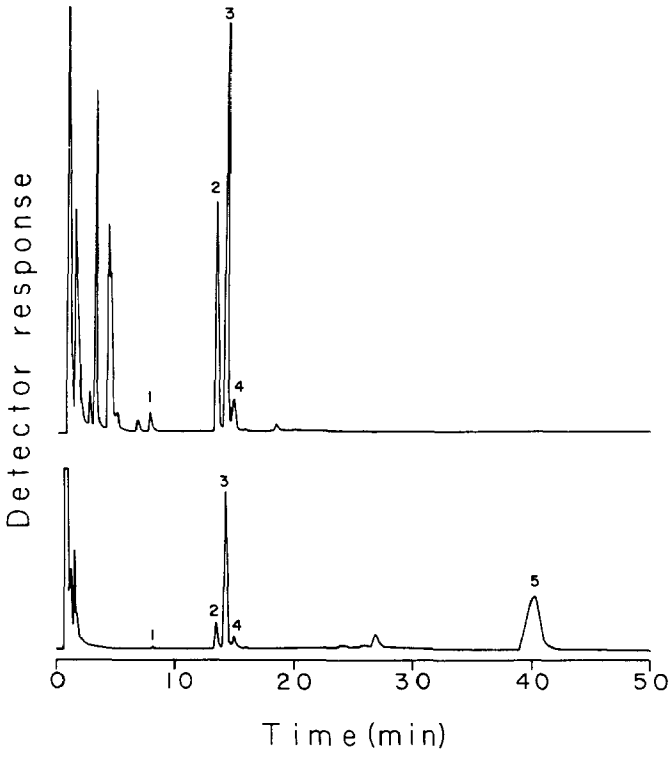
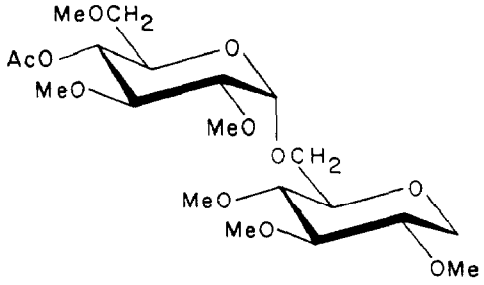


Fig. 3. Gas–liquid chromatograms, on a column (3.18 mm × 2.44 m) of SP-2401, programmed from 110 to 220° at 6°/min, of the partially methylated anhydroalditol acetates derived by reductive cleavage of per-*O*-methylpullulan with $Me_3SiO_3SCF_3$ as the catalyst (upper) and $BF_3 \cdot Et_2O$ as the catalyst (lower). The numbered peaks were identified as follows: (1) 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol, **1**; (2) 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol, **4**; (3) 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol, **3**; (4) 5-*O*-acetyl-1,4-anhydro-2,3,6-tri-*O*-methyl-D-glucitol, **6**, and (5) 6-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-methyl-α-D-glucopyranosyl)-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol, **7** Peaks eluted prior to Peak 1 were present in a reagent control prepared without polysaccharide. Unnumbered peaks were not identified.

by its ^{1}H-n.m.r. spectrum, obtained for a sample isolated by preparative g.l.c. With $Me_3SiO_3SCF_3$ as the catalyst (see Fig. 3, upper), the major products were compounds **4** (Peak 2) and **3** (Peak 3), as expected. Integration of these peaks, and correction for molar response[2,5], gave the mole fractions listed in Table I. 4-Linked D-glucopyranosyl residues in the polysaccharide gave 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**3**), as well as a small proportion of compound **6**, as expected. The combined mole fraction of compounds **3** and **6** (0.64) was, within experimental error, twice the mole fraction of compound **4** (0.33), formed from 6-linked D-glucopyranosyl, residues. Thus, the expected 1:2 molar ratio of 6-linked:4-linked D-glucopyranosyl residues was obtained. A small proportion (2%) of 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**, Peak 1), arising from non-reducing D-glucopyranosyl groups, was also detected in the $Me_3SiO_3SCF_3$-

catalyzed reductive cleavage, as well as a trace of an unidentified di-*O*-acetylanhydro-di-*O*-methylalditol.

Substantially different results were obtained when the reductive cleavage of per-*O*-methylpullulan was performed with $BF_3 \cdot Et_2O$ as the catalyst (see Fig. 3, lower, and Table I). In this experiment, substantially smaller proportions of **3** and **4** were formed, relative to those observed in the $Me_3SiO_3SCF_3$-catalyzed reaction, and a major, new component was observed at a much longer retention-time (Peak 5, Fig. 3). Analysis of the column effluent by chemical-ionization mass spectrometry (c.i.m.s.) with ammonia as the reagent gas revealed that the newly formed component had a molecular weight of 452, which corresponds to that of a mono-*O*-acetylhexa-*O*-methyl derivative of a disaccharide-anhydroalditol. The 300-MHz ^{1}H-n.m.r. spectrum of this component identified it as 6-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-methyl-α-D-glucopyranosyl)-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**7**). As expected, based on c.i.m.s. analysis, the ^{1}H-n.m.r. spectrum contained one *O*-acetyl



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and six methoxyl resonances. Resonances were also observed for H-1*e* of the 1,5-anhydro-D-glucitol moiety (δ 4.02, dd, $J_{1e,1a}$ 11.1, $J_{1e,2}$ 4.8 Hz), H-1 of the α-linked D-glucopyranosyl group (δ 5.04, d, $J_{1,2}$ 3.5 Hz), and H-4 of the α-linked D-glucopyranosyl group (δ 4.91, t, $J_{3,4} = J_{4,5} = 9.8$ Hz). The single-proton triplet at δ 4.91 unequivocally established that the acetyl group is present at O-4 rather O-6 of the α-D-glucopyranosyl group. In the absence of other data, however, the chemical shift (δ 5.04) of H-1 of the α-D-glucopyranosyl group does not unequivocally establish that it is linked at O-6 rather than O-4 of the 1,5-anhydro-D-glucitol moiety; *i.e.*, it is also possible that reductive cleavage of per-*O*-methylpullulan could yield the isomeric disaccharide anhydroalditol 1,5-anhydro-2,3,6-tri-*O*-methyl-4-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-methyl-α-D-glucopyranosyl)-D-glucitol. That Peak 5 (Fig. 3) is, indeed, that for compound **7** can be established by examining the stoichiometry of the other products formed in the $BF_3 \cdot Et_2O$-catalyzed, reductive cleavage. The other major product of reductive cleavage is 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**3**). As pullulan contains one 6-linked and two 4-linked D-glucopyranosyl residues in its repeating unit, these data indicate that Peak 5 (Fig. 3) must be derived from one 4-linked residue and one 6-linked residue of the

repeating unit. The observed mole fractions of reductive-cleavage products are in agreement with the structure proposed for the disaccharide anhydroalditol. From the data (Table I), the ratio of 4-linked:6-linked residues was calculated to be 1.86:1 [(0.42 + 0.03 + 0.37) ÷ (0.07 + 0.37)]. Although this ratio is slightly lower than the 1.94:1 obtained when the reductive cleavage was conducted with $Me_3SiO_3SCF_3$ as the catalyst, some uncertainty exists with regard to the molar response factor of compound **7**; a calculated[5] value of 1.92 relative to compound **3** was used. Clearly, however, these data establish that the 6-linkage of per-*O*-methylpullulan is quite resistant to reductive cleavage when $BF_3 \cdot Et_2O$ is used as the catalyst.

DISCUSSION

With the exception of laminaran, the D-glucans examined in this study met the criterion that led to the development of the reductive-cleavage method, namely, the inability of standard methylation analysis to distinguish between 4-linked aldopyranosyl and 5-linked aldofuranosyl residues in a polysaccharide. In our initial studies, per-*O*-methylated polysaccharides that contained 4-linked D-glucopyranosyl residues were, indeed, found to give 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**3**) as the major product of reductive cleavage, but the isomeric anhydroalditol 5-*O*-acetyl-1,4-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**6**) was also formed in variable but quite significant proportions (10–20%). A careful study of the reductive cleavage of per-*O*-methylated cyclomaltohexaose[4] demonstrated that the formation of **6** was attributable to the presence of water in the reaction mixture, leading to *hydrolysis* of the per-*O*-methylated polysaccharide and subsequent reductive cleavage of 2,3,6-tri-*O*-methyl-D-glucose to produce both **3** and **6**. Although it was shown that the formation of **6** could be minimized (2%) by carrying out reductive cleavage in the presence of CaH_2, we sought to develop, for achieving reductive cleavage, a more-convenient experimental procedure that would also minimize the artifactual formation of **6** from 4-linked D-glucopyranosyl residues.

In this study, reductive cleavages with $Me_3SiO_3SCF_3$ as the catalyst were conducted by adding the premixed reagents (Et_3SiH and catalyst) in dry dichloromethane to the per-*O*-methylated polysaccharides that had been dried under high vacuum. Under these conditions, all of the D-glucans studied gave the expected derivatives of 1,5-anhydro-D-glucitol, and, in those cases where 4-linked D-glucopyranosyl residues were present, the formation of **6** was minimized (typically, <4%). The stock solution was found to be stable for at least one month when stored in a serum-capped ampoule at 4° over Drierite; *i.e.*, analysis of per-*O*-methylpullulan using the freshly prepared stock solution and with the same solution of reagents one month later gave identical results.

Reductive cleavages with $BF_3 \cdot Et_2O$ as the catalyst could also be conducted by adding a dichloromethane solution of the reducing agent and catalyst to the

permethylated polysaccharide, but, in this case, the best results were obtained with freshly mixed reagents. Reductive cleavage of per-*O*-methylpullulan was found to yield small proportions of unidentified products when performed several days after preparation of the dichloromethane solution of reducing agent and catalyst, and therefore, reductive cleavage using premixed reagents was abandoned.

For the per-*O*-methylated D-glucans examined in this study, $BF_3 \cdot Et_2O$ was clearly inferior to $Me_3SiO_3SCF_3$ in catalyzing reductive cleavage. The failure of $BF_3 \cdot Et_2O$ to catalyze the reductive cleavage of per-*O*-methylcellulose and laminaran was unexpected. In both cases, the addition of trifluoroacetic acid to the reaction mixture, to aid dissolution of the fully methylated polysaccharide, resulted in reductive cleavage to some extent, but the product mixtures were complex. In both reactions, products were formed that had molecular weights and electron-impact mass spectra corresponding to partially methylated alditol acetates[7]. The failure of $BF_3 \cdot Et_2O$ to catalyze the reductive cleavage of β-D-mannopyranosyl residues linked at both O-2 and O-6 was noted earlier[2], although it was not established whether the lack of cleavage was due to linkage configuration or linkage position, or both. In this study, it was demonstrated that α-(1→6)-linked D-glucopyranosyl residues are quite resistant to cleavage with $BF_3 \cdot Et_2O$ as the catalyst, however. The resistance of 6-linked hexopyranosyl residues to $BF_3 \cdot Et_2O$-catalyzed, reductive cleavage could be quite useful in structural studies. Similarly, if further studies demonstrate that the resistance of β-linked pyranosyl residues to $BF_3 \cdot Et_2O$-catalyzed reductive cleavage is general, another means for carrying out regioselective cleavage of polysaccharides will be available.

We conclude from the results of these studies, as well as those obtained in our previous studies with D-mannans[2] and D-fructans[3], that $Me_3SiO_3SCF_3$-catalyzed reductive cleavage is an effective technique for simultaneously establishing the ring form and the linkage positions of all residues in a polysaccharide. It also appears that $BF_3 \cdot Et_2O$-catalyzed reductive cleavage will serve as a useful, complementary procedure because of its greater specificity.

EXPERIMENTAL

General. — N.m.r. spectra were recorded with a Nicolet NT-300 n.m.r. spectrometer; ^{1}H-N.m.r. spectra, recorded with $CDCl_3$ as the solvent, were referenced to internal tetramethylsilane, and ^{13}C spectra, also recorded with $CDCl_3$ as the solvent, were referenced to the central, ^{13}C signal (δ 77.00) of chloroform. Analytical g.l.c. was performed on a Hewlett–Packard F and M Model 810 chromatograph equipped with a flame-ionization detector and a stainless-steel column (3.18 mm × 2.44 m) of 10% of SP2401 on 100–120 Supelcoport (Column *1*). Preparative g.l.c. was performed on a Varian VO-P chromatograph equipped with a thermal conductivity detector and the following stainless-steel columns: Column *2* (6.35 mm × 1.83 m), 10% of SE-30 on Chromosorb, and Column *3* (6.35 mm × 3.66 m), the same. G.l.c.–m.s. analyses were performed with a Finnigan 4000 mass

spectrometer equipped with a VG Multispec data system. Column effluents were analyzed by chemical-ionization (c.i.) mass spectrometry (m.s.) (g.l.c.–c.i.m.s.) with ammonia as the reagent gas, wherein characteristic (M + 1) and (M + 18) ions were detected, and by electron-impact (e.i.) m.s. (g.l.c.–e.i.m.s.), in order to verify that eluted components had mass spectra identical to those of independently synthesized standards. All g.l.c. samples were also "spiked" with authentic standards, in order to verify their comigration. Authentic standards were prepared by standard protecting-group strategies. Intermediates were checked for the completeness of protection, or deprotection, by ^{1}H-n.m.r. spectroscopy. Elemental analyses were performed by M-H-W Laboratories, Inc., Phoenix, AZ, on samples purified by preparative g.l.c. in Column *2*. All methylations were conducted by the procedure described by Hakomori[8]. Acetylations were performed in 1:1 (v/v) acetic anhydride–pyridine for 1 h at 100°. Compound **6** was prepared as previously described[4].

Reductive cleavage with Et_3SiH and $Me_3SiO_3SCF_3$. — A stock solution of Et_3SiH and $Me_3SiO_3SCF_3$ in CH_2Cl_2 was prepared in a 10-mL ampoule that had previously been silylated inside by treating it with 10% of Me_2SiCl_2 in toluene for 1.5 h, and then washed with dry methanol, dried at 150°, and cooled in a desiccator. To the ampoule were sequentially added CaH_2 (0.2 g), CH_2Cl_2 (9.0 mL), Et_3SiH (0.8 mL), and $Me_3SiO_3SCF_3$ (1.0 mL). The ampoule was capped with a rubber septum, and then vented through a syringe filled with Drierite for 1 h, to allow H_2 gas to escape. The ampoule was then stored, desiccated, at 4°.

Reductive cleavages were conducted by adding the per-*O*-methylated polysaccharide (5 mg) and a small stirring-bar to a Wheaton V-vial equipped with a Teflon lined screw-top. The vial and contents were kept under high vacuum for 2 h, and then the Et_3SiH–$Me_3SiO_3SCF_3$ stock solution (0.28 mL) was added (starting concentrations: ~0.1M in glucan acetal, 0.5M in Et_3SiH, and 0.5M in $Me_3SiO_3SCF_3$). The vial was now capped, and the mixture was stirred for 20 h at room temperature, at which time 13 μL of Ac_2O (5 equiv./equiv. of acetal) was added. Stirring was continued for 20 h at room temperature, and the reaction was then quenched by the addition of 0.5 mL of saturated aqueous sodium hydrogencarbonate. The biphasic reaction mixture was stirred for 1 h (mild evolution of gas occurs), and the aqueous layer was carefully removed. Saturated aqueous $NaHCO_3$ (0.5 mL) was added, and stirring was continued for 30 min. The aqueous layer was removed, and the CH_2Cl_2 solution was used directly for g.l.c. analysis.

Reductive cleavage with Et_3SiH and $BF_3 \cdot Et_2O$. — Reductive cleavages with $BF_3 \cdot Et_2O$ as the catalyst were conducted by a modification of the procedure reported previously[2]. A 5-mg sample of the per-*O*-methylated polysaccharide and a small stirring-bar were added to a V-vial, and the contents were dried as described before. Dichloromethane (0.25 mL, predried with CaH_2), Et_3SiH (20 μL), and redistilled $BF_3 \cdot Et_2O$ (15 μL) were sequentially added, and the vial was capped. After the mixture had been stirred for 20 h at room temperature, MeOH (0.25 mL) was added, and the mixture was de-ionized by adding Dowex-AG501 X-8-D resin,

a few beads at a time, until the blue color of the resin was retained. The resin was removed by filtration and washed with MeOH (~3 mL), and the filtrate was collected in a 5-mL, round-bottomed flask. Solvents were removed by evaporation under vacuum at 30°, taking precautions not to allow evaporation to dryness to occur, and most of the remaining solvent was removed by purging under a stream of dry nitrogen. Subsequent acetylation of the product was accomplished as previously reported[2].

1,5-Anhydro-2,3,4,6-tetra-O-*methyl*-D-*glucitol* (**1**). — Direct methylation[8] of 1,5-anhydro-D-glucitol[9] (300 mg) gave **1** (319 mg, 79%) as a volatile oil. Purification on silica gel with elution with 1:1 (v/v) chloroform–hexane (R_F 0.22) afforded g.l.c.-pure material (Column *1*, 170°); $[\alpha]_D^{23}$ +40° (*c* 0.14, $CHCl_3$); lit.[10] +67° (neat); ^{1}H-n.m.r. ($CDCl_3$): δ 3.00–3.65 (complex, 7 H, H-1*a*,2,3,4,5,6,6′), 3.39, 3.46, 3.53, 3.63 (4 s, 12 H, 4 MeO), and 4.05 (dd, 1 H, *J* 5.0, 11.0 Hz, H-1*e*); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 221 (100) and 238 (65); g.l.c.–e.i.m.s.: *m/z* 41 (14), 45 (48), 71 (30), 73 (24), 75 (51), 88 (100), 101 (54), 111 (12), 115 (18), 143 (25), 175 (24), and 188 (10).

3-O-*Acetyl-1,5-anhydro-2,4,6-tri*-O-*methyl*-D-*glucitol* (**2**). — Reduction of 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl bromide[11] with lithium aluminum hydride in dry ether afforded 1,5-anhydro-3-*O*-benzyl-D-glucitol (54%), which was converted into **2** by sequential methylation[8], catalytic hydrogenation, and acetylation (7% overall yield). For **2**: $[\alpha]_D^{23}$ +65.5° (*c* 0.10, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.14 (s, 3 H, AcO), 3.10–3.68 (complex, 6 H, H-1*a*,2,4,5,6,6′), 3.38, 3.41, 3.42 (3 s, 9 H, 3 MeO), 4.13 (dd, 1 H, *J* 5.0, 11.0 Hz, H-1*e*), and 5.04 (t, 1 H, *J* 9.2 Hz, H-3); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 249 (16) and 266 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (58), 58 (19), 59 (15), 69 (10), 71 (22), 74 (42), 75 (50), 101 (31), 114(10), 143 (19), 158 (7), 171 (2), 184 (1), 188 (3), and 203 (1).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 52.88; H, 8.13.

4-O-*Acetyl-1,5-anhydro-2,3,6-tri*-O-*methyl*-D-*glucitol* (**3**). — Acetylation of 1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol[12] afforded **3** in 80% yield, $[\alpha]_D^{23}$ +27.9° (*c* 1.2, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.10 (s, 3 H, AcO), 3.10–3.60 (complex, 6 H, H-1*a*,2,3,5,6,6′), 3.35, 3.48, 3.53 (3 s, 9 H, 3 MeO), 4.11 (dd, 1 H, *J* 5.1, 11.2 Hz, H-1*e*), and 4.81 (t, 1 H, *J* 9.2 Hz, H-4); ^{13}C-n.m.r. ($CDCl_3$): δ 20.8 (C-2 of acetyl), 58.7, 59.3, 60.1 (methoxyls), 67.6, 70.7, 72.1, 77.7, 79.3 (C-1,2,3,5,6), 84.9 (C-4), and 169.7 (C-1 of acetyl); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 249 (30) and 266 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (49), 58 (37), 59 (19), 69 (8), 71 (16), 74 (11), 75 (14), 85 (11), 87 (15), 97 (18), 103 (7), 111 (6), 129 (11), 143 (8), 145 (3), 171 (7), and 203 (3).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 52.96; H, 8.15.

6-O-*Acetyl-1,5-anhydro-2,3,4-tri*-O-*methyl*-D-*glucitol* (**4**). — Compound **4** was prepared from 1,5-anhydro-D-glucitol[9] by successive tritylation[13], methylation[8], detritylation[13], and acetylation. Purification of the intermediate 1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol was accomplished by chromatography on silica gel with elution with 1:100 (v/v) methanol–chloroform. For **4**: $[\alpha]_D^{23}$ +75° (*c* 0.10, $CHCl_3$);

^{1}H-n.m.r. ($CDCl_3$): δ 2.10 (s, 3 H, AcO), 3.00–3.34 (complex, 5 H, H-1*a*,2,3,4,5), 3.48, 3.53, 3.65 (3 s, 9 H, 3 MeO), 4.06 (dd, 1 H, *J* 4.9, 11.1 Hz, H-1*e*), 4.17 (dd, 1 H, *J* 5.5, 11.9 Hz, H-6), and 4.32 (dd, 1 H, *J* 2.1, 11.9 Hz, H-6′); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 249 (25) and 266 (100); g.l.c.–e.i.m.s.: *m/z* 43 (82), 45 (43), 58 (45), 59 (20), 69 (13), 70 (14), 71 (56), 73 (20), 75 (82), 87 (100), 88 (53), 101 (53), 129 (10), 130 (20), 143 (3), 156 (6), 175 (1), 186 (1), 188 (3), and 216 (1).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.04; H, 8.06.

3,6-Di-O-acetyl-1,5-anhydro-2,4-di-O-methyl-D-glucitol (**5**). — 3-*O*-Benzyl-D-glucopyranose[11] was converted into 3-*O*-benzyl-2,4-di-*O*-methyl-D-glucopyranose by successive tritylation[13], methylation[8], and detritylation[13]. Successive *O*-debenzylation[14] and acetylation of the last product afforded 1,3,6-tri-*O*-acetyl-2,4-di-*O*-methyl-D-glucopyranose, which was converted into 3,6-di-*O*-acetyl-2,4-di-*O*-methyl-α-D-glucopyranosyl bromide by treatment[12] with 30% HBr in HOAc (2 mL) in CH_2Cl_2 (7 mL) for 2 h at 0°. Hydrogenolysis of the D-glucosyl halide in dry ethyl acetate in the presence of diethylamine, with platinum as the catalyst[9], afforded **5**, which was obtained in analytically pure form by preparative g.l.c. on Column *3* at 190°. For **5**: $[\alpha]_D^{23}$ +55.4° (*c* 0.60, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.11, 2.15 (2 s, 6 H, 2 AcO), 3.15–3.58 (complex, 4 H, H-1*a*,2,4,5), 3.39, 3.41 (2 s, 6 H, 2 MeO), 4.13 (dd, 1 H, *J* 4.8, 10.8 Hz, H-1*e*), 4.20 (dd, 1 H, *J* 5.3, 12.0 Hz, H-6), 4.32 (dd, 1 H, *J* 2.2, 12.0 Hz, H-6′), and 5.07 (t, 1 H, *J* 9.1 Hz, H-3); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 277 (23) and 294 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (14), 58 (14), 69 (10), 71 (13), 74 (33), 75 (28), 87 (14), 101 (24), 111 (4), 114 (4), 116 (5), 117(9), 125 (7), 130 (4), 143 (3), and 156 (7).

Anal. Calc. for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 51.99; H, 7.35.

6-O-(4-O-Acetyl-2,3,6-tri-O-methyl-α-D-glucopyranosyl)-1,5-anhydro-2,3,4-tri-O-methyl-D-glucitol (**7**). — Compound **7** was isolated by preparative g.l.c. of Peak 5 (Fig. 3, lower) on Column *2* at 225°. For **7**: ^{1}H-n.m.r. ($CDCl_3$): δ 2.09 (s, 3 H, AcO), 3.34, 3.47, 3.48, 3.51, 3.55, 3.65 (6 s, 18 H, 6 MeO), 3.00–3.90 [complex, 12 H, H-1*a*,2,3,4,5,6,6′ (anhydroglucitol), H-2,3,5,6,6′ (Glc)], 4.02 [dd, 1 H, *J* 4.8, 11.1 Hz, H-1*e* (anhydroglucitol)], 4.91 [t, 1 H, *J* 9.8 Hz, H-4 (Glc)], and 5.04 [d, 1 H, *J* 3.5 Hz, H-1 (Glc)]; g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 470 (100).

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