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Reductive cleavage of permethylated polysaccharides with borane–methyl sulfide complex and butyltin trichloride

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Abstract

Several per-*O*-methylated monosaccharides and polysaccharides were used as models in an attempt to identify more convenient reagents for accomplishing reductive cleavage of glycosidic linkages. Included in the model studies were methyl α- and β-D-glucopyranoside, methyl α- and β-D-mannopyranoside, and methyl α- and β-D-ribofuranoside. These studies led to the identification of a new promoter, butyltin trichloride, for carrying out reductive cleavage when borane–methyl sulfide complex was used as the reducing agent. These reagents were found to accomplish the reductive cleavage of per-*O*-methylated amylose, cellulose, and pullulan to give only the expected derivatives of 1,5-anhydro-D-glucitol. These reagents also accomplished reductive cleavage of per-*O*-methylated inulin to give only the expected derivatives of 2,5-anhydro-D-mannitol and 2,5-anhydro-D-glucitol. Reductive cleavage using these reagents is easy to perform, and subsequent acetylation of the products is readily accomplished in situ.

Keywords: Reductive cleavage; Polysaccharide; Glycoside; Borane–methyl sulfide

1. Introduction

The reductive-cleavage method [1–3] can be used to simultaneously establish the identity, ratio, linkage position(s), and ring form of glycosyl residues in glycans containing a wide variety of monosaccharide types. This method, which involves the regiospecific hydrogenation of all glycosidic carbon–oxygen bonds in fully methylated polysaccharides, originally employed triethylsilane as the reducing agent and either boron trifluoride etherate [1], trimethylsilyl trifluoromethanesulfonate [4], or a mixture [5] containing 5 equiv of trimethylsilyl methanesulfonate and 1 equiv of boron trifluoride etherate as the promoter. The last two promoters effect complete reductive cleavage of various glycosidic linkages, but boron trifluoride etherate, on the other hand, is very selective [6,7], and this selectivity has proven to be useful for sequence analysis [8]. All three promoters have been used successfully for the analysis of positions of linkage and ring form in residues (e.g., 4-linked aldopyranosides and 5-linked aldofuranosides) where standard methylation analysis would fail. However,

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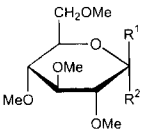
all three promoters are very sensitive to water and, therefore, must be redistilled and carefully stored before use to exclude water. Even if precautions to exclude water are taken, small proportions of ring-contraction products are formed in the reductive cleavage of 4-linked aldopyranosides when either boron trifluoride etherate or trimethylsilyl trifluoromethanesulfonate is used as the promoter [6,9].

More recently, we have sought conditions for carrying out reductive cleavage that are milder and that utilize reagents that are easier to handle. Our initial studies [10] established that borane–complexes such as borane–methyl sulfide and borane–trimethylamine in the presence of boron trifluoride etherate were very effective in this regard, and these reagents were found to be superior to others for the analysis of sialic acid-containing carbohydrates [11]. Reductive cleavages employing these reagents required 24 h or more, however, prompting a further search for promoters that could accomplish the reaction in a shorter period of time but yet be desirable in terms of ease of handling, fidelity of cleavage, and ease of subsequent acetylation of products and reaction workup. Described herein are the results of a study employing several model methyl glycosides and polysaccharides that led to the identification of a new promoter, butyltin trichloride, having such attributes.

2. Results

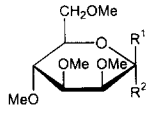
Fully methylated methyl α- and β-D-glucopyranoside, methyl α- and β-D-mannopyranoside, and methyl α- and β-D-ribofuranoside were chosen for study in an attempt to identify new promoters for accomplishing reductive cleavage. Initial experiments were carried out using titanium tetrachloride, titanium(IV) isopropoxide, or mixtures of these two compounds as the promoter and either triethylsilane or borane–methyl sulfide complex [$Me_2S \cdot BH_3$] as the reducing agent. Unfortunately, these experiments were unsuccessful, either giving anomerization, decomposition of starting materials, or no reaction (results not reported). However, when tin(IV) chloride was used as the promoter in the presence of $Me_2S \cdot BH_3$, reductive cleavage of permethylated glucopyranosides **1** and **2** was complete within minutes to give the desired product **3**. Reductive cleavage of permethylated pullulan under the same conditions was complete within an hour, but significant amounts of demethylated products were also observed (results not reported). In an attempt to avoid demethylation, the less reactive promoter butyltin trichloride was used and, indeed, this tin complex was found to accomplish reductive cleavage without demethylation. Selected model compounds were therefore subjected to reductive cleavage in the presence of 5 equiv each of $Me_2S \cdot BH_3$ and $BuSnCl_3$, and the kinetics of product formation was established by GLC. Based upon the results so obtained, selected permethylated polysaccharides were reductively cleaved, and the products, after acetylation, were analyzed by GLC and were identified by comparison of their electron-ionization mass spectra, chemical-ionization mass spectra and retention indices to those of authentic standards [12–14].



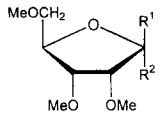
1 R^1 = H, R^2 = OMe
2 R^1 = OMe, R^2 = H
3 $R^1 = R^2$ = H

Kinetics of reductive cleavage of model glycosides. —Shown in Fig. 1 is the time course of product formation during the reductive cleavage of fully methylated methyl α-D-glucopyranoside (**1**, Fig. 1A), methyl β-D-glucopyranoside (**2**, Fig. 1B), methyl α-D-mannopyranoside (**4**, Fig. 1C), methyl β-D-mannopyranoside (**5**, Fig. 1D), methyl α-D-ribofuranoside (**7**, Fig. 1E), and methyl β-D-ribofuranoside (**8**, Fig. 1F) in the presence of $Me_2S \cdot BH_3$ and $BuSnCl_3$ at room temperature. As is evident in Fig. 1, all glycosides underwent reductive cleavage to give the respective anhydroalditol (**3**, **6**, or **9**) as the exclusive product; i.e., no acyclic products were formed. The reactions occurred at substantially different rates, however. Fully methylated methyl α-D-glucopyranoside (**1**) and methyl β-D-glucopyranoside (**2**) were reductively cleaved at about the same rate, and both reactions were complete after 3 h (see Fig. 1A and B, respectively). Reductive cleavage of fully methylated methyl α-D-mannopyranoside (**4**) was considerably slower, however, requiring nearly 5 h for completion (Fig. 1C). The corresponding β anomer **5**, in contrast, underwent reductive cleavage quite rapidly, as noted by the complete disappearance of starting material in less than 1 h (Fig. 1D). Considerable (~ 20%) anomerization to the α anomer **4** occurred in the latter reaction, however, so complete reductive cleavage required about 3 h. The two ribofuranosyl anomers **7** and **8** underwent reductive cleavage ex-

tremely rapidly, as expected, being complete when first analyzed (15 min) (Fig. 1E and F). It should be pointed out that all products (**3**, **6**, and **9**) were stable in the reaction mixtures even after 24 h of reaction.



4 R^1 = H, R^2 = OMe
5 R^1 = OMe, R^2 = H
6 R^1 = R^2 = H



7 R^1 = H, R^2 = OMe
8 R^1 = OMe, R^2 = H
9 R^1 = R^2 = H

Polysaccharides.—Several polysaccharides were chosen for study in order to establish the appropriate conditions for workup of the reductive-cleavage reaction and acetylation of the products and, also, to determine whether the expected products were formed in the expected ratios. In particular, the D-glucans, amylose, cellulose, and pullulan, were chosen because of their content of 4-linked D-glucopyranosyl residues that were known [6] to be susceptible to ring contraction under certain reductive-cleavage conditions. The D-fructan, inulin, was chosen because of its content of acid-sensitive D-fructofuranosyl residues.

Amylose, cellulose, and pullulan.—Amylose and cellulose are linear polysaccharides comprised of α-(1 → 4)- and β-(1 → 4)-linked D-glucopyranosyl residues, respectively, whereas pullulan is comprised

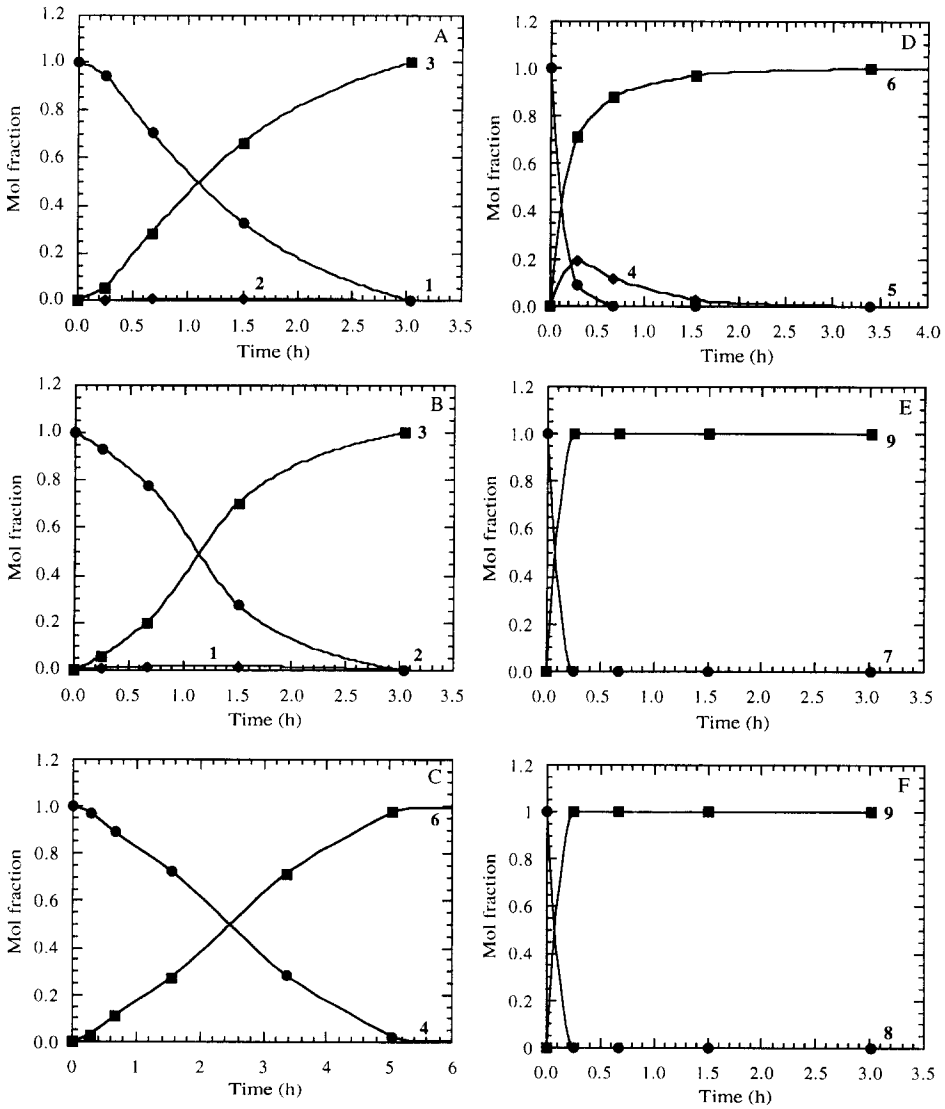
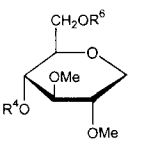
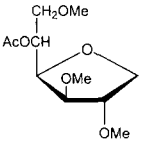


Fig. 1. Time course for reductive cleavage of compounds **1**, **2**, **4**, **5**, **7**, and **8** in the presence of butyltin trichloride and borane–methyl sulfide complex ($Me_2S \cdot BH_3$) at room temperature. **(A)** methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (**1**); **(B)** methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (**2**); **(C)** methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside (**4**); **(D)** methyl 2,3,4,6-tetra-*O*-methyl-β-D-mannopyranoside (**5**); **(E)** methyl 2,3,5-tri-*O*-methyl-α-D-ribofuranoside (**7**), and **(F)** methyl 2,3,5-tri-*O*-methyl-β-D-ribofuranoside (**8**).

of a trisaccharide repeating unit containing two α-(1 → 4)- and one α-(1 → 6)-linked D-glucopyranosyl residues. All three polysaccharides were methylated fully, and the per-*O*-methylated polysaccharides were subjected to reductive cleavage in dichloromethane for 3 h in the presence of 5 equiv each (per equiv of acetal) of $Me_2S \cdot BH_3$ and $BuSnCl_3$. An aliquot of each reaction mixture was then quenched by the addition of aqueous sodium hydrogencarbonate, and the dichloromethane-soluble products were acetylated by treatment with acetic anhydride and 1-methylimidazole. Another aliquot of each reaction mixture was treated directly with acetic anhydride for 1–2 h, then extracted with aqueous sodium hydrogencarbonate. Given in Table 1 are the mole fractions of products obtained by the two acetylation procedures, as well as the values previously obtained using other promoters and/or reducing agents. As is evident in Table 1, reductive cleavage of the permethylated glucans with $Me_2S \cdot BH_3$ in the presence of $BuSnCl_3$ gave the expected products, and in no case was product **12**, arising from 4-linked D-glucopyranosyl residues via ring contraction, observed. Furthermore, in situ acetylation appeared to be just as effective as separate acetylation in that the mole fractions of products derived from the three polysaccharides by the two procedures were essentially the same. For all three polysaccharides, the mole fractions of products obtained by reductive cleavage with $Me_2S \cdot BH_3$ and $BuSnCl_3$ were in good agreement with the values obtained under other reductive-cleavage conditions [5,6,10]. In one case (cellulose), however, reductive cleavage with $Me_2S \cdot BH_3$ and $BuSnCl_3$ was not complete in 3 h, as evidenced by the presence of a very small amount of a disaccharide–anhydroalditol. Chemical ionization (NH_3) mass spectrometry revealed that the latter product had a molecular weight of 452, which corresponds to that of 1,5-anhydro-4-*O*-(4-*O*-acetyl-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-methyl-D-glucitol. No dimeric products were observed in the reductive cleavage of either amylose or pullulan, however.



10 R^4 = Ac, R^6 = Me
11 R^6 = Ac, R^4 = Me



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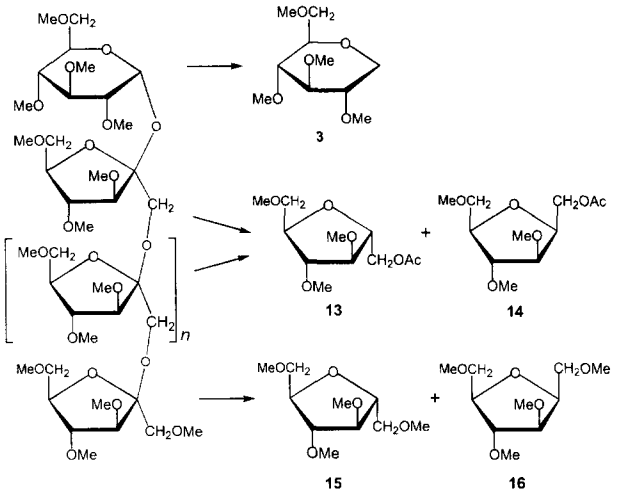
Inulin.—Chicory-root inulin, which was used in this study, is a (2 → 1)-linked, D-fructofuranose polymer terminated at its 'reducing end' by a D-glucopyranosyl group (see Scheme 1). Reductive cleavage of per-*O*-methylated inulin was therefore expected to give compound **3**, derived from the D-glucopyranosyl

Table 1
Mole fractions of products (compounds **3** and **10–12**) derived by reductive cleavage of per-*O*-methylated amylose, cellulose, and pullulan

D-Glucan	Reducing agent/promoter	Mole fraction			
		3	**10**	**11**	**12**
Amylose	$Me_2S \cdot BH_3/BuSnCl_3$ [a]	0.03	0.97	–	–
	$Me_2S \cdot BH_3/BuSnCl_3$ [b]	0.03	0.97	–	–
	$Et_3SiH/Me_3SiOSO_2CF_3$ [c]	tr	0.94	–	0.04
	$Et_3SiH/Me_3SiOSO_2Me + BF_3 \cdot OEt_2$ [d]	tr	0.95	–	–
Cellulose	$Me_2S \cdot BH_3/BuSnCl_3$ [a]	0.06	0.94	–	–
	$Me_2S \cdot BH_3/BuSnCl_3$ [b]	0.03	0.97	–	–
	$Et_3SiH/Me_3SiOSO_2CF_3$ [c]	tr	0.89	–	0.04
	$Et_3SiH/Me_3SiOSO_2Me + BF_3 \cdot OEt_2$ [d]	tr	0.95	–	–
	$Me_2S \cdot BH_3/BF_3 \cdot OEt_2$ [e]	0.014	0.986	–	–
Pullulan	$Me_2S \cdot BH_3/BuSnCl_3$ [a]	0.03	0.66	0.31	–
	$Me_2S \cdot BH_3/BuSnCl_3$ [b]	0.04	0.66	0.30	–
	$Et_3SiH/Me_3SiOSO_2CF_3$ [c]	0.02	0.62	0.33	0.02
	$Et_3SiH/Me_3SiOSO_2Me + BF_3 \cdot OEt_2$ [d]	0.03	0.63	0.29	< 0.01
	$Me_2S \cdot BH_3/BF_3 \cdot OEt_2$ [e]	0.033	0.645	0.322	tr

[a] Products were acetylated by treating an aliquot with acetic anhydride and 1-methylimidazole.
[b] Products were acetylated by adding acetic anhydride directly to the reaction mixture.
[c] Data from ref. [6].
[d] Data from ref. [5].
[e] Data from ref. [10].



Scheme 1.

group, compounds **15** and **16**, derived from the D-fructofuranosyl group at the nonreducing end, and compounds **13** and **14**, derived from the (2 → 1)-linked β-D-fructofuranosyl residues (Scheme 1). Indeed, these were the only products observed when the fully methylated polysaccharide was subjected to reductive cleavage for 30 min with $Me_2S \cdot BH_3$ and $BuSnCl_3$ (Table 2). Interestingly, products **13** and **14**, arising from the (2 → 1)-linked D-fructofuranosyl residues, were formed in a ratio (48:43, respectively), quite different than under other reductive-cleavage conditions. However, the combined mole fraction (0.91) of **13** and **14**, arising from the (2 → 1)-linked D-fructofuranosyl residues, and the combined mole fraction (0.06) of **15** and **16**, derived from the nonreducing D-fructofuranosyl group, were in good agreement with the values obtained under other reductive-cleavage conditions (Table 2). Similarly, the mole fraction (0.03) of the product (**3**) derived from the terminal D-glucopyranosyl group was the same as previously obtained (Table 2).

3. Discussion

Although butyltin trichloride has been used in a variety of other organic transformations [15–17], it has not been used as a promoter for the reductive cleavage of glycosides or other acetals. Based upon the studies reported herein, butyltin trichloride and borane–methyl sulfide complex are very effective reagents for accomplishing the reductive cleavage of permethylated carbohydrates. The reagents are readily available and can be used without further purification or any special precautions. Furthermore, reductive cleavage using these reagents is quite rapid, and acetylation of the products is readily accomplished in situ simply by adding acetic anhydride. These reagents also accomplish reductive cleavage of 4-linked D-glucopyranosyl residues to give the expected product **10** and none of the ring contraction product **12** that is observed in $Me_3SiOSO_2CF_3$- or $BF_3 \cdot OEt_2$-promoted reactions when traces of water are present. Reductive cleavage using these reagents can therefore be accomplished relatively rapidly, conveniently, and with excellent fidelity. Further studies will be undertaken in order to establish the generality of this procedure with respect to the analysis of sugars of other classes.

4. Experimental

General.—Butyltin trichloride, obtained from Aldrich, was stored in the original bottle under nitro-

Table 2
Mole fractions of products (compounds **3** and **13–16**) derived by reductive cleavage of per-*O*-methylated inulin

Reducing agent/promoter	Mole fraction				
	3	**13**	**14**	**15**	**16**
$Me_2S \cdot BH_3/BuSnCl_3$ [a]	0.02	0.48	0.43	0.03	0.03
$Me_2S \cdot BH_3/BuSnCl_3$ [b]	0.03	0.48	0.43	0.03	0.03
$Et_3SiH/Me_3SiOSO_2CF_3$ [c]	0.05	0.67	0.22	0.04	0.01
$Et_3SiH/Me_3SiOSO_2Me + BF_3 \cdot OEt_2$ [d]	0.03	0.75	0.17	0.04	0.01
$Me_2S \cdot BH_3/BF_3 \cdot OEt_2$ [e]	0.027	0.430	0.473	0.032	0.038

[a] Products were acetylated by treating an aliquot with acetic anhydride and 1-methylimidazole.
[b] Products were acetylated by adding acetic anhydride directly to the reaction mixture.
[c] Data from ref. [12].
[d] Data from ref. [5].
[e] Data from ref. [10].

gen at room temperature in a Drierite container. Borane–methyl sulfide complex was obtained from Aldrich as a 1 M solution in CH_2Cl_2 and was stored in a Drierite container at 4 °C. Dichloromethane was distilled from CaH_2 and stored over CaH_2. Acetic anhydride was distilled from, and stored over, 4 Å molecular sieves under nitrogen at room temperature.

Instrumentation.—Analytical GLC was performed using a Hewlett–Packard 5890 gas–liquid chromatograph using the same columns and conditions as previously described [18], except that for routine analysis, the DB-5 column was programmed from 80 to 300 °C at 6 °C/min with no initial hold. GLC–MS analyses were performed using a Finnegan-MAT 95 high-resolution, double-focusing, reverse-geometry mass spectrometer equipped with a Hewlett–Packard 5890A Series II gas–liquid chromatograph and a Digital Equipment Corporation model 2100 workstation. Chemical ionization mass spectra were acquired using NH_3 as the reagent gas under the same conditions as previously reported [18].

Synthesis.—Permethylated methyl α- and β-D-glucopyranoside, methyl α- and β-D-mannopyranoside, and methyl α- and β-D-ribofuranoside were prepared from the respective methyl glycosides by methylation as described by Ciucanu and Kerek [19]. Methyl α-D-ribofuranoside and the corresponding β anomer were synthesized from D-ribose according to the methods of Angyal et al. [20] and Barker and Fletcher [21], respectively, and the products were purified by elution through a cation-exchange (Ca^{2+}) column [20]. Polysaccharides were methylated by the method of Ciucanu and Kerek [19].

Reductive cleavage with $Me_2S \cdot BH_3$ and $BuSnCl_3$.—A sample (5 mg) of the per-*O*-methylated saccharide and a small stirring bar were added to a micro reaction V-vial, then CH_2Cl_2 (1.1 mL, predried with CaH_2), $Me_2S \cdot BH_3$ (5 equiv/equiv of acetal), and butyltin trichloride (5 equiv/equiv of acetal) were sequentially added. The vial was capped, and the contents were stirred at room temperature for the indicated periods of time (see text), then two 100-μL aliquots were transferred via syringe to two 4-mL screwcap vials. Acetic anhydride (55 μL) was added to one vial and, after stirring for 1–2 h, the reaction was quenched with 1 mL of satd aq $NaHCO_3$ and extracted twice with 0.5-mL portions of CH_2Cl_2. Cold satd aq $NaHCO_3$ (1 mL) was added to the vial containing the other 100-μL aliquot of the reductive cleavage reaction, and, after thorough mixing, the contents were extracted twice with 0.5-mL portions of CH_2Cl_2. The CH_2Cl_2 extracts were carefully dried under a stream of N_2, and the product was acetylated by adding 55 μL of acetic anhydride and 25 mL of 1-methylimidazole. After reacting for 10 min, the reactions were processed as previously described [18]. The clear dichloromethane solutions of the products were then analyzed by GLC and GLC–CIMS and the integral values of all GLC peaks (flame-ionization detection) were corrected for molar response by the effective carbon-response method [7,22].

Acknowledgements

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