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MODEL STUDIES ON THE ANALYSIS OF PYRUVIC ACID ACETAL-CONTAINING POLYSACCHARIDES BY THE REDUCTIVE-CLEAVAGE METHOD*

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ABSTRACT

The 4,6-*O*-(1-methoxycarbonylethylidene), -(hydroxyisopropylidene), and -(methoxyisopropylidene) acetals of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside were subjected to reductive cleavage in the presence of triethylsilane and trimethylsilyl methanesulfonate–boron trifluoride etherate ($Me_3SiOMs–BF_3 \cdot Et_2O$), $BF_3 \cdot Et_2O$, or trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) and the mole fractions of products were determined as a function of reaction time. The 4,6-(1-methoxycarbonylethylidene) acetal was quite stable to reductive-cleavage conditions but isomerization of the initial *R,S* mixture of diastereomers to the more-stable *S* diastereoisomer was noted. In addition, a slow, regiospecific, reductive ring-opening of the acetal was observed to give 6-*O*-[1-(methoxycarbonyl)ethyl] derivatives. The 4,6-(hydroxyisopropylidene) acetal was very unstable under reductive-cleavage conditions. Both $Me_3SiOMs–BF_3 \cdot Et_2O$ and $Me_3SiOSO_2CF_3$ catalyzed complete removal of the group, *via* the intermediate 6-[1-(hydroxymethyl)ethyl] ether, but $BF_3 \cdot Et_2O$ gave a mixture of products. The 4,6-(methoxyisopropylidene) acetal was also very labile under reductive-cleavage conditions; $Me_3SiOMs–BF_3 \cdot Et_2O$ catalyzed complete removal of the acetal, *via* the intermediate 6-[1-(methoxymethyl)ethyl]ether, but the intermediate ether was quite stable in the presence of either $BF_3 \cdot Et_2O$ or $Me_3SiOSO_2CF_3$. It is concluded from these studies that polysaccharides bearing 4,6-*O*-(1-carboxyethylidene) substituents can be analyzed directly by sequential permethylation and reductive cleavage. It is proposed that the identity of the substituted monomer and the positions of substitution of the acetal can be determined by sequential permethylation, ester reduction, and reductive cleavage.

INTRODUCTION

In an attempt to simplify the structural characterization of complex carbo-

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hydrates, we are developing for determination of structure a new technique that we refer to as the reductive–cleavage method[1]. The method has already been used for the determination of ring forms and positions of linkage in glycans containing a wide variety of monosaccharide residues[2–8], and for establishing the positions of substitution of attached methyl[9], ethyl[9], and benzyl[10] ether groups. The method has more recently been used[11] to establish the identities of constituent monosaccharides, their sequence, and, in combination with ^{1}H-n.m.r. spectroscopy, their anomeric configurations. In further developing this method, we are examining the fate of various monosaccharides and attached substituents that are frequently encountered in polysaccharides. In this report, we describe the results obtained from a study employing compounds that model the various structural states that might be encountered in the analysis of polysaccharides containing pyruvic acid acetal (1-carboxyethylidene) substituents.

RESULTS

Because we envisaged that reductive-cleavage analysis of polysaccharides containing pyruvic acid acetal substituents might potentially be accomplished directly after (*a*) permethylation, (*b*) sequential permethylation and reduction of ester groups, or (*c*) sequential carboxyl-reduction and permethylation, model compounds were chosen that contained 1-methoxycarbonylethylidene, hydroxyisopropylidene, and methoxyisopropylidene substituents*. Reductive cleavage of each compound was carried out with triethylsilane and each of the catalysts previously reported, namely, boron trifluoride etherate[1] ($BF_3 \cdot Et_2O$), trimethylsilyl trifluoromethanesulfonate[12] ($Me_3SiOSO_2CF_3$), and a mixture[13] containing 5 equivalents of trimethylsilyl methanesulfonate and 1 equivalent of $BF_3 \cdot Et_2O$ per equivalent of acetal. The products so obtained were identified by comparison of their g.l.c. retention-times to those of authentic standards, or by isolation and characterization, except where noted. Their ratios were established by integration of g.l.c. profiles and correction for molar response[2,14]. In addition, the reaction products were acetylated, and the g.l.c. retention-times and chemical ionization (c.i.) and electron impact (e.i.) mass spectra (m.s.) of the acetyl derivatives were compared to those of authentic standards.

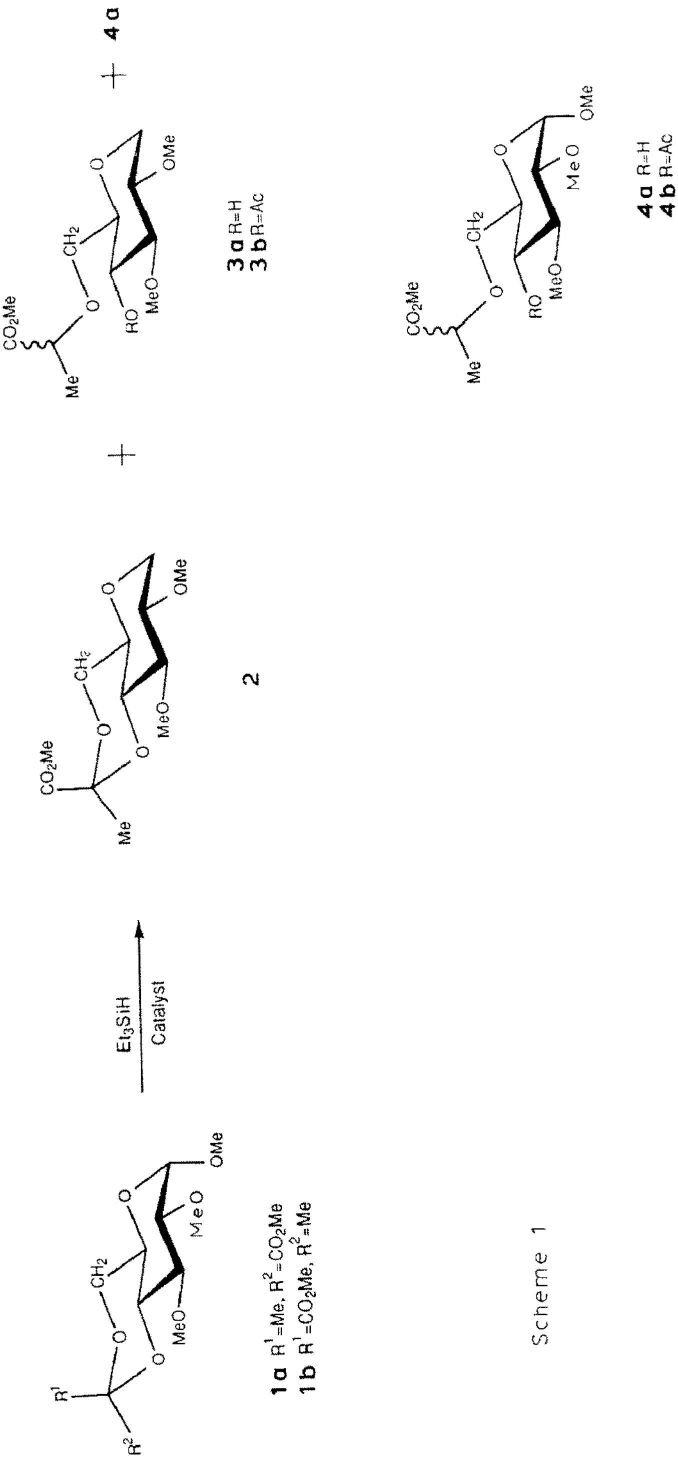
*Reductive cleavage of methyl 4,6-*O*-[(*R*)- and -(*S*)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-α-*D*-glucopyranoside* (**1a,b**). — Shown in Scheme 1 are the structures of compound **1** and the products observed upon reductive cleavage. Compound **2** was observed as a consequence of reductive cleavage of the glycoside, whereas compound **3a** arose as a result of reductive cleavage of the glycoside and reductive ring-opening of the 4,6-*O*-(1-methoxycarbonylethylidene) group. Compound **3a** was characterized on the basis of the c.i. and e.i. mass spectra of its

*The terms *hydroxyisopropylidene, methoxyisopropylidene,* and *acetoxyisopropylidene* are used here as descriptive names for the *1-(hydroxymethyl)ethylidene, 1-(methoxymethyl)ethylidene,* and *1-(acetoxymethyl)ethylidene* groups, respectively.

acetate (**3b**). The c.i. mass spectrum of **3b** contained ions at *m/z* 321 ($M + H^+$) and *m/z* 338 ($M + NH_4^+$), establishing that it had a molecular weight of 320. Its e.i. mass spectrum contained fragment-ions at *m/z* 203, arising from loss of C-6 and the attached ether substituent, and at *m/z* 171 and *m/z* 143, arising from the further loss of methanol and acetic acid, respectively. The e.i. data therefore establish that the 1-methoxycarbonylethyl group is located at O-6 rather than at O-4. Reductive ring-opening of the 4,6-*O*-(1-methoxycarbonylethylidene) group, but not reductive cleavage of the glycoside, gave **4a**, which was characterized as its acetate (**4b**) by g.l.c.–e.i.m.s. and g.l.c.–c.i.m.s. The c.i. mass spectrum of **4b** contained ions at *m/z* 351 ($M + H^+$) and *m/z* 368 ($M + NH_4^+$), establishing its molecular weight as 350. The e.i. mass spectrum of **4b** contained important fragment-ions at *m/z* 319, due to loss of the glycosidic *O*-methyl group, and at *m/z* 233, due to loss of C-6 and the attached ether substituent. Compound **4b** is therefore also identified as the O-6 regioisomer.

These products were formed in substantially different proportions, however, depending upon the elapsed time of the reaction and the catalyst employed (see Fig. 1). When Me_3SiOMs–$BF_3 \cdot Et_2O$ was used as the catalyst (see Fig. 1A), compound **2** was the major product (79% at 4 h) and compound **3a** was the minor product (20% at 4 h). In addition, rapid isomerization of the *R* diastereomer (**1a**) of the starting material to the more stable *S* diastereomer **1b** was noted during the course of the reaction. Very different results were obtained when $BF_3 \cdot Et_2O$ was used as the catalyst (see Fig. 1B); *i.e.*, isomerization of the starting material to the more-stable *S* diastereomer (**1b**) was observed but only traces of **3a** (6%) and **4a** (5%) were observed after reaction for 6 h. Reductive cleavage of **1a,b** in the presence of $Me_3SiOSO_2CF_3$ (Fig. 1C) gave results very similar to those obtained with $BF_3 \cdot Et_2O$, except that isomerization of the starting material to the more-stable diastereomer (**1b**) was more rapid. Small proportions of **2** (5%) and **3a** (6%) were also observed after reaction for 3 h. In all of these reactions, small proportions (typically <5%) of an uncharacterized product were observed. The unknown material was found by c.i.m.s. to have the same molecular weight as compound **2**, and its g.l.c. retention time did not change after acetylation. This uncharacterized product is most probably the *R* diastereomer of compound **2**. As it was not characterized further and was available in such small amounts, it is not indicated in Fig. 1.

*Reductive cleavage of methyl 4,6-*O*-[(R)- and (S)-1-(hydroxymethyl)ethylidene]-2,3-di-*O*-methyl-α-*D*-glucopyranoside* (**5a,b**). — Shown in Scheme 2 are the structures of compound **5** and the products observed upon reductive cleavage. Compound **6a** was formed as a result of reductive cleavage of the glycoside and reductive ring-opening of the 4,6-(hydroxyisopropylidene) acetal, whereas compound **7a** was formed by reductive ring-opening of the hydroxyisopropylidene acetal but not by reductive cleavage of the glycoside. Compounds **8a** and **9a** were both formed as a result of complete removal of the hydroxyisopropylidene group, but differed in whether reductive cleavage of the glycoside had (compound **8a**) or had not (compound **9a**) occurred. In no experiment was a product observed


1a R1=Me, R2=CO2Me
1b R1=CO2Me, R2=Me
Et3SiH
Catalyst
2
3a R=H
3b R=Ac
4a
4a R=H
4b R=Ac

Scheme 1

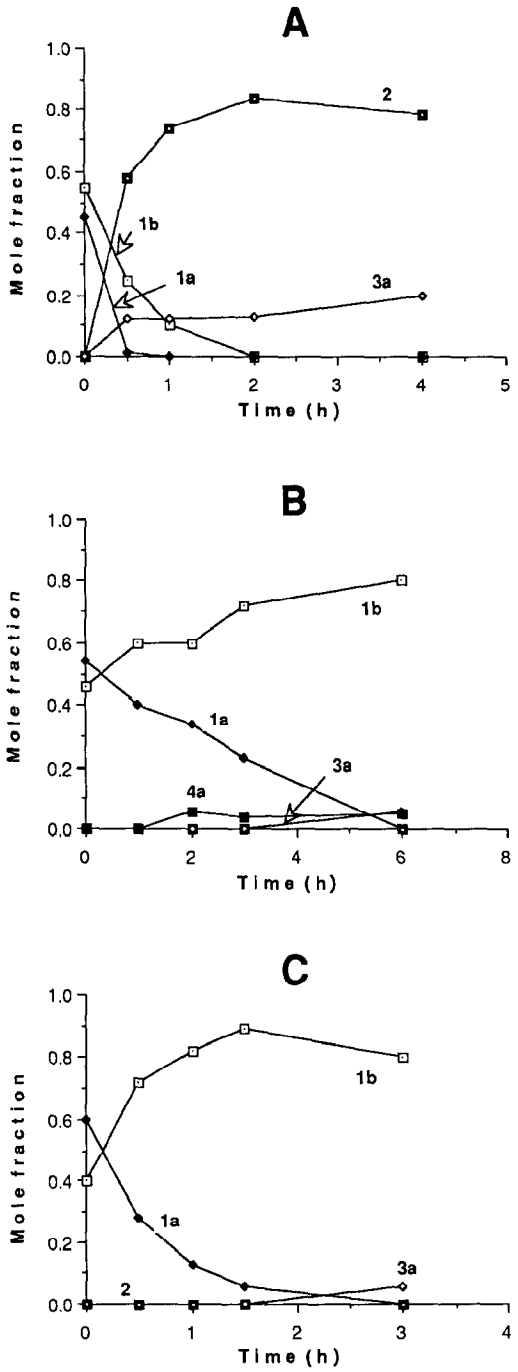


Fig. 1. Time course of product formation during reductive cleavage of methyl 4,6-*O*-[(*R*)- and (*S*)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside (**1a,b**) in the presence of (A) Me_3SiOMs–$BF_3 \cdot Et_2O$, (B) $BF_3 \cdot Et_2O$, and (C) $Me_3SiOSO_2CF_3$. [See Scheme 1.]

Et3SiH
Catalyst
5a R1=Me, R2=CH2OH
5b R1=CH2OH, R2=Me
6a R=H
6b R=Ac
7a R=H
7b R=Ac
8a R=H
8b R=Ac
9a R=H
9b R=Ac

Scheme 2

wherein the hydroxyisopropylidene group was present. Again, the proportions of these products differed substantially, depending upon the elapsed time of the reaction and the catalyst employed (see Fig. 2). When $Me_3SiOMs–BF_3 \cdot Et_2O$ was used as the catalyst (see Fig. 2A), the reaction was essentially complete within 3 h, and compound **8a** was observed as the major product (83%). Compounds **6a** and **9a** were also present in small proportions (4 and 2%, respectively) after reaction for 3 h. In contrast, compound **8a** was not formed in the $BF_3 \cdot Et_2O$-catalyzed reductive cleavage. Instead, the major products were compounds **6a**, **7a**, and **9a**. The proportion of **9a** present in the reaction mixture was relatively constant over time, whereas those of **6a** and **7a** were inversely proportional over time. It was concluded from these results that **9a** arose as a result of rapid *hydrolysis* of the 4,6-*O*-(hydroxyisopropylidene) group, presumably due to the presence of traces of water in the reaction mixture. Once formed, **9a** was fairly stable to further reductive cleavage. Compound **7a**, in contrast, underwent reductive cleavage of the glycoside to yield **6a**. The $Me_3SiOSO_2CF_3$-catalyzed reductive cleavage of **5a,b** gave results (see Fig. 2C) very similar to those obtained with $Me_3SiOMs–BF_3 \cdot Et_2O$; *i.e.* compound **8a** was the major product but small amounts of **6a** and **9a** were observed during the course of the reaction.

*Reductive cleavage of methyl 4,6-*O*-[(R)- and (S)-1-(methoxymethyl)ethylidene]-2,3-di-*O*-methyl-α-*D*-glucopyranoside* (**10a,b**). — Given in Scheme 3 are the structures of compound **10** and the products observed upon reductive cleavage. Compound **12a** arose as a result of reductive ring-opening of the methoxyisopropylidene acetal, whereas **11a** was formed from **12a** by subsequent reductive cleavage of the glycoside. Compounds **8a** and **9a** arose as a result of complete removal of the methoxyisopropylidene group, but differed in whether reductive cleavage of the glycoside had (compound **8a**) or had not (compound **9a**) occurred. In no experiment was a product observed wherein the methoxyisopropylidene group was present. As expected, the proportions of these products differed substantially, depending upon the elapsed time of the reaction and the catalyst employed. In the $Me_3SiOMs–BF_3 \cdot Et_2O$-catalyzed reaction (see Fig. 3A), **8a** was the major product (90% at 3 h), but **9a** and **11a** were also observed during the course of the reaction. These results are therefore very similar to those obtained in the cleavage of **5a,b**. When $BF_3 \cdot Et_2O$ was used as the catalyst (see Fig. 3B), compound **12a** was the major product throughout the reaction, but the proportion of **12a** slowly decreased concomitant with an increase in that of **11a**. Small amounts of **9a** were also observed during the $BF_3 \cdot Et_2O$-catalyzed reaction, but the amount of **9a** formed was relatively constant throughout the reaction, as was also noted in the $BF_3 \cdot Et_2O$-catalyzed cleavage of **5a,b**. In the $Me_3SiOSO_2CF_3$-catalyzed cleavage of **10**, compound **11a** was the major product (63%) and compound **8a** was the minor product (26%) after reaction for 6 h. These results are the opposite of those obtained in the $Me_3SiOSO_2CF_3$-catalyzed cleavage of **5a,b** (see Fig. 2C), wherein **8a** was observed as the major product and the ether derivative (**6a**) was the minor product.

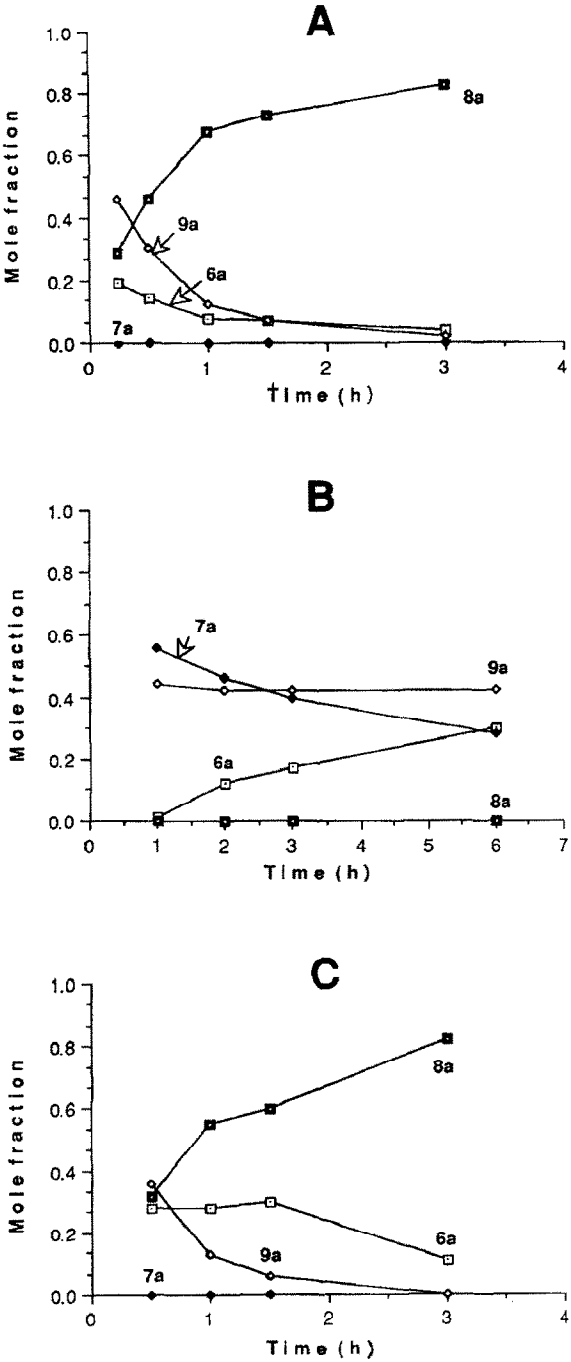
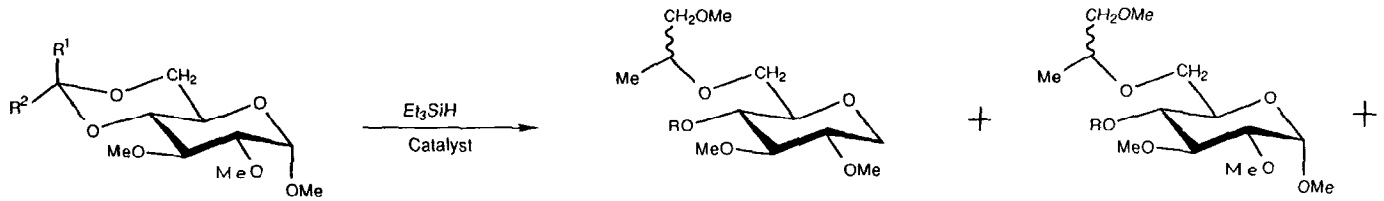


Fig. 2. Time course of product formation during reductive cleavage of methyl 4,6-*O*-[(*R*)- and (*S*)-1-(hydroxymethyl)ethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside (**5a,b**) in the presence of (A) Me_3SiOMs–$BF_3 \cdot Et_2O$, (B) $BF_3 \cdot Et_2O$, and (C) $Me_3SiOSO_2CF_3$. [See Scheme 2.]



10 a R^1=Me, R^2=CH_2OMe
10 b R^1=CH_2OMe, R^2=Me

Scheme 3

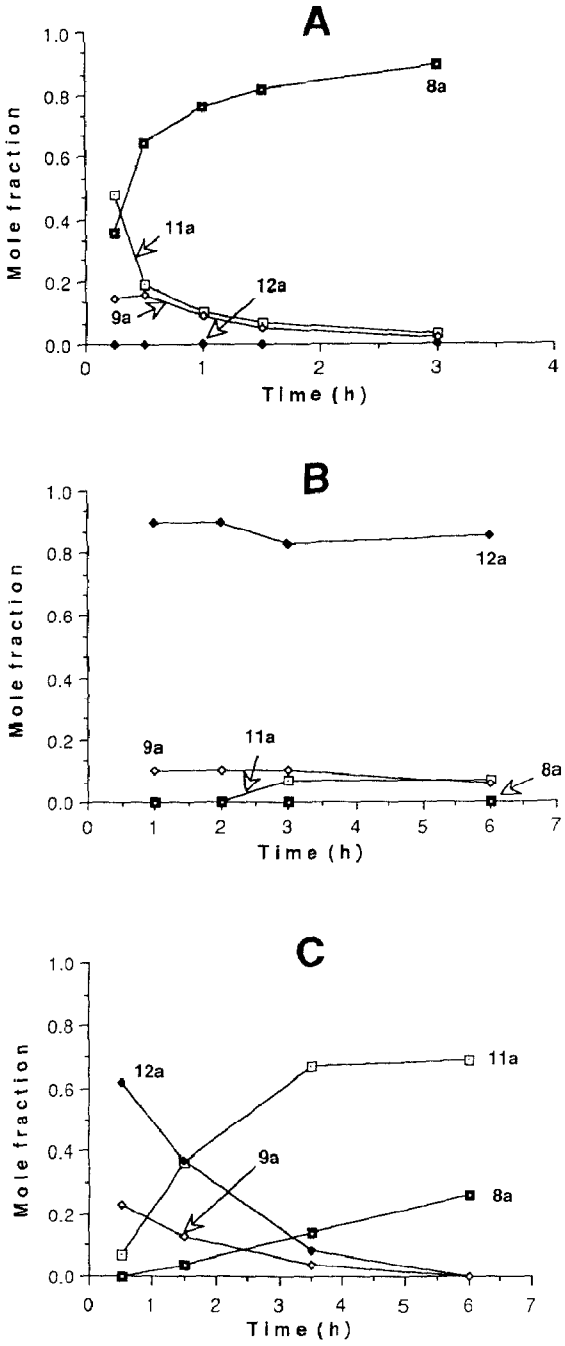


Fig. 3. Time course of product formation during reductive cleavage of methyl 4,6-*O*-[(*R*)- and (*S*)-1-(methoxymethyl)ethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside **(10a,b)** in the presence of (A) Me_3SiOMs–$BF_3 \cdot Et_2O$, (B) $BF_3 \cdot Et_2O$, and (C) $Me_3SiOSO_2CF_3$. [See Scheme 3.]

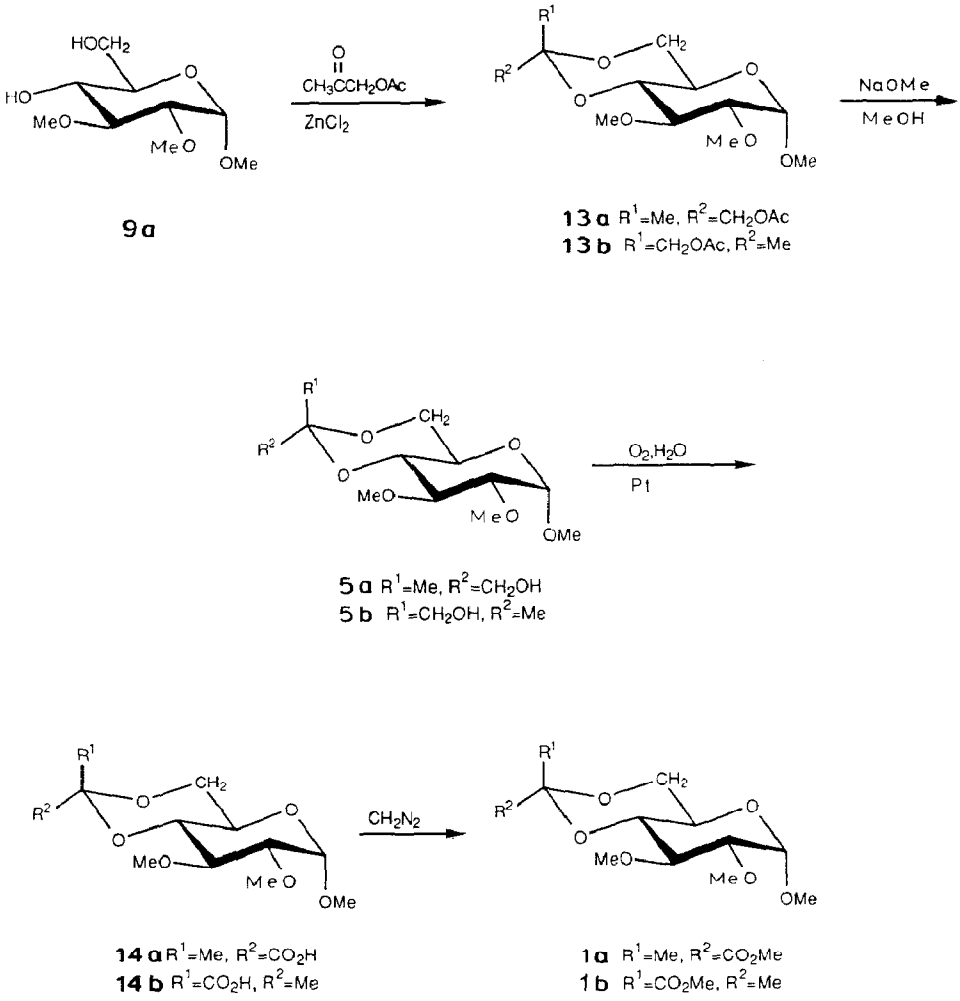
Synthesis. — Methyl 4,6-*O*-[(*R*)- and (*S*)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside (**1a** and **1b**, respectively) and methyl 4,6-*O*-[(*R*)- and (*S*)-1-(hydroxymethyl)ethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside (**5a** and **5b**, respectively) were synthesized as shown in Scheme 4. Reaction of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (**9a**) with acetoxyacetone in the presence of zinc chloride as described by Gorin and Ishikawa[15] gave a 3:2 mixture of **13a** and **13b**, in 35% yield. The diastereomers were distinguished by the ^{1}H-chemical shift of the *C*-methyl group of the acetoxyisopropylidene acetal as described by Garegg, *et al.*[16] for the related derivatives unmethylated at O-2 and O-3; *i.e.*, the *C*-methyl resonance at higher field was assigned to the equatorial acetalic methyl group of the *S* diastereomer (**14b**). *O*-Deacetylation (sodium methoxide in methanol) of the mixture of **13a** and **13b** gave **5a,b** in 77% yield. Compounds **1a,b** were prepared from the **5a,b** mixture by sequential oxidation[17] and esterification (diazomethane).

Methyl 4,6-*O*-[(*R*)- and (*S*)-1-(methoxymethyl)ethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside (**10a** and **10b**) were synthesized from **13** by conducting the acetalation with methoxyacetone in the presence of sulfuric acid and molecular sieves. The *R* and *S* diastereomers (**10a** and **10b**, respectively) were distinguished by the chemical shifts of the acetalic methyl groups[16].

1,5-Anhydro-4,6-*O*-[(*S*)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-D-glucitol (**2**) was prepared by reductive cleavage of **1a,b** in the presence of Me_3SiOMs–$BF_3 \cdot Et_2O$. The c.i. mass spectrum of the product indicated that it possessed a molecular weight of 276, as expected, and its ^{1}H-n.m.r. spectrum contained characteristic resonances for the equatorial[16] acetalic methyl group (δ 1.54), the *O*-methyl groups, and H-1*e* of the 1,5-anhydro-D-glucitol moiety (δ 4.04, dd, *J* 4.6, 10.6 Hz).

6-*O*-[(*R*)- And (*S*)-1-(acetoxymethyl)ethyl]-4-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-D-glucitol (**6b**) and methyl 6-*O*[(*R*)- and (*S*)-1-(acetoxymethyl)ethyl]-4-*O*-acetyl-2,3-di-*O*-methyl-α-D-glucopyranoside (**7b**) were prepared by reductive cleavage of methyl 4,6-*O*-[(*R*)- and (*S*)-1-(acetoxymethyl)ethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside (**13a,b**). The mixture of acetyl derivatives (**13a,b**) was used in preference to the corresponding alcohols (**5a,b**) in both syntheses in order to minimize hydrolysis of the 4,6-*O*-(hydroxyisopropylidene) group (see Fig. 2B). In order to derive **6b**, the reductive cleavage of **13a,b** was carried out for 22 h in the presence of $BF_3 \cdot Et_2O$, and the product was acetylated. Analysis of the purified product by ^{1}H-n.m.r. spectroscopy revealed the expected resonances, especially noteworthy being the triplets (*J* 9.2 Hz) due to the ester methine H-4 resonances of the two diastereomers; the 1-(acetoxymethyl)ethyl group is thus on O-6. Mass spectrometry (e.i.) confirmed this result, as evidenced by the presence of an ion at *m/z* 203, arising from loss of C-6 and the attached group. In order to derive **7b**, the reductive cleavage of **13a,b** was carried out for 10 min in the presence of $BF_3 \cdot Et_2O$, and the product was acetylated. N.m.r. and mass-spectral data were in accord with the structure indicated, for the same reasons as were given for compound **6b**.

4-*O*-Acetyl-1,5-anhydro-6-*O*-[(*R*)- and (*S*)-1-(methoxymethyl)ethyl]-2,3-di-



Scheme 4

O-methyl-D-glucitol (**11b**) and methyl 4-*O*-acetyl-6-*O*-[(*R*)- and (*S*)-1-(methoxymethyl)methyl]-2,3-di-*O*-methyl-α-D-glucopyranoside (**12b**) were prepared by reductive cleavage of **10a,b**. Reductive cleavage of **10a,b** for 40 h in the presence of $BF_3 \cdot Et_2O$, followed by acetylation yielded **11b**. The ^{1}H-n.m.r. spectrum of **11b** contained triplets (J 9.4 Hz) at δ 4.82 and δ 4.83 arising from the ester methine proton (H-4) of the two diastereomers, thus establishing the regiochemistry of reductive ring-opening of the methoxyisopropylidene acetal. The e.i. mass spectrum of **11b** contained a fragment-ion at m/z 203, arising from loss of C-6 and the attached substituent, which also established the regiochemistry of reductive opening of the methoxyisopropylidene acetal. Reductive cleavage of **10a,b** for 5 min in the presence of $BF_3 \cdot Et_2O$, followed by acetylation, yielded **12b**. The ^{1}H-n.m.r. and e.i. mass spectra of **12b** confirmed its structure, for the reasons given for **11b**.

DISCUSSION

Ideally, the analysis of pyruvic acid acetal constituents of polysaccharides could be accomplished by direct reductive cleavage of the fully methylated polymer, presuming that 1-methoxycarbonylethylidene groups were stable to the conditions used to effect reductive cleavage of glycosides. The stability[5,7,8] of carboxylic acid esters to reductive cleavage suggested that direct analysis of a methylated unknown might be possible, but no precedent existed for analysis of the acetal function of such derivatives by this procedure.

Indeed, esterified pyruvic acetals were found to be quite stable to conditions that accomplished reductive cleavage of glycosides (see Fig. 1). Regardless of the catalyst employed, the major products (**1b, 2**) contained an intact 1-methoxycarbonylethylidene group. Isomerization at the acetal carbon atom was observed in these reactions, to give, in all cases, products having the methoxycarbonyl group in the more stable axial orientation (*S* diastereomers). The reductive-cleavage method, therefore, cannot be used to establish the chirality of the original pyruvic acid acetal. Small amounts of products (**3a, 4a**) were formed in these reactions as a result of reductive opening of the 4,6-*O*-(1-methoxycarbonylethylidene) ring. This reaction was regiospecific, as only the 6-*O*-(1-methoxycarbonylethyl) regioisomer was detected. Reductive opening of the acetal ring was not unexpected because an acyclic oxonium ion, which is an obligatory intermediate in the reaction, is also an obligatory intermediate in the isomerization reaction.

Although the foregoing studies demonstrated that polysaccharides bearing 4,6-*O*-(1-carboxyethylidene) substituents could be analyzed by sequential methylation and reductive cleavage, the determination of the identity of the monomer that was substituted by the pyruvic acid acetal, its ring form, and the positions of attachment of the pyruvic acid acetal would require the laborious synthesis of appropriately substituted anhydroalditols as standards. The recent demonstration[11] that partially methylated and partially benzoylated anhydroalditols could be characterized by ^{1}H-n.m.r. spectroscopy to reveal their identity, ring form, and positions of substitution of ether and ester groups suggested an alternative to synthesis for characterization of methylated, pyruvic acid acetal-containing anhydroalditols. A method for complete removal of the 1-methoxycarbonylethylidene group from the fully methylated monomer was therefore sought. Expecting that the *carboxyl-reduced* pyruvic acid acetal would be much less stable toward reductive cleavage, the 4,6-(hydroxyisopropylidene) (**5a,b**) and 4,6-(methoxyisopropylidene) (**10a,b**) derivatives were examined. Derivatives of the former type could be derived by reduction[18] of a fully methylated polysaccharide with $LiAlH_4$, whereas derivatives of the latter type could be derived by carboxyl-reduction prior to methylation[19]. Indeed, the hydroxyisopropylidene and methoxyisopropylidene acetals were found to be very labile to reductive cleavage (see Figs. 2 and 3). In both cases, regiospecific reductive opening of the acetal ring occurred rapidly to give 6-*O*-[1-(hydroxymethyl)ethyl] and 6-*O*-[1-(methoxymethyl)ethyl] derivatives,

respectively. When $Me_3SiOMs–BF_3 \cdot Et_2O$ was used to catalyze reductive cleavage, both ether derivatives were cleaved to give the glycol **8a** as the major product (Figs. 2A and 3A). The glycol **8a** was also the major product in the $Me_3SiOSO_2CF_3$-catalyzed cleavage (see Fig. 2C) of the 6-*O*-[1-(hydroxymethyl)ethyl] derivative, but the related 6-*O*-[1-(methoxymethyl)ethyl] derivatives was relatively stable under these conditions (see Fig. 3C).

These studies lead us to propose the following method for the analysis of polysaccharides containing pyruvic acid acetal substituents. In one experiment, the fully methylated polysaccharide is subjected to total reductive cleavage in the presence of either $Me_3SiOMs–BF_3 \cdot Et_2O$ or $Me_3SiOSO_2CF_3$ as the catalyst. Analysis of the acetylated product by g.l.c.–c.i.m.s. would reveal the presence of partially methylated anhydroalditols containing 1-methoxycarbonylethylidene substituents, because of the characteristic molecular weights of such derivatives. In a second experiment, the fully methylated polysaccharide is reduced with $LiAlH_4$ prior to total reductive cleavage. Analysis of the acetylated product by g.l.c.–e.i.m.s. and –c.i.m.s., or of the benzoylated product by h.p.l.c. and ^{1}H-n.m.r. spectroscopy[11], would reveal the presence of a newly formed anhydroalditol derivative whose identity would establish the positions of substitution of the pyruvic acid acetal substituents. Alternatively, the second experiment could be performed by mildly acidic workup of the $LiAlH_4$ reduction reaction in order to remove the newly formed hydroxyisopropylidene derivative completely. Subsequent reductive cleavage could thus not give rise to intermediate 1-(hydroxymethyl)ethyl ether derivatives (*e.g.* **6a**) that could complicate the analysis.

EXPERIMENTAL

General. — ^{1}H-N.m.r. spectra were recorded with IBM NR-300 and IBM NR-200 n.m.r. spectrometers for solutions in $CDCl_3$, and were referenced to internal tetramethylsilane. G.l.c.–m.s. analyses were performed by using either a Finnigan 4000 mass spectrometer equipped with a VG Multispec data system or a VG Analytical LTD Model VG 7070E-HF high resolution, double-focusing mass spectrometer. Column effluents were analyzed by c.i. mass spectrometry with ammonia as the reagent gas and by e.i. mass spectrometry. Listed are those ions, lying below *m/z* 150 that comprise 10% or more of the intensity of the base peak and those ions above *m/z* 150 that are prominent regardless of their absolute intensity. Analytical g.l.c. was performed with a Hewlett–Packard Model 5890A gas–liquid chromatograph equipped with a Hewlett–Packard 3392A integrator, a flame ionization detector, and a J. and W. Scientific DB-5 fused-silica capillary column (0.25 mm × 30 m). The temperature of the column was held at 110° for 2 min and then programmed to 300° at 6°/min. Medium-pressure liquid chromatography (m.p.l.c.) was carried out in an instrument consisting of a Rheodyne 7125 injector, Eldex model B-100-S4 pump, Scientific Systems model LP-21 pulse dampener, Waters Associates differential refractometer, and ChiraTech Scientific

Instruments, Inc. ultraviolet absorption monitor. Chromatography was conducted on glass columns (4 × 35 cm or 2.1 × 30 cm) packed with 40A silica gel (35–70 mesh) from Merck. Compounds of interest were identified by g.l.c. of collected fractions. Elemental analyses were performed by M-H-W Laboratories, Inc., Phoenix, Arizona, on samples purified by m.p.l.c.

Triethylsilane, trimethylsilyl trifluoromethanesulfonate, boron trifluoride etherate, methanesulfonic acid, chlorotrimethylsilane, methoxyacetone, hydroxyacetone, and methyl iodide were obtained from Aldrich Chemical Co. Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside was obtained from Sigma Chemical Co. Dowex AG501 X-8(D) mixed-bed ion-exchange resin was obtained from Bio-Rad Laboratories. Trimethylsilyl methanesulfonate was prepared as previously described[13]. Acetoxyacetone was prepared by acetylation (acetic anhydride–pyridine) of 1-hydroxyacetone. Methyl 2,3-di-*O*-methyl-α-D-glucopyranoside[20] (**9a**) was prepared from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside by successive methylation and hydrolysis with 50% acetic acid for 2 h at 80°. Acetylation (pyridine–acetic anhydride) of **9a** gave **9b**. 4,6-Di-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-D-glucitol (**8b**) was available from previous work[8].

Methylation was carried out by a modification of the Hakomori[21] procedure as previously described[9].

Reductive cleavages were carried out in Wheaton V vials equipped with Teflon-lined screw caps or in 5–10 mL, round-bottomed flasks. The glassware was silylated inside by treating with 10% Me_2SiCl_2 in toluene for 2 h, washed successively with methanol and toluene, and dried in an oven. The sample (10–15 mg) to be analyzed and a small stirring bar were placed in the vial, and the sample was dissolved in sufficient dry CH_2Cl_2 to give a 0.1M solution. Triethylsilane (10 equiv., 5 equiv. per equiv. of acetal) was added to the vial, followed sequentially by either (*a*) $BF_3 \cdot Et_2O$ (2 equiv.) and Me_3SiOMs (10 equiv.), (*b*) $BF_3 \cdot Et_2O$ (10 equiv.), or (*c*) $Me_3SiOSO_2CF_3$ (10 equiv.). The container was then sealed and the contents stirred for the indicated period of time at room temperature. Reactions were quenched by the addition of MeOH (1 mL), and then deionized with Dowex AG501 X-8(D) mixed-bed ion-exchange resin. The resin was removed by filtration, washed with a small amount of MeOH, and the solution concentrated and either analyzed directly or evaporated to dryness and the residue acetylated with 1:1 (v/v) acetic anhydride–pyridine (5-fold excess) overnight at room temperature, and then processed as previously reported[2].

*Methyl 4,6-*O*-[(R)- and (S)-1-(acetoxymethyl)ethylidene]-2,3-di-*O*-methyl-α-D-glucopyranoside* (**13a,b**). — Compound **9a** (1.6 g) was dissolved in 13.5 g of acetoxyacetone, freshly fused and powdered $ZnCl_2$ (4 g) was added, and the mixture was shaken[15] for 22 h. The reaction was quenched by the addition of an excess of saturated aqueous $NaHCO_3$, the mixture filtered, and the filtrate extracted five times with CH_2Cl_2. Evaporation of the combined extracts and evaporation under diminished presure yielded **13a,b** (35%) as a syrup. Analysis by g.l.c. revealed the presence of components having retention times of 16.9 (**13b**,

39%) and 17.6 min (**13a**, 61%); ^{1}H-n.m.r.: δ 1.41 (s, 1.2 H, acetal CH_3, **13b**), 1.50 (s, 1.8 H, acetal CH_3, **13a**), 2.09, 2.10 (2 s, 3 H, CH_3CO), 3.20–4.00 (complex, 15 H, H-2,3,4,5,6,6′, 3 OCH_3), 4.07 (s, 1.2 H, acetal CH_2, **13a**), 4.33, 4.42 (2 d, 0.8 H, *J* 11 Hz, acetal CH_2, **13b**), 4.79 (d, 0.4 H, *J* 3.7 Hz, H-1, **13b**), and 4.81 (d, 0.6 H, *J* 3.7 Hz, H-1, **13a**); for **13a**: g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 289 (57), 306 (38), 321 (80), and 338 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (28), 69 (12), 71 (10), 73 (14), 75 (41), 85 (11), 88 (51), 101 (26), 155 (5), 159 (2), 161 (4), 173 (1), 183 (2), 215 (4), and 247 (16); for **13b**: g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 289 (57), 306 (38), 321 (100), and 338 (65); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (29), 69 (12), 71 (13), 73 (15), 75 (53), 85 (12), 88 (67), 101 (42), 155 (5), 159 (2), 161 (3), 173 (2), 183 (1), 215 (3), and 247 (8).

Anal. Calc. for $C_{17}H_{24}O_8$: C, 52.49; H, 7.55. Found: C, 52.38; H, 7.55.

*Methyl 4,6-*O*-[(*R*)- and (*S*)-1-(hydroxymethyl)ethylidene]-2,3-di-*O*-methyl-α-*D*-glucopyranoside* (**5a,b**). — *O*-Deacetylation of **13a,b** with NaOMe in MeOH for 2 h, followed by deionization with Dowex AG501 X-8 (D) and evaporation under vacuum gave **5a,b** as a syrup (77%). Analysis by g.l.c. revealed the presence of components having retention times of 13.9 (**5b**, 36%) and 14.3 min (**5a**, 64%); ^{1}H-n.m.r.: δ 1.42 (s, 1.2 H, acetal CH_3, **5b**), 1.48 (s, 1.8 H, acetal CH_3, **5a**), 1.99 (t, 1 H, *J* 6.7 Hz, O*H*), 3.24–3.93 (complex, 17 H, H-2,3,4,5,6,6′, acetal CH_2, 3 OCH_3), and 4.81, 4.82 (2 d, 1 H, *J* 3.7 Hz, H-1); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 247 (60), 264 (10), 279 (15), and 296 (100); g.l.c.–e.i.m.s.: *m/z* 43 (74), 45 (53), 55 (10), 57 (14), 59 (12), 69 (19), 71 (18), 73 (22), 75 (67), 85 (19), 88 (100), 99 (13), 101 (76), 141 (17), 145 (16), 155 (10), 161 (8), 173 (3), 183 (3), 215 (6), and 247 (16).

Anal. Calc. for $C_{12}H_{22}O_7$: C, 51.79; H, 7.97. Found: C, 51.90; H, 8.18.

*Methyl 4,6-*O*-[(*R*)- and (*S*)-1-carboxyethylidene]-2,3-di-*O*-methyl-α-*D*-glucopyranoside* (**14a,b**). — Compound **5a,b** was oxidized to the 1-carboxyethylidene derivative by using a wet preparation of platinum, as described by Gorin *et al.*[17]. Deionization left a syrup (56%) which crystallized; ^{1}H-n.m.r.: δ 1.60 (s, 1.3 H, acetal CH_3, **14b**), 1.74 (s, 1.7 H, acetal CH_3, **14a**), 3.27–4.10 (complex, 15 H, H-2,3,4,5,6,6′, 3 OCH_3), and 4.82 (d, 1 H, *J* 3.8 Hz, H-1).

Anal. Calc. for $C_{12}H_{20}O_8$: C, 49.31; H, 6.90. Found: C, 49.11; H, 6.78.

*Methyl 4,6-[(*R*)- and (*S*)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-α-*D*-glucopyranoside* (**1a,b**). — Treatment[17] of **14a,b** with ethereal diazomethane gave **1a,b** in quantitative yield. Analysis by g.l.c. revealed components having retention times of 14.6 (**1b**, 46%) and 15.2 min (**1a**, 54%); ^{1}H-n.m.r.: δ 1.54 (s, 1.3 H, acetal CH_3, **1b**), 1.70 (s, 1.7 H, acetal CH_3, **1a**), 3.19–4.13 (complex, 18 H, H-2,3,4,5,6,6′, CO_2CH_3, 3 OCH_3), 4.78 (d, 0.4 H, *J* 3.7 Hz, H-1, **1b**), and 4.80 (d, 0.6 H, *J* 3.7 Hz, H-1, **1a**); for **1a**: g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 324 (100); g.l.c.–e.i.m.s.: *m/z* 43 (81), 45 (34), 59 (11), 69 (19), 71 (14), 73 (19), 75 (58), 85 (22), 88 (100), 99 (14), 101 (57), 117 (13), 141 (27), 145 (24), 155 (15), 215 (10), and 247 (27); for **1b**: g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 324 (100); g.l.c.–e.i.m.s.: *m/z* 43 (82), 45 (34), 59 (10), 69 (21), 71 (17), 73 (18), 75 (70), 85 (24), 88 (100), 99 (14), 101 (38), 117

(13), 141 (27), 145 (22), 155 (15), 215 (9), and 247 (32).

Anal. Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.78; H, 7.29.

Methyl 4,6-O-[(S)-1-methoxycarbonylethylidene]-2,3-di-O-methyl-α-D-glucopyranoside (**1b**). — The **1a,b** mixture was subjected to reductive cleavage for 24 h in the presence of Et_3SiH (10 equiv.) and $BF_3 \cdot Et_2O$ (10 equiv.), worked up in the usual way, and the product purified by m.p.l.c. with 1:1 hexane–ethyl acetate, affording **1b** as a clear syrup (78%). Analysis by g.l.c. revealed a single peak with a retention time of 14.6 min; ^{1}H-n.m.r.: δ 1.54 (s, 3 H, acetal C*H*$_3$), 3.18–4.01 (complex, 6 H, H-2,3,4,5,6,6'), 3.41, 3.51, 3.62 (3 s, 9 H, 3 C*H*$_3$O), 3.83 (s, 3 H, CO_2CH_3), and 4.78 (d, 1 H, *J* 3.7 Hz, H-1).

Anal. Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.84; H, 7.34.

Methyl 4,6-O-[(R)- and (S)-1(methoxymethyl)ethylidene]-2,3-di-O-methyl-α-D-glucopyranoside (**10a,b**). — Methoxyacetone (10 equiv.) and 1 drop of concentrated H_2SO_4 were added to compound **9a** (1 equiv.), and the mixture plus molecular sieves was shaken for 24 h, kept for 24 h at room temperature, filtered, and the filtrate diluted with CH_2Cl_2. Sufficient pyridine was added to neutralize the acid, and the mixture was washed twice with water, dried (Na_2SO_4), filtered, and evaporated to give **10a,b** as a clear syrup in 15–20% yield. Analysis by g.l.c. revealed the presence of two components having retention times of 13.8 (**10b**, 35%) and 14.1 min (**10a**, 65%); ^{1}H-n.m.r.: δ 1.40 (s, 1 H, acetal C*H*$_3$, **10b**), 1.50 (s, 2 H, acetal C*H*$_3$, **10a**), 3.24–3.94 (complex, 20 H, H-2,3,4,5,6,6', acetal C*H*$_2$, 4 C*H*$_3$O), 4.80 (d, 0.33 H, *J* 3.7 Hz, H-1, **10b**), and 4.81 (d, 0.67 H, *J* 3.7 Hz, H-1, **10a**); for **10a**: g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 173 (100), 261 (6), 278 (14), 293 (18), and 310 (10); g.l.c.–e.i.m.s.: *m/z* 43 (93), 45 (100), 55 (14), 57 (11), 59 (14), 69 (26), 71 (25), 73 (21), 75 (72), 81 (12), 85 (22), 88 (72), 99 (14), 101 (56), 141 (19), 145 (18), 155 (11), 183 (6), 215 (8), and 247 (43); for **10b**: g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 173 (100), 261 (6), 278 (15), 293 (16), and 310 (11); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (67), 69 (21), 71 (14), 73 (12), 75 (50), 85 (16), 88 (28), 101 (22), 141 (15), 145 (15), 155 (8), 183 (3), 215 (5), and 247 (27).

Anal. Calc. for $C_{13}H_{24}O_7$: C, 53.41; H, 8.27. Found: C, 53.61; H, 8.19.

Methyl 4-O-acetyl-6-O-[(R)- and (S)-1-(acetoxymethyl)ethyl]-2,3-di-O-methyl-α-D-glucopyranoside (**7b**). — Compound **13a,b** was subjected to reductive cleavage for 10 min in the presence of 10 equiv. each of Et_3SiH and $BF_3 \cdot Et_2O$, and the mixture was processed in the usual way. Acetylation afforded impure **7b** in 67% yield. M.p.l.c. on silica gel using 1:1 (v/v) hexane–ethyl acetate as the eluant afforded pure **7b**. Analysis by g.l.c. revealed the presence of two components with retention times of 19.71 and 19.79 min; ^{1}H-n.m.r.: δ 1.15, 1.18 (2 d, *J* 5.9 Hz, 3 H, C*H*$_3$CH), 2.07 (s, 3 H, C*H*$_3$CO), 2.08, 2.09 (2 s, 3 H, C*H*$_3$CO), 3.25–3.74 (complex, 6 H, H-2,3,5,6,6', CH$_3$C*H*), 3.43 (s, 3 H, OC*H*$_3$), 3.51 (s, 6 H, 2 C*H*$_3$O), 4.03 (d, 2 H, *J* 4.5 Hz, C*H*$_2$OCOCH$_3$), and 4.85–4.97 (complex, 2 H, H-1, 4); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 333 (11), 350 (32), 365 (3), and 382 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (15), 59 (12), 75 (44), 88 (53), 101 (19), 233 (2), 235 (3), 241 (2), and 273 (1).

Anal. Calc. for $C_{16}H_{28}O_9$: C, 52.74; H, 7.75. Found: C, 52.88; H, 7.78.

*6-O-[(R)- And (S)-1-(acetoxymethyl)ethyl]-4-O-acetyl-1,5-anhydro-2,3-di-O-methyl-*D*-glucitol* (**6b**). — Compound **13a,b** was subjected to reductive cleavage for 22 h in the presence of 10 equiv. each of Et_3SiH and $BF_3 \cdot Et_2O$, and the mixture was worked up in the usual way, to give impure **6b**. The product was partitioned between CH_2Cl_2 and H_2O, and the CH_2Cl_2 layer was subjected to m.p.l.c. with 1:99 MeOH–$CHCl_3$ as the eluant, to give **6a**. Acetylation of the latter gave pure **6b** as a syrup. Analysis of **6b** by g.l.c. revealed two components, with retention times of 18.07 and 18.15 min; ^{1}H-n.m.r.: δ 1.14, 1.17 (2 d, 3 H, J 6.8 Hz, CH_3CH), 2.06 (s, 3 H, CH_3CO), 2.09, 2.10 (2 s, 3 H, CH_3CO), 3.09–3.62 (complex, 7 H, H-1*a*,2,3,5,6,6′, CH$_3$C*H*), 3.46, 3.52, (2 s, 6 H, 2 CH_3O), 4.00–4.11 (complex, 3 H, H-1*e*, CH_2OCOCH$_3$), and 4.82, 4.84 (2 t, 1 H, J 9.2 Hz, H-4); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 335 (9), and 352 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (16), 58 (23), 59 (10), 71 (11), 85 (11), 97 (17), 101 (33), 111 (14), 125 (11), 129 (11), 143 (12), 171 (15), 203 (9), and 217 (34).

Anal. Calc. for $C_{15}H_{26}O_8$: C, 53.88; H, 7.84. Found: C, 54.02; H, 7.91.

*Methyl 4-O-acetyl-6-O-[(R)- and (S)-1-(methoxymethyl)ethyl]-2,3-di-O-methyl-α-*D*-glucopyranoside* (**12b**). — Compound **10a,b** was subjected to reductive cleavage for 5 min in the presence of 10 equiv. each of Et_3SiH and $BF_3 \cdot Et_2O$ and the product was acetylated, giving **12b** in 72% yield. Analysis by g.l.c. revealed only traces of impurities and a major component with a retention time of 17.2 min; ^{1}H-n.m.r.: δ 1.13 (d, 3 H, J 6.2 Hz, CH_3CH), 2.08 (s, 3 H, CH_3CO), 3.25–3.64 (complex, 8 H, H-2,3,5,6,6′, CH$_3$C*H*, CH_2OCH$_3$), 3.34, 3.43 (2 s, 6 H, 2 CH_3O), 3.52 (s, 6 H, 2 CH_3O), and 4.83–4.96 (complex, 2 H, H-1,4); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 213 (100), 305 (7), 322 (22), 337 (14), and 354 (88); g.l.c.–e.i.m.s.: *m/z* 43 (75), 45 (83), 59 (43), 71 (14), 73 (53), 75 (73), 85 (10), 87 (10), 88 (100), 102 (13), 203 (2), 207 (4), 213 (2), and 234 (4).

Anal. Calc. for $C_{15}H_{28}O_8$: C, 53.56; H, 8.39. Found: C, 53.79; H, 8.47.

*4-O-Acetyl-1,5-anhydro-6-O-[(R)- and (S)-1-(methoxymethyl)ethyl]-2,3-di-O-methyl-*D*-glucitol* (**11b**). — Compound **10a,b** was subjected to reductive cleavage for 40 h in the presence of 10 equiv. each of Et_3SiH and $BF_3 \cdot Et_2O$, and the product (**11a**, 75% yield) was acetylated to give **11b** as a syrup. Analysis by g.l.c. revealed only traces of impurities and a major product with a retention time of 15.6 min; ^{1}H-n.m.r.: δ 1.12 (d, 3 H, J 6.3 Hz, CH_3CH), 2.08 (s, 3 H, CH_3CO), 3.09–3.55 (complex, 9 H, H-1*a*,2,3,5,6,6′, CH$_3$C*H*, CH_2OCH$_3$), 3.32, 3.43, 3.46 (3 s, 9 H, 3 CH_3O), 4.10, 4.11 (2 dd, 1 H, J 4.7, 11.0 Hz, H-1*e*), and 4.82, 4.83 (2 t, 1 H, J 9.4 Hz, H-4); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 307 (80), and 324 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (76), 58 (19), 59 (11), 69 (15), 71 (18), 73 (28), 75 (19), 85 (12), 88 (20), 97 (15), 101 (20), 111 (22), 117 (13), 125 (29), 143 (9), 171 (4), 203 (3), 217 (61), and 261 (4).

Anal. Calc. for $C_{14}H_{26}O_7$: C, 54.89; H, 8.55. Found: C, 54.82; H, 8.75.

*1,5-Anhydro-4,6-O-[(S)-1-methoxycarbonylethylidene]-2,3-di-O-methyl-*D*-glucitol* (**2**). — Reductive cleavage of **1a,b** for 1 h in the presence of Et_3SiH (10

equiv.), Me_3SiOMs (10 equiv.), and $BF_3 \cdot Et_2O$ (2 equiv.) gave **2** as the major product. The pure compound was obtained by m.p.l.c., using 1:1 (v/v) hexane–ethyl acetate as the eluant. Analysis by g.l.c. revealed a single product with a retention time of 12.3 min; ^{1}H-n.m.r.: δ 1.54 (s, 3 H, acetal CH_3), 3.48, 3.65 (2 s, 6 H, 2 CH_3O), 3.83 (s, 3 H, CO_2CH_3), 3.23–3.75 (complex, 7 H, H-1*a*,2,3,4,5,6,6′), and 4.04 (dd, 1 H, *J* 4.6, 10.6 Hz, H-1*e*); g.l.c.–c.i.m.s. (NH_3, positive): *m/z* 294 (100); g.l.c.–e.i.m.s.: *m/z* 43 (100), 45 (23), 58 (12), 69 (24), 117 (11), 125 (10), 185 (1), and 217 (27).

Anal. Calc. for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 52.05; H, 7.29.

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