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Sulfuric acid-catalyzed acetolysis of anomeric ethyl 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosides: kinetics and mechanism

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Abstract

Kinetics of the acetolysis and concomitant anomerization of ethyl 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranosides in a mixture of acetic anhydride, acetic acid, and sulfuric acid have been studied. The progress of the reactions was followed by gas chromatography, and the rate constants of the partial reactions were calculated on the basis of the time-dependent product distributions obtained. The mechanisms of the reactions are discussed.

Keywords: Acetolysis; Anomerization; Glucopyranosides; Kinetics

1. Introduction

The *O*-glycosidic bonds of polymeric carbohydrates are frequently cleaved with the aid of electrophilic solvolysis, such as acid-catalyzed hydrolysis, methanolysis, or acetolysis [1–4]. The most commonly used medium for the acetolysis is a mixture of acetic anhydride, acetic acid, and sulfuric acid, in which the monomeric units are released as an anomeric mixture of peracetates. Since the relative rates of acetolysis usually differ from those of hydrolysis, these two methods provide a complementary set of results for the structural elucidation of oligo- and poly-saccharides [5,6].

The methods used to follow the progress of acetolysis have thus far been limited to changes in optical rotation [7,8] and reductive ability [9] of the reaction solution, while

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separation techniques and NMR spectroscopy have been exploited in the product analysis [10,11]. Since the reaction consists of several parallel and consecutive partial processes, quantitative data on the time-dependent distribution of all the intermediates and products appear desirable. The present paper is aimed at partly filling this gap in information. Acetolysis and concurrent anomerization reactions of ethyl 2,3,4,6-tetra-*O*-acetyl-α- (**1**) and -β-D-glucopyranoside (**2**) have been followed by gas chromatography in a mixture of acetic anhydride, acetic acid, and sulfuric acid. The rate constants of the partial reactions have been calculated, and the mechanisms of the reactions are discussed.

2. Results and discussion

Acetolyses of ethyl 2,3,4,6-tetra-*O*-acetyl-α- (**1**) and -β-D-glucopyranoside (**2**) were carried out in a mixture of acetic anhydride and acetic acid (50:50, v/v) containing 1%

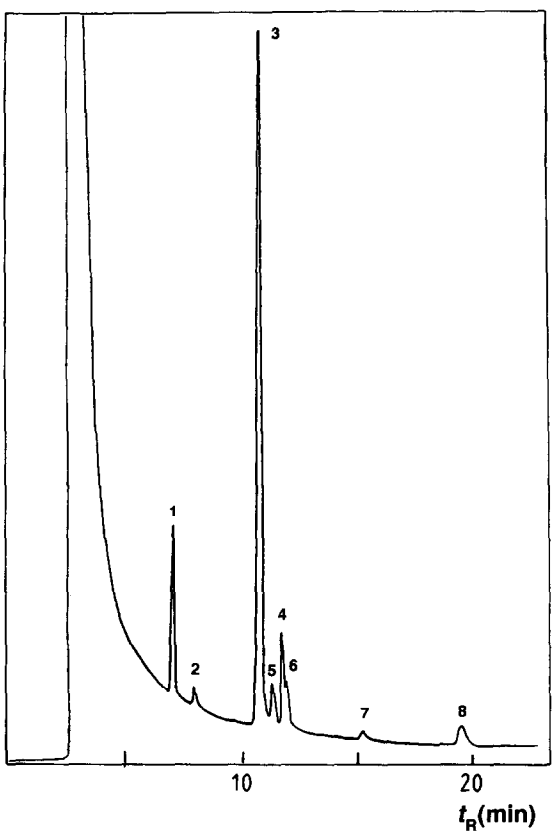
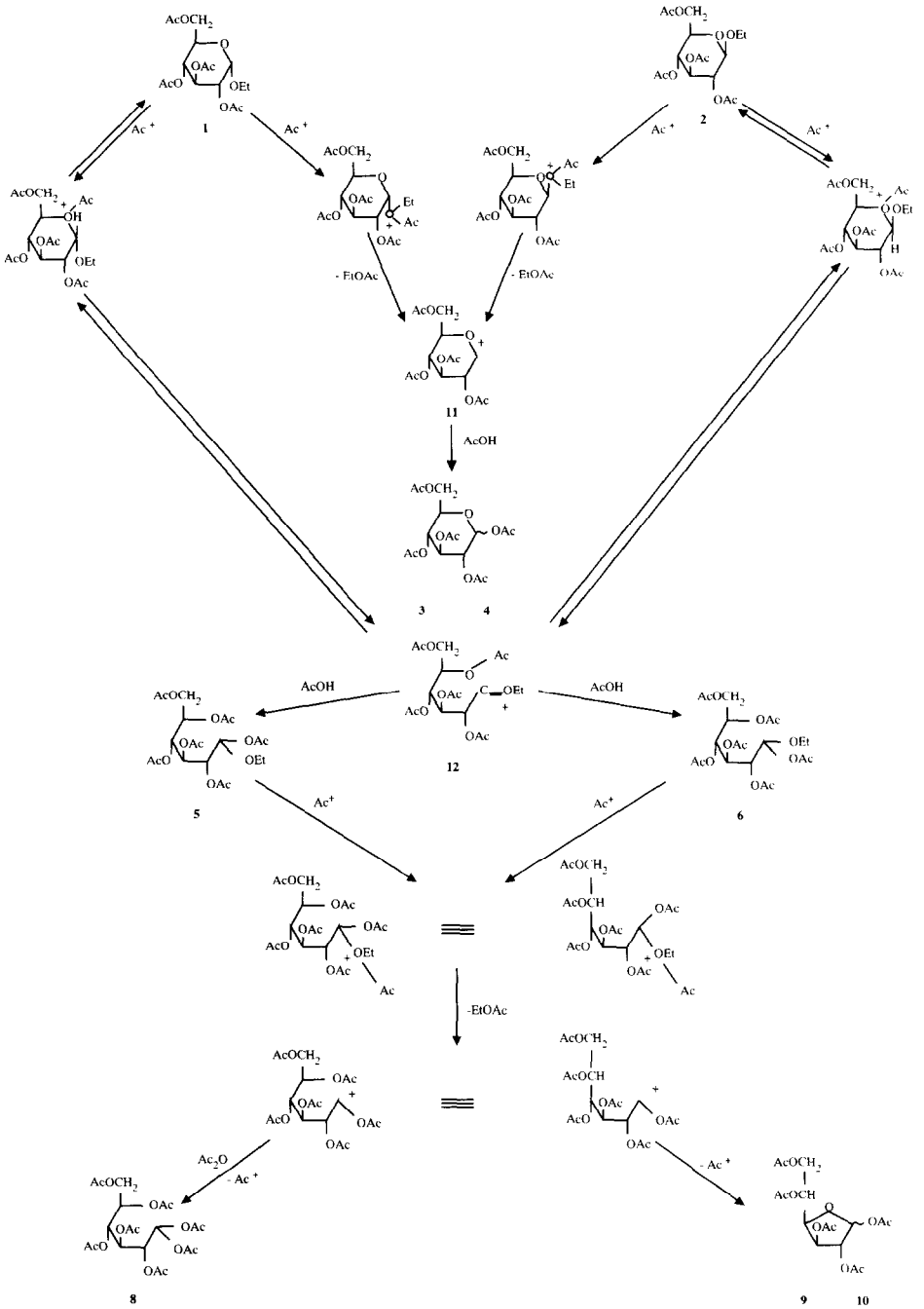


Fig. 1. GC chromatogram of the product mixture after 72-h acetolysis of ethyl tetra-*O*-acetyl-α-D-glucopyranoside (**1**) in a mixture of acetic acid and acetic anhydride (50:50, v/v), containing 1% sulfuric acid, at 25°C. For the structures of the compounds, see Scheme 1. The nature of compound **7** is unknown.



Scheme 1.

Table 1
Time-dependent product distribution for the acetolysis of ethyl tetra-*O*-acetyl-α-D-glucopyranoside (**1**) in a mixture of acetic acid and acetic anhydride (50:50, v/v), containing 1% sulfuric acid, at 25°C [a]

t (h)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0.167	93.1	6.9						
0.33	90.9	8.1	0.3	0.4	0.1	0.1		
0.50	88.6	10.1	0.3	0.7	0.2	0.2		
1.00	88.8	9.4	0.6	0.9	0.2	0.2		
2.0	87.2	9.2	1.4	1.6	0.3	0.3		
3.0	85.2	9.8	2.3	1.6	0.5	0.5		
6.0	79.9	8.3	7.3	2.5	1.0	1.0		
10.0	70.0	8.1	14.1	3.5	2.1	2.1		
24.0	48.6	5.2	33.8	4.7	3.4	3.4		0.9
48.0	28.1	2.3	57.8	4.7	2.9	2.6	0.4	1.2
72.0	14.4	1.7	65.0	8.0	4.2	2.7	0.9	3.0

[a] Given as mol%. For the structures of the compounds, see Scheme 1.

sulfuric acid, and the progress of the reaction was followed by gas chromatography using a flame-ionization detector. Fig. 1 shows as an illustrative example the chromatogram obtained after 72-h incubation at 25°C, when the α-anomer (**1**) was used as the starting material. Spiking with authentic samples revealed the presence of ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**2**), anomeric 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranoses (**3**,**4**), diastereomeric acetyl ethyl acetals (**5**,**6**) of 2,3,4,5,6-penta-*O*-acetyl-D-glucose, 1,1,2,3,4,5,6-hepta-*O*-acetyl-*aldehydo*-D-glucose hydrate (**8**), and an unidentified product **7**, in addition to the starting material (Scheme 1). Only traces of anomeric 1,2,3,5,6-penta-*O*-acetyl-D-glucofuranoses (**9**,**10**) could be detected.

Tables 1 and 2 and Figs 2 and 3 record the time-dependent distributions of the predominant products on using either the α- (**1**) or β-anomer (**2**) of ethyl D-glucopyranoside tetraaacetate as the starting material. These data clearly show that mutual

Table 2
Time-dependent product distribution for the acetolysis of ethyl tetra-*O*-acetyl-β-D-glucopyranoside (**2**) in a mixture of acetic acid and acetic anhydride (50:50, v/v), containing 1% sulfuric acid, at 25°C [a]

t (h)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0.083	46.8	50.6	1.7	0.7	0.2	0.2		
0.167	57.6	39.6	1.5	0.8	0.1	0.3		
0.33	74.3	22.5	1.6	1.1	0.2	0.3		
0.50	84.5	13.4	1.0	0.7	0.2	0.2		
1.00	88.0	9.0	1.3	1.0	0.3	0.3		
2.0	85.2	9.4	2.7	1.5	0.5	0.6		
3.0	84.9	9.4	3.4	1.2	0.5	0.5		
6.0	78.5	9.0	7.8	2.5	1.1	1.1		
10.0	70.0	8.5	15.3	2.9	1.6	1.6		
24.0	49.7	5.8	31.5	5.7	3.6	3.0	0.2	0.7
48.0	38.1	4.6	43.2	6.2	3.5	2.5	0.4	1.5
72.0	14.7	1.6	64.7	7.6	3.8	4.4	1.1	2.2

[a] Given as mol%. For the structures of the compounds, see Scheme 1.

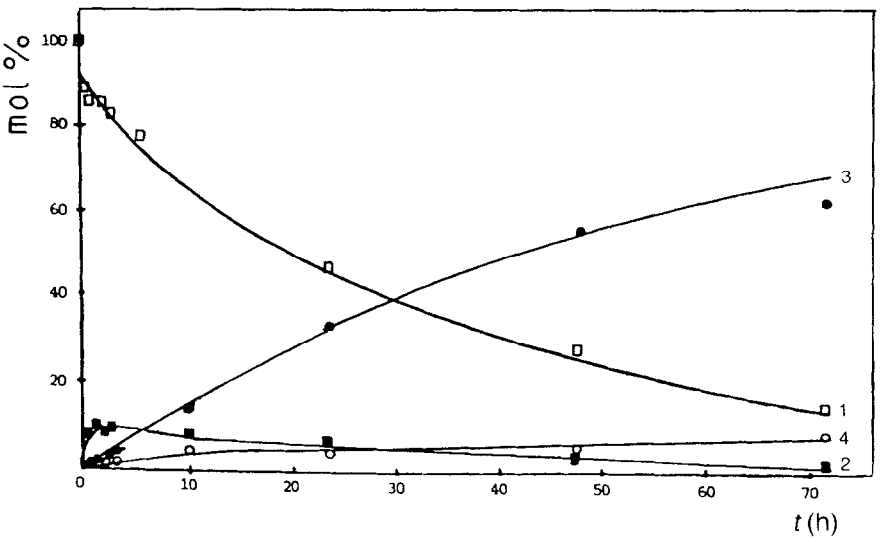


Fig. 2. Time-dependent product distribution for the acetolysis of ethyl tetra-*O*-acetyl-α-D-glucopyranoside (**1**) in a mixture of acetic acid and acetic anhydride (50:50, v/v), containing 1% sulfuric acid, at 25°C. For the structures of the compounds, see Scheme 1.

isomerization of **1** and **2** precedes acetolysis to anomeric pentaacetates (**3**,**4**). This anomerization undoubtedly proceeds by formation and reclosure of a 2,3,4,5,6-penta-*O*-acetyl-1-*O*-ethyl-D-glucitol-1-*C*-ylium ion (**12** in Scheme 1). In theory the anomerization

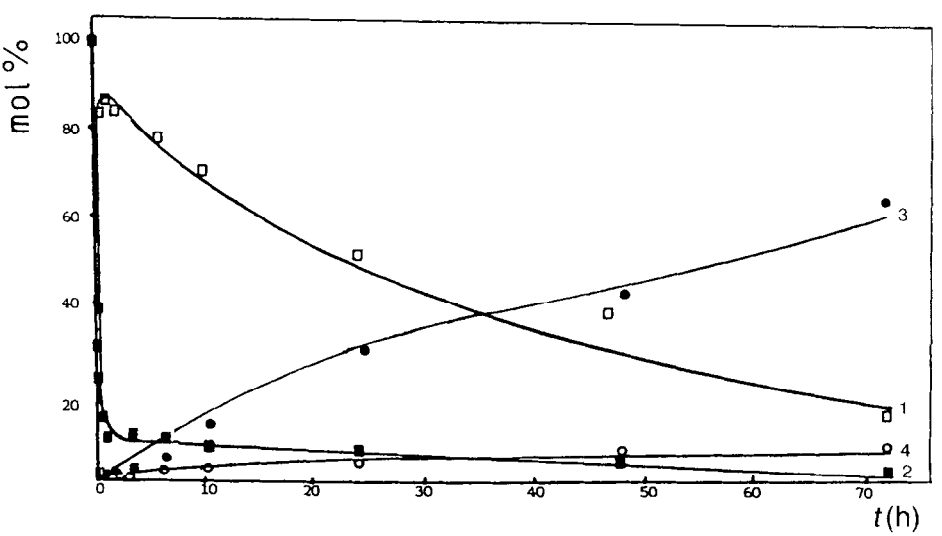


Fig. 3. Time-dependent product distribution for the acetolysis of ethyl tetra-*O*-acetyl-β-D-glucopyranoside (**2**) in a mixture of acetic acid and acetic anhydride (50:50, v/v), containing 1% sulfuric acid, at 25°C. For the structures of the compounds, see Scheme 1.

Table 3
Time-dependent product distribution for the acetolysis of diastereomeric acetyl ethyl acetals (**5** and **6**) of 2,3,4,5,6-penta-*O*-acetyl-D-glucose in a mixture of acetic acid and acetic anhydride (50:50, v/v), containing 1% sulfuric acid, at 25°C [a]

t (h)	5	6	7	8	9	10
0	62.1	37.9				
1.00	64.9	35.1				
3.0	62.1	33.2	1.2	3.6		
24.0	59.1	35.9	1.4	1.6		
48.0	54.8	31.0	2.0	11.4	0.7	0.1
72.0	44.8	28.2	4.9	20.6	0.9	0.6
144.0	24.8	15.2	7.6	53.5	1.5	0.4

[a] Given as mol%. For the structures of the compounds, see Scheme 1.

could also take place via reversible formation of a 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosylium ion (**11** in Scheme 1). In other words, released ethyl acetate would compete with acetic acid for **11**. The concentration of ethyl acetate is, however, so low that this alternative may be excluded.

The acyclic oxocarbenium ion intermediate **12** may be trapped, not only intramolecularly by an acetate oxygen group, but also by acetic acid, resulting in the appearance of the diastereomeric acetyl ethyl acetals, **5** and **6**. Formation of acetyl alkyl acetals on acetolysis of alkyl glycosides has been known since the early studies of Montgomery et al. [12], and more recently shown by McPhail et al. [10]. In order to find out whether the formation of the acetyl ethyl acetals (**5**,**6**) from **1** and **2** is reversible, the acetolysis of **5** and **6** was studied independently. As seen from Table 3 and Fig. 4, the main product of their acetolysis is 1,1,2,3,4,5,6-hepta-*O*-acetyl-*aldehydo*-D-glucose hydrate (**8**). Additionally, the unidentified product **7** mentioned above and traces of anomeric 1,2,3,5,6-penta-*O*-acetyl-D-glucofuranoses (**9**,**10**) were detected. Neither ethyl glucopyranosides (**1**,**2**) nor their furanoid counterparts were formed. Evidently the large excess of acetic acid present makes the reaction from **12** to **5** and **6** virtually irreversible, and hence the only reaction detected is displacement of ethoxide ion, either intermolecularly by acetic anhydride, giving **8**, or intramolecularly by the 4-OAc group, giving **9** and **10**. Whether the reaction takes place via a carbenium ion intermediate, as depicted in Scheme 1, or by a direct acetylium ion-assisted displacement of acetylated ethoxy group remains an open question.

The minimal reaction scheme needed to describe the kinetics for the acetolysis of **1** and **2** is depicted in Scheme 2, and the values obtained for the partial reactions involved are listed in Table 4. The following conclusions may be drawn. (*i*) Anomerization of ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**1**) to its β-anomer proceeds 30 times, and anomerization of the β-anomer to the α-anomer 300 times, as fast as the acetolysis of the anomeric mixture of **1** and **2** to anomeric glucose pentaacetates (**3** and **4**) or acyclic acetyl ethyl acetals (**5** and **6**). Accordingly, both **1** and **2** must be able to undergo relatively rapid ring opening to the acyclic oxocarbenium ion **12**. (*ii*) Formation of the anomeric mixture of glucose pentaacetates (**3** and **4**) via the 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosylium ion (**11**) is five times as fast as that of the acetyl ethyl acetals (**5** and **6**)

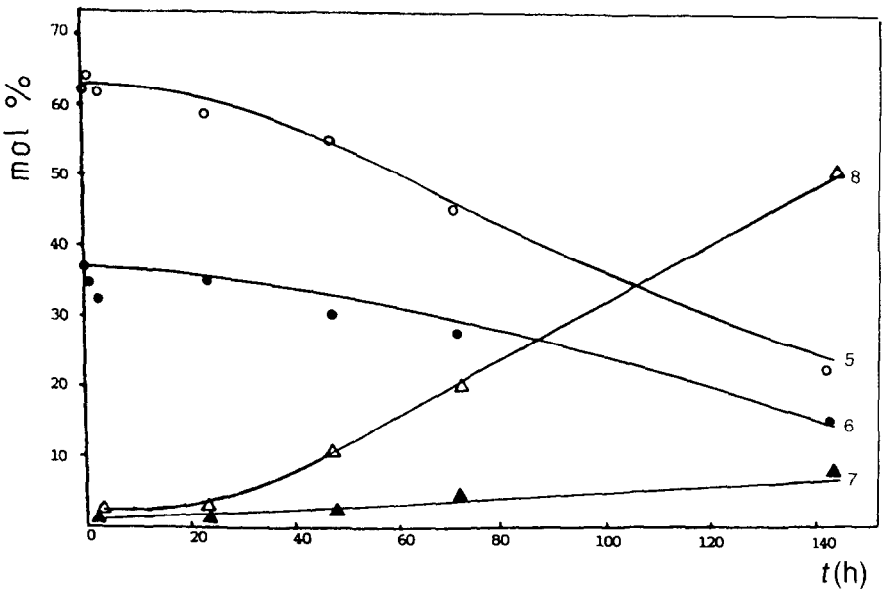
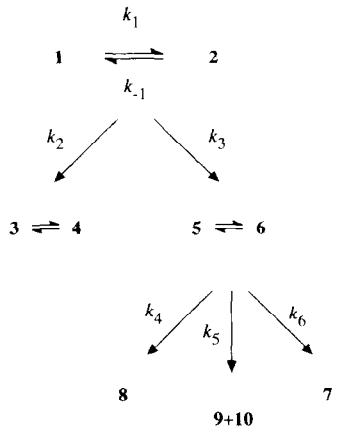


Fig. 4. Time-dependent product distribution for the acetolysis of diastereomeric acetyl ethyl acetals (**5** and **6**) of 2,3,4,5,6-penta-*O*-acetyl-D-glucose in a mixture of acetic acid and acetic anhydride (50:50, v/v), containing 1% sulfuric acid, at 25°C. For the structures of the compounds, see Scheme 1.

via the 2,3,4,5,6-penta-*O*-acetyl-1-*O*-ethyl-D-glucitol-1-*C*-ylium ion (**12**). This observation does not, however, allow straightforward conclusions concerning the relative stability of **11** and **12**, since the formation of **12** from **1** and **2** is a reversible reaction, whereas the formation of **11** from the same compounds may be expected to be virtually irreversible, as long as the substrate concentration remains low. (*iii*) Acetolysis of the



Scheme 2.

Table 4
First-order rate constants for the partial reactions involved in the acid-catalyzed acetolysis of ethyl 2,3,4,6-tetra-*O*-acetyl-α- (**1**) and -β-D-glucopyranoside (**2**) in a mixture of acetic anhydride and acetic acid (50:50, v/v) containing 1% sulfuric acid at 25°C [a]

Rate constant	$10^6\ k\ (s^{-1})$
k_1	170 ± 10
k_{-1}	1600 ± 100
k_2	5.5 ± 0.3
k_3	1.1 ± 0.2
k_4	1.2 ± 0.1
k_5	0.06 ± 0.02
k_6	0.23 ± 0.05

[a] For the rate constants, see Scheme 2.

acetyl ethyl acetals gives only acyclic and furanoid acetates, consistent with the observations of McPhail et al. [10]. The intermolecular displacement of the ethoxy group by acetic anhydride is 20 times as fast as the intramolecular attack of the 4-OAc group. Neither furanoid nor pyranoid glucosides were formed. (*iv*) Formation of acetyl ethyl acetals (**5** and **6**) from **1** and **2** is approximately as rapid as their acetolysis.

Acetolysis of glycosides via open-chain intermediates has previously been suggested [9–11,13], but not unanimously accepted [14]. The present results lend further considerable support to the mechanism via an acyclic oxocarbenium ion. The studies of McPhail et al. [10] on ferric chloride-promoted acetolysis of anomeric methyl 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosides have suggested that only the β-anomer gives an acyclic oxocarbenium ion. The results of the present work do not corroborate this argument. Possibly, the nature of the electrophile employed as the promoter affects the competition between the reactions occurring via acyclic and cyclic intermediates. The other known examples of electrophilic solvolysis of glycosides proceeding, at least partially, via acyclic oxocarbenium ions include acid-catalyzed hydrolysis [15–17] and methanolysis [18] of alkyl aldofuranosides, acid-catalyzed methanolysis of model pyranosides [19], and Lewis acid mediated alcoholysis and thiolysis of methyl glycopyranosides [20].

3. Experimental

General procedures.—The purity of the compounds prepared (**1–6**, **8**) was checked by gas chromatography on a 30-m DB-Wax capillary column under isothermic conditions (215°C). The intermediates and products of the acetolysis reaction were identified by co-injections with authentic samples. To verify the identification, one acetolysis mixture was analyzed on a gas chromatograph attached to a mass spectrometer. The mass spectra were recorded on a Micromass 16 F mass spectrometer (ionizing energy, 70 eV) connected to a VG data system. Acetic acid and acetic anhydride used in the preparation of the acetolysis mixtures were distilled prior to use.

*Ethyl 2,3,4,6-tetra-*O*-acetyl-α- (**1**) and -β-D-glucopyranoside* (**2**).—The glycosides **1** and **2** were obtained by acetylating an anomeric mixture of ethyl D-glucopyranosides

[21] with Ac_2O in pyridine [22], and separating the anomers by HPLC on an RP-18 column (3:2 MeOH–water).

1,2,3,4,6-Penta-O-acetyl-α- (**3**) *and -β-D-glucopyranose* (**4**).—These were prepared as described by Hudson and Dale [23].

(1R)- (**5**) *and (1S)-1,2,3,4,5,6-Hexa-O-acetyl-D-glucose ethyl hemiacetal* (**6**) [24].—2,3,4,5,6-Penta-*O*-acetyl-D-glucose diethyl acetal [25] (12 mmol, 5.7 g) was treated with Ac_2O (50 mL) in the presence of Dowex 50-X2 (1 g, 100–200 mesh, H^+ form). The mixture was kept at 65°C for 1.5 h, after which the resin was filtered off, and the solution was concentrated to an oil under reduced pressure. Fractionation by HPLC on an RP-18 column (3:2 MeOH–water) afforded a mixture of **5** and **6** as a yellow syrup. The identity of the hemiacetals was verified by mass spectrometry.

*1,1,2,3,4,5,6-Hepta-O-acetyl-*aldehydo-D-*glucose hydrate* (**8**) [12].—The mixture of **5** and **6** (4 mmol, 2.0 g) was dissolved in a mixture of Ac_2O and AcOH (50 mL; 7:3, v/v) containing 4% sulfuric acid. After 24 h at room temperature, the solution was neutralized with a saturated solution of NaOAc in AcOH and evaporated to dryness. The crude product was recrystallized from aqueous ethanol, yielding 0.3 g of white crystals (mp 95°C).

1,2,3,5,6-Penta-O-acetyl-α- (**9**) *and -β-D-glucofuranose* (**10**) [26].—3,5,6-Tri-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose was hydrolyzed to an anomeric mixture of 3,5,6-tri-*O*-acetyl-D-glucofuranoses and then subjected to acetylation with a mixture of Ac_2O and pyridine [27–30]. The crude product obtained was fractionated by HPLC on a Si_{60} column (3:2 toluene–diethyl ether). Gas chromatography of the main fraction showed the presence of two components, which were identified as **9** and **10** by ^1H NMR and mass spectroscopy.

Kinetic measurement.—Acetolyses were carried out in sealed tubes immersed in a water bath thermostated at 25°C. The tubes, each containing 1 mL of a 28 μM solution of the starting material in the mixture of AcOH, Ac_2O, and H_2SO_4 (1% H_2SO_4 in a 1:1, mixture of AcOH and Ac_2O), were removed at suitable intervals. An aliquot of 0.1 mL was withdrawn, neutralized with a saturated solution of NaOAc in AcOH, and centrifuged. The supernatant solution was chromatographed on a 30-m DB-Wax capillary column under isothermic conditions (215°C) using flame-ionization detection. The peaks were assigned by spiking with authentic samples. The signal areas were assumed to be proportional to the concentrations because of the similar chemical nature of all the compounds involved. First-order rate constants for the mutual anomerization of **1** and **2** were calculated by eqs (1) and (2), where x_2 is the mole fraction of **2** in the mixture of **1** and **2** at time t, and x_e is the equilibrium value that x_2 approaches (**2** is the starting material). The sum of k_2 and k_3 was

$$k_1 + k_{-1} = t^{-1} \ln(1 - x_e)/(x_2 - x_e) \quad (1)$$

$$k_1/k_{-1} = x_e/(1 - x_e) \quad (2)$$

calculated from the disappearance of [**1**] + [**2**] by the integrated first-order rate equation, and the value obtained was bisected to the rate constants of the parallel reactions with the aid of the concentration ratio of the products, ([**3**] + [**4**])/([**5**] + [**6**]). A similar approach was applied to obtain the rate constants k_4, k_5, and k_6.

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