Carbohydrate Research, 32 (1974) 287–298

ACETOLYSIS OF DISACCHARIDES: COMPARATIVE KINETICS AND MECHANISM

LOUIS ROSENFELD AND CLINTON E. BALLOU

Department of Biochemistry, University of California, Berkeley, California 94720 (U. S. A.)

(Received July 30th, 1973; accepted September 24th, 1973)

ABSTRACT

The initial acetolysis rates of several disaccharides were compared using an assay procedure which involves adding portions of the reaction mixture to an alkaline sodium borohydride solution. After reduction, glycosidically-linked hexose was determined by the phenol–sulfuric acid method. For D-glucose disaccharides, β linkages were cleaved faster than α linkages, suggesting anchimeric assistance from the *trans* C-2 acetoxyl group. The acetolysis reaction rates for the various β-linked D-glucose disaccharides decreased in the order $(1\rightarrow6) \gg (1\rightarrow3) > (1\rightarrow2) > (1\rightarrow4)$. For the α-linked disaccharides, the order was $(1\rightarrow6) \gg (1\rightarrow4) > (1\rightarrow3) > (1\rightarrow2)$. The acetolysis rates for D-mannose disaccharides were in the order α-$(1\rightarrow6) \gg \alpha$-$(1\rightarrow3) > \beta$-$(1\rightarrow4) > \alpha$-$(1\rightarrow2)$. Turanose (3-*O*-$\alpha$-D-glucopyranosyl-D-fructose) was cleaved at a much faster rate than either D-mannobiose or D-glucobiose with α-$(1\rightarrow2)$ or α-$(1\rightarrow3)$ linkages. A reaction mechanism is supported which features an acyclic intermediate, and, for certain disaccharides, C-2 acetoxyl anchimeric assistance.

INTRODUCTION

Relative rates of glycosidic-bond cleavage by acetolysis[1] have been inferred from the products resulting from the acetolysis of various polysaccharides. For example, the acetolysis of yeast mannans[2–4], dextrans[5–15], amylopectin[16,17], and glycoproteins[18] has shown that $(1\rightarrow6)$ bonds are cleaved preferentially. Investigations[6,19] of the relative rates of acetolysis of some disaccharides have confirmed this conclusion.

Because of its complexity, the acetolysis reaction is difficult to follow by the change in optical rotation[20–22], and most reducing sugar assays are not reliable enough to measure a doubling in reducing power when small amounts of rare disaccharides are used. Therefore, for following the reaction we have developed a general technique that allows direct measurement of the amount of disaccharide, oligosaccharide, or polysaccharide remaining, without interference from released monosaccharides. This procedure involves adding samples of the acetolysis reaction to an alkaline borohydride solution, allowing time for reduction to occur, and then measuring the remaining glycosidically-linked hexose with the phenol–sulfuric acid method[23].

This paper reports a study of the relative rates of disaccharide acetolysis. In agreement with the conclusions from previous studies[6,19,21,22], our results show that acetolysis rates are related to the number of electrophilic groups in the sugar at the reducing end of the disaccharides, and that the rates depend on the anomeric configuration and the configuration at other centers in the sugar units. The results serve to define some practical limitations of the reaction and to suggest specific applications that may be useful in structural determinations on natural products.

EXPERIMENTAL

General. — Descending paper chromatograms were eluted with ethyl acetate–acetic acid–formic acid–water[24] (18:3:1:4, v/v). Whatman No. 1 and 3MM filter papers were used for analytical and preparative chromatography, respectively. Hexoses and hexitols were detected by the alkaline silver nitrate procedure[25]. Disaccharides were isolated by chromatography on a 4×200-cm column of Bio-Gel P-2 (200–400 mesh, Bio-Rad Laboratories, Richmond, California 94804). The carbohydrate content in the effluent was measured by the phenol–sulfuric acid technique[23].

Materials. — Disaccharides used for this study are listed in Table I. Crystalline kojibiose and nigerose octaacetates were gifts of Dr. K. Matsuda, Tohoku University, Sendai, Japan. Sophorose was obtained from Dr. Matsuda and from Dr. I. J. Goldstein, University of Michigan, Ann Arbor, Michigan.

Laminarabiose was prepared by partial acid hydrolysis of insoluble laminaran (Pierce Chemical Co., Rockford, Illinois 61105), following the procedure of Barry and McCormick[26]. After 5 h, the hydrolyzate was neutralized with excess barium carbonate, centrifuged, concentrated, and purified by gel filtration on a 4×200-cm column of Bio-Gel P-2.

Controlled acetolysis of *Saccharomyces cerevisiae* (Red Star) yeast mannan[27], deacetylation of the product with sodium methoxide in dry methanol, and separation of the resulting oligosaccharides by gel chromatography yielded the disaccharide 2-*O*-α-D-mannosyl-D-mannose and the tetrasaccharide α-D-Man-(1→3)-α-D-Man-(1→2)-α-D-Man-(1→2)-D-Man. 6-*O*-α-D-Mannopyranosyl-D-mannose was prepared by partial acetolysis of the polysaccharide residue from *Arthrobacter* GJM-1 cultural filtrates, grown on yeast mannan, as described by Jones and Ballou[28]. A 6-hour acetolysis (40°; 10:10:1, v/v, acetic anhydride–acetic acid–conc. sulfuric acid) of mannan from ivory nut shavings (*Phytelephas macrocarpa*)[29] was used for the preparation of 4-*O*-β-D-mannopyranosyl-D-mannose; gel filtration, followed by preparative paper chromatography, yielded the disaccharide; the optical rotation, measured on a Bendix 1100 Polarimeter, gave $[\alpha]_{546}^{25}$ +10.9° (lit.[30]: $[\alpha]_D$ −5.2→ −8.5°).

All other disaccharides were obtained from commercial sources: Maltose, cellobiose, gentiobiose, and turanose from Pfanstiehl Laboratories Inc. (Waukegan, Illinois 60085), isomaltose from Pierce Chemical Co., lactose from Merck, and melibiose from Eastman Kodak Co. (Rochester, New York 14650).

TABLE I

ACETOLYSIS RATE CONSTANTS

Sugar	*Structure*	k (h^{-1})	*Relative* k[a]
5:5:2 System			
Kojibiose	α-D-Glc-*p*-(1→2)-D-Glc	0.022	0.18
Sophorose	β-D-Glc-*p*-(1→2)-D-Glc	0.16	1.4
Nigerose	α-D-Glc-*p*-(1→3)-D-Glc	0.032	0.27
Laminarabiose	β-D-Glc-*p*-(1→3)-D-Glc	0.23	1.9
Maltose	α-D-Glc-*p*-(1→4)-D-Glc	0.12	1.0[a]
Cellobiose	β-D-Glc-*p*-(1→4)-D-Glc	0.14	1.2
Isomaltose	α-D-Glc-*p*-(1→6)-D-Glc	0.95	7.9
Gentiobiose	β-D-Glc-*p*-(1→6)-D-Glc	k_1 6.9	58
		k_2 1.0	8.7
D-Mannobiose	α-D-Man-*p*-(1→2)-D-Man	0.025	0.21
D-Mannotetraose	α-D-Man-*p*-(1→3)-α-D-Man-*p*-(1→2)-α-D-Man-*p*-(1→2)-D-Man	k_1 0.35	2.9
		k_2 0.027	0.23
D-Mannobiose	β-D-Man-*p*-(1→4)-D-Man	0.17	1.4
Lactose	β-D-Gal-*p*-(1→4)-D-Glc	0.49	4.1
10:10:1 System			
Maltose	α-D-Glc-*p*-(1→4)-D-Glc	0.021	1.0[a]
Isomaltose	α-D-Glc-*p*-(1→6)-D-Glc	0.27	13
Gentiobiose	β-D-Glc-*p*-(1→6)-D-Glc	k_1 1.2	57
		k_2 0.30	14
D-Mannobiose	α-D-Man-*p*-(1→6)-D-Man	1.26	60
Melibiose	α-D-Gal-*p*-(1→6)-D-Glc	0.87	41
Turanose	α-D-Glc-*p*-(1→3)-D-Fru	1.5	71

[a]Relative *k*: Maltose acetolysis rate is defined as 1.0.

Acetolysis measurements. — The disaccharide (10 mg) was weighed into a 13 × 100-mm, screw-capped test tube and acetylated with dry pyridine and acetic anhydride (1 ml each) for 15 min at 100°. After cooling, the reaction was stopped by addition of an equal volume of water. When the mixture had again cooled, the solvents were removed at 50° on a rotary evaporator at reduced pressure. Rate measurements were sensitive to traces of pyridine, which neutralized part of the sulfuric acid catalyst. To remove all of the pyridine, the acetylated sugar was dissolved in benzene (1.5 ml), and the benzene layer was washed successively with an equal volume of water, M hydrochloric acid, M sodium hydrogen carbonate solution, and finally water. The benzene was evaporated and the excess of water was removed azeotropically by repetitive evaporation of benzene.

The acetylated sugar, prepared as just described, was dissolved in acetic anhydride (1.0 ml). The tube was cooled in ice, and a mixture of glacial acetic acid (1.0 ml) and 0.10 ml ("10:10:1 system") or 0.40 ml ("5:5:2 system") of concentrated sulfuric acid was added. The solution was mixed, while the tube remained in ice, and three 50-μl samples were removed and treated as follows (zero time sample). At zero time, the capped test tubes were placed in a water bath at 40±0.2°. Samples (50 μl) were removed in triplicate at various times and each was added, with thorough mixing

and washing of the micropipet, to a matched 18 × 150-mm test tube containing a solution (1.0 ml) of 2M sodium hydroxide and 0.5M sodium borohydride in 10% aqueous methanol. Care was taken to rinse the sides of the test tube with the solution it contained. The sodium hydroxide neutralized the acid and saponified the acetylated sugar, while the sodium borohydride reduced to polyols the remaining disaccharide and any monosaccharide released by the acetolysis reaction. After being kept at least 12 h at room temperature, the reduced mixture was treated[23] with 4% phenol (2 ml) and concentrated sulfuric acid (5 ml). After 30 min, the cooled tubes were placed directly into a Bausch and Lomb Spectronic 20 Spectrophotometer and the absorbance was read at 490 nm. Only the nonreducing residue of the hexose-containing disaccharide produced color at 490 nm, and it represented the amount of disaccharide remaining at a given time. Figure 1 shows a standard curve for maltose octaacetate in the system with and without addition of sodium borohydride. When less sugar was used, the amounts of the acetolysis reagents were reduced proportionately. Temperatures higher than 40° caused the rapid conversion of sulfuric acid into sulfoacetic acid, which lowered the reaction rate.

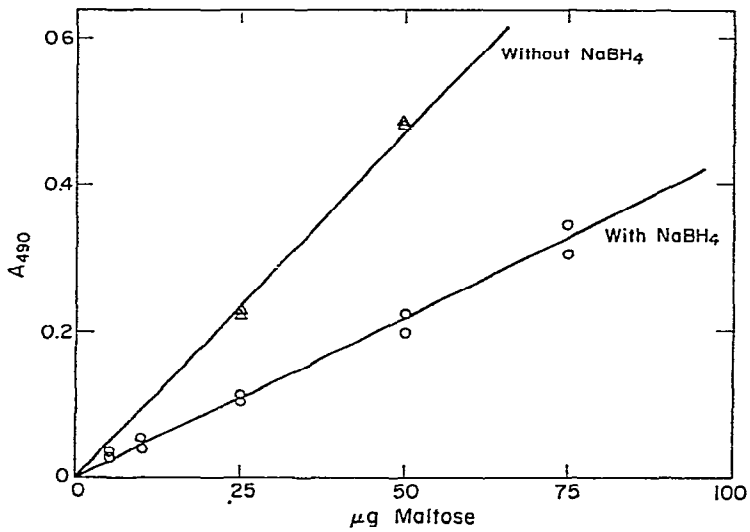


Fig. 1. Standard curves for maltose octaacetate in the assay system described in the text: (○) with and (△) without sodium borohydride.

Measurement of percent anomerization. — Shortly after the last sample was removed, an equal volume of pyridine was added to stop the reaction. The solvents were evaporated, the residue was dissolved in benzene (1.5 ml), and the benzene layer was washed as previously described. After removal of the benzene by evaporation, the excess of water was removed by azeotropic distillation with benzene. Deacetylation was accomplished with 0.04M sodium methoxide in dry methanol for 30 min and was terminated with AG 50 (H^+) ion-exchange resin (Bio-Rad Laboratories). The resulting sugar mixture was then reduced with a sodium borohydride–sodium boro-

tritide (New England Nuclear, Boston, Massachusetts 02118, 200 Ci/mole) mixture[28] in 50mM ammonium hydrogen carbonate solution overnight. The reduction was stopped and cations were removed with AG 50 (H^+) ion-exchange resin, and the resin was filtered off and washed with 50% aqueous methanol. Borate ions were removed by repetitive addition and evaporation of methanol. The reduced carbohydrates were chromatographed on 2.5-cm strips of Whatman No. 1 paper for 5 days. The strips were cut into 1-cm pieces which were placed in scintillation vials. Water (0.1 ml) was placed on the pieces of paper to elute the products and Bray's solution[31] (10 ml) was added. The vials were counted in a Packard Tri-Carb Scintillation Spectrometer. This procedure only measured the extent of anomerization at the end of the acetolysis reaction, where anomerization had presumably reached equilibrium.

RESULTS AND DISCUSSION

Rate constants. — The conclusion that the acetolysis reaction was pseudo first-order with respect to disaccharide concentration in the 5:5:2 system is supported by the observation that two different concentrations of maltose gave parallel straight lines when reported on semilogarithmic paper (Fig. 2). The rate constant, k, was determined graphically, employing the equation

$$k = \frac{1}{t} \ln \frac{A_0}{A_t}$$

where A_0 and A_t represent the absorbance at 490 nm at times zero and t, respectively. The reaction did not give useful data for times longer than about 15 h, since the sulfoacetic acid that was formed[32] reduced the calatyst concentration. The data were plotted against time on semi-logarithmic paper, the half-life was determined from this plot, and the rate constant was calculated. Only data in the initial linear portion of the curves were used, owing to the occurrence of unknown side-reactions which became more significant with time.

Two sugars, gentiobiose and D-mannotetraose, gave biphasic curves when the data were treated in the just described manner (Figs. 3 and 4). Gentiobiose was free of isomaltose at the beginning of the reaction, but it anomerized to isomaltose (see below), and a change in the slope was observed after 40 min in the 10:10:1 system (Fig. 3), at which time the acetolysis rate for isomaltose predominated. In the 5:5:2 system, the rate changed after 1.5 min, while the first sample was being taken (Fig. 5). The initial slope was attributed to the acetolysis of gentiobiose and the second to the acetolysis of isomaltose. D-Mannotetraose has one (1→3) linkage and two (1→2) linkages. Assuming that these linkages are attacked independently, the initial slope represents a mixture of the rates of acetolysis of both type of linkages, whereas the second slope represents the slower acetolysis of the (1→2) linkage. The first rate constant was calculated by subtracting the extrapolated A_{490} of the second slope from the A_{490} of the initial slope at given times before the slope changed (Fig. 4).

Rate constants are given in Table I. Two different acetolysis systems were used.

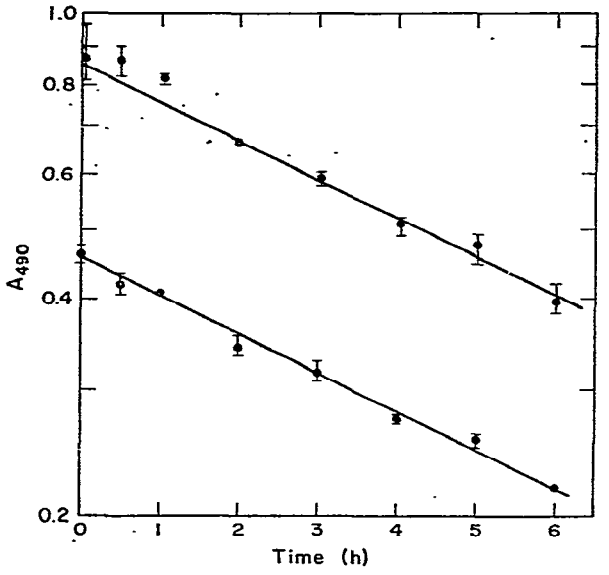


Fig. 2. Acetolysis of two different initial concentrations of maltose acetate at 40° in the 5:5:2 system. The circles represent the average of three readings, and the bars show the range.

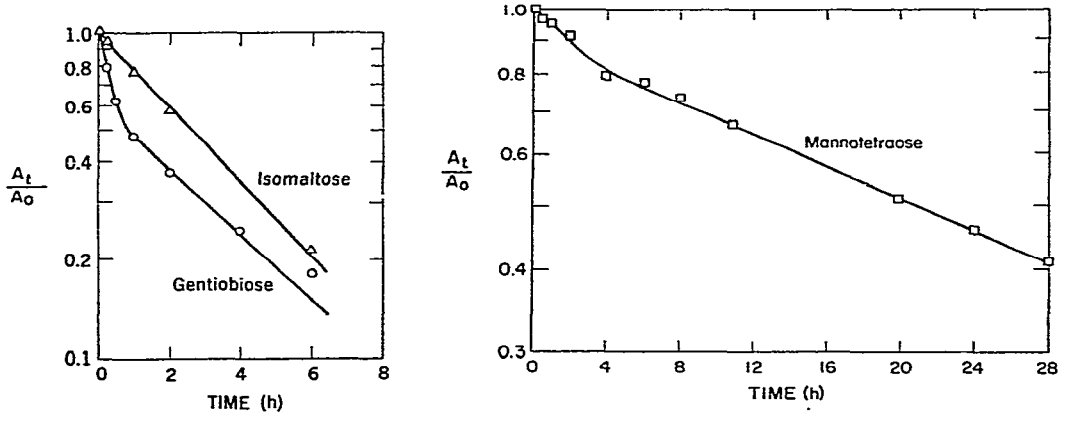


Fig. 3. Acetolysis of gentiobiose (○) and isomaltose (△) at 40° in the 10:10:1 system. The gentiobiose rate curve is biphasic, with the slower slope approximating that for the isomaltose curve. Each point represents the average of three readings.

Fig. 4. Acetolysis at 40° of D-mannotetraose in the 5:5:2 system.

One was 5:5:2 (v/v) acetic anhydride–acetic acid–concentrated sulfuric acid for the more resistant disaccharides; the other was 10:10:1 (v/v) acetic anhydride–acetic acid–concentrated sulfuric acid for the more reactive disaccharides. Data from the two systems were correlated by the relative rate column.

Several conclusions are suggested by the data of Table I. First, as expected, the rates of acetolysis depend on the kind of linkage (see Figs. 3, 5, and 6). For homolo-

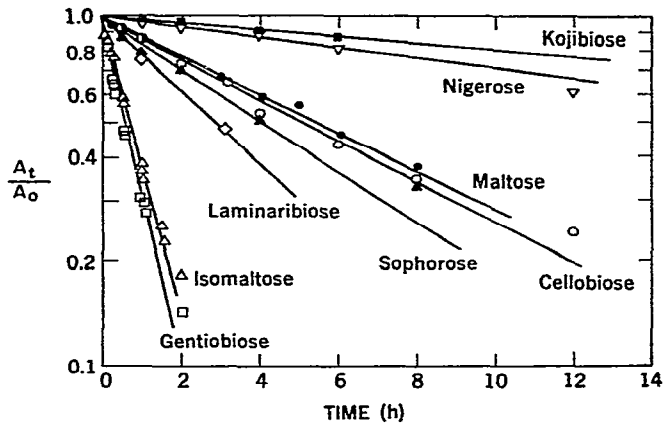


Fig. 5. Acetolysis of the eight isomeric reducing D-glucobioses at 40° in the 5:5:2 system. Each point represents the average of three readings except for gentiobiose and isomaltose, where all readings are shown. The gentiobiose curve is biphasic and changed slope before the first sample could be taken: ■, kojibiose; ▽, nigerose; ●, maltose; ○, cellobiose; ▲, sophorose; ◊, laminarabiose; △, isomaltose; □, gentiobiose.

gous D-glucose-containing disaccharides, β linkages were cleaved 1.2 to 8 times faster than α linkages. The order of acetolysis rates for α-linked D-glucose disaccharides was (1→6) ⩾ (1→4) > (1→3) > (1→2), in agreement with the work of Matsuda[6,19]. For β linkages, the order was (1→6) ⩾ (1→3) > (1→2) > (1→4). The acetolysis of gentiobiose was 41 times faster than that of sophorose and 320 times faster than that of kojibiose, in the 5:5:2 system.

α-D-Mannose disaccharides were acetolyzed in the same order as the α-linked D-glucobioses. 6-*O*-α-D-Mannopyranosyl-D-mannose was cleaved about 280 times faster than 2-*O*-α-D-mannopyranosyl-D-mannose, which accounts for the highly successful use of this reaction for selective degradation of yeast mannans[2–4]. In

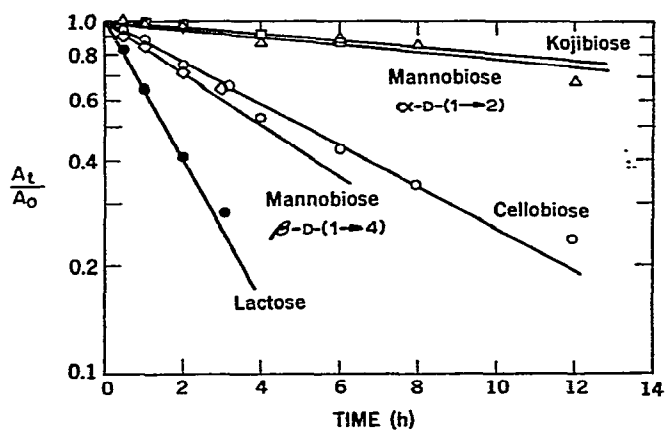


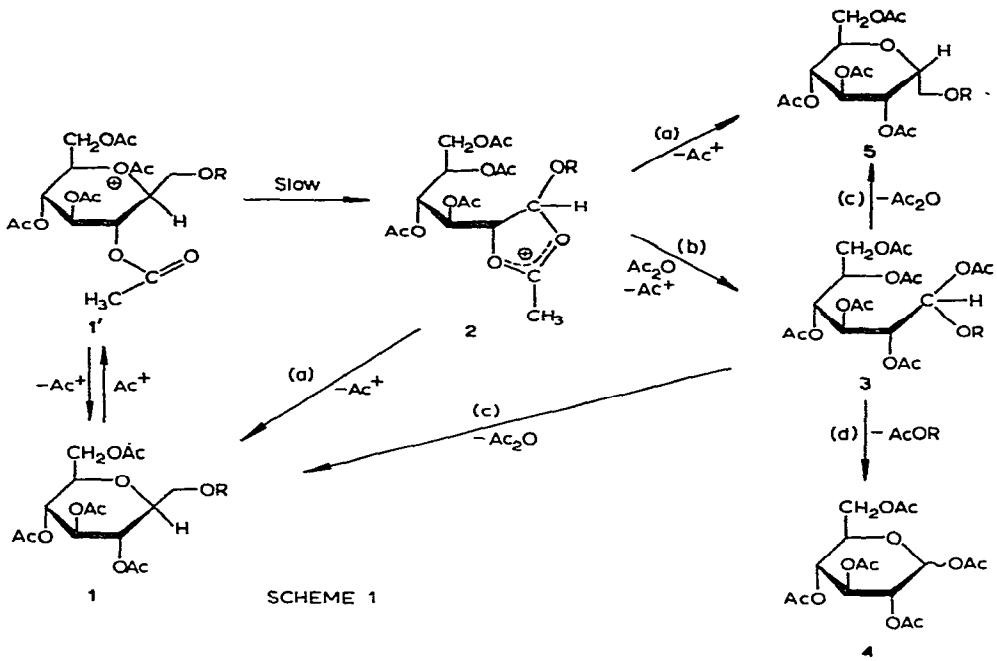
Fig. 6. Acetolysis rate curves for disaccharides containing different sugars in the 5:5:2 system. □, kojibiose; △, α-D-(1→2)-mannobiose; ○, cellobiose; ◊, β-D-(1→4)-mannobiose; ●, lactose.

contrast, the difference in rates between α-(1→6) and α-(1→3)-linked D-mannobioses was only a factor of 21, in agreement with the report[33] that α-(1→3) linkages were more labile than α-(1→2) linkages in D-mannooligosaccharides. 4-*O*-β-D-Mannopyranosyl-D-mannose was acetolyzed more slowly than the α-(1→3)-linked D-mannobiose, an observation that agrees with the mechanism proposed below.

Table I also demonstrates that acetolysis rates depend on the composition of the disaccharide, since the hexose at both the nonreducing end and the reducing end had an effect on the rates for disaccharides having the same linkage. For β-(1→4)-linked disaccharides, the relative rates were D-galactose > D-mannose > D-glucose at the nonreducing end (Fig. 6). For the α-(1→2)-linked sugars, D-mannobiose was cleaved slightly faster than D-glucobiose. Although the differences are small, these results agree with those of Smith and Srivastava[34], which suggested that the linkage between two D-mannose residues was split more easily than that between a D-glucose and a D-mannose residue. Turanose (3-*O*-α-D-glucopyranosyl-D-fructose) was cleaved much faster than the other α-(1→2) and α-(1→3)-linked disaccharides studied (D-mannobiose and D-glucobiose), showing that the sugar residue at the reducing end also influenced the rate.

Mechanism. — Our results are consistent with the mechanism shown in Scheme I. The attacking species is assumed to be the acetylium cation $[CH_3CO]^+$, formed from the reaction of acetic anhydride with sulfuric acid[1]. This cation, shown in Scheme I as Ac^+, may attack the ring oxygen (O-5) of the nonreducing sugar, as suggested by Lindberg[21]. For β-linked D-glucobioses, anchimeric assistance by the C-2 acetoxyl group may help open the ring and stabilize the charge in a rate-determining step, producing intermediate **2**. Steric factors may prevent such participation by the C-2 acetoxyl group of α-linked D-glucobioses. Ring closure (a) followed by a loss of Ac^+ would yield **1** or **5** (anomerization). Alternatively, the anomeric carbon of intermediate **2** may be subjected to nucleophilic attack by acetic anhydride (Ac_2O) with subsequent loss of an acetylium cation (b). The acetal group at C-1 may then be attacked by O-5 with elimination of either acetic anhydride (c, anomerization) or AcOR (d, acetolysis). This is an extension of the acyclic mechanism for anomerization proposed by Lindberg[21] and incorporates mechanistic features presented by Capon[35] for the acyclic, acid-hydrolysis mechanism. It assumes that ring opening is the rate determining step. Another mechanism proposed by Lemieux[36] has not been completely ruled out by this study, although it assumes that the rate determining step is attack on the glycosidic oxygen atom.

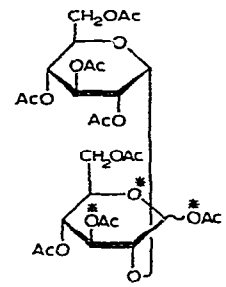
Acid hydrolysis, which is not affected significantly by the electronic nature of the aglycon of glycosides[35] nor by the reducing end sugar of disaccharides[37], is believed to follow a mechanism involving a cyclic intermediate[38]. In contrast, acetolysis rates of glycosides[22] and disaccharides (Table I) are very sensitive to electronegative groups linked to the aglycon and to the reducing sugar residue. Lindberg[22] has shown that, under acetolysis conditions, acetylated alkyl glycosides that have electronegative groups located, on the aglycon residue, two carbon atoms away from the glycosidic oxygen atom were anomerized and acetolyzed more slowly than those in which the

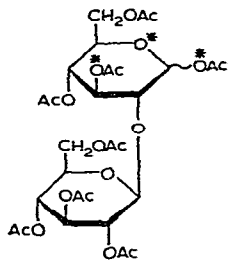


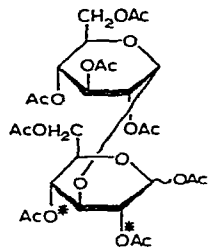
SCHEME 1

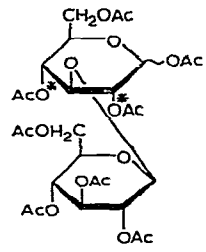
electronegative group was located farther away or in which the aglycon was an unsubstituted alkyl group. Acetylated disaccharides linked (1→2), (1→3), (1→4), and (1→6) differ in the number and type of electron-withdrawing groups that are attached near the glycosidic bond, on the sugar, at the reducing end. (See next page). These linkages have 3, 2, 2, and 1 electrophilic groups, respectively, located two carbon atoms away from the glycosidic oxygen atom. The ring oxygen atom is one of these groups for (1→2), (1→4), and (1→6) linkages, whereas the remainder are the more electrophilic acetoxyl groups[21,22]. The above interpretation is consistent with the finding of Matsuda[6] that trehalose is acetolyzed more slowly than kojibiose.

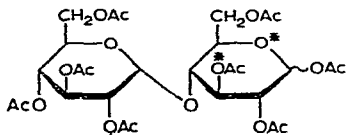
The acetolysis rates shown in Table I generally agree with the predictions based on these electronic considerations. However, β-linked D-glucobioses apparently are less importantly affected by the electrophilic nature of the reducing end sugar, probably because C-2 anchimeric assistance predominates to stabilize the charge (Scheme I). This is suggested by the observation that β linkages are acetolyzed faster than α linkages for the D-glucobiose series. Moreover, since 4-*O*-β-D-mannopyranosyl-D-mannose was acetolyzed more slowly than the α-(1→3)-linked D-mannobiose, it is possible that anchimeric assistance also occurs in the α-D-mannobiose series. Since disaccharides having a D-galactose residue on the nonreducing end were acetolyzed faster than homologous disaccharides that have a D-glucose or D-mannose residue at that position, participation by the acetoxyl group on C-4 may have occurred. The latter type of intermediate has been proposed to account for the specificity of Koenigs–Knorr reactions of L-fucose[39].

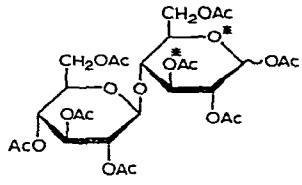

α-D-(1→2) (Kojibiose)

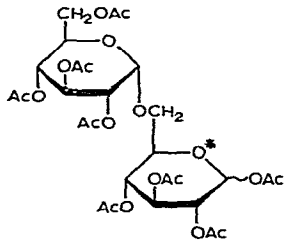

β-D-(1→2) (Sophorose)

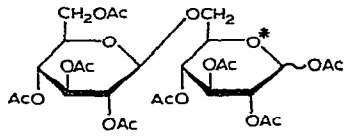

α-D-(1→3) (Nigerose)


β-D-(1→3) (Laminaribiose)


α-D-(1→4) (Maltose)


β-D-(1→4) (Cellobiose)


α-D-(1→6) (Isomaltose)


β-D-(1→6) (Gentiobiose)

In addition to the electronic terms just discussed, steric factors may contribute to the significantly higher acetolysis rates of (1→6) linkages. Thus, O-5 may be able to reattack C-1 of intermediate 3 (Scheme I) more easily when there is less steric hindrance from the ring of the reducing sugar residue.

Extent of anomerization. — Table II shows the extent of anomerization for the disaccharides remaining at the termination of each reaction. Anomerization usually proceeds in a manner such that the substituents at C-1 and C-5 of hexose residues assume the *trans* configuration[36]; therefore, one would expect a β-D-linked glucoside or disaccharide to anomerize to the α-D form. Similarly, an α-linked D-mannoside would be expected to be stable to anomerization. Table II generally supports this rule, but in addition α-linked isomaltose, melibiose, and turanose showed a small degree of anomerization and these were also acetolyzed rapidly. Lindberg[21,22,40] has noted a correlation between rates of acetolysis and anomerization, an observation that suggests that both reactions proceed through the same intermediate. Scheme I shows

TABLE II

EXTENT OF ANOMERIZATION

Sugar	*Duration of acetolysis (h)*	*Anomerization (%)*
5:5:2 System		
Kojibiose	27.0	<5
Nigerose	27.0	<5
Laminarabiose	8.3	37
Cellobiose	12.1	15
Isomaltose	4.2	5.5
Gentiobiose	4.2	83
Mannobiose α-D-(1→2)	24.2	<5
Lactose	8.25	63
10:10:1 System		
Maltose	48.2	<5
Isomaltose	12.4	5.4
Gentiobiose	12.5	84
Mannobiose α-D-(1→6)	8.4	9.7
Melibiose	10.5	80
Turanose	8.3	88

that anomerization can occur from either intermediate **2** or **3**. If so, then anomerization may occur before acetolysis, as has been shown for alkyl glycosides[21,40] and for gentiobiose (Table I).

Applications of acetolysis to polysaccharides. — Acetolysis under controlled conditions has been used successfully to cleave (1→6) linkages of various polysaccharides in preference to other linkages[1–19]. From the results presented in this paper and elsewhere[1,21,22,35,36], it is possible to suggest how this specificity arises. With due regard for anomerization[21,22] and epimerization[41,42], the acetolysis reaction may now be extended rationally for cleaving heteropolysaccharides that have favored linkages other than (1→6). For example, the structures of galactomannans and galactoglucans might be studied using acetolysis as a structural probe. If the proper conditions can be found, the selective degradation of yeast D-glucan[43] by acetolysis may also be feasible. The latter project is currently under investigation.

Note added in proof (Received December 17th, 1973). Since writing this report, other related work has come to our attention. The article by Govorchenko and Ovodov[44] on the acetolysis of neutral disaccharides generally agrees with our results, although it appears that they did not realize that gentiobiose was rapidly isomerized to isomaltose. β-β-Trehalose, which is most rapidly acetolyzed in their system, shows the extreme example of anchimeric assistance, whereas α-α-trehalose is an extreme example of the electronegative effect described herein. The results in two other papers concerned with the acetolysis of disaccharide derivatives[45,46] are also rationalized on the basis of the strength, number and position of electronegative groups.

ACKNOWLEDGMENTS

The authors thank Drs. K. Matsuda and I. J. Goldstein for supplying the rare

disaccharides listed in the text. They also thank Mr. Peter Lipke, Dr. Max Tate, Dr. Gary Gray, and Dr. J. F. Kirsch for advice and criticism.

REFERENCES

1 R. D. GUTHRIE AND J. F. MCCARTHY, *Advan. Carbohyd. Chem.*, 22 (1967) 11.
2 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 34 (1956) 1796.
3 J. K. N. JONES AND W. H. NICHOLSON, *J. Chem. Soc.* (1958) 27.
4 Y. C. LEE AND C. E. BALLOU, *Biochemistry*, 4 (1965) 257.
5 K. MATSUDA, H. WATANABE, AND K. ASO, *Tohoku J. Agr. Res.*, 12 (1961) 351.
6 K. MATSUDA, H. WATANABE, K. FUJIMOTO, AND K. ASO, *Nature*, 191 (1961) 278.
7 K. MATSUDA, K. FUJIMOTO, AND K. ASO, *Tohoku J. Agr. Res.*, 12 (1961) 359.
8 K. FUJIMOTO, K. MATSUDA, AND K. ASO, *Tohoku J. Agr. Res.*, 13 (1962) 55.
9 I. J. GOLDSTEIN AND W. J. WHELAN, *J. Chem. Soc.* (1961) 170.
10 M. TORII, E. A. KABAT, AND S. BEYCHOK, *Arch. Biochem. Biophys.*, 103 (1963) 283.
11 H. SUZUKI AND E. J. HEHRE, *Arch. Biochem. Biophys.*, 104 (1964) 305.
12 D. ABBOT AND H. WEIGEL, *J. Chem. Soc., C* (1966) 816.
13 B. A. LEWIS, M. J. ST. CYR, AND F. SMITH, *J. Org. Chem.*, 33 (1968) 3139.
14 F. YAMAUCHI AND K. MATSUDA, *Agr. Biol. Chem.* (Tokyo), 33 (1969) 103.
15 K. SAKAKIBARA, M. TORII, A. MISAKI, AND H. MIYAJI, *Carbohyd. Res.*, 25 (1972) 443.
16 M. L. WOLFROM, J. T. TYREE, T. T. GALKOWSKI, AND A. N. O'NEILL, *J. Amer. Chem. Soc.*, 73 (1951) 4927; M. L. WOLFROM AND N. E. FRANKS, *Methods Carbohyd. Chem.*, 4 (1964) 269.
17 A. THOMPSON, M. L. WOLFROM, AND E. J. QUINN, *J. Amer. Chem. Soc.*, 75 (1953) 3003.
18 B. BAYARD, B. FOURNET, S. BOUQUELET, G. STRECKER, G. SPIK, AND J. MONTREUIL, *Carbohyd. Res.*, 24 (1972) 445.
19 K. FUJIMOTO, K. MATSUDA, AND K. ASO, *Tohoku J. Agr. Res.*, 13 (1962) 61.
20 K. FREUDENBERG AND K. SOFF, *Ber.*, 69 (1936) 1245; *Chem. Abstr.*, 27 (1937) 1504.
21 B. LINDBERG, *Acta Chem. Scand.*, 3 (1949) 1153.
22 B. LINDBERG, *Acta Chem. Scand.*, 3 (1949) 1350.
23 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
24 M. H. SAIER, JR. AND C. E. BALLOU, *J. Biol. Chem.*, 243 (1968) 4319.
25 W. E. TREVELEYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature*, 166 (1950) 444.
26 V. C. BARRY AND J. E. MCCORMICK, *Methods Carbohyd. Chem.*, 1 (1962) 328.
27 J. KOCOUREK AND C. E. BALLOU, *J. Bacteriol.*, 100 (1969) 1175.
28 G. H. JONES AND C. E. BALLOU, *J. Biol. Chem.*, 244 (1969) 1052.
29 G. O. ASPINALL, E. L. HIRST, E. G. V. PERCIVAL, AND I. R. WILLIAMSON, *J. Chem. Soc.* (1953) 3184.
30 G. O. ASPINALL, R. B. RASHBROOK, AND G. KESSLER, *J. Chem. Soc.* (1958) 215.
31 G. A. BRAY, *Anal. Chem.*, 1 (1960) 279.
32 E. A. JEFFERY AND D. P. N. SATCHELL, *J. Chem. Soc.* (1962) 1913.
33 W. C. RASCHKE AND C. E. BALLOU, *Biochemistry*, 11 (1972) 3807.
34 F. SMITH AND H. C. SRIVASTAVA, *J. Amer. Chem. Soc.*, 78 (1956) 1404.
35 B. CAPON, *Chem. Rev.*, 69 (1969) 407.
36 R. U. LEMIEUX, *Advan. Carbohyd. Chem.*, 9 (1954) 1.
37 M. L. WOLFROM, A. THOMPSON, AND C. E. TIMBERLAKE, *Cereal Chem.*, 40 (1963) 82.
38 B. E. C. BANKS, Y. MEINWALD, A. J. RHIND-TUTT, I. SHEFT, AND C. A. VERNON, *J. Chem. Soc.* (1961) 3240.
39 M. DEJTER-JUSZYNSKI AND H. M. FLOWERS, *Carbohyd. Res.*, 23 (1972) 41.
40 J. JANSON AND B. LINDBERG, *Acta Chem. Scand.*, 14 (1960) 877.
41 P. JERKEMAN, *Acta Chem. Scand.*, 17 (1963) 2769.
42 G. J. F. CHITTENDEN, *Carbohyd. Res.*, 22 (1972) 491.
43 A. MISAKI, J. JOHNSON, JR., S. KIRKWOOD, J. V. SCALETTI, AND F. SMITH, *Carbohyd. Res.*, 6 (1968) 150.
44 V. I. GOVORCHENKO AND YU. S. OVODOV, *Khim. Prir. Soedin.* (1972) 256.
45 V. I. GOVORCHENKO, V. I. GORBATCH, AND YU. S. OVODOV, *Khim. Prir. Soedin.* (1972) 258.
46 V. I. GOVORCHENKO, V. I. GORBATCH, AND YU. S. OVODOV, *Carbohydr. Res.*, 29 (1973) 421.