Carbohydrate Research, 185 (1989) 249–260
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE REVERSION REACTIONS OF D-GLUCOSE DURING THE HYDROLYSIS OF CELLULOSE WITH DILUTE SULFURIC ACID

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(Received December 3rd, 1987; accepted for publication in revised form, August 10th, 1988)

ABSTRACT

The reversion products formed during the acid hydrolysis of Avicel to D-glucose, under conditions envisaged for the industrial conversion of woody biomass into monomeric sugars, have been determined by using gas–liquid chromatography. Avicel was hydrolyzed in dilute sulfuric acid (0.26–1.27 wt.%) between 160 and 250° in small (3 mm, i.d.) glass tubes at a 3:1 liquid-to-solid ratio. The anhydro sugars, levoglucosan and 1,6-anhydro-β-D-glucofuranose, were produced in the ratio of 7:3 and constituted >50% of the total yield of reversion products. The yield of anhydro sugar followed equilibrium kinetics, and reached 6% at maximum yields (50%) of D-glucose. Isomaltose and gentiobiose were the most preponderant disaccharides found among the reversion products, constituting together ~25% of the reversion products. The (1→2)- and (1→3)-linked α-disaccharides preponderated over their β counterparts. The total yields of reversion products approached 10% on the basis of the D-glucose theoretically available.

INTRODUCTION

The acid hydrolysis of woody biomass has been intensively studied as a method of providing monomeric sugars for fermentation to desirable bulk chemicals. Such products as ethanol can be used as substitutes or diluents for liquid petroleum fuels. Although the enzymic conversion of lignocellulosics into monomeric sugars is an area of active research[1,2], and the economics of enzymic conversion continue to improve[3], a two-stage hydrolysis process with dilute sulfuric acid appears to be the technology closest to actual commercial development in the United States[4,5]

Cellulose is saccharified to D-glucose in the second stage of the two-stage

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process. Yields of D-glucose of up to 55%, based on cellulose, have been reproducibly obtained with the use of a plug-flow reactor operated at a high solids-content and with dilute sulfuric acid as the catalyst[6,7]. Under the industrial hydrolysis conditions envisaged (0.7–1.2% sulfuric acid, 3:1 liquid-to-solid ratio, and 190–250°), D-glucose reacts to form numerous dehydration[8,9] and reversion products[10,11]. Although the dehydration reactions of D-glucose have been investigated with respect to the acid hydrolysis of cellulose[12], the reversion reactions of D-glucose have not received the same attention.

The acidic medium required for cellulose hydrolysis provides the conditions necessary for the random attack of available hydroxyl groups on hemiacetal carbon atoms, *i.e.*, reversion. Because there is a high concentration of D-glucose present during cellulose conversion processes, intra- and inter-molecular attack by available hydroxyl groups will both produce anhydro sugars and disaccharides. The hydrolytic conditions prevalent during cellulose hydrolysis severely limit oligosaccharide formation. The reversion products are non-fermentable, but they can be converted into D-glucose by a secondary hydrolysis, and thus are a potential source of additional D-glucose. However, for practical purposes they constitute loss of fermentable D-glucose during any dilute-acid hydrolysis sequence. The yields of reversion products, as well as the kinetics of the reversion reaction with a cellulose substrate, have not yet been reported. Therefore, it was of interest to determine the extent of reversion during cellulose hydrolysis as a function of reaction time, temperature, and acidity. A previously reported reduction–permethylation technique[13] was utilized to provide reaction-product samples which were amenable to quantitative analysis by gas–liquid chromatography. The results from our analysis of the reversion reactions are the subject of this report.

EXPERIMENTAL

Materials. — All solutions were prepared from de-aerated Milli-Q water (Millipore; Bedford, MA). Avicel, a microcrystalline cellulose, was used for all reactions and contained 95.4% total of D-glucose by h.p.l.c. wood-sugar analysis[14]. All compounds and reagents employed in this investigation were available from major chemical-supply houses, and were used without further purification.

Cellulose hydrolysis. — Tared reaction-tubes (3 mm, i.d.) were charged with Avicel (50–70 mg) and dried *in vacuo* overnight at 70°. The tubes were cooled in a desiccator and weighed to determine the amount of Avicel within the tube. The appropriate amount of dilute sulfuric acid (±5 μL) was added to bring about a 3:1 liquid-to-solid ratio. The tubes were then sealed under an argon atmosphere and the contents mixed to assure a homogeneous mixture. The hydrolysis reactions were performed by immersing these tubes in a molten-salt bath whose temperature was maintained to within ±0.05°. The reaction time was that which elapsed between immersion in the salt bath and subsequent quenching in an adjacent water-bath. The reaction tubes were then frozen (−70°) and subjected to the workup and analysis procedures. Three tubes were used for each hydrolysis reaction.

Product workup and analysis. — The reaction mixture was quantitatively removed from the reaction tubes and placed in a tared centrifuge tube. The solid and liquid reaction-products were then isolated through use of centrifugation. The entire liquid reaction-product was brought to a known volume (10 mL) from which an aliquot (2 mL) was taken for determination of the yield of D-glucose (Beckman D-Glucose Analyzer II, D-glucose oxidase procedure). The solid reaction-products were dried *in vacuo* overnight at 80°. The carbohydrate content of the residue was determined by the Somogyi modification of the Nelson procedure[15,16].

The remaining liquid reaction-product (8 mL) was made neutral with aq. NaOH, freeze-dried, and mixed with methanol. Filtration of the suspension eliminated most of the salts present, as well as furan-type polymers which would interfere with the gas–liquid chromatographic analysis. The methanol-soluble fraction was evaporated under a stream of nitrogen at 30°, desiccated overnight *in vacuo* over P_2O_5, and subjected to the reaction–permethylation procedure for determination of reversion yields.

Reduction–permethylation analysis. — The procedure of Helm *et al.*[13] was used without modification. Yields were determined on the basis of the D-glucose theoretically available.

RESULTS AND DISCUSSION

Reversion-product yields. — The yield of reversion products is a function of the reactivity of an individual hydroxyl group towards a hemiacetal carbon atom, as well as the stability of the resulting glycosidic bond. It has been amply documented[10,11] that the (1→6)-linked disaccharides isomaltose and gentiobiose are the main dimers formed from the reversion of D-glucose. The reversion yields determined for a typical cellulose hydrolysis reaction are shown in Fig. 1. The yield

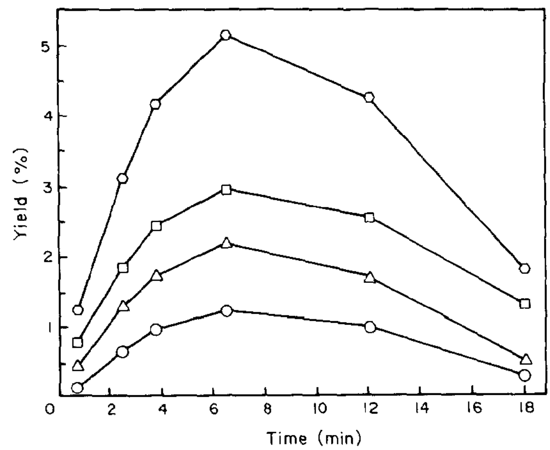

Fig. 1. Yields of reversion products [○, (1→6-linked disaccharides; △, total disaccharides; □, anhydro sugars; and ○, total reversion products] from the acid hydrolysis of Avicel. Reaction conditions: 200°, 0.77% sulfuric acid, 3:1 liquid-to-solid ratio. Yields are given as percentages of theoretically available D-glucose.

values are based on the moles of D-glucose theoretically available from the starting material (Avicel). The yields of reversion products tracked the yields of D-glucose during the cellulose hydrolysis reaction, *i.e.*, the yields went through a maximum. Yields of anhydro sugar were always higher than the total disaccharide yields, regardless of the temperature or acidity used. This is in good agreement with the results of Peat and co-workers[11], who treated 1% D-glucose solutions in 165mM sulfuric acid for up to 10 h at 100°. Both levoglucosan and 1,6-anhydro-β-D-glucofuranose were isolated[11] by these workers. Their yield of anhydro sugars constituted 70% of the total reversion material. Thompson *et al.*[10] treated 30% D-glucose solutions with 82mM HCl at 97°, and found that levoglucosan, the only anhydro sugar isolated, contributed only 3% to the total yield of reversion products. The difference in yields between these two investigations was attributed to the difference in original D-glucose concentrations[11].

The higher concentration of D-glucose in the study of Thompson and co-workers[10] was thought to favor intermolecular condensation-reactions. However, it was determined in our work that the intramolecular condensation reactions are favored, even though D-glucose concentrations are, at times, in excess of 15% (>0.8M). The yields of the disaccharides followed the expected trend, where the (1→6)-linked material preponderates. The (1→3)-linked disaccharides nigerose and laminarabiose are formed in slightly higher yields than the (1→2)-linked dimers kojibiose and sophorose.

For 0.26–1.27% sulfuric acid, the yield of reversion products appears to be independent of acidity, as shown in Fig. 2. This supports the experimental work of Minor[17], who concluded that the reversion reaction can be treated as an equilibrium

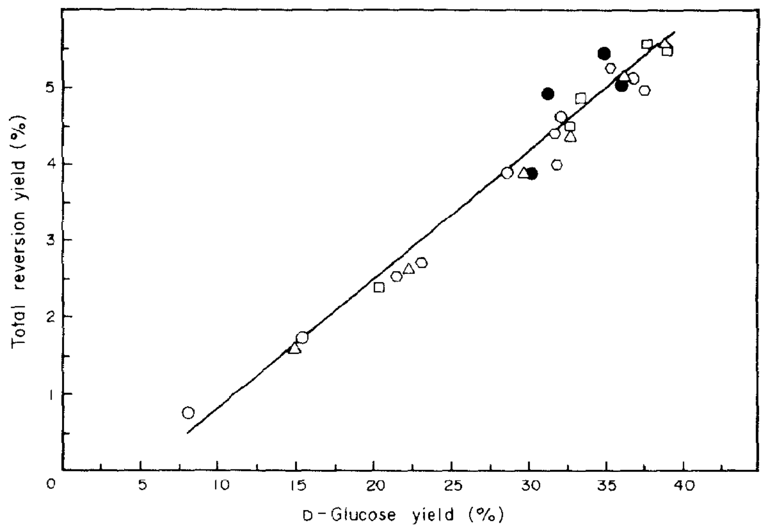

Fig. 2. Reversion product yield with respect to D-glucose yield at several sulfuric acid concentrations: ●, 0.26%; ⬡, 0.51%; □, 0.77%; △, 1.00%; and ○, 1.27%. Reaction conditions: 210°, liquid-to-solid ratio, 3:1.

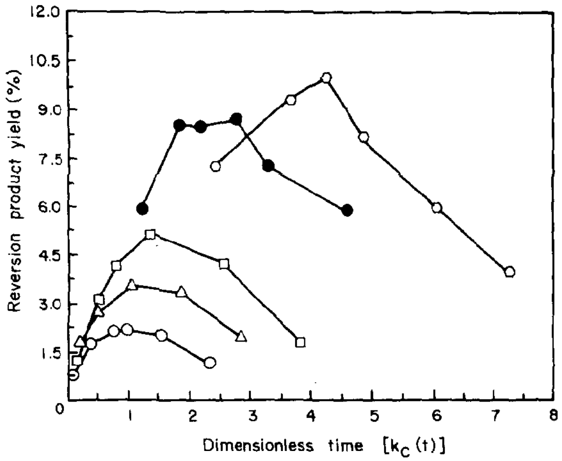

Fig. 3. Total yield of reversion products at several reaction temperatures: ○, 160°; △, 180°; □, 220°; ●, 240°; and ⬡, 250°. Hydrolysis conditions: 0.77% sulfuric acid, 3:1 liquid-to-solid ratio. Yields are given as percentages of theoretically available D-glucose.

reaction. Thermodynamic consistency requires that the yield of an equilibrating product be independent of changes in acidity (assuming that the acid is acting as a catalyst and not as a reactant); only the rates of the two opposing reactions will be affected. The concentrations of the reversion products will therefore depend only on the D-glucose concentration. Maximum yields of D-glucose were relatively independent of the acid concentration investigated in this work, and thus, the maximum yields of reversion products were approximately the same at all acid concentrations. The linear relationship shown in Fig. 2 corresponds to a regression coefficient of 0.984.

Whereas the yields of D-glucose were relatively independent of the acid concentrations employed in this work, the yields were very much dependent upon the reaction temperature. This is because the increase in the pseudo-first-order rate-constant for cellulose hydrolysis (D-glucose appearance) with increase in temperature is greater than the increase in the rate of D-glucose disappearance. Therefore, as the reaction temperature is increased[18], a higher maximum yield of D-glucose results, within a shorter period of time, and the yield of reversion product should increase with increase in the reaction temperature. The yield of reversion products as a function of reaction time and temperature is shown in Fig. 3. The effect of the increased concentration of D-glucose on the proportion of reversion material formed is quite evident. During cellulose hydrolysis at 160 and 250°, the maximum yields of D-glucose occur at 6 h and 14 s, respectively. In order to plot data for all reaction temperatures in one Figure, the reaction time for each hydrolysis reaction was multiplied by the pseudo-homogeneous, cellulose-hydrolysis first order rate-constant (k_c) determined at each reaction temperature to give units of dimension-

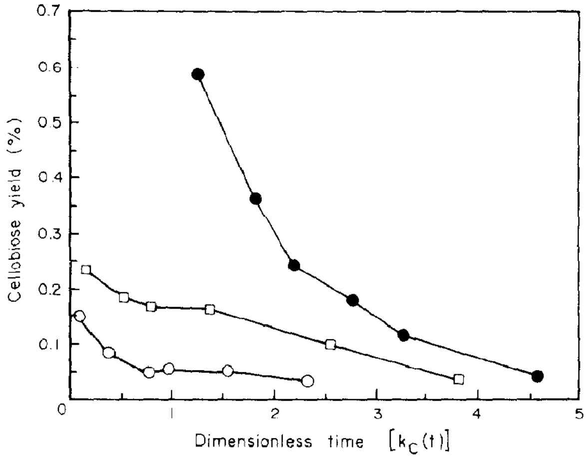

Fig. 4. The effect of reaction time and temperature (●, 240°; □, 180°; and ○, 160°) on cellobiose yield.

less time, k_c(t). The maximum yield of total reversion products determined in this work was 10% at 250°. In all cases, the anhydro sugars accounted for at least 50% of the total reversion yield.

Disaccharide-linkage ratios. — The yield of each individual disaccharide formed *via* reversion depends upon the relationship between hydroxyl reactivity, glycosidic-bond stability, and the resistance of D-glucose to decomposition in aqueous acidic solutions at elevated temperatures. For all disaccharide pairs except the (1→6)-linked dimers, the β linkages are the most stable under acid conditions[19]. It is therefore surprising that, among the disaccharides formed during the hydrolysis of cellulose with dilute sulfuric acid, the α-linked ones preponderate, except in the cellobiose–maltose pair. The yield of cellobiose was typically higher than that of maltose, especially during the early stages of cellulose hydrolysis, as cellobiose can arise from cellulose hydrolysis, as well as from reversion. The yield of cellobiose was nevertheless included in the total reversion yield as it satisfied the requirement of being non-fermentable but potential D-glucose. The yield of cellobiose for several reaction conditions is displayed in Fig. 4. The yield was highest early in the reaction, when there was still a considerable proportion of cellulose remaining. It is important, however, that the cellobiose yields are never greater than 1%. This result suggests that the yield of cello-oligosaccharides is quite low during the acid hydrolysis of cellulose.

For reactions that were performed at 210°, the ratios of configurational isomers were independent of the acid concentration, as shown in Table I. These results are consistent with those of Peat *et al.*[11], who reported higher yields of nigerose and isomaltose than of the corresponding β-disaccharides (laminarabiose and gentiobiose, respectively). Thompson and coworkers[10] reported a higher yield of maltose than of cellobiose from the acid treatment of a 30% D-glucose solution.

TABLE I

PAIRWISE RATIOS OF VARIOUS REVERSION PRODUCTS COMPARED WITH THE RATIOS OF THEIR ACID STABILITIES

Products compared	*Ratio*[a] *±s.d.*	*Preponderant product (%)*	*Ratio of acid stabilities*[b]
Levoglucosan and 1,6-anhydro-β-D-glucofuranose	2.49 ±0.10	71 ±1	
Kojibiose (α) and sophorose (β) [(1→2)-linked]	1.38 ±0.13	58 ±5	0.58
Nigerose (α) and laminarabiose (β) [(1→3)-linked]	1.22 ±0.10	55 ±4	0.65
Isomaltose (α) and gentiobiose (β) [(1→6)-linked]	1.50 ±0.07	60 ±3	1.44

[a]Ratios determined at 210° from the cellulose hydrolyzates resulting from treatment with 0.26–1.27 wt. % sulfuric acid. Standard deviation based on 27 different reactions. [b]From ref. 19. Product stability ratio is defined as $(1/k_\alpha)/(1/k_\beta)$ for hydrolysis at 99.5° in 0.1M HCl. If $k_\alpha < k_\beta$, the stability ratio is >1.

The inverse of the relative hydrolysis rates for the individual disaccharide pairs determined[19] at 99.5° in 0.1M HCl are shown in Table I for comparative purposes. It would be expected that, if the yield of a particular disaccharide pair was dependent to a great extent on the acid stability of the glycosidic bond, the product ratio would be similar to the ratio predicted from the acid stability data. Assuming that the relative hydrolysis rates do not change significantly with temperature, the β-disaccharides would be present in higher yields (with the exception of isomaltose and gentiobiose). The "acid hydrolysis ratios" match the determined product-ratios only for the (1→6)-linked disaccharides, those of the other disaccharide pairs being considerably different.

Mora *et al.*[20,21] and Bishop[22] reported that the optical rotations of water-soluble polymers produced by acid reversion under anhydrous conditions are higher than the equilibrium values for D-glucose and D-xylose, respectively. The production of polymers having a high optical rotation indicates a propensity for the formation of α linkages. Mild hydrolysis of the D-glucose reversion-polymers with acid returned the equilibrium optical rotation of the solution to the value for D-glucose[21]. These results, together with those for the formation of disaccharides reported herein, indicate that, during acid-catalyzed reversion, the formation of an α linkage is favored over that of a β linkage. The exact mechanistic reasons why the one linkage is favored over the other is still open to conjecture.

Anhydro sugar product ratios. — Upon being heated with acids, hexoses will form equilibrium mixtures that contain one, or several, anhydrides[23]. The equilibrium anhydride concentration will be dependent upon the anhydro sugar stability under acidic conditions, as well as on the conformational properties of the free hexose in solution. Under the conditions of the acid hydrolysis of cellulose (*i.e.*, high temperature and acid catalysis), the rate of mutarotation will increase to the point that, for the purposes of this work, D-glucose in solution can probably be considered to be at instantaneous, tautomeric equilibrium[24].

Because the formation of the furanose forms of D-glucose is an endothermic reaction, increase in the temperature will increase the equilibrium concentration of the furanoses to some degree, as was observed for *aldehydo*-D-glucose by Hayward and Angyal[25]. However, as the effect of temperature on the equilibrium optical rotation is slight[26], and the dynamic concentration of the furanose forms of D-glucose in aqueous solutions is[27] $<0.2\%$ at 43°, the increase in concentration is probably minimal.

This reasoning suggests that a small proportion of D-glucose will be in the furanose form, which is required in order to effect formation of the anhydrofuranose. The concentration of the pyranose forms is almost 100 times that of the furanose forms, and yet levoglucosan and the anhydrofuranose were formed in the ratio of 2.5:1 (see Table I). As the pyranose–furanose transition occurs very rapidly, it may be conjectured that depletion of the furanose pool by additional reactions (*i.e.*, acid-catalyzed reversion) should be augmented by the rapid formation of more furanose material to maintain the equilibrium. If the formation of the anhydrofuranose is rapid as well, an overall equilibrium will be established that can be expressed in terms of the concentrations of D-glucose and anhydrofuranose[23].

The acid stability of the anhydrofuranose has been reported to be relatively high, considering the strained structure. Dimler *et al.*[28] found that a 2% solution of this material remained unchanged after treatment with 0.2M HCl for 24 h at 25°. These conditions would have hydrolyzed at least 50% of a methyl D-glucofuranoside solution. Thus, rapid pyranose–furanose transition and bond formation, along with the stability[29] of the subsequent anhydrofuranose form may account for the unexpectedly high yield.

Thus, it is tentatively proposed that formation of the anhydro sugars during the hydrolysis of cellulose with dilute sulfuric acid can be depicted as shown in Eqs. *1–3*, where the overall equilibrium constants for each reaction can be described by the concentrations of D-glucose and the individual anhydro sugars in solution. The equilibrium constant for total anhydro sugars is the sum of the individual equilibrium constants.

$$\text{Levoglucosan} \overset{K(Lg)}{\leftrightarrows} \begin{array}{c}\text{D-glucose at}\\ \text{tautomeric}\\ \text{equilibrium}\end{array} \overset{K(AGf)}{\rightleftarrows} \text{anhydroglucofuranose,}$$

where

$$K(Lg) = [\text{levoglucosan}]/[\text{D-glucose}] \tag{1}$$

$$K(AGf) = [\text{anhydrofuranose}]/[\text{D-glucose}] \tag{2}$$

$$K(\text{total anhydro sugars}) = K(Lg) + K(AGf) \tag{3}$$

Reversion kinetics. — In the foregoing discussion, it was suggested that the reversion reactions of D-glucose under cellulose-hydrolysis conditions can be considered as an equilibrating system. The Gibbs–Helmholtz equation relates the equilbrium constant (K) for a reversible reaction to the standard heat of reaction (ΔH°) as shown in Eq. *4*. Integration and substitution of an overall constant, C, results in an Arrhenius-type expression (Eq. *5*), where K is the ratio of the concentration of product (reversion material) to that of reactant (D-glucose).

$$d(\ln K)/dT = \Delta H^\circ/(RT^2) \tag{4}$$

$$K = C[\exp(-\Delta H^\circ/RT)] \tag{5}$$

The data obtained for the determination of the overall heat of reaction for anhydro sugar formation is shown in Fig. 5 (line B). The equilibrium constant increases with an increase in temperature, as would be expected for an endothermic reaction. The slope of the line corresponds to a standard heat of reaction of 24 $kJ.mol^{-1}$ (5730 cal/mole) (correlation coefficient, $r = 0.991$).

A true disaccharide equilibrium constant should follow the relationship $K = [\text{Disaccharide}]/[\text{D-Glucose}]^2$, but this was not the case. The total reversion constants displayed in Fig. 5 (line A) are based on the relationship: K = [Reversion Product]/[D-Glucose]. Thus, the expression for total reversion products is of empirical value only. The empirical, standard heat of reaction based on the slope of line A was 18.11 $kJ.mol^{-1}$ (4325 cal/mole; $r = 0.974$). The reason for the lack of correlation of the disaccharide yields with the square of the concentration of D-glucose is not known for certain. The disaccharides are thought to undergo dehydration reactions, due to the availability of a reducing end-group. A fraction of the newly formed disaccharides may enter into standard dehydration-reaction

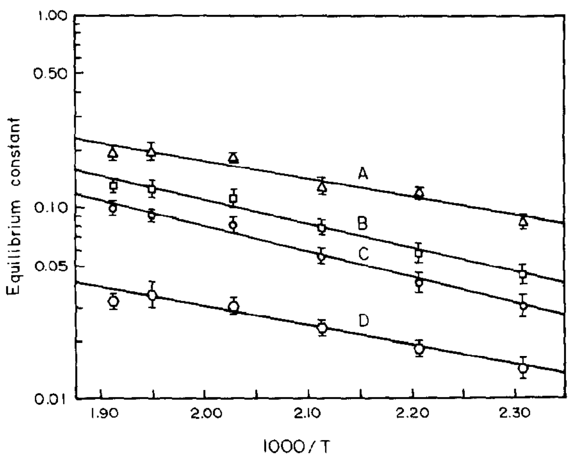

Fig. 5. The temperature-dependence of the reversion equilibrium constants: A, total reversion products (empirical relationship); B, total anhydro sugar; C, levoglucosan; and D, 1,6-anhydro-D-glucofuranose. Absolute temperature (T) in kelvins.

pathways, although no furanoid glycosides could be detected by g.l.c.–m.s. Disaccharide dehydration may prevent an equilibrium between D-glucose and disaccharides from being established. It is also possible that the summation of all of the yields of the individual disaccharides increased the experimental error to the point that a relationship was masked. However, the use of individual disaccharide-yield data in the theoretical disaccharide equilibrium expression was unsuccessful as well. The best available equations representing the thermodynamics of the reversion reactions are presented in Eqs. *6* and *7* for T in K, and R in cal/mole.K.

$$K(\text{Anhydro sugars}) = [\text{Anhydro sugar}]/[\text{D-Glucose}]$$
$$K(\text{Anhydro sugars}) = 35.0 \exp(-5730/RT) \tag{6}$$

$$K(\text{Total reversion}) = [\text{Total reversion products}]/[\text{D-Glucose}]$$
$$K(\text{Total reversion}) = 13.6 \exp(-4325/RT) \tag{7}$$

Anhydro sugar thermodynamics. — The coupling of the two anhydro sugars into one overall equilibrium expression (Eq. *6*) is a simplification of the actual reaction pathway. As was seen in Scheme 1, it was proposed that the levoglucosan and the anhydrofuranose arise from D-glucose at tautomeric equilibrium. There are, therefore, two equilibrium expressions that were combined in generating Eq. *6*. It is of interest to decompose the overall expression into its component parts, and solve for the thermodynamic properties of each system. Because it is assumed that the pool of intermediates which generates the individual anhydro sugars is constant, due to the fast mutarotation, an equilibrium expression can be generated for each pathway by determining the concentrations of D-glucose and the particular D-glucose anhydride.

The equilibrium constants determined for each anhydro sugar are shown in Fig. 5 (lines C and D). The expressions containing the heats of reaction for each equilibrium are as follows.

$$K(\text{Levoglucosan}) = 35 \exp(-6170/RT) \qquad r = 0.993 \tag{8}$$

$$K(\text{Anhydrofuranose}) = 3.2 \exp(-4630/RT) \qquad r = 0.980 \tag{9}$$

The reaction for the formation of levoglucosan is slightly more endothermic than that for the anhydrofuranose.

Numerical values for the thermodynamic parameters of the anhydro sugar equilibria are given in Table II. The Gibbs free-energy changes for each reaction are quite similar, but anhydrofuranose formation is slightly the higher. The higher heat of reaction for the formation of levoglucosan is compensated by the higher entropy of reaction. The $\Delta G°$ values calculated in this work for the formation of levoglucosan from D-glucose is significantly less than that calculated by Angyal and Dawes[23] (25.9 $kJ.mol^{-1}$) for the reaction of D-glucose at 100° in 0.25M H_2SO_4.

TABLE II

THERMODYNAMICS OF EQUILIBRIUM FOR THE D-GLUCOSE–ANHYDRO SUGAR[a] TRANSFORMATION

Temperature (°C)	*ΔG° (kJ/mole)*		*ΔS° (J/mole.K)*	
	LG	*AGf*	*LG*	*AGf*
160	13.0	15.3	29.7	9.2
180	12.1	15.1	30.1	9.2
200	11.4	14.7	30.5	9.6
220	10.3	14.2	31.4	10.5
240	10.2	14.3	30.5	10.0
250	10.0	14.9	29.7	8.4

[a]LG, levoglucosan (ΔH° = 25.8 kJ/mole); AG*f*, 1,6-anhydro-β-D-glucofuranose (ΔH° = 19.4 kJ/mole).

Comparison of kinetic equations with those in the literature. — Peat *et al.*[11] studied the reversion reaction of 1% D-glucose solutions in 165mM sulfuric acid at ~100°. The yields of reversion products were observed to increase over the length of time investigated. Pretreatment of a 6% D-glucose solution with 90% formic acid for ~40 min at 98°, followed by treatment with 165mM sulfuric acid of a 1% D-glucose solution for 1.5 h gave higher reversion yields. If it is assumed that this yield represents the equilibrium concentration; the overall equilibrium constant found for anhydro sugars was 0.014. The insertion into Eq. *6* of 373K, determined in this work for the anhydro-sugar equilibrium constant, results in a value of 0.016.

Ough and Rohwer[30] investigated the levoglucosan yield present during the hydrolysis of commercial starch over broad ranges of concentration and acidity, and found a relatively constant yield at maximum D-glucose yield (K = 0.026). Because all reactions were performed at one temperature (147°), the equations determined in this work can again be tested. On the assumption that the value reported by Ough and Rohwer[30] represented both levoglucosan and the anhydrofuranose, Eq. *6* predicts an equilibrium constant of 0.037. If only levoglucosan was detected, and Eq. *8* is used, a value of 0.024 is obtained.

CONCLUSIONS

The reversion reaction, as it occurs during the high-temperature hydrolysis of Avicel with dilute sulfuric acid, has been studied by use of quantitative gas–liquid chromatography. The overall yield of the anhydro sugars was higher than that of disaccharides and the total reversion yield approached 10% at 50% D-glucose yields. The α-linked position-isomeric disaccharides preponderated over their β-linked counterparts.

The reaction system was, with reasonable success, modeled by equilibrium expressions relating the reversion yield to the D-glucose concentration. Disaccharides failed to fit the theoretical relationship of [Disaccharide]/[D-Glucose]^2, and this was presumed to be due to disaccharide dehydration-reactions. The formation of anhydro sugars was characterized as two separate equilibrium-reactions, and the thermodynamic parameters of each were ascertained. Levoglucosan and 1,6-anhydro-β-D-glucofuranose were formed in the ratio of 2.5:1, which was independent of reaction acidity. The relatively high yield of the anhydrofuranose was attributed to a combination of the high rates of mutarotation and glycosidic-bond formation, as well as to its stability under the conditions studied.

ACKNOWLEDGMENTS

We thank Dr. James L. Minor for his cooperation during the performance of this work, and Marilyn Effland for determination of the remaining cellulose. R.F.H. was supported by the USDA Special Grants Program, the College of Agricultural and Life Sciences, and the Graduate School, University of Wisconsin-Madison.

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