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RATE OF HYDROLYSIS OF XYLO-OLIGOSACCHARIDES IN DILUTE SULFURIC ACID

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ABSTRACT

The kinetics of hydrolysis in dilute sulfuric acid of xylo-oligosaccharides ranging between the di- and penta-oligosaccharides has been studied. One of the two terminal bonds and each internal bond of all xylo-oligosaccharides tested were hydrolyzed at the same rate. The hydrolytic rate of the other terminal bond was the same as that of xylobiose, which was 1.8 times greater than that of an internal bond. The rates of hydrolysis of xylo-oligosaccharides have been described as functions of the reaction temperature and concentration of sulfuric acid. It has been shown that the yield of xylose in hydrolysis of xylo-oligosaccharides by sulfuric acid may be calculated from the ratio (=1.8) of the rate for xylobiose to that of an internal bond and the empirical equation that describes the rate-constant for xylobiose.

INTRODUCTION

We have been working on problems of production of xylose from woods and agricultural residues by acid hydrolysis[1]. When xylan in these materials is subjected to acid hydrolysis under conditions so mild that no part of the cellulose is attacked, xylan dissolves to give oligosaccharides. The reaction conditions favorable for hydrolysis of xylan cause only incomplete hydrolysis of the soluble xylo-oligosaccharides, and a secondary hydrolysis is necessary to achieve an increase in the yield of xylose. To find optimal conditions for this secondary hydrolysis, information on the reaction rates is necessary. Although it is known that the bond at the nonreducing end-group cleaves faster than the other bonds in hydrolysis of cello-[2,3] and malto-oligosaccharides[2,4], the rate of hydrolysis of xylo-oligosaccharides is unknown and there is still no direct experimental observation on the hydrolysis rate of internal bonds. This paper describes the rates of hydrolysis, in dilute sulfuric acid, of xylo-oligosaccharides ranging between di- and penta-oligosaccharides.

MATERIALS AND METHODS

Materials. — Crystalline xylobiose, xylotriose, xylotetraose, and xylopentaose were prepared as described in previous papers[5,6].

Hydrolysis. — A solution (1 mL) of 10 g of sugar per L in 0.025 to 0.5M sulfuric acid was put into a small glass tube. The tube was sealed in a flame and kept in a glycerol bath at 80–120° for a predetermined period of time. Measurement of the reaction time was started after 80 sec had elapsed from the time when the tube was put into the bath; preliminary experiments had shown that 80 sec was required to reach the desired temperature of the contents in the tube. After the appropriate time-interval, the tube was rapidly cooled and the contents were neutralized and analyzed.

Decomposition of the xylose produced is so slow that it may be ignored under the conditions used in this work[7].

Analysis. — Xylobiose, xylotriose, and xylotetraose were determined by g.l.c.[5] and xylopentaose by paper partition chromatography (p.p.c.). The g.l.c. system used was not effective for the determination of xylopentaose.[5] In p.p.c., the sugar extracted from the spot that migrated with 6:4:3 butanol–pyridine–water solvent was determined by the orcinol–hydrochloric acid method[8]. Hydrolysis of xylobiose was monitored by the hypoiodite method, as modified by Hatanaka[9]. As shown in Fig. 1, the same results for hydrolyses of xylobiose and xylotetraose were obtained by three separate methods.

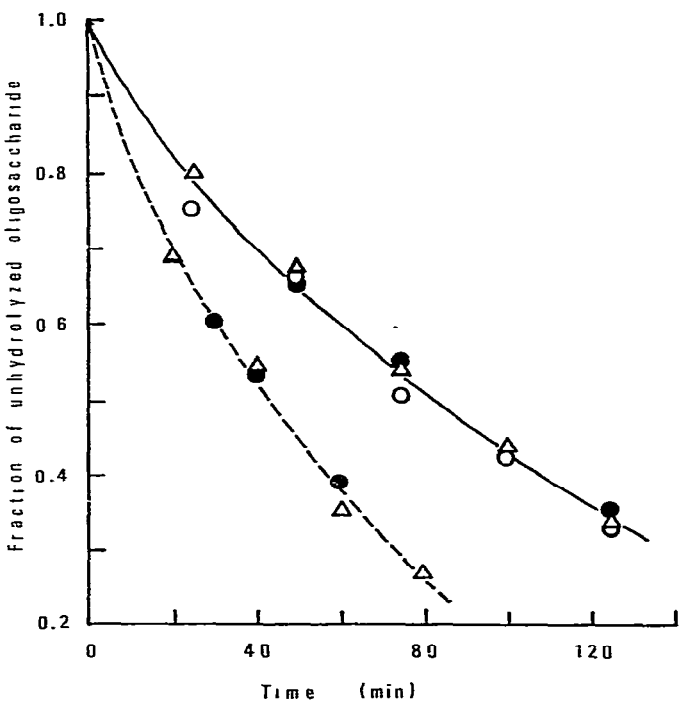

Fig. 1. Hydrolysis of xylo-oligosaccharides in 0.025M H_2SO_4 at 100°, monitored by three separate methods. ○, hypoiodite method; △, p.p.c.; ●, g l c.; ——, xylobiose; - - -, xylotetraose.

Notation of hydrolytic rate-constants. — Hydrolytic rate-constants are defined as follows:

$$X\overset{k_5^{(1)}}{-\!-}X\overset{k_5^{(2)}}{-\!-}X\overset{k_5^{(3)}}{-\!-}X\overset{k_5^{(4)}}{-\!-}X^*,$$

$$X\overset{k_4^{(1)}}{-\!-}X\overset{k_4^{(2)}}{-\!-}X\overset{k_4^{(3)}}{-\!-}X^*,\quad X\overset{k_3^{(1)}}{-\!-}X\overset{k_3^{(2)}}{-\!-}X^*,\text{ and }X\overset{k_2}{-\!-}X^*,$$

where X—X—X—X—X* and so on, are, in turn, xylopentaose, xylotetraose, xylotriose, and xylobiose; X* is the reducing end-group, and $k_5^{(1)}$ and so on, are the first-order reaction rate constants for hydrolysis of the different glycosidic bonds in the sugars. Furthermore, $k_5 = k_5^{(1)} + k_5^{(2)} + k_5^{(3)} + k_5^{(4)}$, $k_4 = k_4^{(1)} + k_4^{(2)} + k_4^{(3)}$, and $k_3 = k_3^{(1)} + k_3^{(2)}$, and when the differences between internal bonds are ignored, $k_{int} = k_5^{(2)} = k_5^{(3)} = k_4^{(2)}$.

RESULTS AND DISCUSSION

If we suppose that the hydrolysis of xylopentaose, xylotetraose, xylotriose, and xylobiose operates as a system of consecutive and parallel, first-order, irreversible reactions and proceed along the paths represented in Fig. 2, then k_5, k_4, k_3, k_2, $k_4^{(2)}$, and $(k_5^{(2)} + k_5^{(3)})$ may be described by the following equations:

$$X_n = X_{n0}\exp(-k_n t) \text{ (where } n = 5, 4, 3, \text{ or } 2) \quad (1),$$

$$X_3 = X_{40}[-(k_4 - k_4^{(2)})/(k_4 - k_3)][\exp(-k_4 t) - \exp(-k_3 t)] \quad (2),$$

$$X_2 = X_{40}[-A\exp(-k_4 t) - B\exp(-k_3 t) + (A + B)\exp(-k_2 t)] \quad (3)$$

{where $A = [(2k_4 - k_3)k_4^{(2)} - k_3 k_4]/[(k_4 - k_3)(k_4 - k_2)]$
and $B = (k_3 k_4 - k_3 k_4^{(2)})/[k_4 - k_3)(k_3 - k_2)]$}, and

$$X_4 = X_{50}[(k_5^{(2)} + k_5^{(3)} - k_5)/(k_5 - k_4)][\exp(-k_5 t) - \exp(-k_4 t)] \quad (4),$$

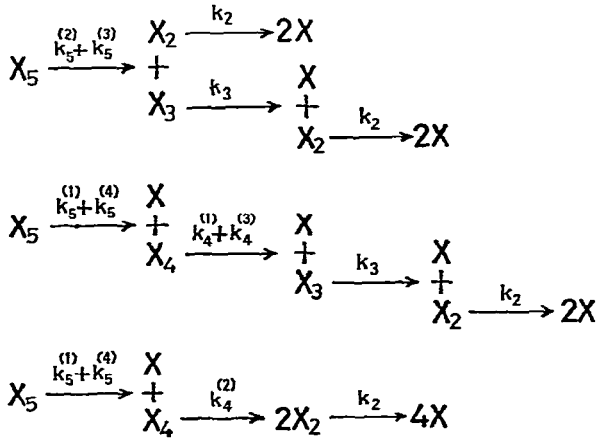

Fig. 2. Hydrolysis paths for xylopentaose. X_5, xylopentaose; X_4, xylotetraose; X_3, xylotriose; X_2, xylobiose; X, xylose.

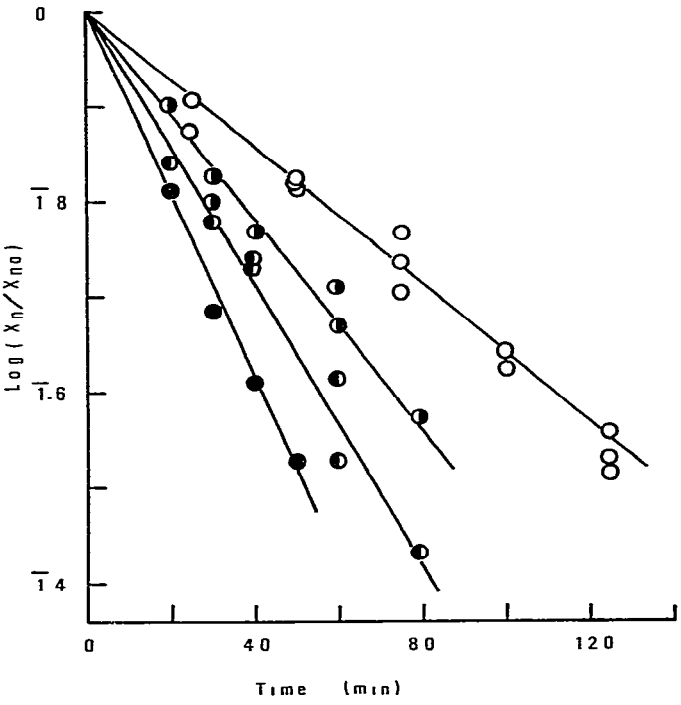

Fig. 3. Rates of hydrolysis of xylo-oligosaccharides in 0 025M H_2SO_4 at 100°. ●, xylopentaose; ◐, xylotetraose; ◑, xylotriose; ○, xylobiose, X_n. conc. of unhydrolyzed sugar after hydrolysis; X_{no}, initial conc. of the sugar.

where X_5 and so on, are concentrations (mol/L) of (for instance) xylopentaose after a period of time, t (min), and X_{50}, and so on, their initial concentrations (mol/L)

Determination of rate constants. — After an oligosaccharide (1 g/100 mL) had been hydrolyzed in 0.025, 0.05 and 0.25M sulfuric acid at 80 and 100° for different periods of time, the oligosaccharide still remaining was determined. When the logarithm of the ratio of concentration of sugar remaining unchanged after hydrolysis (X_5, X_4, X_3, or X_2) to the initial sugar concentration (X_{50}, X_{40}, X_{30}, or X_{20}) was plotted against time, a straight line was obtained for each set of reaction conditions. For example, Fig. 3 shows the results in 0.025M sulfuric acid at 100°. This result shows that hydrolysis of xylo-oligosaccharides proceeds as a first-order reaction. The reaction rate-constants calculated from the data by use of Eq. *1* are listed in Table I.

After xylopentaose and xylotetraose had been separately hydrolyzed in 0.025, 0.05, and 0 25M sulfuric acid at 80 and 100° for different periods of time, the xylotetraose (X_4, mol/L) produced from xylopentaose (X_{50}, mol/L) was determined, as well as xylotriose (X_3, mol/L) and xylobiose (X_2, mol/L) from xylotetraose (X_{40}, mol/L). By use of Eqs. *2*, *3*, and *4*, values of ($k_5^{(2)} + k_5^{(3)}$) and $k_4^{(2)}$ were obtained from X_4/X_{50}, X_3/X_{40}, and X_2/X_{40} at different times, together with the constants k_5, k_4, k_3, and k_2. Under each set of hydrolytic conditions, the magnitudes of each $k_4^{(2)}$ value

TABLE I

THE FIRST-ORDER REACTION RATE-CONSTANTS FOR HYDROLYSIS OF XYLO-OLIGOSACCHARIDES IN 0 025 TO 0 250M SULFURIC ACID AT 80 TO 100°

Rate constant	*80°*	*100°*		
	0.25M H_2SO_4 (min^{-1})	*0 25M H_2SO_4 (min^{-1})*	*0 05M H_2SO_4 (min^{-1})*	*0.025M H_2SO_4 (min^{-1})*
$k_4^{(2)}$ (from Eq. *2*)	0.0043	0 060	0 013	0 0046
$k_4^{(2)}$ (from Eq. *3*)	0 0046	0 065	0 0097	0 0041
$(k_5^{(2)} + k_5^{(3)})/2$	0 0050	0 069	0 0099	0.0046
Average (k_{int})	0 0046	0 065	0 011	0 0044
	(0 0051)[a]	(0 069)	(0 011)	(0 0047)
k_2	0 0080	0.12	0 019	0 0084
	(0 0091)	(0 12)	(0 019)	(0 0085)
k_3	0 012	0 19	0 029	0 013
	(0 014)	(0.19)	(0.030)	(0 013)
k_1	0 017	0 23	0 039	0 017
	(0 017)	(0.26)	(0 040)	(0 018)
k_5	0 021	0 30	0 050	0 022
	(0 022)	(0 33)	(0 050)	(0 023)
k_2/k_{int}	1.74	1 85	1.73	1.91

[a]Calculated values based on Eq. *5* are given in parentheses

from Eqs. *2* and *3* at different times were nearly equal each to one another. The average gave the two $k_4^{(2)}$ values in Table I. This result shows that an internal bond in xylotetraose is hydrolyzed according to the first-order reaction already described. It was correct to assume the same relation for $(k_5^{(2)} + k_5^{(3)})$. As the $(k_5^{(2)} + k_5^{(3)})/2$ and $k_4^{(2)}$ values determined under every set of hydrolysis conditions are nearly equal, the average of these values gives the rate constant for hydrolysis of internal bonds, k_{int}. The values of k_{int} under various hydrolytic conditions are shown in Table I. Fig. 4 shows that the curves drawn from k_{int} and other rate constants in Table I by use of Eqs. *2*, *3*, and *4* fit in with the observed values of X_4/X_{50}, X_3/X_{40}, and X_2/X_{40}.

Under every set of hydrolytic conditions, the values of $(k_5 - k_2)/3$, $(k_4 - k_2)/2$, and $(k_3 - k_2)$ (where 3, 2, and 1 describe the number of internal bonds plus one), agreed with the value of k_{int}. The average of the k_2/k_{int} values obtained under different hydrolytic conditions is nearly equal to 1.8. The results show that one of the two terminal bonds and each internal bond of a xylo-oligosaccharide are hydrolyzed at the same rate (rate constant = k_{int}) and the hydrolytic rate of the other terminal bond is the same as that given by xylobiose (rate constant = k_2), which is 1.8 times larger than that of an internal bond. This observation is in line with data on the hydrolytic rates of cellulose, starch, and D-glucose-containing oligosaccharides[2-4]. The present result does not reveal directly which of the two terminal bonds

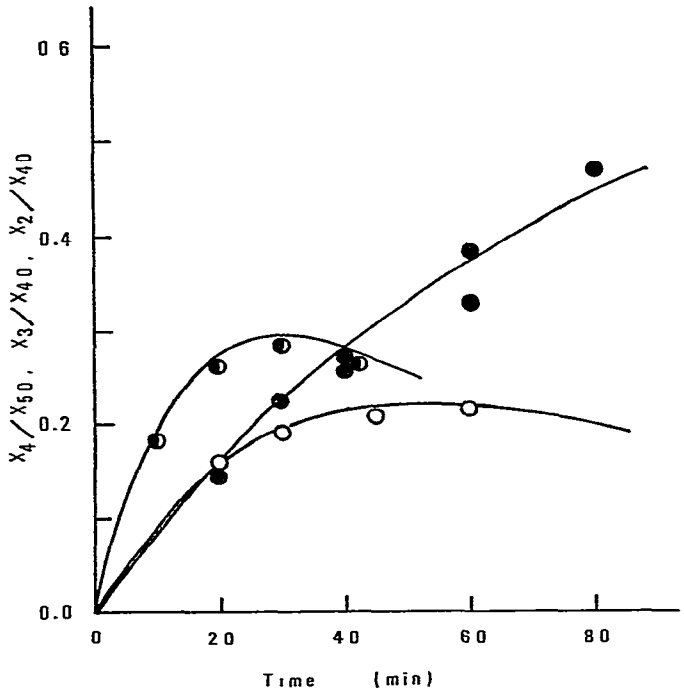

Fig. 4. Degradation of xylo-oligosaccharides to lower oligosaccharides. ○, xylotetraose (X_4) from xylopentaose (X_{50}) in 0.25M H_2SO_4 at 80°; ◐, xylotriose (X_3) from xylotetraose (X_{40}) in 0.05M H_2SO_4 at 100°; ●, xylobiose (X_2) from xylotetraose (X_{40}) in 0.025M H_2SO_4 at 100°. ——, calculated from rate constants in Table I by use of Eqs. *2*, *3*, and *4*.

splits faster than the other, but it fits our purpose. The fact shows that the hydrolytic rate-constant for any xylo-oligosaccharide may be derived from that of xylobiose.

Rate constants as a function of temperature and acid concentration. — Rate constants of hydrolysis of xylobiose were determined in sulfuric acid of different concentrations at various temperatures. When the logarithm of the rate constant was plotted against the reciprocal of the absolute temperature, parallel straight lines were obtained at successive intervals, as shown in Fig. 5. The activation energy of the reaction, 34,000 cal.mol^{-1}, was obtained from the gradient of the straight lines. The constant that describes the effect of acid concentration, 1.16, was obtained from the distances between the straight lines. The following experimental equation, which describes the hydrolytic rate-constant, was obtained from these constants and $k_2/k_{int} = 1.8$:

$$k_n = (1.27 \cdot n - 0.24) \cdot 10^{19} \cdot C^{1.16} \cdot \exp(-34000/(RT)) \qquad (5),$$

where n is the degree of polymerization of the xylooligosaccharide, C the concentration of sulfuric acid described by normality, N ($[H^+]$), T the absolute temperature, K, and R the gas constant, 1.987 cal.deg^{-1}.mol^{-1}. The values of k_n calculated from Eq. *5* agreed with the observed values shown in Table I, where the calculated values are indicated in parentheses.

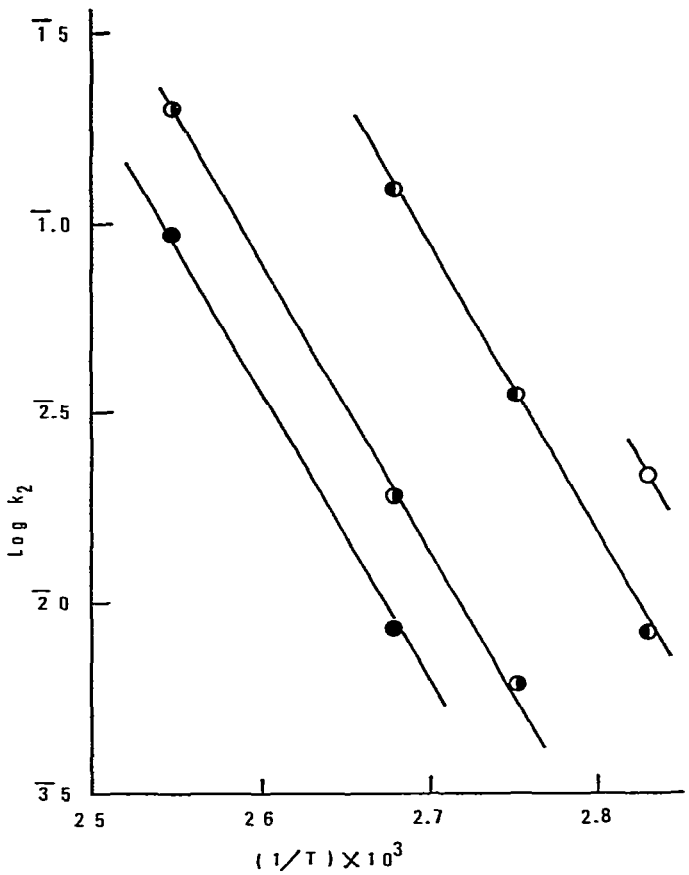

Fig. 5. Hydrolytic rate-constants for xylobiose as a function of temperature ○, 0 5M H_2SO_4; ◐, 0.25M H_2SO_4; ◑, 0.05M H_2SO_4; ●, 0.025M H_2SO_4 ——, calculated from Eq. *5*.

Yield of xylose from xylo-oligosaccharides. — Calculation now gives details of the release of xylose with time during the hydrolysis of xylo-oligosaccharides. Examples in Fig. 6 show that there is a good agreement between the observations and calculations based on Eqs. *5–8*, which express the yield (X) of xylose as a fraction of initial potential xylose according to Fig. 2:

X from xylopentaose,

$$X = 1 + 0.098\exp[(-4.8/1.8)k_2t] - 0.806\exp[(-3.8/1.8)k_2t] + 2.56\exp[(-2.8/1.8)k_2t] - 2.85\exp(-k_2t) \quad (6);$$

X from xylotetraose,

$$X = 1 - 0.360\exp[(-3.8/1.8)k_2t] + 1.82\exp[(-2.8/1.8)k_2t] - 2.46\exp(-k_2t) \quad (7);$$

and X from xylotriose,

$$X = 1 + 0.867\exp[(-2.8/1.8)k_2t] - 1.87\exp(-k_2t) \quad (8).$$

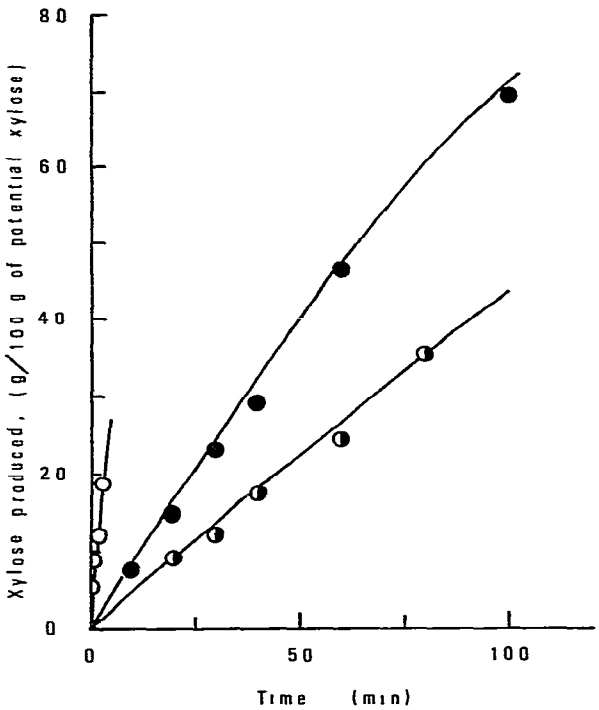

Fig. 6. The cleavage of xylo-oligosaccharides into xylose in dilute sulfuric acid at 100°. ◑, xylotriose, 0.025M H_2SO_4; ●, xylotetraose, 0.05M H_2SO_4; ○, xylopentaose, 0 25M H_2SO_4. ——, calculated from Eqs. *5–8*.

Equations *5–8* may be used to determine the reaction times required for complete hydrolysis of xylopentaose, and its homologs, in sulfuric acid of different concentrations at various temperatures.

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