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ACID HYDROLYSIS OF (1→4)-α-D-GLUCANS

PART II. ANALYSIS OF PRODUCTS FROM RADIOACTIVE OLIGOSACCHARIDES*,**

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ABSTRACT

The rate constants for hydrolysis of the individual glycosidic bonds of maltotriose and maltohexaose have been determined by using radioactive compounds. The results are consistent with data from non-radioactive experiments. The hydrolysis can be described by using two rate constants. The rate constant for the bond at the non-reducing end of the chain is 1.8 times the value for the other bonds. Energies and entropies of activation for scission of these bonds have been calculated and compared with those of the α- and β-Schardinger dextrins. It is speculated that different mechanisms of hydrolysis may exist for the linear and cyclic polymers.

INTRODUCTION

Previous investigations on the acid hydrolysis of (1→4)-α-D-glucans have usually been limited to dealing with polydispersed systems from which only average rate-constants could be obtained. Even for pure, unlabeled oligosaccharides containing two or more glycosidic bonds, it is in principle impossible to measure the rate constants in such a way as to distinguish between bonds closer to the reducing end or closer to the non-reducing end. However, in the present study we have been able to determine the rate constant for each glycosidic bond by using pure maltotriose and maltohexaose radioactively labeled in the reducing D-glucose residue.

MATERIALS

Radioactive oligosaccharides. — Maltotriose and maltohexaose labeled with ^{14}C exclusively in the D-glucose residue at the reducing end were donated by Dr. M. Abdullah. They were prepared by the method of French *et al.*[1].

Non-radioactive oligosaccharides. — Maltotriose was donated by Dr. M. Abdullah. Maltohexaose was isolated from a hydrolyzate of β-Schardinger dextrin by paper chromatography. Dr. J. Robyt donated the Schardinger dextrins.

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METHODS

Ascending paper chromatography. — Whatman No. 3-MM chromatography paper was cut into sheets 13″ high × 14″ wide. The samples were spotted on the paper and the chromatograms were irrigated 3 times with either hot 70% 1-propanol or hot 3:2:2 1-butanol–pyridine–water[2].

Radioautography. — Radioactive chromatograms were taped to Kodak, No Screen X-ray film and exposed for about 6 days. The film was developed and the chromatogram was aligned with it to locate the radioactive areas.

Scintillation counting. — Radioactive spots and blanks were cut out of the chromatograms and counted in a Packard Tricarb Liquid Scintillation Spectrometer[3].

Gas-flow counting. — Aliquots of the initial digest were pipetted onto copper planchets and evaporated to dryness. Samples and standards were counted in a gas-flow counter and the values were used to calculate specific activity and radioautogram exposure-time.

Total carbohydrate determination. — Concentration of carbohydrates was determined by the phenol–sulfuric acid method[4].

EXPERIMENTAL

Radioactive carbohydrate (10 μl) was added to 5 mg of carrier carbohydrate in 0.8 ml of water, and 0.2 ml of 25 mM sulfuric acid was added. An aliquot of 100 μl was diluted to 10 ml for analysis of total carbohydrate and radioactivity. Samples of approximately 75 μl were pipetted into each of 12 capillary tubes, which were then sealed. The capillaries were fastened to glass rods by pieces of rubber tubing, with two capillaries per rod. The capillaries were placed in a water bath at 75, 85, or 95° for hydrolysis. At desired times two capillaries were removed and placed in ice–water to quench the hydrolysis. They were then labeled and stored at 4° until all of the samples had been taken. The capillary seals were then broken and the digests were neutralized with a few μl of pyridine. The hydrolyzates were removed with capillary pipette and spotted onto paper chromatograms. For comparison, non-radioactive α- and β-Schardinger dextrins were hydrolyzed at 75, 85, and 95° by the procedures described in the previous paper[5].

RESULTS AND DISCUSSION

Radioactive maltotriose and maltohexaose were labeled exclusively in the reducing D-glucose moiety. The number of moles was thus proportional to the number of radioactive counts per min. The sum of the counts per min. for the hydrolytic products and the substrate remaining was proportional to the number of moles of initial substrate. End-labeled substrates are hydrolyzed uniquely, enabling the bonds to be differentiated (see "mechanisms" below). Labeling in the reducing end provides a clear means of testing the hypothesis that bonds at the non-reducing end are cleaved faster than the rest. In such a case, most of the label remains initially with the largest

product molecule. A label at the non-reducing end would yield D-glucose, whose significance is masked by the formation of D-glucose from other products.

The hydrolysis is described by plots of the mole fraction of radioactive product, C_n, against corrected time, θ. The use of θ not only makes the derivation of the kinetic equations simpler, but allows one to determine the rate-constant fractions, which are not sensitive to small variations in acidity and temperature. Equations describing the hydrolysis, assuming only two rate constants, are given below. Calculated and experimental values are given in Tables I–V, and are in good agreement.

TABLE I

RADIOACTIVE PRODUCTS OF HYDROLYSIS OF MALTOTRIOSE[a]

t	θ	C_1		C_2	
		Exp.	*Calc.*	*Exp.*	*Calc.*
210	0.0985	3.195	3.50	6.185	5.87
210	0.1154	3.993	4.11	6.904	6.97
455	0.2033	7.184	7.24	11.22	11.1
455	0.2629	9.869	9.41	13.25	13.7
525	0.2751	9.865	9.84	14.19	14.2
525	0.3525	12.98	11.8	16.98	17.9
715	0.3751	13.40	13.4	17.89	17.8
715	0.4928	17.69	17.5	21.23	21.3
1440	0.8865	31.34	31.0	27.46	27.7
1440	0.9621	33.13	33.4	28.13	28.3

[a]Hydrolytic conditions: 95.0°, 5.0 mM sulfuric acid, 4910 μg/ml; 94.2 d.p.m./μg; all C values are × 100; t = time in min.

TABLE II

RADIOACTIVE PRODUCTS OF HYDROLYSIS OF MALTOTRIOSE[a]

t	θ	C_1		C_2	
		Exp.	*Calc.*	*Exp.*	*Calc.*
0	0.0069	0.233	0.24	0.453	0.44
0	0.0076	0.246	0.26	0.511	0.50
210	0.1083	3.775	3.97	6.481	6.30
210	0.1104	3.829	3.93	6.619	6.52
445	0.2157	7.789	7.71	11.61	11.7
445	0.2213	8.094	7.90	11.75	12.0
575	0.2761	10.07	9.86	14.06	14.3
575	0.2820	10.36	10.1	14.21	14.5
710	0.3369	12.07	12.1	16.53	16.6
710	0.3421	12.33	12.2	16.65	16.8
1440	0.6767	24.30	23.8	24.87	25.4
1440	0.6976	24.53	24.7	25.68	25.5

[a]Hydrolytic conditions: 95.0°, 5.0 mM sulfuric acid, 4910 μg/ml; 236 d.p.m./μg; all C values are × 100; t = time in min.

TABLE III

RADIOACTIVE PRODUCTS OF HYDROLYSIS OF MALTOTRIOSE[a]

t	θ	C_1		C_2	
		Exp.	*Calc.*	*Exp.*	*Calc.*
0	0.0099	0.340	0.37	0.643	0.61
0	0.0114	0.388	0.42	0.743	0.72
180	0.0368	1.346	1.36	2.261	2.25
180	0.0363	1.358	1.35	2.204	2.21
420	0.0710	2.707	2.64	4.149	4.22
420	0.0701	2.564	2.60	4.207	4.17
660	0.1034	3.817	3.84	6.007	5.98
660	0.1076	4.024	3.99	6.180	6.22
840	0.1290	4.846	4.80	7.279	7.31
840	0.1285	4.792	4.77	7.270	7.29
1440	0.2015	7.536	7.59	10.71	10.7
1440	0.2108	7.946	7.82	11.06	11.2

[a]Hydrolytic conditions: 85.0°, 5.0 mM sulfuric acid, 4730 μg/ml; 235 d.p.m./μg; all C values are × 100; t = time in min.

TABLE IV

RADIOACTIVE PRODUCTS OF HYDROLYSIS OF MALTOTRIOSE[a]

t	θ	C_1		C_2	
		Exp.	*Calc.*	*Exp.*	*Calc.*
0	0.0147	0.382	0.54	1.077	0.92
0	0.0137	0.433	0.51	0.930	0.85
720	0.0427	1.546	1.58	2.632	2.61
720	0.0396	1.478	1.46	2.397	2.42
1440	0.0677	2.431	2.51	4.115	4.04
1440	0.0644	2.380	2.39	3.864	3.85
1800	0.0837	3.109	3.17	4.920	4.92
1800	0.0813	3.078	3.01	4.735	4.80
2160	0.0945	3.512	3.50	5.512	5.52
2160	0.0996	3.709	3.69	5.770	5.79
2880	0.1284	4.835	4.76	7.217	7.29
2880	0.1289	4.742	4.79	7.347	7.31

[a]Hydrolytic conditions: 75.0°, 5.0 mM sulfuric acid, 4730 μg/ml; 235 d.p.m./μg; all C values are × 100; t = time in min.

Figs. 1–4 show the calculated curves and the experimental points. The limit of the ratio of product formed to substrate used up, as θ goes to zero, is equal to the rate-constant fraction (see Appendix). The plot of this ratio for small values of θ approximates a straight line (Fig. 5) and is a quicker, but not so precise, way of determining the rate-constant fractions.

Rate constants are consistent among the radioactive-tracer experiments and also with other experimental data[5] (Table VI). The constants for maltose and the

TABLE V

RADIOACTIVE PRODUCTS OF HYDROLYSIS OF MALTOHEXAOSE[a]

t	θ	C_1		C_2		C_3		C_4		C_5	
		Exp.	*Calc.*	*Exp.*	*Calc.*	*Exp.*	*Calc.*	*Exp.*	*Calc.*	*Exp.*	*Calc.*
0	0.0097	0.022	0.12	0.212	0.15	0.206	0.20	0.102	0.20	0.432	0.30
0	0.0103	0.021	0.11	0.211	0.18	0.190	0.18	0.093	0.17	0.504	0.33
230	0.1734	2.860	2.93	2.906	2.83	2.820	2.81	2.652	2.62	4.678	4.73
230	0.1759	2.892	3.02	2.908	2.86	2.791	2.78	2.675	2.74	4.861	4.73
370	0.2718	4.636	4.61	4.360	4.35	4.098	4.12	4.040	4.01	6.668	6.71
370	0.2773	4.664	4.72	4.529	4.39	4.126	4.21	4.130	4.09	6.774	6.80
525	0.3901	6.375	6.47	6.528	6.36	5.423	5.47	5.383	5.38	8.864	8.63
525	0.3988	6.625	6.76	6.179	6.14	5.529	5.75	5.556	5.48	8.999	8.76
715	0.5259	8.666	8.89	7.762	7.83	7.041	7.17	6.816	6.74	10.62	10.3
715	0.5536	9.256	9.38	8.202	8.16	7.265	7.47	6.971	6.95	10.82	10.6
1440	1.0715	18.24	17.7	13.74	13.8	11.19	11.5	10.00	9.94	12.59	12.7
1440	1.0785	18.47	18.0	13.92	13.8	11.11	11.5	10.01	9.96	12.49	12.7

[a]Hydrolysis conditions: 95°, 5.0 mM sulfuric acid, 4160 μg/ml; 500 d.p.m./μg; all C values are × 100; t = time in min.

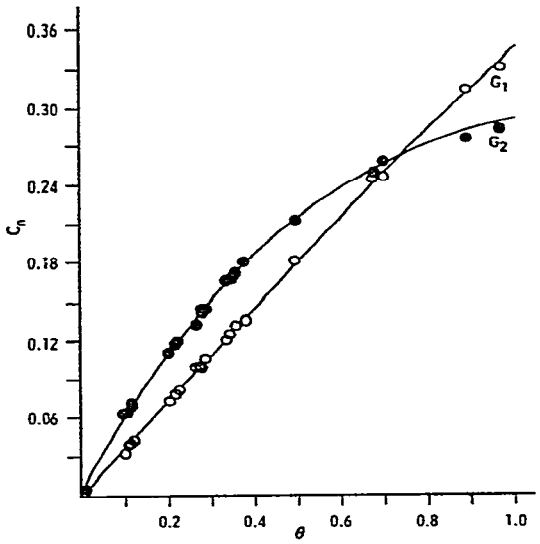
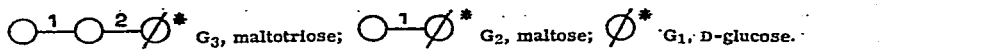


Fig. 1. Mole fraction of radioactive products against corrected time from maltotriose digests at 95°. Theoretical curves and experimental points.

bonds at the non-reducing end of maltotriose and maltohexaose are essentially the same and are about 1.8 times larger than the rate constants of the other glycosidic bonds in maltotriose, maltohexaose, amylose, and amylodextrin. This difference between the glycosidic bonds is in agreement with work done on cellotriose[6] and isomaltotriose[7].

Substrate models:



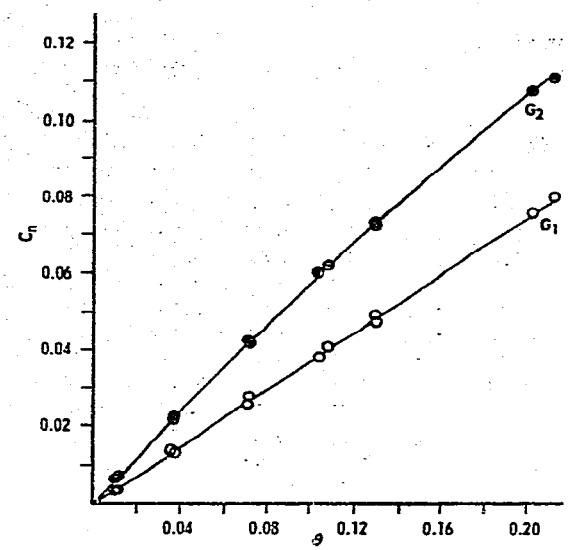


Fig. 2. Mole fraction of radioactive against corrected time from maltotriose digests at 85°. Theoretical curves and experimental points.

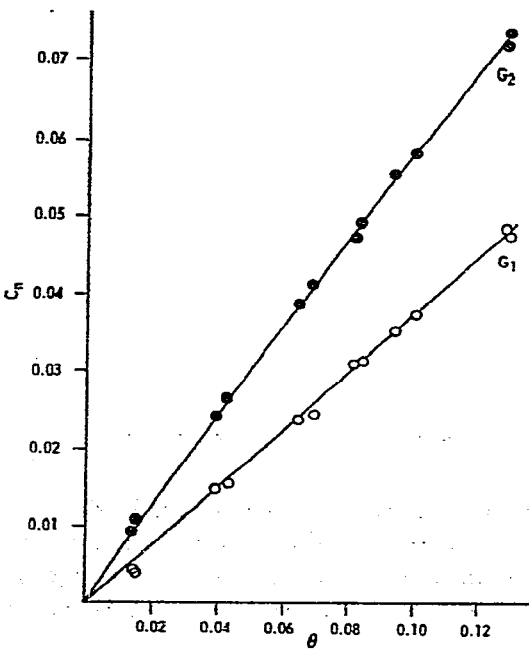


Fig. 3. Mole fraction of radioactive products against corrected time from maltotriose digests at 75°. Theoretical curves and experimental points.

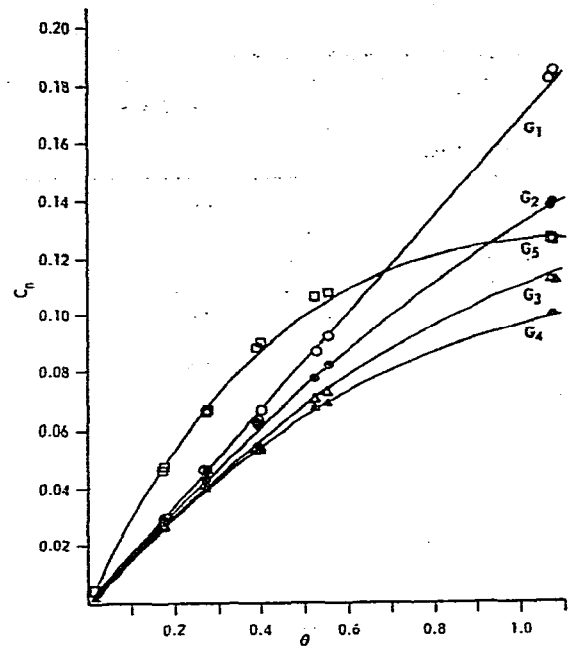


Fig. 4. Mole fraction of radioactive products against corrected time from maltohexaose digests at 95°. Theoretical curves and experimental points.

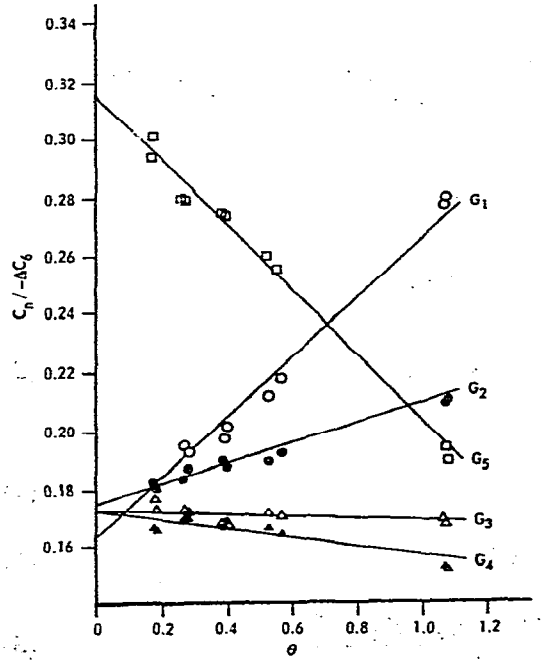


Fig. 5. Ratio of mole fraction of radioactive products to mole fraction of radioactive maltohexaose used up, against corrected time. Intercepts give rate-constant fractions.

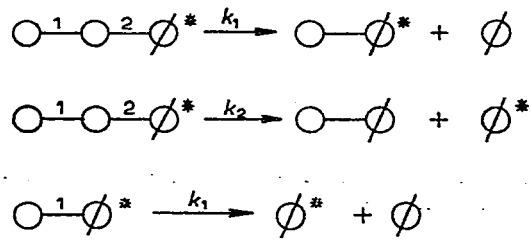
TABLE VI

RATE CONSTANTS AND RATE-CONSTANT FRACTIONS FOR BONDS IN LINEAR POLYMERS

Substrates	*Non-reducing end bond*		*Other bonds*		*Temp (°C)*
	$k \times 10^4$ *min*$^{-1}$	K_1	$k \times 10^4$ *min*$^{-1}$	K_2	
Maltose	3.13[a]				95.0
Maltotriose	3.05	0.648	1.66	0.352	95.0
Maltotriose	0.857	0.630	0.503	0.370	85.0
Maltotriose	0.249	0.630	0.146	0.370	75.0
Maltohexaose	3.29[a]	0.316	1.78[a]	0.171	95.0
Amylodextrin			1.94[a]		95.0
Amylose			1.64[a]		95.0

[a]Data from previous paper (Ref. 5).

Mechanisms:



Differential equations based on models:

Let $\theta = (k_1+k_2)t$, corrected time; $K_1 = \dfrac{k_1}{k_1+k_2}$, $K_2 = \dfrac{k_2}{k_1+k_2}$, rate-constant fractions; and $C_3^0 = 1$

$$\frac{dC_3}{d\theta} = -C_3 \tag{1}$$

$$\frac{dC_2}{d\theta} = -K_1(C_2-C_3) \tag{2}$$

$$\frac{dC_1}{d\theta} = K_1 C_2 + K_2 C_3 \tag{3}$$

Solutions to differential equations:

$$C_3 = e^{-\theta} \tag{4}$$

$$C_2 = \frac{K_1}{K_2}(e^{-K_1\theta} - e^{-\theta}) \tag{5}$$

$$C_1 = 1 - \frac{K_1 e^{-K_1\theta} + (K_2-K_1)e^{-\theta}}{K_2} \tag{6}$$

See Appendix for equations for maltohexaose.

The kinetic curves for maltotriose at 75, 85, and 95° are shown in Fig. 6. The energy and entropy of activation of each of the maltotriose bonds are determined by

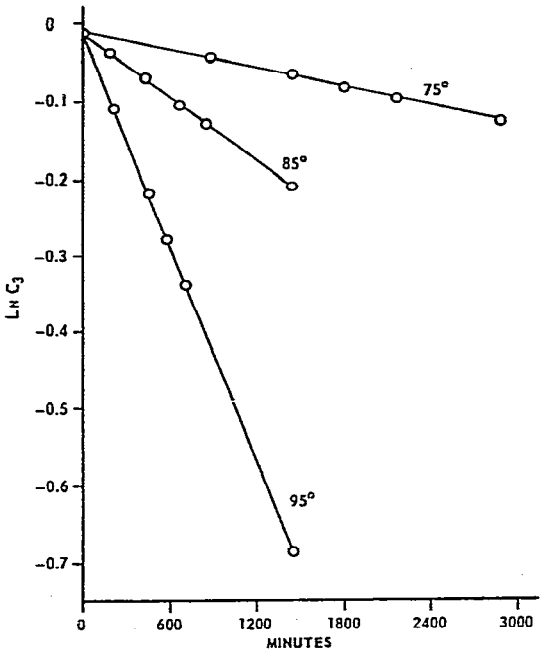


Fig. 6. Kinetic plots of hydrolyses of radioactive maltotriose at various temperatures. $k_{95} = 4.71 \pm 0.11 \times 10^{-4}$ min^{-1}; $k_{85} = 1.36 \pm 0.06 \times 10^{-4}$ min^{-1}; $k_{75} = 0.395 \pm 0.021 \times 10^{-4}$ min^{-1}.

dividing the rate constants by the hydrogen-ion activity to obtain the reduced rate-constants (k_r) and by utilizing Eqns. *7* and *8* (k_B is the Boltzmann constant).

$$k_r = Ae^{-E/RT} \tag{7}$$

$$A = \frac{k_B T}{h} e^{\Delta S^*/R} \tag{8}$$

The Arrhenius plot is shown in Fig. 7. The energy and entropy of activation for the maltotriose bonds are given in Table VII, where they are compared with values for Schardinger dextrins.

The entropy of activation for the bond at the reducing end of maltotriose is smaller than the value for that at the non-reducing end. Assuming that the hydrolysis proceeds through a carbonium–oxonium ion intermediate, the ring undergoing reaction would have to pass through a half-chair conformation[8]. The lower entropy of activation for the bond at the reducing end suggests that a glucosydic bond at O-4, like a hydroxymethyl group at C-5, hampers the transition to this half-chair conformation[9].

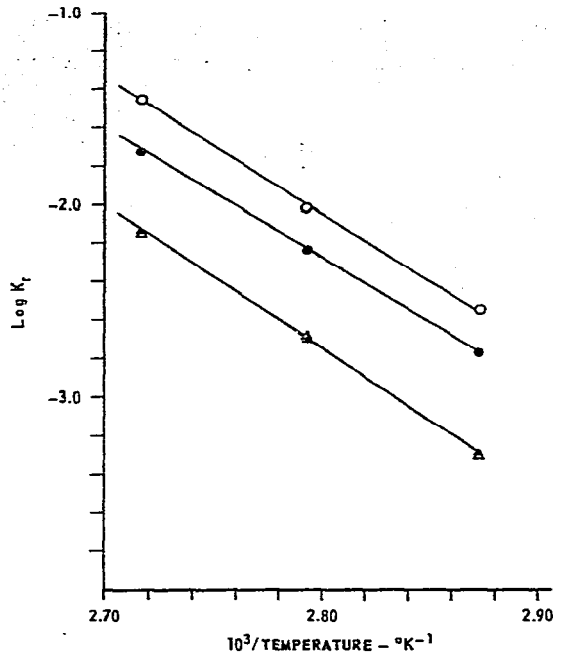


Fig. 7. Common logarithm of reduced rate-constants against reciprocal temperature in degrees Kelvin. Bond at non-reducing end of maltotriose, open circles; Bond at reducing end of maltotriose filled circles; β-Schardinger dextrin, open triangles; α-Schardinger dextrin, filled triangles.

TABLE VII

ENERGIES AND ENTROPIES OF ACTIVATION[a]

Bond	*Temp.* (°C)	$k_r \times 10^2$ *min*$^{-1}$	*E* (*kcal.mole*$^{-1}$)	σ_E	*ΔS* at 100 °C* (*cal.deg*$^{-1}$*.mole*$^{-1}$)	σ_S
	95.0	3.48				
Non-reducing	85.0	0.977	31.9	0.9	12.8	0.1
	75.0	0.284				
	95.0	1.89				
Reducing	85.0	0.574	31.0	0.2	9.12	0.02
	75.0	0.166				
	95.0	0.716				
β-Dextrin	85.0	0.208	34.1	1.0	15.7	0.1
	75.0	0.0493				
	95.0	0.738				
α-Dextrin	85.0	0.194	34.4	0.4	16.5	0.0
	75.0	0.0496				

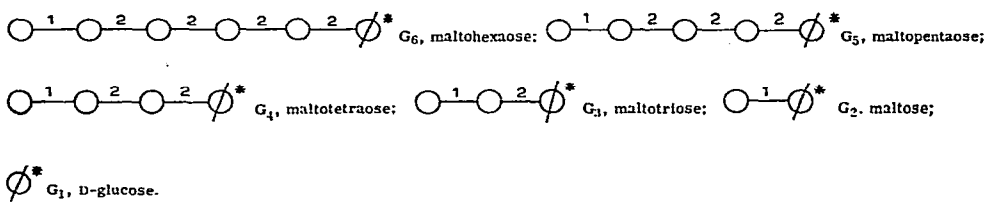
[a] $[H^+] = 0.00877$ for all temperatures.

The rate constants, and energy and entropy of activation, for the bonds in the Schardinger dextrins might be expected to be similar to the values for the bond at the reducing-end of maltotriose. The molecules all have bulky groups attached to C–4 that could hamper the transition to the half-chair conformation. In fact, the rate

constants for the Schardinger dextrins are smaller and their energies and entropies of activation, larger. The entropies of activation are even larger than that for the bond at the non-reducing end. A higher entropy may suggest an intermediate more loosely structured[8]. Such an intermediate may be the acyclic carbonium ion formed by attack of a hydrogen ion on the ring oxygen atom. Examination of a space-filling, molecular model of the α-Schardinger dextrin suggests that the formation of a cyclic carbonium–oxonium ion intermediate would produce great strain in the molecule and result in a rigidly structured intermediate. An acyclic intermediate is consistent with the space-filling molecular model and the thermodynamic data. However, it may be that the entropy of reaction for the protonation of the glycosidic oxygen atom, which is included in the observed entropy of activation, is larger for the cyclic Schardinger dextrins than for the linear compounds[10].

APPENDIX

Substrate models:



Differential equations based on models:

Let $\theta = (k_1+4k_2)t$, corrected time; $K_1 = \frac{k_1}{k_1+4k_2}$, $K_2 = \frac{k_2}{k_1+4k_2}$, rate-constant fractions; and $C_6^0 = 1$.

$$\frac{dC_6}{d\theta} = -C_6 \tag{9}$$

$$\frac{dC_5}{d\theta} = -(1-K_2)C_5+K_1C_6 \tag{10}$$

$$\frac{dC_4}{d\theta} = -(1-2K_2)C_4+K_1C_5+K_2C_6 \tag{11}$$

$$\frac{dC_3}{d\theta} = -(K_1+K_2)C_3+K_1C_4+K_2(C_5+C_6) \tag{12}$$

$$\frac{dC_2}{d\theta} = -K_1(C_2-C_3)+K_2(C_4+C_5+C_6) \tag{13}$$

Solutions to differential equations:

Let $M = \frac{K_1}{K_2}$.

$$C_6 = e^{-\theta} \tag{14}$$

$$C_5 = M(e^{-(1-K_2)\theta} - e^{-\theta}) \tag{15}$$

$$C_4 = \tfrac{1}{2}(M^2-1)\,e^{-\theta} - M^2\,e^{-(1-K_2)\theta} + \tfrac{1}{2}(M^2+1)\,e^{-(1-2K_2)\theta} \tag{16}$$

Let

$$A = -\tfrac{1}{6}(M^3-3M+2);\ B = \tfrac{1}{2}M(M^2-1);\ C = -\tfrac{1}{2}M(M^2+1);\ D = \tfrac{1}{6}(M^3+3M+2).$$

$$C_3 = Ae^{-\theta} + Be^{-(1-K_2)\theta} + Ce^{-(1-2K_2)\theta} + De^{-(K_1+K_2)\theta} \tag{17}$$

Let $F = -\tfrac{1}{4}(MA + \frac{B}{M} - M + 1);\ G = -(F + MA - \frac{BC}{M^2} - MD).$

$$C_2 = Fe^{-\theta} + MAe^{-(1-K_2)\theta} - \frac{BC}{M^2}\,e^{-(1-2K_2)\theta} - MDe^{-(K_2+K_2)\theta} + Ge^{-K_1\theta} \tag{18}$$

$$C_1 = 1 - (C_2 + C_3 + C_4 + C_5 + C_6) \tag{19}$$

$$\lim_{\theta \to 0} \frac{C_5}{-\Delta\, C_6} = K_1 \tag{20}$$

$$\lim_{\theta \to 0} \frac{C_4}{-\Delta\, C_6} = \lim_{\theta \to 0} \frac{C_3}{-\Delta\, C_6} = \lim_{\theta \to 0} \frac{C_2}{-\Delta\, C_6} = \lim_{\theta \to 0} \frac{C_1}{-\Delta\, C_6} = K_2 \tag{21}$$

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