

COMPARATIVE HYDROLYSIS RATES OF THE REDUCING DISACCHARIDES OF D-GLUCOPYRANOSE¹

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ABSTRACT

The hydrolytic reaction velocities of the eight reducing D-glucopyranose disaccharides have been determined at 80° and $99.5 \pm 0.1^\circ\text{C}$. in 0.1*N* hydrochloric acid solution. These disaccharides are: kojibiose [$\alpha\text{-D-(1} \rightarrow 2)$], sophorose [$\beta\text{-D-(1} \rightarrow 2)$], nigerose [$\alpha\text{-D-(1} \rightarrow 3)$], laminaribiose [$\beta\text{-D-(1} \rightarrow 3)$], maltose [$\alpha\text{-D-(1} \rightarrow 4)$], cellobiose [$\beta\text{-D-(1} \rightarrow 4)$], isomaltose [$\alpha\text{-D-(1} \rightarrow 6)$], and gentiobiose [$\beta\text{-D-(1} \rightarrow 6)$]. The reactions of the more readily available sugars maltose, cellobiose, isomaltose, and gentiobiose were followed by optical rotation in a concentration of 2%; those of the others were followed by copper reducing values using the Somogyi method, with 0.01% to 0.02% sugar concentrations. From these data the molar activation energies were calculated.

During the past several years considerable information concerning the types of chemical linkages in various polysaccharides has been obtained by fragmentation studies. In such work the polymer is hydrolyzed in acid solution, in concentrations which would allow only a negligible amount of reversion, and the known fragments are then isolated and identified. The linkages found in the fragments so obtained can then be assured to have been preformed in the polymer. The success of this method depends largely upon the ability to predict the extent of hydrolysis necessary to produce maximum amounts of the desired fragments. Thus the isolation of isomaltose from the acid hydrolysates of glycogen (7) and amylopectin (10) succeeded only after statistical analysis revealed that maximum amounts of the desired fragment would be obtained when the hydrolysis had proceeded to 89 and 91%, respectively. Such calculations are based in part upon the relative rates of hydrolysis of the chemical linkages in the polymer. The general application of this principle to other polymers would

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require a knowledge of the relative hydrolysis rates of all the D-glucose disaccharides which could be expected to result from the fragmentation of D-glucans. The hydrolysis rates of maltose (2,5-7), cellobiose (2,3), gentiobiose (2), and isomaltose (7), under various conditions of temperature and concentration, have been reported previously. Accurate hydrolysis data are needed for all the reducing disaccharides of D-glucopyranose, under comparable conditions.

Materials and Methods

The specific reaction rate constants were calculated by the first-order reaction formula:

$$k = \frac{2.303}{t} \log_{10} \frac{R_0 - R_\infty}{R_t - R_\infty}$$

in which R_0 , R_t , and R_∞ are measurable values related to the concentration of disaccharide at the start, during, and at the end of the reaction, and t is time. The R values were determined either in terms of optical rotations or in ml. of sodium thiosulfate solution equivalent to the reducing sugar in an aliquot portion of reaction mixture.

The Arrhenius energy of activation constant E was calculated by means of the form of the Arrhenius equation (1):

$$E = \log_{10} \frac{k_2}{k_1} \cdot \frac{2.303 RT_1 T_2}{T_2 - T_1}$$

in which R = the molar gas content, and

k = the specific reaction constant at temperature T .

The disaccharides sophorose, nigerose, laminaribiose, maltose, cellobiose, and gentiobiose used in these studies were prepared in this laboratory. Kojibiose was furnished to us by K. Matsuda.

The hydrolysis at 80°C. was carried out in a thermostatted water bath. Two-gram portions of maltose, cellobiose, isomaltose, and gentiobiose were each dissolved in 100 ml. 0.1N hydrochloric acid solution in volumetric flasks and placed in the water bath. The optical rotations were measured at initiation of the reaction, and samples were removed at intervals and observed until no further change could be noted in the rotational values. Kojibiose, nigerose, and laminaribiose in 0.02% and sophorose in 0.01% concentrations were each dissolved in 250 ml. of 0.1N hydrochloric acid in volumetric flasks and placed in the water bath. Aliquot portions of 5 ml. were analyzed by the Somogyi (4) copper reduction method at the initiation of the reaction and at intervals throughout until no further change took place. The reaction rates were calculated for each of the analyses. A boiling water bath at a pressure ranging from 745 to 750 mm. (99.5° ± 0.1°C.)

was used as a heating medium at the higher temperature. The solutions were sealed in tubes and immersed in the bath. The average values, together with the mean variations, are recorded in Table I and summarized in Figs. 1 and 2. Table I also records the molar activation energies found.

Results and Discussion

The activation energies for maltose, cellobiose, and gentiobiose were in good agreement with the values for these substances reported by Moelwyn-Hughes (2) and for maltose by Sharples (3) (Table I).

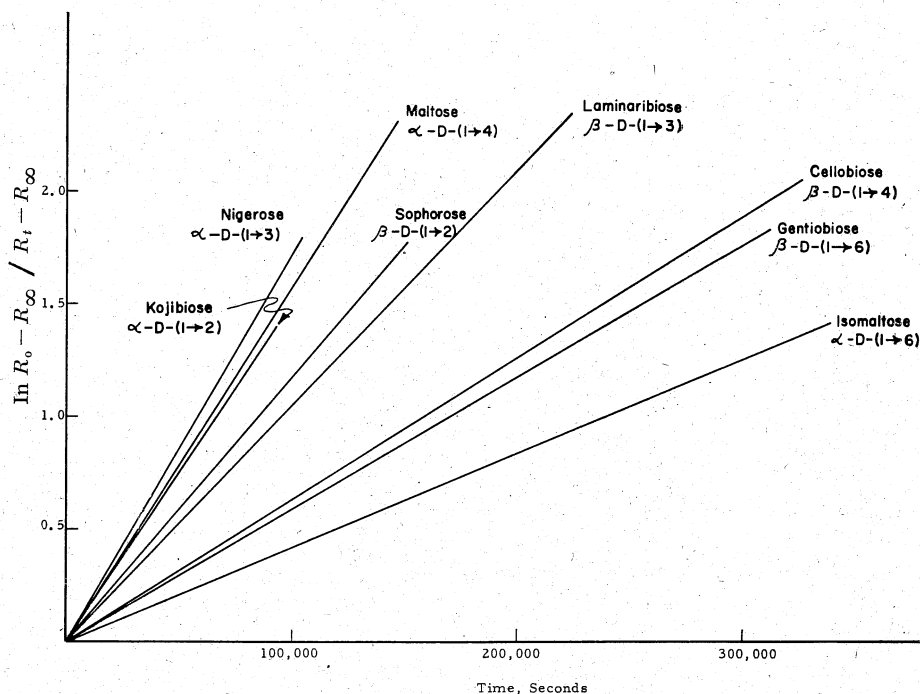


Fig. 1. Hydrolysis velocities of reducing disaccharides of D-glucose in 0.1N HCl at 80°C. Concentrations: maltose, cellobiose, isomaltose, and gentiobiose, 2%; sophorose 0.01%; nigerose, laminaribiose, and kojibiose, 0.02%.

Nigerose has been isolated, in low yield, as a fragmentation product of starch (8) and glycogen (9). Our data show that while isomaltose should be readily isolable from such hydrolysates, the hydrolytic rates of maltose and nigerose are so similar and rapid that the amount of nigerose isolable will always be well below that actually present.

The hydrolytic rate data (Figs. 1 and 2), at both temperatures,

TABLE I
HYDROLYSIS RATE DATA FOR THE REDUCING DISACCHARIDES OF
D-GLUCOPYRANOSE IN 0.1*N* HYDROCHLORIC ACID

SUBSTANCE	$k_1 \times 10^{-5}/$	MEAN VAR.	$k_2 \times 10^{-5}/$	MEAN VAR.	MOLAR ACTIVATION CONSTANT, <i>E</i>
	SEC.; 80°C.		SEC.; 99.5 ± 0.1°C.		
Kojibiose [α -D-(1 → 2)]	1.46	0.02	17.3	0.40	33,000
Sophorose [β -D-(1 → 2)]	1.17	0.08	10.1	0.15	28,900
Nigerose [α -D-(1 → 3)]	1.78	0.07	14.1	0.50	27,200
Laminaribiose [β -D-(1 → 3)]	0.99	0.04	9.3	0.30	30,000
Maltose [α -D-(1 → 4)]	1.55	0.04	16.3	0.60	31,500 30,970 ^a
Cellobiose [β -D-(1 → 4)]	0.66	0.05	6.6	0.26	30,800 30,710 ^a 31,200 ^b
Isomaltose [α -D-(1 → 6)]	0.40	0.03	5.0	0.19	33,800
Gentiobiose [β -D-(1 → 6)]	0.58	0.02	7.2	0.28	33,750 33,390 ^a

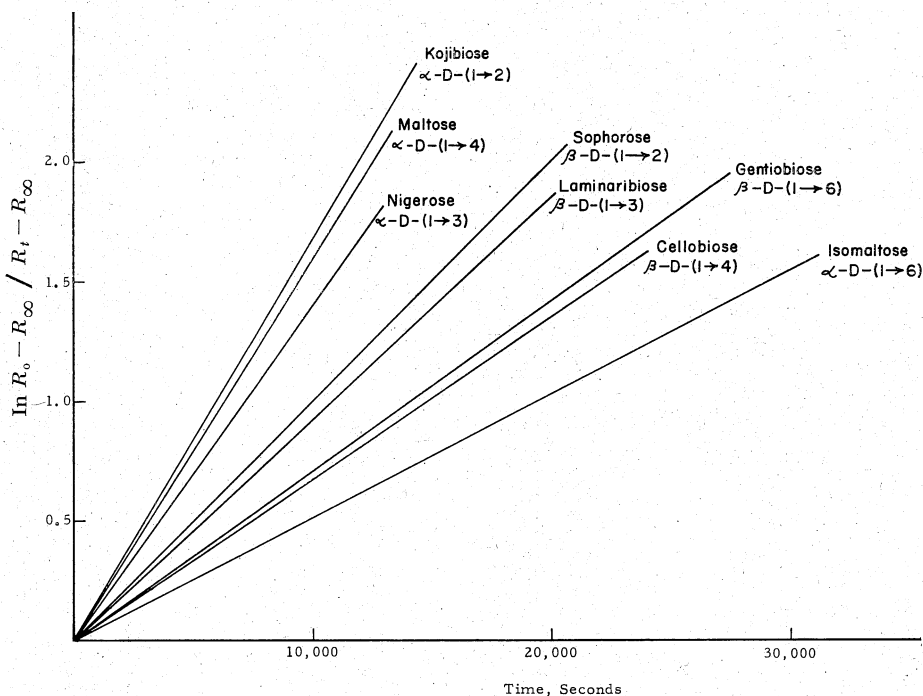
^a Ref. 2.^b Ref. 3.

Fig. 2. Hydrolysis velocities of the reducing disaccharides of D-glucose in 0.1*N* HCl at 99.5° ± 0.1°C. Concentrations: maltose, cellobiose, isomaltose, and gentiobiose, 2%; sophorose 0.01%; nigerose, laminaribiose, and kojibiose, 0.02%.

classify the sugars into three groups, in decreasing order of rates: (a) α -D-(1 \rightarrow 2), α -D-(1 \rightarrow 3), α -D-(1 \rightarrow 4); (b) β -D-(1 \rightarrow 2), β -D-(1 \rightarrow 3); (c) β -D-(1 \rightarrow 4), α -D-(1 \rightarrow 6), β -D-(1 \rightarrow 6). In all cases, save the (1 \rightarrow 6)-linked disaccharides, the α -D linkage is more readily hydrolyzable than the corresponding β -D. Cellobiose seems to be rather anomalous in its difficulty of hydrolysis. All of the molar activation constants are of the same order of magnitude.

Acknowledgment

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³ Similar though not quite comparable data to those herein reported have recently been communicated by Fujimoto, K., Matsuda, K., and Aso, K. *Tohoku J. Agr. Res.* **13**: 61-67 (1962).