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Thermodynamics of hydrolysis of disaccharides

Lactulose, α-D-melibiose, palatinose, D-trehalose, D-turanose and 3-*o*-β-D-galactopyranosyl-D-arabinose

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High-pressure liquid chromatography and microcalorimetry have been used to study the thermodynamics of the hydrolysis reactions of a series of dissaccharides. The enzymes used to bring about the hydrolyses were: β-galactosidase for lactulose and 3-*o*-β-D-galactopyranosyl-D-arabinose; β-glucosidase for α-D-melibiose; β-amylase for D-trehalose; isomaltase for palatinose; and α-glucosidase for D-turanose. The buffer used was sodium acetate (0.02–0.10 M and pH 4.44–5.65). For the following processes at 298.15 K: lactulose(aq) + H_2O(liq) = D-galactose(aq) + D-fructose(aq), $K^\circ = 128 \pm 10$ and $\Delta H^\circ = 2.21 \pm 0.10$ kJ mol^{-1}; α-D-melibiose(aq) + H_2O(liq) = D-galactose(aq) + D-glucose(aq), $K^\circ = 123 \pm 42$ and $\Delta H^\circ = -0.88 \pm 0.50$ kJ mol^{-1}; palatinose(aq) + H_2O(liq) = D-glucose(aq) + D-fructose(aq), $\Delta H^\circ = -4.44 \pm 1.1$ kJ mol^{-1}; D-trehalose(aq) + H_2O(liq) = 2 D-glucose(aq), $K^\circ = 119 \pm 10$ and $\Delta H^\circ = 4.73 \pm 0.41$ kJ mol^{-1}; D-turanose(aq) + H_2O(liq) = D-glucose(aq) + D-fructose(aq), $\Delta H^\circ = -2.68 \pm 0.75$ kJ mol^{-1}; and 3-*o*-β-D-galactopyranosyl-D-arabinose(aq) + H_2O(liq) = D-galactose(aq) + D-arabinose(aq), $K^\circ = 107 \pm 10$ and $\Delta H^\circ = 2.97 \pm 0.10$ kJ mol^{-1}. These six processes correspond, respectively, to the hydrolysis of the following linkages: galactose-fructose (1 → 4), galactose-glucose (1 → 6), glucose-fructose (1 → 6), glucose-glucose (1 → 1′), glucose-fructose (1 → 3), and galactose-arabinose (1 → 3). Using available data in the literature, the heat capacity changes for these processes are found to be in the range −36 to −69 J mol^{-1} K^{-1}. The available thermodynamic data on the hydrolysis of disaccharides is summarized. It is found that the entropy changes for the hydrolysis of disaccharides are in the range 31–56 J mol^{-1} K^{-1} and are well represented by an average value of 40 ± 7 J mol^{-1} K^{-1}. The abnormally high enthalpy of hydrolysis of aqueous sucrose (−14.96 kJ mol^{-1}) can be explained by consideration of the enthalpy of conversion of fructopyranose to fructofuranose.

1. Introduction

This study is a continuation of earlier research [1–11] on the thermodynamics of reactions involving carbohydrates. The motivation for these studies stems primarily from the fundamental role these substances play in living systems as well as their potential use in processes in which biomass is converted to useful chemicals and fuel. The disaccharides are particularly interesting, since relatively few monosaccharides occur free in nature but are found joined to one another by glycosidic linkages. Thus, the thermodynamic data obtained for these reactions is useful both in examining the efficiencies and energy requirements of specific processes and in predicting the direction of these chemical reactions. The utility of this type of information in understanding metabolic processes

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has been discussed in the classic monograph of Krebs et al. [12].

While the amount of available thermodynamic data on hydrolysis processes is extremely limited [8,13], our earlier results [9–11] indicated that the entropy changes for the hydrolysis reactions of disaccharides were reasonably constant (30–43 J mol^{-1} K^{-1}). Such a rule of constancy could prove very useful in the development of methods for estimating thermodynamic data for other processes and compounds of a similar nature. Thus, it was also desired to obtain additional data to further test this empirical observation. Towards this end microcalorimetry and high-pressure liquid chromatography have been used, respectively, for enthalpy and equilibrium measurements of the enzyme-catalyzed hydrolyses of the following disaccharides [1]: lactulose, α-D-melibiose, palatinose, α-D-melibiose, palatinose, D-trehalose, D-turanose, and 3-*o*-β-D-galactopyranosyl-D-arabinose.

2. Experimental

The materials used in this study were obtained [2] from Sigma with only a few exceptions. The α-D-glucose was obtained from the National Institute of Standards and Technology, the D-fructose and D-galactose were from Pfanstiehl, and the sodium acetate was from J.T. Baker. The carbohydrates used in this investigation and their moisture contents in mass percent, as determined by Karl Fischer titration, are: D-arabinose, $\leqslant 0.03$; D-fructose, $\leqslant 0.03$; α-D-glucose, $\leqslant 0.03$; D-galactose, 0.10; lactulose, 0.26; α-D-melibiose, 2.0; palatinose, 5.9; D-trehalose, 11.0; D-turanose, 0.56; and 3-*o*-β-D-galactopyranosyl-D-arabinose, 1.4. These moisture contents were applied as corrections to both the equilibrium and calorimetric measurements. All of the carbohydrates were found to be pure using the chromatographic procedures described below. The enzymes used to bring about the hydrolyses were: β-galactosidase (EC 3.2.1.23) for lactulose and 3-*o*-β-D-galactopyranosyl-D-arabinose; β-glucosidase (EC 3.2.1.21) for α-D-melibiose; β-amylase (EC 3.2.1.2) for D-trehalose; oligo-1,6-glucosidase (EC 3.2.1.10), commonly called isomaltase, for palatinose; and α-glucosidase (EC 3.2.1.20) for D-turanose. These enzymes were either in powdered or in lyophilized forms. Control experiments demonstrated that they presented no interferences with the chromatographic measurements. It should be noted that the usefulness of these enzymes was determined in an empirical manner using actual lots of commercial enzymes. Thus, the effectiveness of a particular enzyme in a few cases (e.g., β-amylase for D-trehalose and β-glucoside for α-D-melibiose) may have been due to contaminating enzymes in the lot of enzyme used. The important point is that the thermodynamic parameters determined herein are all state functions and are independent of the actual pathway or mechanism of the reaction. Thus, the experimental results obtained in these experiments are independent of the nature of the catalyst used to bring about the actual reaction. Also, the thermodynamic parameters determined for the reactions studied herein pertain to the overall hydrolysis processes which automatically include any effects due to anomerization and isomerization. A detailed analysis of these processes in terms of the actual anomeric forms involved requires information on both the Gibbs energies and enthalpies of these subsidiary processes which is not available for most of the systems studied herein. Nevertheless, a partial analysis is performed in this paper for the hydrolysis of sucrose to D-glucose and D-fructose.

A Dionex BioLC equipped with an HPIC-AS6A anion-exchange column and a pulsed amperometric detector were used to measure the amounts of

[1] Systematic names of these disaccharides are: lactulose, 4-*o*-β-D-galactopyranosyl-α-D-fructose; α-D-melibiose, 6-*o*-α-D-galactopyranosyl-D-glucose; palatinose, 6-*o*-α-D-glucopyranosyl-D-fructose; D-trehalose, 1-*o*-α-D-glucopyranosyl-α-D-glucopyranoside; and D-turanose, 3-*o*-α-D-glucopyranosyl-D-fructose. The disaccharide 3-*o*-β-D-galactopyranosyl-D-arabinose has no common name. Palatinose is also called isomaltulose.

[2] Certain commercial materials and products are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.

carbohydrates present in solution. The mobile phases, flow rates, and approximate retention times for the hydrolysis studies of the disaccharides follow. For D-trehalose, the mobile phase was 60 mM NaOH, flow rate of 0.6 ml/min, and retention times of 3.6 min for D-trehalose and 6.0 min for D-glucose. For 3-*o*-*β*-D-galactopyranosyl-D-arabinose, the mobile phase was 60 mM NaOH, flow rate of 0.6 ml/min, and retention times of 6.5 min for both D-galactose and D-arabinose and 15 min for 3-*o*-*β*-D-galactopyranosyl-D-arabinose. For lactulose, the mobile phase was 60 mM NaOH, flow rate of 0.7 ml/min, and retention times of 8.0, 9. 0 and 16.0 min for D-galactose, D-fructose and lactulose, respectively. For *α*-D-melibiose, the mobile phase was 50 mM NaOH, flow rate of 0.7 ml/min, and retention times of 6.0–6.5 min. for both D-glucose and D-galactose and 16 min for *α*-D-melibiose. For palatinose, the mobile phase was 60 mM NaOH, flow rate of 0.7 ml/min, and retention times of 6.0, 7.0 and 18.0 min for D-glucose, D-fructose and palatinose, respectively. For D-turanose, the mobile phase was 60 mM NaOH, flow rate of 0.6 ml/min, and retention times of 6.0, 7.0 and 14.0 min for D-glucose, D-fructose and D-turanose, respectively. A Hewlett-Packard HP-1090 liquid chromatograph was also used to determine the extents of the hydrolysis reactions and to check for possible side reactions. A Bio-Rad HPX-87C calcium cation-exchange column was used for the separation and detection was carried out using a refractive index detector. The mobile phase was pure water at a flow rate of 0.6 ml/min. Since, at equilibrium, there is very little disaccharide left in solution, it was necessary to rely upon the more sensitive pulsed amperometric detector for the analysis of the amount of disaccharide remaining in solution. Calibration of the chromatographs was performed daily using synthetic mixtures of the appropriate carbohydrates prepared to have very nearly the composition of the solutions being analyzed.

In the equilibrium studies, solutions were allowed to equilibrate with gentle stirring in thermostatted water baths. In each case equilibrium was approached from two directions, i.e., starting with the disaccharide (the forward direction) and starting with the appropriate monosaccharide(s) (the reverse direction). There was sufficient chromatographic detector sensitivity for the determination of equilibrium constants for the hydrolysis of lactulose, *α*-D-melibiose, D-trehalose and 3-*o*-*β*-D-galactopyranosyl-D-arabinose. However, there was not enough sensitivity to determine the residual amounts of palatinose and D-turanose left and it was therefore not possible to determine equilibrium constants for the hydrolysis of these disaccharides. Equilibration times were 6 days for D-trehalose and *α*-D-melibiose; 10 and 17 days were allowed for 3-*o*-*β*-D-galactopyranosyl-D-arabinose and lactulose, respectively.

The calorimeters are of the heat-conduction type and have calibration constants varying from 17 to 22 W V^{-1}. The sensitivity (units of V W^{-1}) is the inverse of the calibration constant. The sample vessels, which are fabricated from high-density polyethylene, contain two compartments holding approx. 0.55 and 0.45 ml of solution, respectively. The thermopile voltages are amplified by a factor of 10^4, measured using a digital voltmeter, and recorded using a data acquisition system and a microcomputer. A complete description of the calorimeters, their performance characteristics, and the data acquisition and treatment are given in refs. 14 and 15. Measurements of reaction heat were performed by mixing, in the calorimeter, a substrate solution and an enzyme solution. The substrate solution was prepared by dissolving a known amount of disaccharide in a buffer solution. The enzyme solution was prepared by adding the same buffer solution to the appropriate enzyme. The blank heats accompanying the mixing of the enzyme solution with the buffer ranged from -1.50 to $+0.44$ mJ. Control experiments were also performed in which the enzyme solution was mixed with a solution prepared to have the composition of the reaction mixture at equilibrium. These control experiments served to determine if there was any heat associated with the interaction of the enzymes with the substrates which was independent of the heat associated with the chemical reaction. These control experiments yielded results which ranged from -1.05 to $+0.39$ mJ. These heats were applied as corrections to the heats measured for the hydrolysis reactions. The chromatographic procedures described above were

also used to determine the percentages of the disaccharides undergoing hydrolysis when the reactions were carried out in the microcalorimeters. These analyses were performed immediately following the removal of the calorimeter vessels from the microcalorimeters. These percentages are: lactulose, 99.8%; α-D-melibiose, 17–18%; palatinose, 100%; D-trehalose, 93–97%; D-turanose, 51–52%; and 3-*o*-β-D-galactopyranosyl-D-arabinose, 99.8%. The extents of reaction were calculated from these percentages and used to calculate the molar enthalpies of reaction given later in this paper.

3. Results and Discussion

The processes of interest in this investigation are:

$$\text{lactulose(aq)} + H_2O(\text{liq}) = \text{D-galactose(aq)} + \text{D-fructose(aq)} \quad (A)$$

$$\alpha\text{-D-melibiose(aq)} + H_2O(\text{liq}) = \text{D-galactose(aq)} + \text{D-glucose(aq)} \quad (B)$$

$$\text{palatinose(aq)} + H_2O(\text{liq}) = \text{D-glucose(aq)} + \text{D-fructose(aq)} \quad (C)$$

$$\text{D-trehalose(aq)} + H_2O(\text{liq}) = 2\ \text{D-glucose(aq)} \quad (D)$$

$$\text{D-turanose(aq)} + H_2O(\text{liq}) = \text{D-glucose(aq)} + \text{D-fructose(aq)} \quad (E)$$

$$3\text{-}o\text{-}\beta\text{-D-galactopyranosyl-D-arabinose(aq)} + H_2O(\text{liq}) = \text{D-galactose(aq)} + \text{D-arabinose(aq)} \quad (F)$$

[3] The standard state used in this investigation is, for the solute, the hypothetical ideal solution of unit molality. The standard state for the water is the pure solvent and the activity of water was assumed to be unity. While the thermodynamic equilibrium constant is dimensionless, units of mol kg^{-1} were used in all thermodynamic calculations.

Table 1

Equilibrium data for the hydrolysis reactions of disaccharides. Lactulose was hydrolyzed to D-galactose and D-fructose using β-galactosidase; α-D-melibiose to D-galactose and D-glucose using β-glucosidase; D-trehalose to D-glucose using β-amylase; and 3-*o*-β-D-galactopyranosyl-D-arabinose to D-galactose and D-arabinose using β-galactosidase. Equilibration times for these systems ranged from 6 to 17 days. For all reactions the buffer was 0.1 M sodium acetate at pH 5.65. In each case equilibrium was approached from two directions: starting with the disaccharide (the forward direction) and starting with the appropriate monosaccharide(s) (the reverse direction). For those experiments started from the forward direction, the initial concentrations of the disaccharides were approx. 0.050 M; for experiments started from the reverse direction, the (combined) initial concentration(s) of the monosaccharide(s) was 0.10 M. The equilibrium constants given in columns 3 and 4 are the averages of four to six measurements. The equilibrium constants in the last column are the averages of all of the data obtained at the specified temperatures. The uncertainties are 95% confidence limits.

Disaccharide	T/K	K		
		Forward	Reverse	Average
Lactulose	298.15	122±9	133±3	128±10
α-D-Melibiose	298.15	126±10	121±41	123±42
D-Trehalose	286.45	118±6	127±7	122±9
	292.25	119±5	126±5	123±7
	298.15	113±5	124±8	119±10
	304.55	115±5	122±8	118±10
	310.25	113±6	118±5	115±8
3-*o*-β-D-Galacto-pyranosyl-D-arabinose	286.15	115±3	105±8	108±9
	292.15	106±5	109±6	108±8
	298.15	104±4	104±8	104±9

The results of the equilibrium measurements [3] are given in table 1. In all cases equilibrium was approached from two different directions, i.e., starting with only the disaccharide in solution (the forward direction) and starting with only the appropriate monosaccharide(s) in solution (the reverse direction). For D-trehalose and for 3-*o*-β-D-galactopyranosyl-D-arabinose, equilibrium measurements were performed at temperatures other than 298.15 K. Note however, that the random error in the equilibrium measurements is of such a magnitude as to be of little value either in giving us definitive information on the variation of the equilibrium constants with temperature or in calculating an enthalpy change for the process.

Table 2

Enthalpies of hydrolysis of disaccharides at 298.15 K

The concentrations of the disaccharides in solution prior to hydrolysis were in the range 0.015–0.024 M. The hydrolyses of lactulose, α-D-melibiose, D-trehalose, and 3-*o*-β-D-galactopyranosyl-D-arabinose were done using sodium acetate buffer (0.1 M and pH 5.65); sodium acetate buffer (0.02 M and pH 4.44) was also used for the hydrolysis of palatinose and 3-*o*-β-D-galactopyranosyl-D-arabinose. The enzymes used to bring about the hydrolyses and their concentrations in solution were: β-galactosidase (≈ 3 g l^{-1}) for lactulose and 3-*o*-β-D-galactopyranosyl-D-arabinose; β-glucosidase (≈ 10 g l^{-1}) for α-D-melibiose; β-amylase (≈ 4 g l^{-1}) for D-trehalose; isomaltase (≈ 7 g l^{-1}) for palatinose; and α-glucosidase (≈ 4 g l^{-1}) for D-turanose. Appropriate corrections have been applied for both blank heats and incomplete reaction (see section 2). Four to six measurements were performed for each system. The uncertainties are 95% confidence limits.

Disaccharide	ΔH (kJ mol^{-1})
Lactulose	2.21 ± 0.04
α-D-Melibiose	-0.88 ± 0.50
Palatinose	-4.44 ± 1.1
D-Trehalose	4.73 ± 0.41
D-Turanose	-2.68 ± 0.75
3-*o*-β-D-Galacto-pyranosyl-D-arabinose	2.97 ± 0.06

Therefore, based upon the data given in table 1, the following values are adopted for the equilibrium constants for the hydrolysis reactions: $K_A^\circ = 128 \pm 10$, $K_B^\circ = 123 \pm 42$, $K_D^\circ = 119 \pm 10$, and $K_F^\circ = 107 \pm 10$ at 298.15 K. The corresponding Gibbs energy changes for these processes at 298.15 K are: $\Delta G_A^\circ = -12.03 \pm 0.15$, $\Delta G_B^\circ = -11.93 \pm 0.32$, $\Delta G_D^\circ = -11.85 \pm 0.06$, and $\Delta G_E^\circ = -11.56 \pm 0.10$ kJ mol^{-1}. As pointed out in section 2, for both palatinose and D-turanose the extent of reaction was greater than for the four other hydrolysis reactions and the chromatographic sensitivity was not sufficient to allow us to determine equilibrium constants for these hydrolysis reactions. Later, we shall have a basis for estimating values for these equilibrium constants.

The results of the calorimetric measurements are given in table 2. Based upon these results, the following enthalpy changes have been assigned to the hydrolysis reactions: $\Delta H_A^\circ = 2.21 \pm 0.10$, $\Delta H_B^\circ = -0.88 \pm 0.50$, $\Delta H_C^\circ = -4.44 \pm 1.1$, $\Delta H_D^\circ = 4.73 \pm 0.41$, $\Delta H_E^\circ = -2.68 \pm 0.75$, and $\Delta H_F^\circ = 2.97 \pm 0.10$ kJ mol^{-1} at 298.15 K. Here, the uncertainties for the hydrolyses of lactulose and 3-*o*-β-D-galactopyranosyl-D-arabinose have been increased over the statistical 95% confidence limits to allow for possible errors due to sample purity, chromatographic analysis of the extent of reaction, and the adjustment to the standard state. Combination of these enthalpy changes with the Gibbs energy data leads to the following entropy changes: $\Delta S_A^\circ = 47.8 \pm 0.5$, $\Delta S_B^\circ = 37.1 \pm 2.0$, $\Delta S_D^\circ = 55.6 \pm 1.4$, and $\Delta S_E^\circ = 41.70 \pm 0.4$ J mol^{-1} K^{-1} at 298.15 K.

The results presented above are the first direct measurements to be reported on the hydrolysis reactions of these disaccharides. Some comparisons can be made, however, using appropriate thermochemical cycles. For D-trehalose · $2H_2O$(cr), the enthalpy of combustion given by Domalski [16] and based upon the early measurements of Stohmann and Langbein [17] can be combined with the enthalpy of solution measured by Jasra and Ahluwalia [18] to calculate the enthalpy change for the hydrolysis of D-trehalose. The result is $\Delta H_D^\circ = -1.7$ kJ mol^{-1} at 298.15 K. Here, we have assumed that the enthalpy of solution of D-trehalose 'dihydrate' and stated by Jasra and Ahluwalia [18] to be 'trehalose · $1.86H_2O$(cr)' is the appropriate result to use in combination with the enthalpy of combustion of D-trehalose dihydrate as determined by Stohmann and Langbein [17]. In any case the uncertainties in this calculation are large (≥ 20 kJ mol^{-1}) due to both this assumption and, much more likely, to possible random and systematic errors in the enthalpy of combustion data. Thus, while the calculated enthalpy change of -1.7 kJ mol^{-1} is in agreement with the result obtained herein, namely $\Delta H_D^\circ = 4.73 \pm 0.41$ kJ mol^{-1}, it serves only as an approximate verification of our results. Stohmann and Langbein [17] also determined the enthalpy of combustion of anhydrous D-trehalose. Here, however, we do not have an enthalpy of solution to use in a similar calculation.

A second thermochemical pathway uses data reported by Marechal and Belocopitow [19]. They used the enzyme α,α-trehalose phosphorylase (EC

2.4.1.64) and obtained an equilibrium constant for the overall process:

D-trehalose(aq) + orthophosphate(aq)

= D-glucose(aq) + D-glucose 1-phosphate(aq) (G)

Their results were obtained at 310.15 K and in 40 mM imidazole–HCl buffer. The reported equilibrium constants are 4.2 and 17 at pH 6.3 and 7.0, respectively. We apply ionization and temperature corrections [7] to their results and obtain a Gibbs energy change of approx. -3.6 kJ mol^{-1} for the following ionic reaction at 298.15 K:

D-trehalose(aq) + HPO_4^{2-}(aq) = D-glucose(aq)

+ D-glucose 1-phosphate^{2-}(aq) (H)

Here, auxiliary thermodynamic data for the ionizations of D-glucose 1-phosphate and orthophosphate has been taken, respectively, from the measurements of Ashby et al. [20] and from ref. 21. Also, an enthalpy change of 3.7 kJ mol^{-1} for process (H) above was estimated using data for the enthalpy of hydrolysis of glucose 6-phosphate^{2-}(aq) [8] and the enthalpy of hydrolysis of D-trehalose determined herein. Finally, we shall need the Gibbs energy change of -17.95 kJ mol^{-1} [8] for the following process at 298.15 K:

D-glucose 1-phosphate^{2-}(aq) + H_2O(liq)

= D-glucose(aq) + HPO_4^{2-}(aq) (J)

The Gibbs energy change for the hydrolysis of D-trehalose is then calculated to be -25.8 kJ mol^{-1} at 298.15 K using the relationship: $\Delta G^\circ_D = \Delta G^\circ_H + \Delta G^\circ_J$. This corresponds to an equilibrium constant equal to 3.3×10^4. It is not in agreement with the result of $K^\circ_D = 119 \pm 10$ obtained in this study. We believe that there is a large systematic error in the measurements of Marechal and Belocopitow [19]. It is possible that the error may lie in the methods of analysis used, but it should also be noted that they [19] did not demonstrate that their reaction mixture was at equilibrium by approaching it from two different directions. Also, the entropy change of 102 J mol^{-1} K^{-1} that is calculated for the hydrolysis of D-trehalose using the adjusted result of Marechal and Belocopitow [19] and enthalpy of hydrolysis of D-trehalose determined herein is unreasonable in comparison to the entropies of hydrolysis of the other disaccharides for which data exist (see table 3).

Jasra and Ahluwalia [18] report apparent molar heat capacities for α-D-melibiose(aq), D-trehalose(aq) and D-turanose. Their results can be

Table 3

Thermodynamic parameters for hydrolysis of disaccharides at 298.15 K

Disaccharide	K°	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})	ΔC_p° (J mol^{-1} K^{-1})	References
Cellobiose	> 155	< −12.5	−2.43 ± 0.31	⩾ 34	≈ 2	9
Gentiobiose	17.6 ± 0.7	−7.11 ± 0.10	2.58 ± 0.32	32.5 ± 1	≈ −41	9
Isomaltose	17.3 ± 0.4	−7.06 ± 0.05	5.86 ± 0.54	43 ± 2	≈ 44	9
Lactose	35 ± 3	−8.81 ± 0.20	0.44 ± 0.10	31.0 ± 0.8	≈ 0	10
Lactulose	128 ± 8	−12.03 ± 0.15	2.21 ± 0.04	47.8 ± 0.5		this work
Maltose	> 513	< −15.5	−4.55 ± 0.10	⩾ 37	≈ 13	9, 22
α-D-Melibiose	123 ± 17	−11.93 ± 0.32	−0.88 ± 0.55	37.1 ± 2	−69	this work and 18
Palatinose			−4.44 ± 1.1			this work
Sucrose	(4.44 ± 0.5) × 10^4	−24.5 ± 1.0	−14.96 ± 0.16	31.9 ± 3.4	58 ± 14	11
D-Trehalose	119 ± 3	−11.85 ± 0.060	4.73 ± 0.41	55.6 ± 1.4	−59	this work and 18
D-Turanose			−2.68 ± 0.75		−36	this work and 18
3-*o*-β-D-galacto-pyranosyl-D-arabinose	106 ± 5	−11.56 ± 0.10	2.97 ± 0.06	41.7 ± 0.4		this work

combined with the apparent molar heat capacity data [8,21] for D-glucose(aq), D-fructose(aq) and H_2O(liq) to calculate heat capacity changes for the hydrolysis reactions of these three disaccharides. The results are $\Delta C_p^\circ = -69$, -59 and -36 J mol^{-1} K^{-1} at 298.15 K for the hydrolysis of α-D-melibiose(aq), D-trehalose(aq) and D-turanose(aq), respectively.

The thermodynamic parameters for the hydrolysis reactions of disaccharides are summarized in table 3. Also included are data for systems which have been studied previously [9–11,22]. An important feature of table 3 is found by examination of the entropy changes for the hydrolysis reactions. The range of values is 32–56 J mol^{-1} K^{-1} and the average is 40 J mol^{-1} K^{-1} with an average deviation of 7 J mol^{-1} K^{-1}. This remarkable constancy in the entropy of hydrolysis can prove useful in the estimation of other thermodynamic parameters. For example, it was not possible to measure equilibrium constants for the hydrolysis of palatinose(aq) and D-turanose(aq). Using the measured enthalpies of hydrolysis of these compounds and the average entropy change for the other hydrolysis reactions (40 J mol^{-1} K^{-1}) leads to Gibbs energy changes of -16.4 and -14.6 kJ mol^{-1} for the hydrolysis of palatinose(aq) and D-turanose(aq), respectively. The corresponding equilibrium constants are $K_C^\circ = 740$ and $K_E^\circ = 360$. A similar calculation leads to equilibrium constants of 330 and 770, respectively, for the hydrolysis of cellobiose and maltose at 298.15 K. These estimates are consistent with our earlier study [9] where, because of product inhibition of enzyme activity, it was only possible to determine that the equilibrium constants were greater than certain stated values. It is also worth noting that the quantity $T\Delta S$ is approximately equal to -12 kJ mol^{-1} at 298.15 K for these hydrolysis reactions. Thus, these processes are essentially entropically driven in the direction of hydrolysis to the monosaccharides.

A principal contribution to the entropy changes accompanying these hydrolysis reactions is from the increase in translational entropy due to the production of two carbohydrate molecules from a single disaccharide. For the systems studied herein, this effect is expected to be essentially constant. The other principal contributions to the entropy change would be due to vibrational and rotational effects. Clearly, the effects of hydration of the reactants and products would have to be accounted for in any actual calculation and one would also need accurate information on those molecular frequencies having low wave numbers. Thus, while the information needed for such a detailed calculation is not available, the relative constancy of the entropy changes accompanying these hydrolysis reactions is consistent with the view that the entropic contributions due to vibrational and rotational effects is reasonably constant from system to system.

The thermodynamic parameters given in table 3 can also be used to predict the temperature dependency of the equilibrium constants and the enthalpy changes using the following equations:

$$\Delta G_T^o = -RT\ln K = \Delta H_\theta^o + \Delta C_p^\circ(T-\theta) + T(\Delta G_\theta^\circ - \Delta H_\theta^\circ)/\theta - T\Delta C_p^\circ \ln(T/\theta) \quad (1)$$

$$\Delta H_T^\circ = \Delta H_\theta^o + \Delta C_p^\circ(T-\theta) \quad (2)$$

Here, T is the thermodynamic temperature, θ denotes the reference temperature (298.15 K), and R is the gas constant (8.31451 J mol^{-1} K^{-1}). The heat capacity changes are assumed to be constant over the temperature range of interest. Examination of the data in table 3 shows that the heat capacity changes are in the range -69 to $+58$ J mol^{-1} K^{-1} for these hydrolysis reactions. Thus, neglect of the heat capacity change could cause possible errors of about 1.8 kJ mol^{-1} in both the Gibbs energy and enthalpy changes in adjusting the data to temperatures 25 K away from the reference temperature of 298.15 K.

In table 3 the enthalpy changes are, with the exception of the enthalpy of hydrolysis of sucrose, rather small and in the range -4.6 to $+5.9$ kJ mol^{-1}. A possible explanation for the relatively large enthalpy of hydrolysis of sucrose is to be found in the fact that when it is hydrolyzed the products formed are D-glucose and D-fructofuranose. The fructofuranose will then convert to the equilibrium mixture of the pyranose and furanose forms in solution. This conversion of furanose to pyranose forms is accompanied by an

enthalpy change of -15.2 kJ mol^{-1} at 298.15 K [8]. Using this value and the percent compositions of the anomeric forms [8,23] present at equilibrium in solution, i.e. fructopyranose is 72% and fructofuranose is 28%, leads to a contribution of $\{-15.2 \times 0.72\}$ or -10.9 kJ mol^{-1} to the enthalpy of hydrolysis of aqueous sucrose. Thus, if this pyranose/furanose equilibrium were not present, the enthalpy of hydrolysis of sucrose would be equal to $\{-14.96-(-10.9)\}$ or -4.1 kJ mol^{-1}. This value would be in the range of the enthalpies of hydrolysis of the other disaccharides. This rationalization, however, requires somewhat closer scrutiny in terms of the following discussion.

Four of the disaccharides shown in table 3 yield fructose when hydrolyzed. These four disaccharides are sucrose, lactulose, palatinose, and D-turanose. Only for sucrose, however, is the enthalpy of hydrolysis significantly different from the existing body of data. X-ray crystallographic data exist for the crystalline forms of all of these substances. Analysis of this data shows that in D-turanose, the fructose is in the pyranose ring form [24,25]. The fructose exits, however, in the furanose form in both sucrose [26] and palatinose [27]. Fructose is present in crystalline lactulose [28] as a mixture of the β-fructofuranose, α-fructofuranose and β-fructopyranose in the ratio 0.745:0.100:0.155. Examination of the Fischer projection formulas of these disaccharides shows that lactulose, palatinose and D-turanose are reducing sugars and thus can undergo rearrangement in solution. Sucrose, however, is a nonreducing sugar and the fructose ring in it cannot undergo any structural rearrangements until the disaccharide bond has been cleaved on hydrolysis. Thus, the structural matters discussed above may indeed be the basis for the relatively large enthalpy of hydrolysis of aqueous sucrose.

4. Summary and significance of results

This paper contains the results of equilibrium and calorimetric experiments on the hydrolysis of several disaccharides: lactulose, α-D-melibiose, palatinose, D-trehalose, D-turanose, and 3-*o*-β-D-galactopyranosyl-D-arabinose. This data and the total of the thermodynamic information (ΔG°, K°, ΔH°, ΔS° and ΔC_p°) in the literature on the hydrolysis of disaccharides are summarized in table 3. Most of this information has been obtained only recently, primarily because of the experimental difficulty of measuring equilibrium constants for processes where most of the reactant, i.e., the disaccharide, is consumed. Based upon examination of these thermodynamic parameters, it is possible to arrive at the following generalizations: the entropy changes accompanying the hydrolysis of disaccharides is remarkably constant (40 J mol^{-1} K^{-1} with an average deviation of 7 J mol^{-1} K^{-1}); and the enthalpies of hydrolysis of disaccharides are small (-5 to $+6$ kJ mol^{-1}) with the exception of sucrose which has an enthalpy of hydrolysis of -15.0 kJ mol^{-1}. The constancy in the entropy of hydrolysis is probably attributable to the predominant effect of the translational contribution and the essential constancy of any vibrational contributions to the differences in entropies between the disaccharide and the monosaccharide(s) formed on hydrolysis. The small differences in the enthalpies of hydrolysis could, in principle, be interpreted using information on the fractions of the various anomeric forms in solution together with the enthalpy differences between the anomeric forms. However, while the fractions of the anomeric forms in solution are known, information on the enthalpy differences is sparse [8]. For sucrose, however, information on the enthalpy change for the pyranose/furanose conversion is available and it has been used to rationalize the relatively large enthalpy change for this hydrolysis reaction.

The essential constancy in the entropy of hydrolysis can prove useful in the estimation of other thermodynamic parameters. Specifically, it was not possible to determine equilibrium constants for the hydrolysis of cellobiose and maltose by direct measurement. However, using the calorimetric enthalpies and estimated entropy changes, reasonable estimates were made for the Gibbs energies and the equilibrium constants for the hydrolysis reactions involving these disaccharides. Using these principles and the data in table 3, similar estimates can be made for the hydrolysis

reactions of a very large number of disaccharides. The value of these estimates are further enhanced by a demonstration (see following paper [22]) that additivity works exceptionally well for predicting thermodynamic parameters for the hydrolysis of oligosaccharides.

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