Carbohydrate Research, 79 (1980) 23–28

THERMAL BEHAVIOR OF SOME REDUCING DISACCHARIDES

JOHN W. LISKOWITZ,
Department of Civil and Environmental Engineering, New Jersey Institute of Technology, Newark, New Jersey 07102 (U.S.A.)
C. EDWIN WEILL, AND BENJAMIN CARROLL
Department of Chemistry, Rutgers, The State University of New Jersey, Newark, New Jersey 07102 (U.S.A.)

(Received July 2nd, 1979; accepted for publication, July 28th, 1979)

ABSTRACT

Thermogravimetry was used to monitor the loss of weight of a series of reducing disaccharides during a heating process. The resulting heating-curves provide a kinetic fingerprint for the individual compounds. It was shown that the configuration of the disaccharides and the nature of the glycosidic bond affect (*a*) the shape of the heating curve, and (*b*) the temperature at which a given disaccharide loses a fixed percentage of its weight. There is also a positive correlation between the thermogravimetric data and the acid-catalyzed hydrolysis of the disaccharides in solution; the disaccharides that lose water at the lowest temperatures are those having the largest rate-constants of acid hydrolysis. Examination of the products of heating indicate polymerization and an intramolecular loss of water, followed by cleavage to form 1,6-anhydroaldohexopyranoses.

INTRODUCTION

The reducing disaccharides lose water when they are heated. This weight loss is part of a process of bond cleavage and bond formation in which such products as polymers[1-3] and anhydro sugars are formed[4-6]. The mechanism of formation of the 1,6-anhydroaldopyranoses has been postulated as involving attack of the oxygen atom of the primary hydroxyl group on the anomeric carbon atom, followed by loss of a molecule of water per molecule[5,6]. When the carbohydrates are subjected to higher temperatures, some of the primary products, such as the anhydrohexoses, are converted into secondary products. Shafizadeh and his colleagues[7], in elegant work, have identified some of the more exotic of these secondary products (such as levoglucosenone, 1,6-anhydro-3,4-dideoxy-β-D-*glycero*-hex-3-enopyranos-2-ulose) that are products of the acid-catalyzed pyrolysis of carbohydrates.

In this laboratory, interest has centered on thermogravimetric analysis in the early stage of the heat treatment, at which time the weight loss is small, and the only volatile product is water. Previous thermogravimetric studies demonstrated that the configuration of a D-aldohexopyranose residue is a factor in the polymerization[8,9]. The purpose of the present investigation was to examine the influence of the type of glycosidic bond and the physical state of the molecules on the thermogravimetric behavior of some disaccharides in the absence of a catalyst.

The disaccharides melibiose hydrate, maltose hydrate, anhydrous lactose, lactose hydrate, and anhydrous cellobiose were selected for study. The weight losses associated with the thermal reactions were measured quantitatively, in an inert atmosphere under dynamic conditions, with a thermal, semimicro balance, and the products of the reaction were analyzed by paper chromatography, and end-group analysis.

EXPERIMENTAL

Equipment. — An Ainsworth Model RU-AU-I automatic, semimicro, recording balance was used in all of the thermogravimetric measurements. A complete description of the apparatus has been published[8].

Materials. — All of the disaccharides were reagent-grade compounds. They were further purified by passage of an aqueous solution through a charcoal–Celite column, the effluent concentrated, and the compound crystallized, and examined for purity by paper chromatography.

Methods. — The initial sample used in all of the thermogravimetric measurements was 556 μmol. The furnace was flooded with argon prior to initiation of the heating process. The temperature was raised at a uniform rate of $6.5°.\text{min}^{-1}$, under a constant flow of argon at a rate of $0.08\ \text{ft}^3.\text{min}^{-1}$.

Descending paper-chromatograms on Whatman No. 1 paper were developed with 6:4:3 (v/v) 1-butanol–pyridine–water, with detection by the alkaline silver nitrate method of Trevelyan *et al.*[10].

The colorimetric procedure of Nussenbaum and Hassid[11] was used in the reducing end-group analyses.

RESULTS AND DISCUSSION

A. Comparison of thermogravimetric curves

The thermogravimetric curves are shown in Fig. 1; two of the most interesting are those for lactose hydrate and anhydrous lactose. If a weight loss of 4 mg is taken as an arbitrary basis for comparison, it is found that this weight loss occurs at ~235° for both of these reactants, and their thermogravimetric curves are quite similar up to this temperature (if allowance is made for the water of hydration in the former); lactose hydrate melts on heating, whereas the anhydrous compound remains solid. Apparently, the water of hydration and the physical state of these disaccharides have little effect upon their thermal reactivity as indicated by thermogravimetric measurements.

Fig. 1 shows a large difference in the thermogravimetric curves of maltose

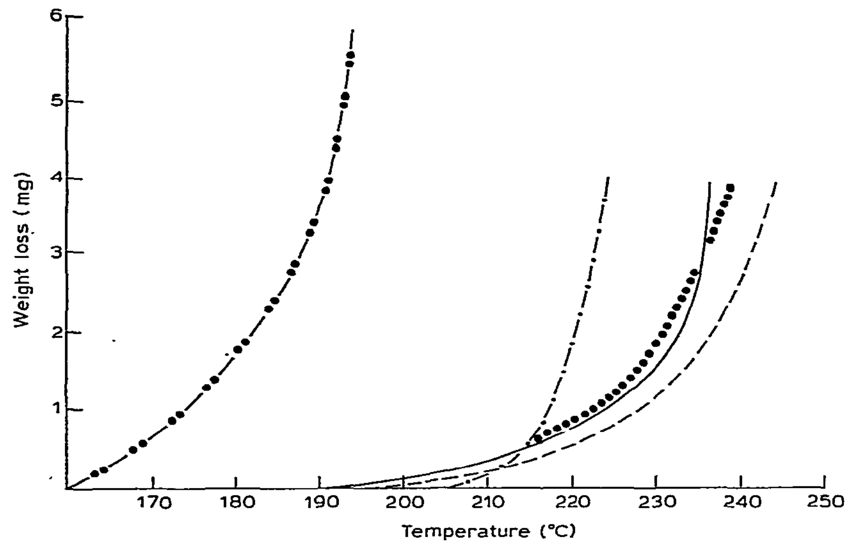

Fig. 1. Thermogravimetric behavior of melibiose, maltose, lactose, and cellobiose. [Anhydrous basis; initial amount of sample, 556 μmol; the samples were heated at 6.5°.min^{-1} in an atmosphere of argon. Key: —··—··—, maltose hydrate; —·—·—, melibiose hydrate; ———, lactose hydrate; ...., lactose (anhydrous); and – – – – –, cellobiose (anhydrous).]

hydrate and cellobiose. Again using the 4-mg weight-loss (on the basis of the anhydrous disaccharides) as the basis of comparison, this weight loss occurs at 190° for maltose, and at 245° for cellobiose. Puddington[12] had also noted this difference, and he attributed the higher stability of the cellobiose to the β-glycosidic bond present in this molecule. Gardiner[6] suggested that the mechanism of pyrolytic cleavage of glycosidic bonds may be similar to the mechanism of the acid-catalyzed hydrolysis of the same bonds. The fact that the rate constant for the acid-catalyzed hydrolysis of maltose is over twice that for cellobiose[13] lends credence to his suggestion.

Lactose [*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose] and cellobiose [*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose] also constitute an interesting pair for comparison. The 4-mg weight-loss for lactose occurs at 235°, ~10° below that observed for cellobiose, and is probably attributable to the D-galactopyranosyl group in lactose. Both α- and β-D-galactose had previously been shown to lose the 4-mg reference-weight at temperatures below those found for the anomers of D-glucose. These results again sustain Gardiner's theory[6] that the mechanisms of pyrolytic cleavage and acid-catalyzed hydrolysis are similar; the rate constant for the acid hydrolysis of lactose is almost twice that of cellobiose.

The behavior of melibiose [*O*-α-D-galactopyranosyl-(1→6)-D-glucopyranose] is more difficult to interpret, because a (1→6)-glycosidic bond is involved; the other disaccharides in this study have (1→4)-bonds. If the 4-mg loss in weight is again used as a reference, it is found that melibiose undergoes this loss at ~223°, compared with 235° for lactose. The loss at the lower temperature for the α-D-linked melibiose seems to be consistent with the results of comparison of maltose and cellobiose, in which case, maltose lost the weight at a temperature well below that of the β-D-linked compound.

The comparison of the thermogravimetric curve of melibiose with that of maltose [*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose] is more difficult to explain. As both of them contain α-D-glycosidic bonds, the presence of the D-galactopyranosyl group in melibiose might have been expected to have caused it to lose weight at the lower temperature. An explanation is possible if the analogy to acid-catalyzed hydrolysis is again used. The rate constant for this acid hydrolysis of maltose is over three times that of its (1→6) analog isomaltose [*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose], suggesting that (1→6)-bonds are more resistant to cleavage.

B. Examination of the products of heating

The samples examined by paper chromatography were heated to a temperature ~20° higher than the reference temperatures shown in Fig. 1. These new temperatures were so chosen as to be high enough to afford an increased yield of some of the initial products, but not so high as to give any significant proportion of the secondary, or more extensively degraded, products.

The results of paper chromatography for three of the disaccharides are shown in Table I; maltose formed an insoluble gel at the more elevated temperature. The respective starting-material constitutes one of the fractions visible in all of the chromatograms, and this was to be expected; even the elevated temperatures used for these samples is not high enough to cause extensive pyrolysis during a short exposure. All of the chromatograms show some 1,6-anhydro-D-aldohexopyranose fraction, having R_{Glc} values considerably greater than 1.0. Such fractions had previ-

TABLE I

CHROMATOGRAPHIC EXAMINATION OF REACTION PRODUCTS[a]

R_{Glc} *values*[b]		
Melibiose	*Cellobiose*	*Lactose*
1.6 m	1.6 w	1.7 m
1.0 s	1.0 ms	1.0 w
0.88 m	0.63 vs	0.88 w
0.69 w	0.50 w	0.54 w
0.45 vs	0.39 m	0.02 s
0.29 s	0.31 m	
0.17 s	0.20 ms	
0.07 vs	0.06 ms	

[a] Standards: D-glucose, 1.0; D-galactose, 0.88; cellobiose, 0.65; lactose, 0.50; and melibiose, 0.44. Intensity of component: m, medium; s, strong; v, very; w, weak. [b] R_{Glc} refers to the distance from the origin relative to D-glucose as unity.

TABLE II

COMPARISON OF OBSERVED AND ESTIMATED PERCENTAGE OF REDUCING END-GROUPS IN THE DISACCHARIDE REACTION-MIXTURES

Disaccharide	*Upper temp. (°C)*	*Percentage of reducing end-groups*	
		Estimated[a]	*Observed*
Lactose hydrate (Calbiochem)	234	61	81
Lactose (anhydrous)	236	61	86
	260[b]	0	8
Cellobiose	245	61	100
	260[b]	13	60
Melibiose	223	61	92
	230[b]	40	43
Maltose	193	41	94

[a]Estimated from thermogravimetric measurements by assuming that, for every molecule of water formed, one end-group reacted. [b]Data taken from Table I. All other numbers refer to Fig. 1.

ously been identified in heated disaccharides[6]. The presence of D-glucose is evident in all of these analyses, and D-galactose in the appropriate ones. Also present are fractions having R_{Glc} values suggestive of tri- and tetra-saccharides and larger polymers.

The percentage of reducing end-groups remaining in the reaction products obtained from the experiments shown in Fig. 1 and Table I are given in Table II. These are compared with the reducing end-groups remaining, as estimated by assuming that the weight loss shown in Fig. 1 is water that results from the intermolecular condensation and intramolecular 1,6-anhydride formation of the disaccharides. In an earlier study[8] with the monosaccharides, it was shown that the reducing end-groups are involved in the intermolecular and intramolecular reactions that involve the loss of water.

Previous work with monosaccharides had demonstrated good agreement between observed and estimated percentages of reducing end-groups. As would be expected, the agreement for the disaccharides in Fig. 1 is not so good. Evidently, the extent of formation of polymer and of 1,6-anhydrohexopyranose is balanced by enough simple cleavage of the disaccharides to raise the value found by end-group analysis. The results are slightly better for the higher-temperature data taken from Table I, but, even in these cases, the formation of polymers and of 1,6-anhydrohexoses is balanced to some extent by the formation of the monomers (D-glucose and D-galactose), which tends to increase the reducing-group value found by analysis. Interestingly, lactose, which shows only small proportions of D-glucose and D-galactose in the heated sample (see Table II), also shows the best agreement between the estimated and the observed values for reducing end-groups.

REFERENCES

1 P. T. Mora, J. W. Wood, and V. M. McFarland, *J. Am. Chem. Soc.*, 82 (1960) 3418–3421.
2 A. Pictet and M. M. Egan, *Helv. Chim. Acta*, 7 (1924) 295–297.
3 F. Micheel and R. Puchta, *Makromol. Chem.*, 48 (1961) 17–23.
4 R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, 64 (1942) 2435–2438.
5 A. Thompson and M. L. Wolfrom, *J. Am. Chem. Soc.*, 80 (1958) 6618–6620.
6 D. Gardiner, *J. Chem. Soc., C*, (1966) 1473–1476.
7 F. Shafizadeh, C. W. Philpot, and N. Ostojic, *Carbohydr. Res.*, 16 (1971) 279–287; F. Shafizadeh and Y. Z. Lai, *J. Org. Chem.*, 57 (1972) 278–284; F. Shafizadeh and P. P. S. Chin, *Carbohydr. Res.*, 46 (1976) 149–154; F. Shafizadeh, R. H. Furneaux, T. T. Stevenson, and T. G. Cochran, *ibid.*, 61 (1978) 519–528.
8 J. W. Liskowitz and B. Carroll, *Carbohydr. Res.*, 5 (1967) 245–255.
9 J. W. Liskowitz and B. Carroll, *J. Macromol. Sci. Chem.*, A2 (1968) 1139–1147.
10 W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature (London)*, (1950) 116.
11 S. Nussenbaum and W. Z. Hassid, *Anal. Chem.*, 24 (1952) 501–503.
12 I. E. Puddington, *Can. J. Res.*, B26 (1948) 415–431.
13 J. N. BeMiller, *Adv. Carbohydr. Chem.*, 22 (1967) 25–108.