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THERMAL REARRANGEMENTS OF CELLOBIOSE AND TREHALOSE

F. SHAFIZADEH AND Y. Z LAI

Wood Chemistry Laboratory, Department of Chemistry and School of Forestry, University of Montana, Missoula, Montana 59801 (U S A)

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ABSTRACT

The thermal transformations and rearrangements of β-cellobiose and trehalose have been investigated by dynamic thermal analysis and parallel, isothermal, chemical methods The reducing disaccharide showed concurrent melting and thermal anomerization, followed by condensation and ultimate decomposition. The non-reducing disaccharide showed dehydration, melting, polymerization, and decomposition The polymeric materials formed on isothermal heating of cellobiose and trehalose were shown to be randomly linked glucans containing pyranoid and furanoid rings and unsaturated components

INTRODUCTION

Pyrolysis of cellulosic materials is of considerable technological interest because it leads to the formation of volatile degradation products which control the flaming combustion of a variety of natural products[1–3] In this laboratory, the pyrolytic reactions are being investigated to develop a better understanding of the chemistry of combustion and flameproofing of cellulosic materials Since these reactions are highly complex and complicated, a variety of model compounds, including reducing sugars[4,5], glycosides[5–10], and 1,6-anhydro-β-D-glucopyranose (levoglucosan)[11–15], have been used to show how the glycosidic bond is cleaved and the sugar molecule is decomposed These studies have shown that heating of the monosaccharide derivatives results in three different types of thermal transformation At lower temperatures, dehydration, melting, and a variety of other physical transitions usually occur At the intermediate high temperatures above the melting point, free sugars show anomerization and inter- and intra-molecular condensation, whereas the glycosides and anhydro sugars give inter- and intra-molecular transglycosylation products, including the free aglycon Further heating results in the thermal decomposition of the sugar moiety

Combination of the dynamic thermal analysis (d t a) and parallel isothermal chemical methods provides a powerful system for investigating the nature of the complex thermal reactions and their products

This article reports on the thermal analysis of a non-reducing disaccharide, trehalose, and a reducing disaccharide, cellobiose, which serve to bridge the gap between the polysaccharides and the related monosaccharide model compounds

RESULTS AND DISCUSSION

The thermal analysis of cellobiose is illustrated in Fig 1, in which the d t a. curve shows a major endothermic peak at 248°, corresponding to the melting point of the sample The peak for the melting point overlapped with a small, broader endotherm to form a shoulder at 260° and is followed by another endotherm at 330° The last two endotherms are accompanied by a sharp weight-loss, shown by the t g a curve The d t g curve shows the rate of weight-loss, which reaches peaks at 262° and 333°, corresponding to the last two endotherms

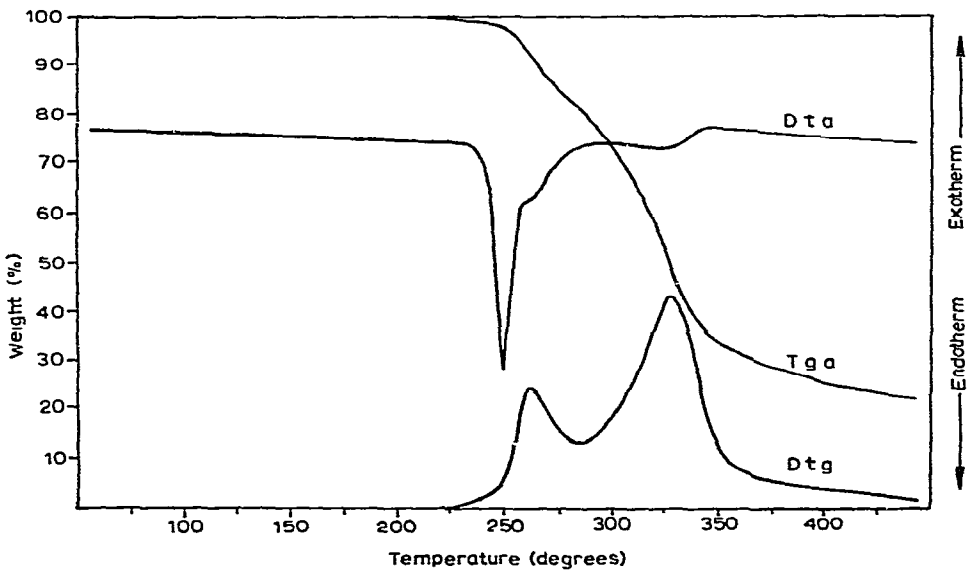

Fig 1 Thermogram of β-cellobiose

It is generally known that reducing sugars seldom display a sharp melting-point, and literature values often cover a wide range Thermal analysis of α-D-xylopyranose has shown that the broad melting-range is due to the equilibration of the α- and β-D forms which takes place simultaneously with the formation of the liquid phase[5] Although β-cellobiose is reported[16] to melt at 225°, its thermal analysis showed a broad melting-endotherm (*cf.* Figs 1 and 2), indicating a simultaneous transformation The course of this transformation was investigated by trimethylsilylation and g l.c analysis of samples heated at temperatures corresponding to different stages in the development of the melting-endotherm in Fig 1 Since the melting-endotherm overlaps with another endotherm, that is accompanied by weight-loss, the resulting g l c data presented in Table I not only show the anomerization of cellobiose but also a gradual disappearance of the molecule However, although most of the cellobiose sample disappears upon heating to 260°, the bulk of the sugar moiety can be recovered as D-glucose after acid hydrolysis (see Table I) Thus, the

endotherm at 260° could be assigned to the condensation of cellobiose molecules The condensation reactions are in turn accompanied by a subsequent decomposition of the sugar moiety Decomposition of the condensation products, which is accompanied by a rapid weight-loss, reaches a maximum rate at 333° and ultimately leaves 25 5% of a relatively stable, carbonaceous residue at 400°

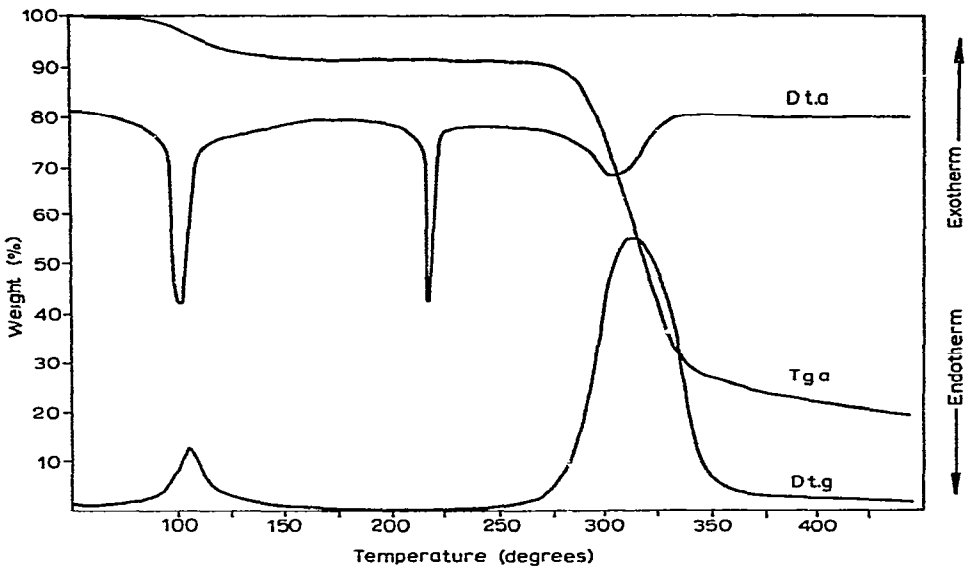

Fig 2 Thermogram of trehalose

Trehalose, as shown in Fig 2, has thermal properties that are entirely different The compound is known to crystallize with two molecules of water[17,18] The loss of the water of crystallization at ~100° has been generally recorded as its melting-point However, depending on the ambient vapor pressure, loss of water and liquefaction could take place at different temperatures The dehydration product forms anhydrous crystals which melt at 215° The complex dehydration and recrystallization characteristics of trehalose will be discussed elsewhere

The melting of trehalose, as seen in Fig 2, is followed by only one major endotherm that is accompanied by a substantial weight-loss reaching the maximum rate (d t g curve) at 313° and leaving a carbonaceous char of ~22 5% at 400° Although the substantial weight-loss and charring represent the degradation of the sugar moiety, g l c analysis of sugar samples heated to various temperatures corresponding to different stages of the final endotherm indicated that the decomposition reactions are preceded by condensation and polymerization of trehalose As seen in Table I, although trehalose rapidly disappears on heating at temperatures above 260°, even at 300° substantial quantities of the D-glucose residues could be recovered by hydrolysis of the heated sample These observations on the thermal behavior of cellobiose and trehalose are in line with the previous results obtained on thermal

TABLE I

ANALYSIS OF THE MIXTURES FORMED ON HEATING OF β-CELLOBIOSE AND TREHALOSE

Disaccharide	*Temperature (degrees)*	*Levoglucosan*	D-*Glucopyranose*		*Remaining cellobiose*				*Remaining trehalose*	D-*Glucose after acid hydrolysis*
			α form	*β-form*	*α-form*	*β form*	*α+β*	$\frac{\alpha \times 100}{\alpha+\beta}$		
β Cellobiose	25				11 6[a]	88 4	100 0	11.6		
	240		0.1	0.1	9 7	76 0	85 7	11.3		
	245	T[b]	0 3	0.3	15 5	66 5	82.0	19 1		
	250	0 1	1 1	1 2	25.5	20 5	46 0	55 4		
	255	0.5	1 2	1 3	12.8	8 5	21 3	60.2		
	260	1 0	0 7	0 7	5 6	3 5	9 1	61.5		70.2
Trehalose	260								101 0	
	270	T	T	T					92 5	
	280	0 1	T	T					85 5	
	290	0 5	T	T					66 5	
	300	1 3	0 1	0 1					7 6	36 8

[a]Percentage yield based on the weight of the sample [b]Trace amounts

anomerization, condensation, and decomposition of reducing sugars, as compared to the transglycosylation and decomposition of the glycosides at relatively higher temperatures

Further information on the nature of thermal polymerization products and reactions was obtained through structural analysis of the polymeric materials formed by isothermal heating of cellobiose and trehalose at 240° and 260°, respectively The resulting mixture, after extraction with methanol, was fractionated by gel filtration on Sephadex G-25 to give the elution patterns shown in Fig 3; these show that both cellobiose and trehalose produce a fraction (*A*) of higher molecular weight which accounts for ~11–16% of the original sample The physical and chemical properties of this product and subsequent fractions (*B*, *C*, and *D*) of lower molecular weight are summarized in Table II

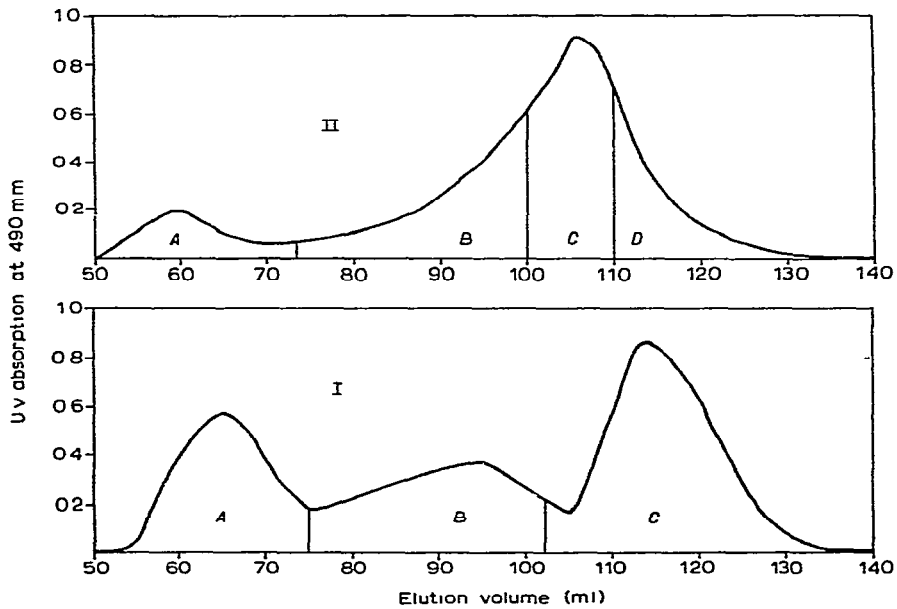

Fig 3 Gel-filtration pattern of the thermal polymerization products of cellobiose (I) and trehalose (II) *A*, polysaccharide fraction, *B*, oligosaccharide fraction, *C* and *D*, disaccharides

The t l c analysis of fraction *A*, derived from both cellobiose and trehalose, showed that it is a completely immobile polysaccharide Fraction *B* was a mixture of oligosaccharides because it showed continuous strips on t l c and contained no starting material on g l c The subsequent fractions *C* and *D* contained mainly the starting materials

Acid hydrolysis of the polysaccharide fraction gave D-glucose, as the only identifiable product, in relatively low yields of 53 5 and 45 6% for cellobiose and trehalose polymers, respectively; this indicates the presence of other units in the polymer The presence of substances other than D-glucose in the polymeric fraction was further confirmed by the observation that the polymer became partially insoluble

TABLE II

FRACTIONATION PRODUCTS OF CELLOBIOSE AND TREHALOSE HEATED AT 240° AND 260°, RESPECTIVELY

Fraction	*Cellobiose*					*Trehalose*				
	Yield	*Color*	$[\alpha]_D^{25}$ *(degrees)*	*Cellobiose content*	D-*Glucose on acid hydrolysis*	*Yield*	*Color*	$[\alpha]_D^{25}$ *(degrees)*	*Trehalose content*	D-*Glucose on acid hydrolysis*
Volatile (weight-loss)	15 4[a]					17 3[a]				
Methanol-soluble	20.3[a]	Brown	+23 9	5.1[b]		22 5[a]	Brown	+24.3	40 3[b]	
Water-soluble	64 3[a]					60 2[a]				
A (polysaccharide)	27 5[b]	Black	+68 1	0	53 5[b]	18 2[b]	Black	+72 0	0	45 6[b]
B (oligosaccharide)	30 3[b]	Brown	+46.8	0	85.8[b]	27 8[b]	Brown	+97.1	0	80.4[b]
C	42.2[b]	Cream		85.8[b]		31 0[b]	Light yellow		23 5[b]	
D						23.0[b]	Light yellow		75.8[b]	

[a]Percentage of the original sample [b]Percentage of the respective fraction.

after the acid treatment. The insoluble residue probably results from further reactions of unsaturated groups in the polymer.

Further evidence for the presence of non-glucose units was provided by the observation that the polymers showed an i r. carbonyl absorption band at 1710 cm^{-1}, u.v. absorption maxima at 220 and 275 nm, and a free-radical signal, detected by e s r. spectroscopy. Similar carbonyl absorption bands have been observed for the products of acid polymerization of D-xylose[19–21], and for the products of thermal polymerization of D-xylose and 4-*O*-methylglucuronoxylan[9]. Originally, they were attributed to the presence of acyclic units derived from the reducing sugar [19–21]. However, since the same bands are observed for the products formed from non-reducing carbohydrates[9], and in view of the considerable evidence for thermal dehydration and rearrangement reactions[4–10] and identification[22] of a 3-deoxy-D-*erythro*-hexosulose from the pyrolysis of D-glucose and cellulose, it has been suggested[9] that the carbonyl absorption could be due to the incorporation of 3-deoxyglycosulose molecules in the polymer or the dehydration and rearrangement of the glycosyl units Units of this type could also account for the u.v. absorption

The oligosaccharide fraction was completely soluble and produced a much higher yield of D-glucose (80–85%) after acid hydrolysis.

The specific rotations of the polysaccharides, from cellobiose (+68 1°) and from trehalose (+72 0°), were similar to those of synthetic glucose polymers[21] having a high content of α-D-glycosidic links These data indicate that 1,6-anhydro sugars could form the intermediate transglycosylation products It is also interesting to note that, when the pyrolysis of cellobiose and trehalose was carried out under vacuum, substantial yields of levoglucosan were obtained[23], because removal of the intermediate compounds from the reaction zone stops further transglycosylation and polymerization reactions.

End-group analysis, involving reduction and acid hydrolysis of the polysaccharides, gave a ratio of D-glucose to D-glucitol of 25–30 1 It should be noted that this value does not represent the true degree of polymerization, since these polymers contain non-glucose units and, possibly, considerable branching and anhydro end-groups[24] Again, as expected, the oligosaccharide fraction (*B*) had a lower ratio (10–12 1) of D-glucose to D-glucitol

Further information on the molecular structure of these polysaccharides and oligosaccharides was obtained by periodate oxidation Fractions *A* and *B* each consumed ~1 6 moles of periodate per "anhydro-D-glucose" unit As in acid hydrolysis, fraction *A* gave an insoluble residue after the oxidation G l c of products obtained on acid hydrolysis of fractions *A* and *B*, after successive periodate oxidation and reduction with borohydride, showed the presence of D-glucose, D-xylose, glycerol, erythritol, ethylene glycol, and trace amounts of D-glucitol, as summarized in Table III. The relative proportions of these products were similar for different fractions of the cellobiose or trehalose polymer, but were quite different for the two types of polymers Cellobiose polymers gave D-glucose as the major product of Smith degradation, whereas trehalose produced more D-xylose.

TABLE III

END-GROUP DETERMINATION AND PERIODATE OXIDATION OF THE THERMAL CONDENSATION PRODUCTS OF CELLOBIOSE AND TREHALOSE

Product	D-*Glucose to* D-*glucitol*	*Periodate consumption*[a]	*Smith degradation products*[b]					
			Ethylene glycol	*Glycerol*	*Erythritol*	D-*Xylose*	D-*Glucitol*	D-*Glucose*
Cellobiose								
Fraction *A*	30 1	1 85						
Soluble part			0 2	0 8	0.3	0 3	T[c]	1
Insoluble part			0 3	0 5	0 2	0.1	T	1
Fraction *B*	12 1	1.77	0 4	3 1	1 3	0.6	T	1
Trehalose								
Fraction *A*	25 1	1.56						
Soluble part			1.3	2 1	0 1	5 0	T	1
Insoluble part			1 0	1 6	0 2	6 0	T	1
Fraction *B*	10 1	1 62	0 2	0.7	0 02	3 2	T	1

[a]Moles of oxidant per D-glucose residue [b]Molar ratios [c]Trace amounts

Formation of unoxidized D-glucose reflects the presence of (1→3)-linkages or multiple-linked units such as (1→2 1→4)-linkages A reducing end-group with the latter type of linkages would be responsible for the formation of D-glucitol Since the starting material had no D-xylose residues, this sugar could be formed from glucofuranose units in which HO-5 and HO-6 were free and HO-2 or HO-3 was blocked Furthermore, glycerol is formed when HO-3 and HO-4 are free, and erythritol is formed when HO-2 and HO-3 are free and HO-4 is blocked The formation of ethylene glycol from normal polysaccharides is rather unusual because it should originate from an acyclic unit substituted at HO-6 but, in this case, it could have been derived from a decomposition product

Various products formed on Smith degradation, and the high proportions of D-xylose and unoxidized D-glucose shown in Table III, indicate that the polymeric materials are randomly linked and highly branched

The above data confirm the results obtained from thermal investigation of D-xylose[5,9] and a variety of glycosides,[5–10] and extend the concepts that have been developed for carbohydrate compounds in general As noted before, heating of free sugars results in thermal polymerization through the reaction of the reducing end-group, whereas heating of the glycosides leads to the formation of the free aglycon and condensation of the glycosyl moiety through a transglycosylation reaction Thus, on heating, cellobiose, trehalose, levoglucosan, and cellulose[25] all provide a mixture of randomly linked, polymeric materials These materials consume ~1 5 moles of periodate, and are qualitatively similar but quantitatively somewhat different For instance, the ratio of D-xylose to D-glucose produced by Smith degradation was 0 3 1 for cellobiose and cellulose, 0 6 1 for levoglucosan, and 5 1 for trehalose This variation could be explained by the fact that inter- and intra-molecular transglycosylation of trehalose should result in the formation of a free sugar, similar to the formation of the free aglycon on thermolysis of glycosides Under the pyrolytic conditions, the free sugar could undergo different types of isomerization, including the formation of furanoid forms that are subsequently condensed and incorporated in the polymeric structure[21 25–27] Since condensation of the reducing end-group in cellobiose takes place ahead of transglycosylation, as shown by the thermal analysis data, the latter reaction could not lead to the formation of a free sugar from cellobiose, nor from levoglucosan or cellulose However, the formation of an intermediate 1,4-anhydro compound, such as 1,4-anhydro-α-D-glucopyranose or 1,6-anhydro-β-D-glucofuranose[25], could account for the lesser proportion of furan-ring structures and D-xylose formation.

EXPERIMENTAL

Analytical methods — The thermal analysis, u v spectroscopy and g l c of carbohydrates, and the Smith-degradation processes were carried out by the methods and equipments described before[5–9] The t l c was carried out on silica IB-F (Bakerflex), using 1-butanol–pyridine–water (6 4 3) The thermal analysis was programmed at the rate of 15°/min

Thermal degradation of cellobiose and trehalose. — Samples of cellobiose and trehalose were obtained from Sigma Chemical Company Small amounts (~2 mg) of the disaccharides were heated, in the d.t a cell, to various temperatures corresponding to the melting or decomposition endotherms, at the rate of 15°/min The heated samples were trimethylsilylated[28] and analyzed for the monomeric compounds, levoglucosan, D-glucose, and the starting materials, cellobiose or trehalose, by g l c The results obtained are given in Table I

Thermal condensation of cellobiose and trehalose — A sample (1 g) of cellobiose or trehalose was placed in a 100-ml flask, which was then flushed with nitrogen and heated in an oven at 240° and 260°, respectively, for 30 min. The resulting weight-loss was then recorded and the heated mixture was extracted with methanol for 4 h The extracted residue (0 6 g) was dissolved in water (5 ml) and chromatographed on column (1 5 × 125 cm) of Sephadex G-25 at room temperature with a flow rate of ~ 10 ml/h. The eluate was collected in fractions of 5–10 ml, and the amount of carbohydrate material in each fraction was determined by the phenol–sulfuric acid method[29] to give the elution patterns shown in Fig 3 The appropriate fractions were combined to give three or four major fractions, as indicated in the chromatograms The materials in each fraction were obtained by concentration of the solution to a small volume followed by freeze-drying Table II summarizes the yields, and physical and chemical properties of the different fractions

Analysis of the polymeric materials — Samples (5 mg) of the polymeric materials were hydrolyzed with boiling M hydrochloric acid (2 ml) for 8 h. D-Glucitol (5 mg) was used as the internal standard. The solutions, after neutralization with Amberlite IR-45(HO^-) resin, were filtered and evaporated to dryness The residues, after trimethylsilylation[28], were analyzed by g l c Small amounts of fraction *A* polysaccharide became insoluble after acid treatment

For determination of the end-groups, samples (5 mg) of the polymeric materials were dissolved in water (5 ml) containing sodium borohydride (20 mg). After 48 h at room temperature, the excess reagent was decomposed by Amberlite IR-120(H^+) resin and the filtered solution was evaporated to dryness The boric acid in the residue was removed as the methyl ester, by repeated evaporation with methanol under diminished pressure. The residual material was then hydrolyzed and analyzed by g l c , as previously described

For periodate-oxidation experiments, solutions of samples (8.0 mg) of each fraction in 15mM sodium metaperiodate (10 ml) were kept in the dark The periodate consumption was determined on aliquots (0.5 ml), which were first diluted to 100 ml with distilled water and then analysed by the spectrophotometric method[30]. Periodate consumption reached a constant value after 4–5 days The oxidation products were identified by using the procedure of Alfes and Bishop[31].

As in the acid hydrolysis, small amounts of fraction *A* polysaccharide became insoluble after the oxidation. This insoluble residue was also collected and analyzed for the oxidation products

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