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SYNTHESIS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF MALTULOSE AND CELLOBIULOSE

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ABSTRACT

The title compounds were prepared in ~90% overall yield by treatment of the appropriate aldodisaccharide (maltose or cellobiose) with boric acid and triethylamine in dilute, aqueous (pH 11, 70°) solution. When sodium hydroxide was substituted for triethylamine, the yields were unaltered, but reaction rates were increased. In addition, in the sodium hydroxide system, reactions at higher concentrations of sugar (up to 40% w/v) could be performed with only slight decreases in overall yields. New methods were developed for the high-performance liquid-chromatographic analysis (Zorbax-NH_2 column, modified with Column Life Extension Agent) of the sugars involved in these isomerizations. Multigram quantities of pure ketodisaccharides were prepared with a simple semi-preparative l.c. system.

INTRODUCTION

As a part of a program to identify new carbohydrates having potential food applications, we have studied two ketodisaccharides, maltulose (4-*O*-α-D-glucopyranosyl-D-fructose) and cellobiulose (4-*O*-β-D-glucopyranosyl-D-fructose). Because neither compound is readily obtainable from natural sources, and the common methods for their synthesis are quite tedious, little is known about their physicochemical properties or their potential food and pharmaceutical applications. Recently, we developed a synthesis for a similar ketodisaccharide, lactulose[1,2] (4-*O*-β-D-galactopyranosyl-D-fructose). That sugar has several unique medical and food uses and is now an important commercial product. Because maltulose and cellobiulose have structures similar to that of lactulose, they may also have useful properties.

Maltulose was first identified as a product from the action of salivary alpha amylase on liver glycogen[3]. Peat *et al.*[3] synthesized it from maltose by a Lobry de Bruyn–Alberda van Ekenstein transformation. That transformation, involving base-catalyzed isomerization of an aldodisaccharide (maltose) into the ketodisaccharide (maltulose) was also the basis for several later syntheses of both maltulose[4–6], and cellobiulose[7,8] from their isomeric aldodisaccharides. These methods, however,

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are far from ideal as the ketoses are produced in low yields, are unstable under the reaction conditions, and must be tediously isolated from a mixture of alkaline degradation-products and families of isomeric mono- and di-saccharides. Mendicino[9] discovered that, in such reactions, the yield of ketose could be improved greatly by the addition of borate to the starting mixture. Borate acts as a ketose-complexation agent that shifts the equilibrium toward the ketose and protects that product from degradation. Because relatively large amounts of borate were found necessary for high yields of ketose (at least a 50:1 borate–sugar ratio), this reagent has been considered impractical for most synthetic purposes. Modifications in which borate is immobilized on anion-exchange resins[10,11], have also been used to prepare both maltulose and cellobiulose, but the yields for these processes were not reported, and substantial quantities of concentrated borate solution were required to elute the firmly bound ketoses from the resin. Other complexation reagents, such as free[12] and column-bound[13] aluminate, have been used to prepare maltulose. Aluminate, however, was very difficult to remove from the final product in the former case and in the latter, the product yields were lower, in part because of increased alkaline-degradation reactions

In this paper, we describe how maltose and cellobiose may be isomerized conveniently and efficiently, in the presence of a minimal amount of boric acid, into maltulose and cellobiulose. We describe first, methods for the semi-preparative isolation and quantitative determination of these ketodisaccharides by high-performance liquid chromatography (l.c.).

RESULTS AND DISCUSSION

Chromatographic isolation and identification of maltulose and cellobiulose. — When maltose or cellobiose were initially isomerized under conditions similar to those described later, chromatographic analysis revealed the presence of at least three minor components, in addition to the newly generated disaccharides. Pure samples of these disaccharides were needed for identification purposes and also as quantitative l.c. standards. Although certain techniques, such as chromatography on charcoal–Celite or separation of the acetylated derivatives on silica gel, might have been useful for purifying the reaction products, we found direct, semi-preparative l.c. on Aminex*-Q-15S resin (Ca^{2+} form) to be ideally suited to this purpose. Fig. 1 illustrates the use of this technique in isolating the major product from the isomerization of cellobiose. Fig. 1A demonstrates the separation of a test mixture of the four sugars expected in the reactions. Fig. 1B is a typical chromatogram of the crude mixture; the shaded region corresponds to the fraction collected. In this manner, 100 mg of a chromatographically pure (Fig. 1C) product could be isolated in <40 min. The isomerization product from maltose was similarly chromatographed to yield the pure

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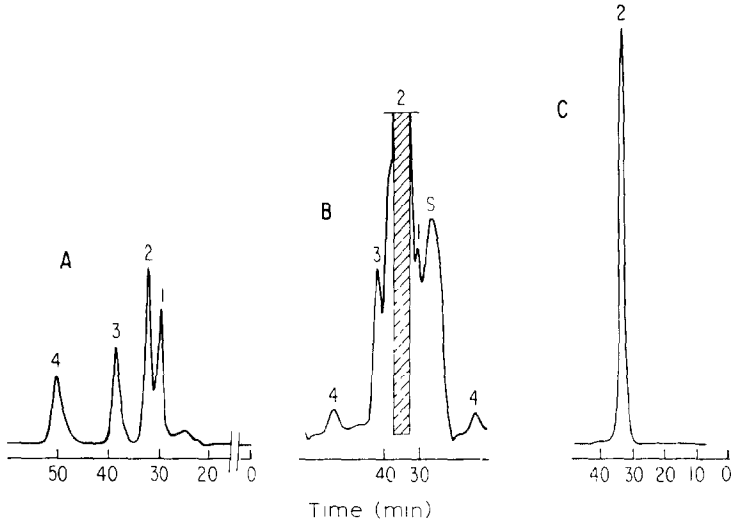

Fig. 1. A. Semi-preparative separation of cellobiose (1), cellobiulose (2), glucose (3), and fructose (4) on an Aminex Q-15S (calcium form) column; flow rate of the mobile phase (water): 0.33 mL/min; sample: 20 μL of solution containing 100 mg/mL of each sugar; detector: differential refractometer. B. Semi-preparative separation, showing the fraction collected (shaded region) from a 200-mg (1 mL) injection of crude isomerized cellobiose; other conditions, same as A; S: peak containing organic and inorganic salts. C. Sample collected from B, chromatographed under the conditions of A.

ketose. Retention data for maltose and its reaction product were similar to those of cellobiose and its reaction product in this chromatographic system. The chromatographic properties of these products give insight into their chemical structures. Sugars are separated on calcium-form cation-exchange resins by size exclusion[14] and by their differing abilities to complex with calcium ions[15]. Glucose and fructose are well separated on thse columns, presumably[15] because fructose exists partially as the β-furanose tautomer, which possesses a pair of *cis*-hydroxyl groups stereochemically favorable for complexing with calcium. Under the conditions of separation, glucose exists solely in pyranose forms, with hydroxyl-group orientations that do not favor complexation with calcium. The major products from the isomerization reactions of these disaccharides are eluted before both monosaccharides and have retention times consistent with those of reducing disaccharides. The fact that these major products are eluted after their corresponding aldodisaccharide is consistent with structures that bear the favorably complexed, reducing fructose residue.

The product derived from maltose was identified as maltulose by its m.p. by elemental analysis of its crystalline monohydrate, and by complete ^{13}C-n.m.r. characterization[16].

The cellobiose derivative was identified as cellobiulose, based on its chromatographic properties, the results of partial acid hydrolysis, elemental analysis of the amorphous solid, and ^{13}C-n.m.r. spectral characterization[16].

Analytical l.c. of ketodisaccharides. — The yields of ketodisaccharides produced

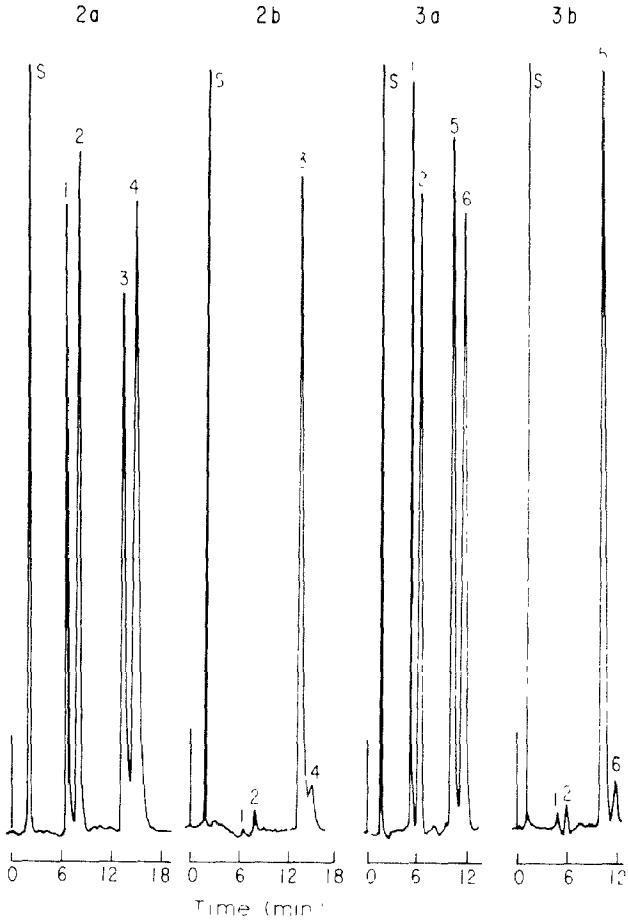

Fig. 2. Separation of fructose (1), glucose (2), maltulose (3), and maltose (4) on Zorbax-NH_2 mobile phase; 19:6 (v/v) acetonitrile–water containing Column Life Extension Agent; flow rate, 1.5 mL/min; refractive-index detection, 8×. a, Standard mixture. b, Profile of maltose isomerization reaction.

Fig. 3. Separation of fructose (1), glucose (2), cellobiulose (5), and cellobiose (6) on Zorbax-NH_2 mobile phase; conditions as in Fig. 2; Flow rate, 2 mL/min. a, Standard mixture b, Profile of cellobiose isomerization reaction

under a variety of experimental conditions were conveniently determined by l.c. Because of poor resolution between aldo- and keto-disaccharides, and the relatively long analysis-time, the Aminex Q-15S column was not used for this purpose. Instead, an aminopropyl bonded-phase, packed column, used in the normal-phase partition mode, was more suited to these analytical separations. Amino-type bonded-phase packings and similar "carbohydrate" columns are now routinely used for separating such sugar mixtures as those of isomeric monosaccharides[17] or soluble oligomers from hydrolyzed polysaccharides[18]. The separation of isomeric disaccharides is challenging because of the extreme similarity of their structures. However, we were

TABLE I

RETENTION AND RESOLUTION OF GLUCOSE, FRUCTOSE, MALTULOSE, AND MALTOSE ON ZORBAX-NH_2 MODIFIED WITH COLUMN LIFE EXTENSION AGENT[a]

Sugar	t_R *(min)*	k[b]	N[c]	R[d]
Fructose	6.8	2.5	3130	2.4
Glucose	8.0	3.3	2050	
Maltulose	13.4	6.0 (4.6)[e]	3200 (3600)	1.6 (1.2)
Maltose	15.0	7.0 (5.1)	2600 (2950)	

[a]Mobile phase same as in Fig. 2. [b]Phase-capacity factors. [c]Number of theoretical plates. [d]Resolution between adjacent pairs of isomers. [e]Values in parentheses are for an identical separation without Column Life Extension Agent.

TABLE II

RETENTION AND RESOLUTION OF GLUCOSE, FRUCTOSE, CELLOBIULOSE, AND CELLOBIOSE ON ZORBAX-NH_2 MODIFIED WITH COLUMN LIFE EXTENSION AGENT[a]

Sugar	t_R *(min)*	k[b]	R[c]
Fructose	5.2	2.7	2.25
Glucose	6.2	3.4	
Cellobiulose	9.9	6.0	1.8
Cellobiose	11.5	7.2	

[a]Mobile phase same as in Fig. 3. [b]Same as Table I. [c]Resolution between adjacent pairs of isomers.

able in a previous study[19] to separate lactose adequately from lactulose. Direct application of that method yielded satisfactory results for the separation of cellobiose from cellobiulose, but was not effective for the maltose–maltulose system. Excellent resolution for both sets of isomers was obtained, however, with the amino column, when an organic amine modifier (Column Life Extension Agent) was added to the mobile phase. Separations of standard mixtures of the four major sugars found in maltose and cellobiose isomerization reactions are shown in Figs. 2a and 3a. Chromatographic profiles of reactions are illustrated in Figs. 2b and 3b. Complete separation, with excellent resolution between adjacent pairs of isomers (Tables I and II) was achieved in 15 min or less for these mixtures.

Amine modifiers of various types have been used to improve the efficiency and/or selectivity of resin[20] and plain silica[21,22] columns for carbohydrate separations. The effect of the Column Life Extension Agent on the aminopropyl column used here is demonstrated in Table II for the separation of maltose and maltulose. Addition of the amine to the mobile phase decreased the column efficiency slightly, but increased the selectivity, resulting in an overall superior resolution between aldo- and

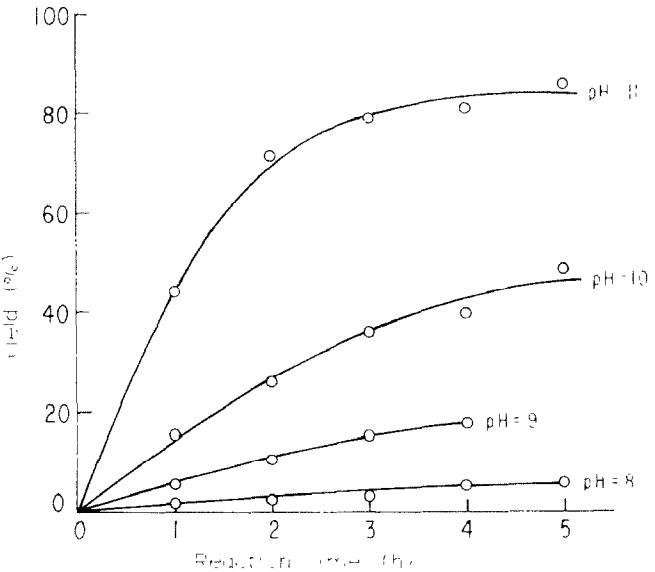

Fig. 4. Yields of cellobiulose from cellobiose. Starting concentration of cellobiose 5% (w/v); boric acid–carbohydrate = 1.1 (molar basis); temperature of reaction, 70 °. The pH was adjusted by addition of triethylamine.

keto-disaccharide. This improvement allowed the quantitative determination of sugars present in disproportionate amounts (Figs. 2b and 3b).

Synthesis of ketodisaccharides — In a previous study[1], we found that lactose could be efficiently converted into lactulose in aqueous solution when boric acid and a tertiary amine were present. As maltose and cellobiose are (1→4)-linked aldo-disaccharides, structurally analogous to lactose, we pursued a similar conversion of these compounds into their corresponding ketodisaccharides. Thus, when cellobiose was allowed to react in dilute solutions (5% w/v) containing boric acid and triethylamine, another disaccharide was generated. This disaccharide, isolated by semi-preparative l.c., was identified as cellobiulose. Both the rate of its formation and the reaction yield increased with the pH of the medium (Fig. 4). When the temperature and concentrations of carbohydrate and boric acid were kept constant, an increase in the concentration of triethylamine (leading to higher pH values) led to higher yields of product. The highest yield observed under those conditions (86%) was at pH 11, after 5 h of reaction. As with lactose[1], these reactions proceeded with very few alkali-catalyzed, degradative side-reactions. Even under the most basic conditions (pH 11) the yield of product was high, and the total weight of sugars present in the final product accounted for ≥97% of the starting amount of cellobiose. The final mixture contained fructose (1%), glucose (3%), cellobiose (10%), and cellobiulose (86%).

The isomerization of maltose into maltulose was also studied. Maltulose so produced was isolated by semi-preparative l.c. and identified as the crystalline mono-

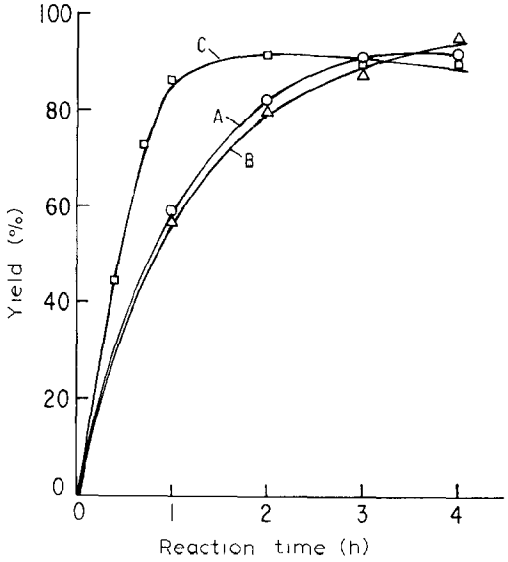

Fig. 5. Yield of maltose from maltose isomerization reactions. Conditions: 5% (w/v) concentration of carbohydrate, pH 11, 70°. A. pH adjusted with triethylamine, vented reaction-vial. B. pH adjusted with triethylamine, sealed vial. C. pH adjusted with dilute sodium hydroxide.

hydrate. Because of the previously demonstrated pH dependence on reaction rate and product yield, the conversion of maltose was studied at pH 11.0 only. The yield of maltulose, from a solution that originally contained 5% (w/v) of maltose, a 1:1 molar ratio of carbohydrate to boric acid, and triethylamine (pH 11) as base, reached a maximum at ~4 h (Fig. 5, curve A). This rate profile was similar to that seen for the conversion of cellobiose. The maximum yield, however, was slightly greater for the conversion of maltose (>92%) than of cellobiose (86%). When this (curve A) reaction was performed, the reaction vessel was warmed to 65° before sealing, and was then vented at hourly intervals when samples were withdrawn. During the initial heating-period, and when the mixture was vented, noticeable amounts of gases were emitted, undoubtedly including vaporized triethylamine (b.p. 88°). To determine whether the small loss of this reagent affected product yield, a similar reaction was performed in which the vessels were sealed before heating and left unvented until analysis. The rate of product formation and final yield were nearly identical (Fig. 5, curve B) however, to the previous, vented reaction (curve A). The pH values of the two mixtures after 4 h of heating were 10.4 (vented) and 10.3 (sealed). Obviously, only a minor proportion of the triethylamine is lost during the warm-up and venting process. When a similar mixture was allowed to react for 4 h without sealing at any time, maltulose was formed in only 15% yield. Therefore, it appears that some closure of the vessel is necessary for optimal yield, but dangerous pressures in the reaction vessel need not be tolerated.

Triethylamine is a convenient catalyst for these isomerizations because it is

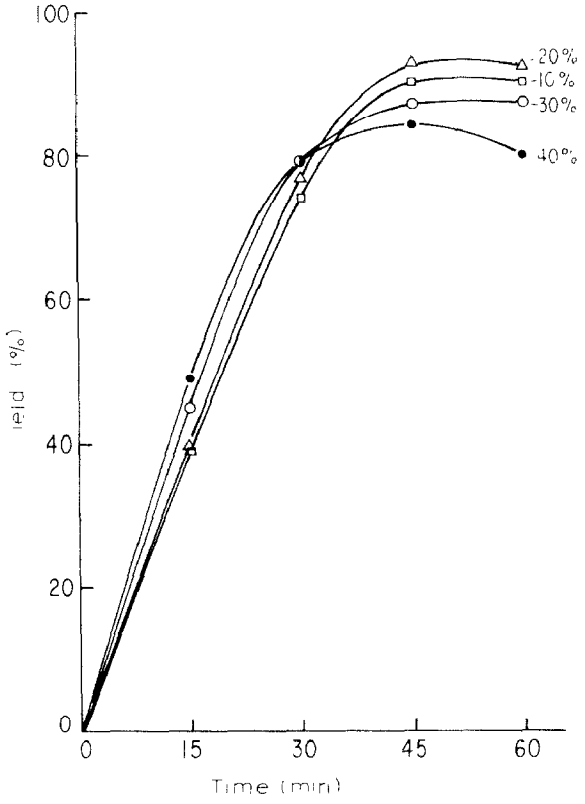

Fig. 6 Effect of starting concentration of maltose on the yield of maltulose. Conditions: pH 11, 70°, pH was adjusted with sodium hydroxide.

suitably basic, cannot cause glycosylamine-mediated, degradation reactions, and is readily recovered and recycled from one reaction to the next. These, and other advantages of this amine have been discussed[1,2]. However, because triethylamine is only slightly soluble in water, we could only conduct reactions under dilute conditions. When higher concentrations of reactant and boric acid were used, not enough triethylamine could be dissolved to reach pH 11 and, hence, optimum reaction conditions were not obtained. As it was of interest to us to prepare ketodisaccharides in more-concentrated reactions, the use of sodium hydroxide as a replacement for triethylamine was investigated. The maltulose synthesis described in Fig. 5 (curves A and B) was repeated, but the pH was adjusted to 11 with dilute sodium hydroxide. The rate of reaction (curve C) was higher than in the previous reactions, but the yield remained nearly the same. Maltose was then isomerized in more-concentrated solutions, from 10 to 40% w/v (Fig. 6). It appears that optimum reaction-times, under these conditions, depend only slightly on the initial concentration of reactant as the maximum yield, in every case, occurred at ~45 min. The yields, however, were highest for the reactions at lower concentration. For example, chromatographic analysis of the 20% (w/v) reaction at 60 min showed fructose (2.7%), glucose (3.7%),

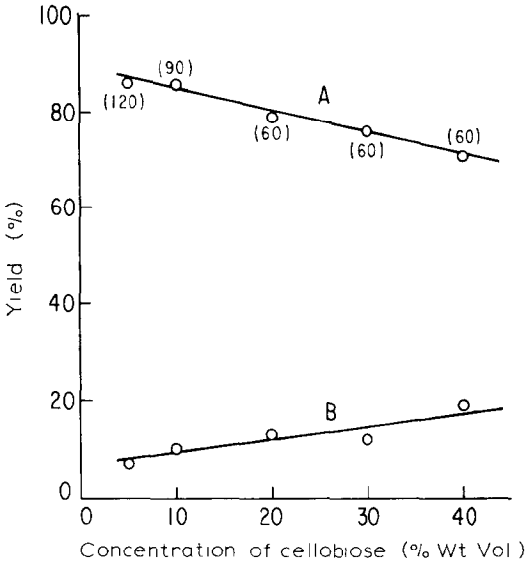

Fig. 7. Effect of starting concentration of cellobiose on yield of cellobiulose; conditions: pH 11, 70°. A. Maximum yield of cellobiulose (reaction time in parentheses). B. Percentage of unidentified materials in sample. pH was adjusted with sodium hydroxide.

maltulose (92.6%), and maltose, <0.1%; unidentified material (by difference) was 1%. Analysis of the 40% reaction at 45 min gave fructose (3.3%), glucose (2.9%), maltulose (84%), maltose (3.1%), and unidentified material (6.7%). The unidentified material is the weight difference between the total dry weight of sample and the total chromatographically determined weights of known sugars in the sample. This material is a combination of unknown sugars or sugar by-products that are not identified and quantitatively determined. It is noteworthy that in all reactions, there was very little drastic degradation of sugar, or production of saccharinic acid. Sample aliquots at various times contained experimentally equal dry weights, even after purification by ion exchange.

The isomerization of cellobiose at elevated concentrations did not proceed quite so readily as that of maltose. The maximum yields at specified reaction-times (Fig. 7, curve A) were proportionally less for the more-concentrated solutions and a larger amount of unidentified products was present in the final mixtures (curve B).

It is noteworthy that, in the preparation of these ketodisaccharides in concentrated solution, the final isolation procedure appears to be especially critical. It was noted that some and perhaps extensive loss of product may occur during the removal of boric acid if the process is not carefully controlled. When this occurred, new and unknown peaks having retention times slightly greater than those of monosaccharides appeared on the chromatogram. It is apparent that these side reactions are partially responsible for the lower yields seen in the more-concentrated reactions, where this problem was noticed more often. With careful technique, however, good yields of both products may be obtained.

It has been shown here that (1→4)-linked aldodisaccharides, in the presence of an equimolar amount of boric acid and a basic catalyst, may be isomerized to ketodisaccharides in nearly quantitative yields. In previous studies where aldodisaccharides were treated with alkali alone[5-7], low yields of the ketoses were produced and much of the sugar was lost through alkaline-degradation reactions. It is apparent that boric acid in this system acts to increase the yield of ketose and to protect this product from alkaline degradation. Boric acid or alkali metal borates have previously[9,10] been used as complexation reagents for similar purposes, but not always with such high-yielding results. The mechanism whereby such complexation reagents as borate increase the yields of a particular reaction product is well known. In such base-catalyzed isomerization reactions as these, a pseudo-equilibrium is established between aldose and ketose and, in some instances, other isomeric compounds. If borate is present, and if it preferentially forms a stable complex with only one of the equilibrating tautomers, the equilibrium will be shifted toward that species. The complexation reagent acts as a trap for that particular product. In practice however, finding conditions that favor complexation of only one member in the reaction scheme is often difficult because of the variety of factors that govern the degree of complexation of sugars with borate. Some of these factors[23], such as overall reaction concentrations, pH, and ratio of carbohydrate to borate, can be experimentally modified to optimize the yield of product. Other factors are uncontrollable, such as the fixed stereochemical arrangement of hydroxyl groups in the product and reactant molecules. Specifically, borate or boric acid forms stable complexes only with sugar-hydroxyl groups in favorable orientations. The stereochemical features of borate–sugar complexation have been studied extensively[23-28]. It is now known that certain arrangements of hydroxyl groups, such as adjacent *cis*-hydroxyl groups on furanose rings, form strong bidentate complexes with borate. These complexes are generally more stable than those derived from borate species reacting with *cis*-hydroxyl groups from pyranose ring-systems. The (1→4)-linked aldodisaccharides used in this study do not contain furanose ring systems, nor can they form them through mutarotation. Only the hydroxyl groups at C-1 and C-2 of the α-D-glucopyranosyl group would be available for borate complexing. This arrangement would lead to a predictably weak borate complex. This assumption has been confirmed in studies involving conductivity measurements[24], electrophoresis[25], and n.m.r. spectroscopy[26]. On the other hand, the isomeric (1→4)-linked ketodisaccharides contain reducing fructosyl groups that exist[16] partially in furanose forms. These ketose products would, therefore, be expected to form strong complexes with borate through the *cis* 2- and 3-hydroxyl groups of the β-D-fructofuranosyl group, or even a stronger tridentate (O-1, O-3, and O-6 of α-D-fructofuranosyl group) complex, as has been proposed for fructose[26] in the presence of borate. We have obtained indirect evidence for ketodisaccharide–borate complexation by observing major changes in the ^{13}C-n.m.r. solution spectra of ketodisaccharides when borate is added. The overall high yields of (1→4)-linked ketodisaccharides in these isomerization reactions is therefore readily explicable from these observations. When the monosaccharides, glucose and galactose[2], and

(1→6)-linked aldodisaccharides (unpublished results) were allowed to react under the same conditions described here, lower yields of the ketoses were produced. In these instances, both reactants and products can exist in furanose forms that contain favorably oriented hydroxyl groups. As both reactant and product are complexed, the equilibrium is not shifted to the extent seen in the (1→4)-linked disaccharide system. It appears that the reagents and conditions used in this study are ideally suited for the preparation of 4-*O*-substituted ketoses.

Of these two ketodisaccharides, only maltulose has as yet been isolated crystalline. Maltulose does not crystallize well in the presence of maltose, or of oligosaccharide impurities[29], and therefore we had to purify the product by semi-preparative l.c. prior to crystallization. Alternatively, the maltose (starting material) could be recrystallized until it was oligosaccharide-free, isomerized, and the resulting maltulose crystallized directly. By the procedures outlined here, it is now possible to readily prepare gram quantities of pure maltulose and cellobiulose.

EXPERIMENTAL

General. — Maltose monohydrate (Sigma), cellobiose (Pfanstiehl Labs.), other sugar standards, boric acid, triethylamine, and sodium hydroxide were all the finest commercial grades available. Amberlite IR-120 (H^+) and Duolite A-561 (free base) resins were used for deionization. Amberlite XAD-4 was used for decolorization of solutions. Melting points are uncorrected. Elemental analyses were performed by a commercial laboratory.

Analytical, high-performance liquid chromatography. — Chromatographic solvents were all l.c. grade. Column Life Extension Agent (Alltech) was added to acetonitrile–water mobile-phase mixtures at a level of 2 drops per L of pre-mixed solvent. Chromatography was performed with a Beckman model 334 system, consisting of a model 421 controller, two model 110A high-pressure pumps, and an Altex universal injector. For sample detection, a Waters model 401 differential refractometer and MFE model 21125B recorder was used. Sugars were quantitatively determined by peak-height analysis and external-standard methodology. Prior to analysis, standard sugar solutions and reaction samples were both dissolved in 1:1 acetonitrile–water at concentrations <20 mg/mL. Response factors for each sugar were calculated by linear-regression analysis of a plot of peak heights versus standard sugar concentrations. Correlation coefficients were, in all instances, >0.999. Standards were injected occasionally during analyses to correct for changes in detector sensitivity. Prior to chromatography, all catalysts and boric acid were removed from samples.

Isolation of ketodisaccharides by semi-preparative, high-performance liquid chromatography. — The previously described chromatographic system was used in conjunction with a 9 × 600-mm, waterjacketed (80°) column packed with Aminex Q-15S (Ca^{2+} form). Pure, degassed water was used as the mobile phase. The deionized samples to be injected were dissolved in water at total sugar concentrations ≤400 mg/mL. A 1-mL injection loop was used. Samples of pure ketodisaccharides were

collected, pooled, and evaporated *in vacuo* in the presence of methanol, to yield light, fluffy powders. Maltulose was crystallized as the monohydrate, according to the procedure of Hodge[12], m.p. 116.5–118.5°. Partial acid hydrolysis of the ketodisaccharide at 80° in 0.1M sulfuric acid, yielded two monosaccharides that had retention times identical to those of fructose and glucose on both Zorbax-NH_2 and Aminex Q-15S columns.

Anal. Calc. for $C_{12}H_{22}O_{11} \cdot H_2O$: C, 40.00; H, 6.72. Found: C, 39.96; H, 6.81.

Cellobiulose was likewise isolated as a white, fluffy, hygroscopic, and amorphous powder. Partial acid hydrolysis yielded the monosaccharides glucose and fructose.

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 41.82; H, 6.52.

Preparation of ketodisaccharides with triethylamine–boric acid at various pH values. — Within an efficient fume hood, four mixtures containing 5.0 g of cellobiose or maltose, 0.90 g of boric acid, and 85 mL of water were titrated in 100-mL beakers to pH 8.0, 9.0, 10.0, or 11.0 with triethylamine. All mixtures were then diluted to 100 mL, and then transferred to 250-mL round-bottom flasks that were immersed in a bath thermostatted at 70°. The vessels were allowed to reach 65° and were then sealed with rubber septa. In another set of experiments, the vessels were sealed before heating to determine whether loss of volatile amines, escaping during the warm-up period, affected reaction yield. The reactions were sampled initially, and during the course of the reaction, by removing 5-mL aliquots. Each aliquot was deionized and decolorized by passage through a column (1 × 15 cm) containing 7.5 mL of IR-120 (H^+), 2.5 mL of Amberlite XAD-4, and 2.5 mL of Duolite A-561 (free base) resins. The column effluent was carefully evaporated *in vacuo* at 30° to a thick syrup, but not to dryness. At this point, 10 mL of abs. methanol were added, and the syrup was then evaporated to dryness. This process was repeated twice more to remove the boric acid as methyl borate. Evaporation of the original column effluent to complete dryness at higher temperatures resulted in the formation of several new and unidentified products. These compounds were not produced when the foregoing precautions were taken during the isolation procedure. The dry sugar residues were then dissolved in equal volumes of acetonitrile–water and analyzed by analytical l.c.

Preparation of ketodisaccharides at elevated concentrations. – Maltose and cellobiose were isomerized to ketodisaccharides at 10, 20, 30, and 40% (w/v) concentrations of starting sugar. Under these conditions, sodium hydroxide was used as the base catalyst. All concentrated reactions were conducted at 70° and pH 11. In a typical reaction at 40% (w/v), 40 g of cellobiose and 7.2 g of boric acid were dissolved in water (30 mL) as the solution was titrated to pH 11 by dropwise addition of 10 mL of 4M sodium hydroxide. This solution was diluted to exactly 100 mL and placed in a 250-mL, round-bottom flask kept at 70°. The flask was sealed and the contents analyzed as described for the previous reactions.

Preparation of crystalline maltulose without semi-preparative chromatography. — Maltose was recrystallized 3 times to remove trace impurities of oligosaccharides[30]. A 20.0-g sample of the purified maltose was mixed with boric acid (3.56 g) and 60 mL of water and then titrated to pH 11 with 4M sodium hydroxide. The entire sample

was diluted to 100 mL and then heated for 3 h at 70° in a 250-mL, septum-sealed, round-bottom flask. The solution was then cooled, poured through a column consisting of 100 mL of IR-120 (H^+), 90 mL of XAD-4, and 70 mL of A-561 (free-base resins). The effluent was collected, evaporated, and treated with methanol as described, to remove boric acid. The residue (17.0 g) was then dissolved in water (80 mL), in a 1-L round-bottom flask that was placed in a thermostatted bath at 45°. Slowly, a total of 500 mL of acetone was added to the flask, which was kept at 45° during, and for 1 h after, the addition. The solution was then allowed to cool slowly to room temperature. The solid material, which crystallized from the solution during the next week, was filtered off; yield 10.25 g. This maltulose contained small amounts of monosaccharide impurities. The filtrate was placed in a 1-L flask again, and acetone was added dropwise until the solution became turbid. As the solution cleared, crystals of maltulose monohydrate deposited on the sides of the flask. This process was repeated until no more maltulose would crystallize. The crystals (3.75 g) were isolated by filtration, m.p. 116–119°. The chromatographic and ^{13}C-n.m.r. spectroscopic properties of this compound were identical to those of the previously described sample of maltulose.

ACKNOWLEDGMENTS

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