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EVIDENCE OF STABLE HYDROGEN-BONDED IONS DURING ISOMERIZATION OF HEXOSES IN ALKALI

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ABSTRACT

Epimeric pairs of aldohexoses and the related ketohexose were isomerized in aqueous KOH at various temperatures and pH values, and the mixtures then analyzed by h.p.l.c. on either a cation-exchange resin or a reversed-phase column. It was found that the proportions of starting aldohexoses remaining after several days often exceeded those of the same that were formed from the epimeric aldoses and the corresponding ketoses. The difference with allose, gulose, and mannose was much larger than with the other aldohexoses. These differences are rationalized by assuming that anomers having the OH groups attached to C-1, C-2, and C-3 in an axial–equatorial–axial or an equatorial–axial–equatorial arrangement form especially stable, hydrogen-bonded ions or molecular complexes that disturb the equilibrium state and affect the isomerization and mutarotation reactions.

INTRODUCTION

The epimerization of monosaccharides in basic media has been extensively studied[1–20]. This reaction proceeds mainly by reversible enolization[1–9], which involves formation of a 1,2-enediol by abstraction of a proton located α to the carbonyl group of the acyclic form of the sugar. Addition of a proton to C-2 of the 1,2-enediol results in the formation of two epimeric aldoses, whereas addition to C-1 yields the related ketose. In the early literature, it was assumed that abstraction of a proton from an acyclic sugar to form the enediol and addition of a proton to the enediol were mediated by the medium. Later, Kieboom and his co-workers[17–19] suggested that an intramolecular transfer of the alpha proton to the negatively charged oxygen atom on C-5 of the pseudocyclic intermediate[10,11] of a pyranose was responsible for the formation of 1,2-enediols. This concept was used to explain why the isomerization of glucose and fructose is faster than that of mannose. An intramolecular aldose–ketose isomerization pathway had been suggested by the Fiesers[12], who proposed that the hydroxyl group on C-2 of an acyclic aldose dissociates, and the α hydrogen atom (with two electrons) undergoes intramolecular transfer to C-1, to yield the corresponding ketose in the acyclic form. Gleason and Barker[13] confirmed the presence of this pathway in the isomerization of ribose to

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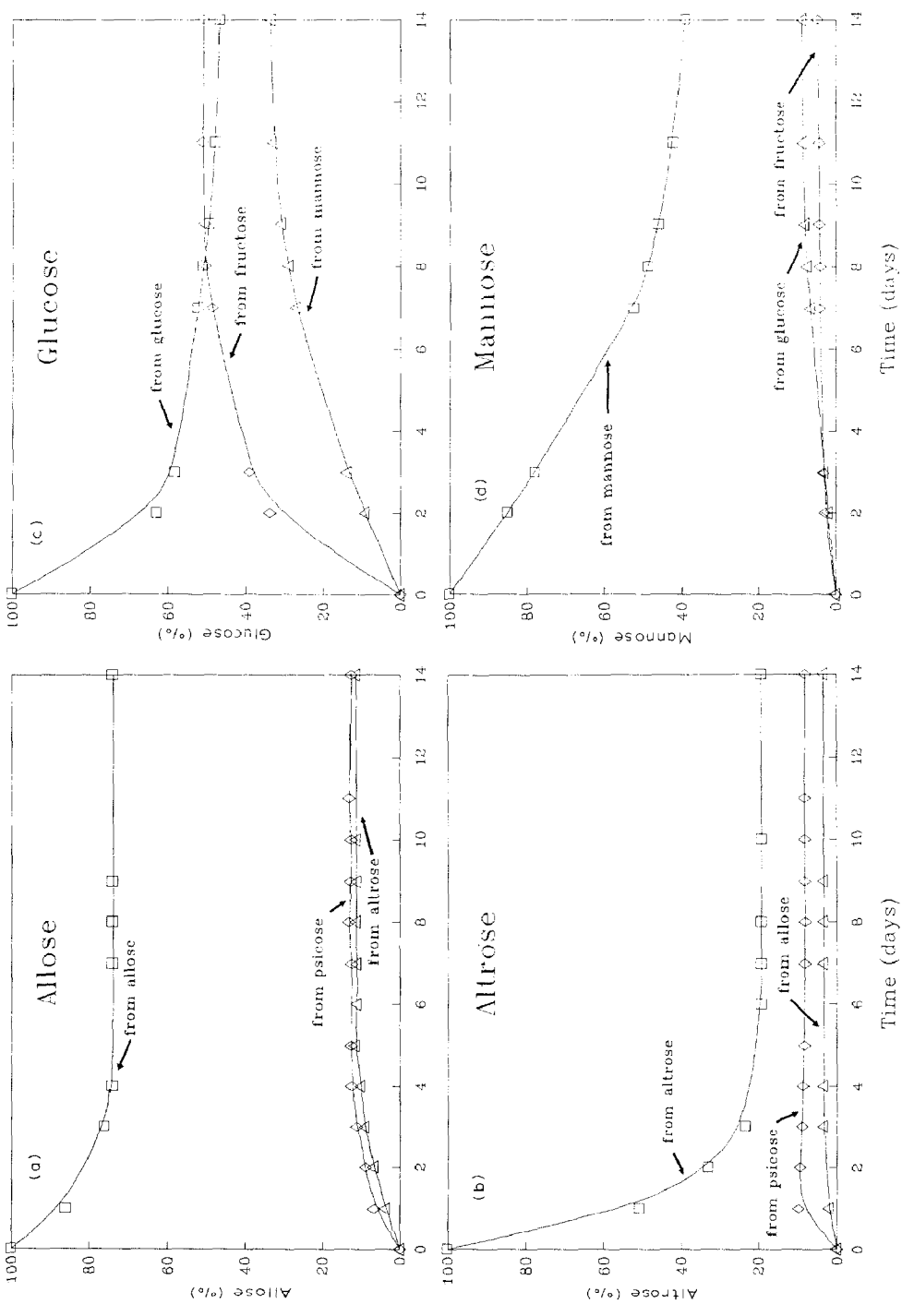
(a)
Allose
from allose
from psicose
from altrose
Allose (%)
(b)
Altrose
from altrose
from allose
from psicose
Altrose (%)
(c)
Glucose
from glucose
from fructose
from mannose
Glucose (%)
(d)
Mannose
from mannose
from glucose
from fructose
Mannose (%)
Time (days)
Time (days)

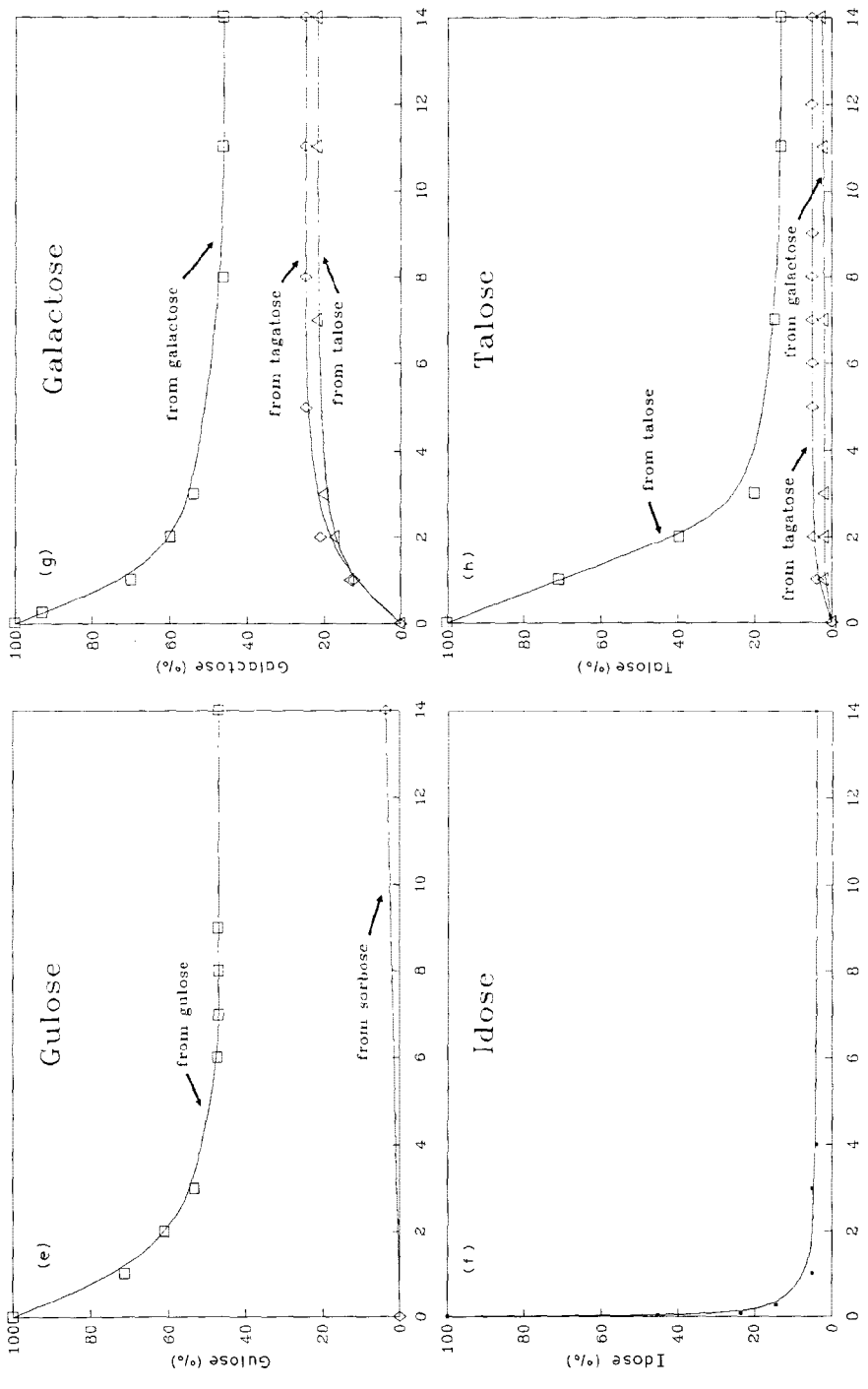

Fig. 1. Isomerization of aldohexoses with aqueous KOH at pH 11.5 and 25°. Graphs show the consumption of each aldohexose (due to isomerization), and its formation from its epimeric aldose and related ketose in the course of 14 days. Percentages are based on the total monosaccharides present in each solution.

arabinose, and designated the process isomerization by hydride transfer. Because these pathways proceed through a common intermediate, namely, the 1,2-enediol in the case of reversible enolization, and the acyclic ketose in the case of isomerization by hydride transfer, one would expect to find at equilibrium the same proportion of the epimeric aldoses and related ketose, irrespective of which sugar is isomerized. According to studies with epimerases under physiological conditions, the equilibrium ratios of glucose:mannose:fructose should be[14] 41:18:41.

DISCUSSION

In a recent study[20], we found that glucose, mannose, and fructose in aqueous KOH at 25° and pH 11.5 do not reach equilibrium in many days. The proportion of sugars found after many days depends on which isomer is used as the substrate. The aim of the present work was to shed light on this result by studying in detail the isomerizations of aldohexoses and related ketoses in alkaline media. To obtain a clear understanding of the reactions, each of the eight aldohexoses and the four related ketoses was isomerized at various temperatures in aqueous KOH having different pH values. The solutions were periodically analyzed by h.p.l.c. in order to monitor changes in the composition of the reaction mixtures.

Fig. 1 summarizes the results of aldohexose isomerizations carried out at pH 11.5 and 25°. Each frame depicts three plots (obtained from three different experiments). The uppermost plot reports the ratio of an aldohexose consumed during isomerization, and the lower two, the ratios of the same aldose found in solution when the epimeric aldose and the related ketose are isomerized. In the systems allose–altrose–psicose, glucose–mannose–fructose, galactose–talose–tagatose, and gulose–idose–sorbose, it may be seen from the curves that after several days, there is nearly always more of the starting aldohexose in solution than is formed from its epimeric aldose and related ketose under like conditions. The largest differences were observed for allose, mannose, and gulose. After isomerization for 14 days, 75% of the initial allose remained in solution, in comparison to the 11 and 13% formed under like conditions from altrose and psicose, respectively. Furthermore, more altrose remained after isomerization than was formed from allose and psicose (19% *versus* 4 and 7%). With glucose, it was found that the proportion of this sugar remaining after isomerization (50%) was nearly equal to that produced from fructose (51%), but exceeded that obtained from mannose (40%). A larger proportion of the initial mannose remained in solution when this sugar was isomerized, compared to that of mannose produced from glucose and fructose (39% *versus* 9 and 5%). Similarly, more galactose remained in solution than was produced from talose and tagatose (50% *versus* 40 and 30%), and more talose remained than was formed from galactose and tagatose (10% *versus* 3.5 and 5%). Likewise, more gulose remained in solution after isomerization (40%), than was produced from sorbose or idose (5 and <1%). The idose results are somewhat questionable, because the only idose available was amorphous and contained a substantial percen-

tage of sorbose; this is not surprising, as idose is slowly converted into sorbose in neutral solutions[21]. Under the conditions of the experiments, it was largely converted into sorbose in two days.

The foregoing results show that the isomerization of aldohexoses is affected by some factor that hampers direct enolization or hydride transfer reactions. We consider that the inhibiting factor is the formation of an inert hydrogen-bonded (Hb) ion or complex. Presumably, stable Hb ions of allose and gulose are formed from the α-pyranose anomers, whereas a stable Hb ion of mannose is formed from the β-pyranose anomer. These anomers have the hydroxyl groups attached to carbon atoms 1, 2, and 3 in axial–equatorial–axial or equatorial–axial–equatorial arrangements, and they form especially stable Hb ions, as well as $CaCl_2$ complexes[22–25].

Many years ago, it was shown[22] that a solution of D-gulose and calcium chloride contains, at all dilutions, two classes of molecule, namely, associated D-gulose–calcium chloride and completely dissociated α- and β-D-gulose in equilibrium. The degree of dissociation is influenced by the concentration; in dilute solution, the complex is largely dissociated into the sugar and calcium chloride, but, at high concentration, it exists mainly as α-D-gulose–$CaCl_2$–H_2O. These results show that a change in the concentration of D-gulose–calcium chloride causes a disturbance in the anomeric equilibrium. The results of the present investigation suggest that hydrogen-bonded, complex ions formed from reducing sugars under alkaline conditions also disturb the equilibrium state. The hydrogen-bonded anomer cannot undergo direct enolization or hydride transfer, whereas the non-bonded anomer can. This accounts for the anomalous behavior of sugars having the mannose, gulose, and allose configurations.

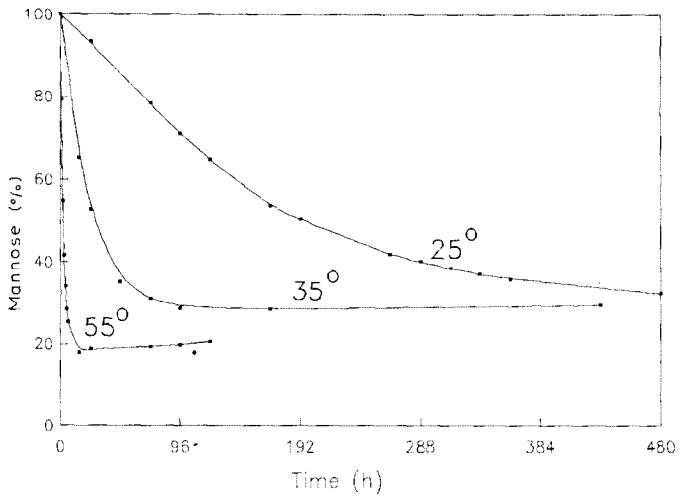

Fig. 2. Isomerization of mannose at different temperatures with aqueous KOH at pH 11.5. Curves show the percentage of mannose remaining, based on the total monosaccharides present in the solution after different time-intervals at 25, 35, and 55°.

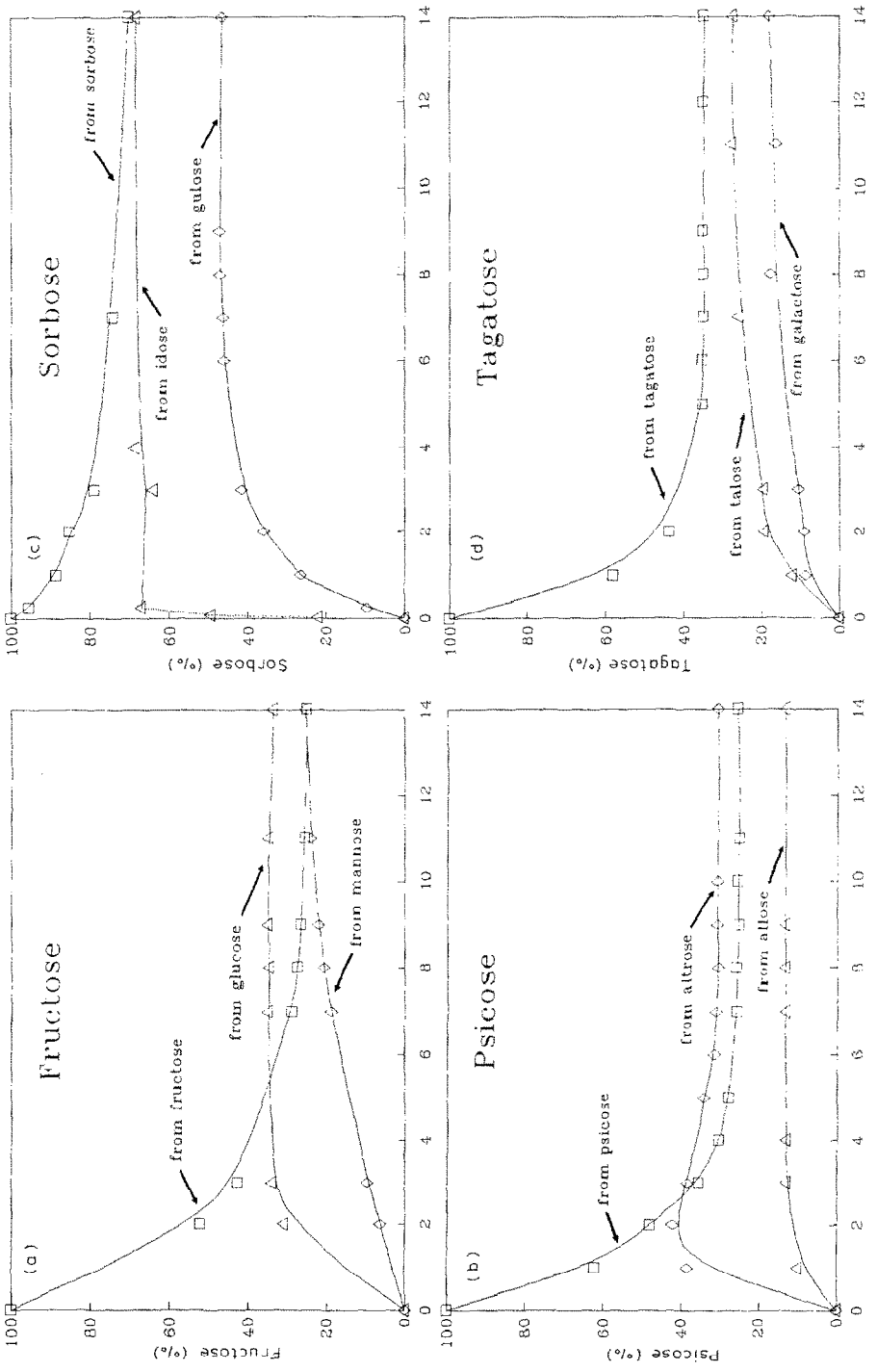

Fig. 3. Isomerization of hexuloses with aqueous KOH at pH 11.5 and 25°. Graphs show the consumption of each hexulose (due to isomerization), and its formation from the two related aldohexoses in the course of 14 days. Percentages are based on the total monosaccharides present in each solution.

The results depicted in Fig. 2 support this hypothesis. The curve for the reaction at 25° shows that many days are required for the isomerization of mannose in anomeric equilibrium, and that the process is incomplete, even after 15 days. At 35°, ~70% of the original mannose is isomerized in 4 days. At 55°, ~80% of the original mannose is consumed in 1 day. The original mannose solution contained ~40% of β-D-mannopyranose. Presumably, the β-pyranose anomer forms the relatively inert Hb ion almost instantly. With long reaction times, or at higher temperatures, the Hb ion is slowly decomposed; the kinetics of the system are complex.

Raising the pH of the reaction mixture increased the proportions of the rearrangement and degradation products, which in turn decreased the total amount of sugars present. Thus, the isomerization of glucose at 25° was not detectable at pH 9.5; it required four weeks to reach a steady state at pH 10.5, and 5 days at pH 11.5. The isomerization of mannose at the same temperature required 14 days at pH 11.5.

It should be noted that, at higher pH or temperature, the proportion of rearrangement products increased dramatically. Thus, for example, after 5 days at 55°, the total monosaccharides remaining in a solution of mannose undergoing isomerization amounted to only 16% of the starting sugar.

The isomerization of ketoses was similar to that of aldoses, but was found to be complicated by their capability to form both 1,2- and 2,3-enediols. Fig. 3 shows that, after two weeks at pH 11.5 and 25°, the proportion of psicose remaining after isomerization (26%) was less than that of psicose formed from altrose (31%) but exceeded the proportion of psicose produced from allose (13%). The proportion of fructose remaining after the same period (*viz.*, 25%) equaled that of fructose formed from mannose, but was less than that obtained from glucose (34%). Similarly, after two weeks, the proportion of sorbose remaining in solution (74%) was slightly higher than that of sorbose formed from idose (68%), but exceeded that produced from gulose (47%). Finally 35% of the tagatose remained after isomerization of this ketose, *versus* 27% and 18% of tagatose produced from talose and galactose, respectively. The higher reactivity of ketoses in general, and their ability to form both 1,2- and 2,3-enediols, probably introduce complicating factors that render the effect of Hb ions less evident with ketoses than with aldoses.

EXPERIMENTAL

Materials. — Commercial samples of certain of the hexoses were used directly (glucose, galactose, fructose, sorbose, and psicose) or after recrystallization (mannose). The allose, altrose, talose, idose, and tagatose used had previously been prepared and purified by one of us (H.S.I.). Gulose was obtained from gulose·$CaCl_2$ by passing an aqueous solution thereof through a column of Rexyn I-300 ion-exchange resin. Idose, which is a syrup, contained sorbose (25%).

Methods. — To study the isomerization of hexoses, the hexose (200 mg) was dissolved in water (10 mL), and treated dropwise with 2M KOH to bring the pH to

11.5. The solutions were kept in a constant-temperature bath under a nitrogen atmosphere, and the pH was adjusted to within 0.1 unit in order to maintain the solutions at the desired pH. The frequency of adjustment depended upon the rate of isomerization. Slow-isomerizing aldoses, such as allose and mannose, needed adjustment every 4 days, whereas rapidly isomerizing aldoses, such as glucose, required daily adjustments. The mixture was analyzed by h.p.l.c. using one or more of the following column systems.

(*a*) The main system consisted of a series of cartridge-type columns purchased from Pierce Chemical Company, Rockford, IL. Two analytical Polypore PB columns were used for the separation of saccharides. These are columns of cation-exchange resin in the Pb^{2+} form, each 22 cm long. They were preceded by two guard columns, a Polypore H (3 cm) and a Polypore AN (3 cm), inserted to avoid damage to the analytical columns by the alkali or the saccharinic acid salts.

(*b*) In this system, the two guard columns were followed in series by one column of Polypore CA cation-exchange resin in the Ca^{2+} form (10 cm long) and one column of Polypore PB (22 cm long). This combination was used to improve the separation of the mannose and fructose peaks, which are not well separated by the lead columns alone.

Both column systems (*a* and *b*) were heated to 60° with a water jacket, and eluted with water. A constant flow-rate of 0.25 mL/min was maintained with a Waters model 501 pump (Millipore, Waters Chromatography Division, Milford, MA).

(*c*) A reversed-phase column (Carbohydrate Analysis from Waters Associates) was used to determine the relative amounts of sorbose and galactose present during the isomerizations of galactose, talose, and tagatose. This was necessary because the galactose and the sorbose peaks were not well resolved with systems *a* and *b*. The reversed-phase column was eluted with 17:3 acetonitrile–water, and cooled to 15° with a water jacket. Although the performance of the column was improved by cooling, it did not match that of either system *a* or *b*.

Aliquots (20–50 μL) were taken with a microliter syringe, and injected directly into the chromatograph through a Rheodyne 7125 injector equipped with a 5-μL loop for cation-exchange columns and a 20-μL loop for the reversed-phase column. The saccharides were detected with a Waters 410 differential refractometer attached to a Hewlett–Packard 3392 integrator. Known weights of authentic sugars were used in order to identify the isomerization products and to determine their concentration. The percentage of each sugar was calculated relative to the total monosaccharides present in solution. In the cation-exchange resin systems *a* and *b*, saccharinic acids were removed by the guard columns prior to chromatography, and, in the reversed-phase system *c*, saccharinic acids were not detected.

The results obtained are depicted in Figs. 1, 2, and 3. The plots were obtained by using Harvard Presentation Graphics software on an IBM-XT computer.

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