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CONTRIBUTION OF THE REACTION PATHWAYS INVOLVED IN THE ISOMERIZATION OF MONOSACCHARIDES BY ALKALI*

HASSAN S. EL KHADEM, SOFIANE ENNIFAR, AND HORACE S. ISBELL
Department of Chemistry, The American University, Washington, D.C. 20016 (U.S.A.)

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ABSTRACT

The isomerization of eight hexoses and four pentoses, in aqueous KOH at pH 11.5 under nitrogen, was monitored by l.c. on cation (Pb^{2+})-exchange and reverse-phase columns. The primary epimerization reaction of pentoses and hexoses accounts for ~90% of the saccharides found in solution after one week. The remaining saccharides were formed in part by fragmentation and recombination. This is indicated by the formation of hexoses (glucose and sorbose) during the isomerization of pentoses, and by the simultaneous formation of 3- and 4-epimeric hexuloses from hexoses. The aldol reaction responsible for the formation of hexoses (glucose, fructose, and sorbose) from glyceraldehyde at pH 11.5 was found to be fast ($t_{1/2}$ = 2 h), which accounts for the rapid fragmentation and recombination observed.

INTRODUCTION

For many years the complex reactions of sugars in alkaline solutions have engaged the attention of chemists, who have proposed several pathways to account for the products formed. It was generally assumed that all the pathways start with the carbonyl form of the sugar. In 1900 Wohl and Neuberg[1] proposed that the interconversion of glucose, mannose, and fructose, observed by Lobry de Bruyn and Alberda van Ekenstein[2], occurs through 1,2-enediol intermediates. Nef[3] extended the idea to include successive enolizations and ketonizations. Abstraction of the hydrogen atom on C-3 of 2-ketohexoses followed by electron shifts affords 2,3-enediols, which can give by the reverse process two 3-epimeric 2-ketoses and two 2-epimeric 3-ketoses. The latter may in turn ionize to yield two 3,4-enediols which can afford all of the ketohexoses and aldohexoses of the D and L configuration. Because the carbon chain is not disrupted during this process, an aldohexose labeled at C-1 will afford isomers having the label at C-1 and C-6. The isomers obtained *via* 3-ketoses and 2,3-enediols would be labeled on C-1, whereas those formed *via* 4-ketoses and 4,5-enediols would be labeled at C-6. Sowden and

*Dedicated to Dr. R. Stuart Tipson.

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Thompson[4] found that D-[1-^{14}C]glucose in alkali gives D- and L-sorbose having 91% of the label at terminal carbon atoms (C-1 and C-6), and only 9% at non-terminal carbons, thus demonstrating that a large proportion of the chain remains intact. Similar results were recently obtained by King-Morris and Serianni[5], who found that D-(1-^{13}C)mannose isomerized in alkali to afford small proportions of glucose, mannose, and fructose enriched with ^{13}C at C-6, but not at carbon atoms 2, 3, 4, or 5. De Wit[6] found that ~1.2M KOH was required to abstract carbon-linked protons in order to afford 1,2-enediols.

Isbell[7] measured the rates of hydrogen exchange during isomerization with alkali, and found that for most monosaccharides, reversible enolization was the major pathway to isomerization.

Another pathway was proposed by Evans[8] to explain the extensive isomerization of hexuloses and aldohexoses that occurs on prolonged treatment with alkali. He suggested that fragmentation by reverse-aldol reactions, followed by recombination by aldol reactions, led to isomerization because the products formed are either achiral or racemize in alkaline media.

A third pathway was discovered by Gleason and Barker[9], who showed that hydride shifts may occur during epimerization. They found that [2-^{3}H]ribose in aqueous alkali affords significant proportions of [1-^{3}H]arabinose. Hydride shifts start with the ionization of a hydroxyl group, followed by migration of a hydride ion to an adjacent carbonyl group (possibly through a cyclic intermediate) to afford the dissociated ketoses. Although hydride shifts that convert ketoses to their 3- and 4-epimers have not been reported, they cannot be excluded.

DISCUSSION

In the present work, the isomerization of monosaccharides was studied under mild conditions that minimize the formation of acidic rearrangement-products. A number of hexoses and pentoses were treated at 25° with aqueous KOH at pH 11.5 under a nitrogen atmosphere for various periods of time. The mixtures were periodically analyzed by l.c., using cation-exchange and reverse-phase columns. Over long periods, the mixtures became colored and showed the presence of saccharinic acids and polymeric compounds, which were not studied here. High-performance liquid chromatography (l.c.) was selected as the analysis method because it is able to detect and quantitate most of the minor constituents produced when monosaccharides are treated with base, and it allows the analysis to be repeated at short time-intervals. The l.c. method is superior to that of gas chromatography because the latter requires derivatization, and often does not detect sugars other than those produced by the epimerization reaction[10].

A close study of the different isomerization pathways reveals that each one has characteristic features which may be used to distinguish it from the others:

(*a*) Order of formation of isomers. Ketoses afford 3-epimeric ketoses and 4-epimeric ketoses successively by reversible enolization, and simultaneously by

the fragmentation pathway. This is because in the first pathway (enolization), the rate-determining step is the abstraction of a proton located α to the carbonyl group. Accordingly, the time needed to form an isomer will increase along with the number of enolizations necessary to produce it. For example, formation of the 3-epimeric 2-ketose requires one enolization and therefore should be much faster than the formation of the 4-epimeric 2-ketoses, which requires two additional enolizations (one to form the 3,4-enediols from the 3-ketoses, and one to form the 2,3-enediols from the latter).

(*b*) Chain extension or contraction: In the reversible-enolization and hydride-shift pathways the carbon chain remains intact, whereas in the fragmentation pathway it may be extended or contracted. For example, an aldopentose yields, by fragmentation, glyceraldehyde and the enediol of glycolaldehyde, which recombine to afford isomeric pentoses. In addition, two molecules of the glyceraldehyde produced may combine to afford hexoses.

(*c*) Carbon-linked protons exchanged: An aldose undergoing isomerization exchanges one carbon-linked proton in the reversible-enolization pathway, more than one proton in the fragmentation pathway, and none in the hydride-shift route. Because the number of protons exchanged increases as the isomerization progresses, the observed number is not definitive of the enolization and fragmentation pathways.

Hexoses. — In theory, the isomerization of a hexose should lead to 8 hexuloses and 16 aldohexoses of the D and L series. However, because enantiomers cannot be separated on the columns used, only 4 ketoses and 8 aldoses could be detected. Half of these were formed in significant proportions, whereas the other half constituted <1% of the sugar mixture (except when glucose was one of these, and then the concentration of this half was higher).

A summary of the composition of the mixture of saccharides obtained from hexoses after a reaction period of two weeks is given in Table I. The most plentiful isomers in the mixture were the starting sugar and the products of the primary epimerization-reaction (the epimeric aldose and the corresponding ketose). These were followed, in terms of abundance, by the 3-epimeric ketose and the two 4-epimeric ketoses. The epimeric aldose and related ketose appeared first, followed by the 3- and 4-epimeric ketoses, which appeared simultaneously. This result suggests that some fragmentation and recombination takes place. Tetroses and octoses were not detected, suggesting that fragmentation between C-2 and C-3 was not important.

Fig. 1 shows the consumption of glucose, mannose, and fructose and the formation of their major isomers with time. After ~2 weeks, no marked change in composition of the reaction mixtures was observed. The curves for the three sugars showed substantial differences.

The isomerization of D-glucose in aqueous KOH maintained at pH 11.5 was relatively rapid. Fructose was the first and the most abundant isomer formed in the mixture. It was detected after one min of reaction, and was followed shortly there-

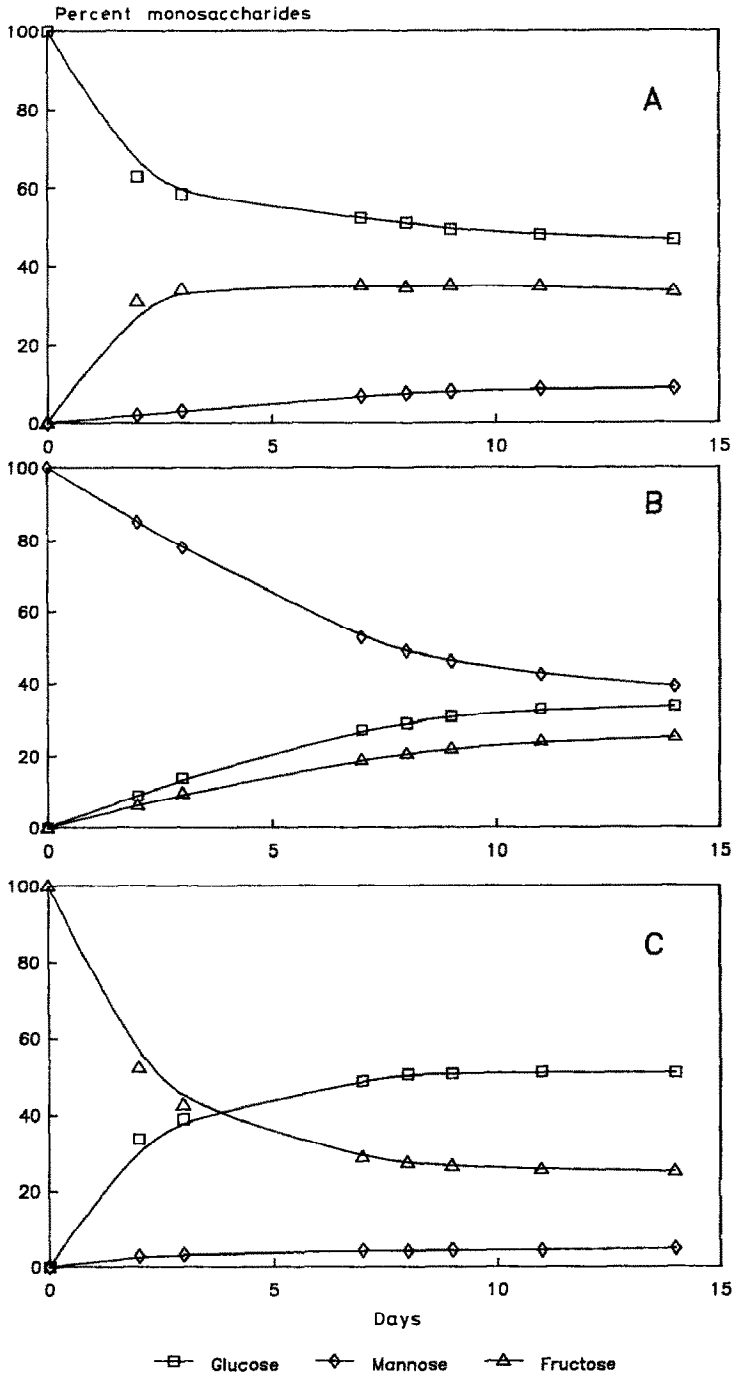

Fig. 1. The isomerization of glucose (A), mannose (B), and fructose (C) at 25° in aqueous KOH at pH 11.5.

TABLE I

COMPOSITION OF THE MIXTURE OBTAINED BY TREATING AQUEOUS SOLUTIONS OF SOME HEXOSES WITH KOH AT pH 11.5 FOR 2 WEEKS

Starting hexose	*Ketoses formed (%)*				*Aldoses formed (%)*		
	Psi	*Fru*	*Sor*	*Tag*	*Glc*	*Others*	
D-Glucose	4.4	33.5	3.6	1.5	50.0	Man	8.4
D-Mannose	3.0	25.3	3.0	<1.0	29.4	Man	39.2
D-Fructose	8.3	25.0	10.0	1.4	51.0	Man	3.7
D-Galactose	1.8	5.1	18.0	15.0	4.1	Gal	50.1[a]
D-Tagatose	0.8	4.6	6.9	33.8	1.0	Gal	43.5[b]
D-Idose	3.1	16.5	60.0	2.1	1.0	Gul	1.0
L-Sorbose	2.0	9.0	64.0	8.0	3.3	Gul	6.6
D-Allose	8.8	2.8	<1.0	<1.0	1.2	All	75.8[c]

[a]Talose, 3.5%. [b]Talose, 3.7%. [c]Altrose, 0.9%.

after by mannose. The importance of the epimerization reaction was apparent from the high ratio of glucose, mannose, and fructose, which after one week amounted to ~90% of the sugar mixture detected. After ~2 h psicose, sorbose, and tagatose were detected in rapid succession. The simultaneous formation of the three ketoses is best explained by fragmentation of the fructose formed during the epimerization reaction. If no fragmentation had occurred, psicose (the 3-epimer) would have appeared before sorbose and tagatose (the 4-epimers).

When a solution of D-mannose in dilute KOH was kept at pH 11.5, the rate of isomerization was lower than that of glucose. The first isomer to appear was glucose, which was detected after 10 min. The amount of fructose formed was less than that obtained from glucose in the same period of time. Small proportions of psicose and sorbose were detected simultaneously, and tagatose was the last hexose to appear. The rate of isomerization of mannose during the first 5 h of reaction was comparable to, although slightly lower than, the rate of hydrogen exchange[7] (based on the exchange of one proton), suggesting that its epimerization was proceeding mainly by reversible enolization.

The relatively low reactivity of mannose in comparison to glucose is not unusual[11]. It may be ascribed to a high energy-barrier in passing from the acyclic form to the enediol[6,10]. It is surprising, however, that after 2 weeks, when the consumption of sugar had nearly ceased, the mixture still contained >35% of mannose. It was expected that at equilibrium the mannose content would be the same as that found in the isomerization of glucose and fructose, i.e. <10%, but this was not the case. This aspect is being investigated further.

The isomerization of D-fructose was faster than that of glucose and mannose. The first isomer to appear in the mixture was glucose, followed by mannose.

Psicose, sorbose, and tagatose were detected at nearly the same time (the first two in significant amounts). After two weeks, fructose yielded more 3- and 4-epimers (20%) than did glucose (10%) and mannose (6%).

The rate of isomerization of L-sorbose was much lower than that of fructose and tagatose. When sorbose was treated with KOH maintained at pH 11.5, three peaks appeared simultaneously; these were gulose/idose (an unresolved peak), tagatose (a 3-epimer), and fructose (a 4-epimer). Presumably, the first two sugars were produced by the epimerization reaction, whereas fructose was probably formed in part by fragmentation and recombination. After 24 h, psicose (another 4-epimer) appeared, and the proportion of tagatose and fructose steadily increased. When L-sorbose was treated at the same pH with $Ca(OH)_2$, instead of KOH, the percentages of the resulting hexoses were substantially different and fructose was produced in a much larger proportion (12%). By contrast, the isomerization of D-idose proceeded rapidly, mostly affording one of the products of the epimerization reaction (sorbose). After 2 h, two-thirds of the starting sugar was converted to sorbose, and after one week practically none of the starting sugar remained.

D-Tagatose isomerized rapidly in the presence of alkali, and after 2 weeks the mixture contained galactose (43.5%), tagatose (33.8%), small proportions of sorbose and fructose (6.9 and 4.6%), and only 3.7% of talose. D-Galactose isomerized more slowly; after two weeks the mixture contained galactose (50.1%), tagatose (15.0%), sorbose (18.0%), fructose (5.0%), and talose (3.5%).

The isomerization of D-allose was extremely slow, and was dominated by the epimerization reaction, yielding principally psicose and a little altrose. The high proportion of allose and the low proportion of idose are noteworthy. The latter may be ascribed to conversion of idose into the thermodynamically more stable ketose (sorbose).

Pentoses. — The isomerization of aldopentoses with dilute KOH at pH 11.5 leads to formation of all of the isomeric aldopentoses. Table II shows the composition of the mixtures produced when the four D-aldopentoses were treated with KOH at pH 11.5 for 1 and 4 weeks, and then analyzed by l.c.

TABLE II

COMPOSITION OF THE SACCHARIDE MIXTURE OBTAINED BY TREATING AQUEOUS SOLUTIONS OF D-PENTOSES WITH KOH AT pH 11.5 FOR 1 AND 4 WEEKS

Starting pentose	*Isomerization products[a] (after 1 week, after 4 weeks)*					
	Aldopentoses (%)				*Hexoses (%)*	
	Ribose	*Arabinose*	*Xylose*	*Lyxose*	*Glucose*	*Sorbose*
Ribose	25.0, 18.7	32.2, 27.3	24.7, 19.9	14.4, 10.8	0.7, 2.9	2.5, 2.9
Arabinose	5.4, 4.6	74.3, 72.1	12.4, 11.9	7.0, 6.7	0.4, 0.8	0.3, 0.3
Xylose	4.8, 3.7	14.8, 15.0	59.3, 54.7	18.6, 18.1	0.3, 1.0	2.3, 2.5
Lyxose	1.8, 3.3	6.7, 6.0	22.1, 21.3	69.5, 66.7	0.1	0.8

[a]Unidentified peaks formed from some pentoses after 4 weeks: ribose, 9.7, 3.2, 2.9%; xylose, 4.1%; Lyxose, 1.6%.

The most striking feature of aldopentose isomerization was the production of hexose. Unlike hexoses, which do not yield appreciable amounts of isomers belonging to other series, aldopentoses afforded glucose and sorbose. Fructose was probably formed, but it could not be detected in the presence of the larger amounts of lyxose, because the two saccharides possess similar retention-times. The production of 6% glucose and sorbose from ribose and 5% from xylose is strong evidence of fragmentation reactions. Thus, glyceraldehyde and its enediol, formed by the reverse-aldol reaction, may combine to yield hexoses. The fragmentation and recombination reaction for ribose is also supported by the extensive and rapid H–T exchange (28 times that of glucose) observed by Isbell *et al.*[12]. Ribose also seems to undergo hydride shifts, as observed by Gleason and Barker[9]. Thus, this pentose presumably isomerizes by all three pathways.

Trioses. — When DL-glyceraldehyde was kept at 25° in aqueous KOH at pH 11.5 under an atmosphere of nitrogen, a rapid reaction occurred, which in 2 h resulted in the conversion of 50% of the glyceraldehyde into glucose, sorbose, and fructose (see Table III). Although the rate of the reverse-aldol reaction responsible for the fragmentation of hexoses and pentoses was not determined, the rapid recombination suggests that the reverse-aldol reaction might also be fast.

CONCLUSIONS

1. The simultaneous formation of the 3- and 4-epimers, and the formation of isomers outside the parent series, are characteristic features of fragmentation and recombination. It was found that when hexoses and pentoses are treated with bases they undergo fragmentation and recombination. This was apparent from the fact that hexoses yield 3- and 4-epimeric ketoses simultaneously, and that pentoses afford hexoses (glucose and sorbose) in addition to pentoses.

2. The formation of hexoses from glyceraldehyde by the aldol reaction proceeded rapidly (50% of the starting material was consumed in 2 h). This might explain, in part, the rapid fragmentation and recombination observed with hexoses and pentoses.

TABLE III

PRODUCTS OF THE ALDOL CONDENSATION OF DL-GLYCERALDEHYDE IN AQUEOUS KOH SOLUTION AT pH 11.5

Composition after:	*5 min*	*1 h*	*2 h*
Glyceraldehyde	92.0%	61.7%	49.1%
Sorbose	2.0%	13.5%	18.8%
Fructose	1.5%	10.1%	14.1%
Psicose	0.7%	4.3%	5.9%
Tagatose		2.4%	2.8%
X[a]	0.4%	2.9%	3.9%
Y[a]	0.8%	2.9%	3.0%
Z[a]		2.0%	2.4%

[a]Retention times of unidentified peaks, relative to glucose: X, 2.02; Y, 1.82; and Z, 1.26.

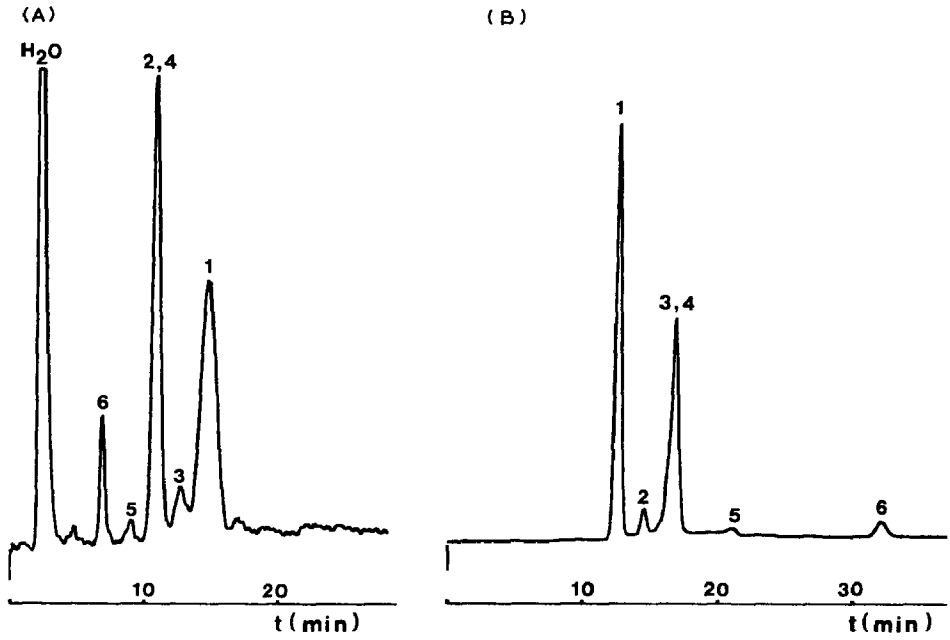

Fig. 2. Liquid chromatograms of a glucose solution kept for 1 week at 25° in aqueous KOH at pH 11.5, and examined on a Carbohydrate Analysis column (A), and an Aminex HPX-87-P column (B): 1, glucose; 2, sorbose; 3, mannose; 4, fructose; 5, tagatose; 6, psicose.

EXPERIMENTAL

Procedures. — The aldopentoses, aldohexoses, and hexuloses (2 g) were quickly dissolved in aqueous KOH and kept at pH 11.5 (100 mL), and 25° under a nitrogen atmosphere. The pH of the mixture was measured periodically and adjusted as necessary. Aliquots (2 mL) were withdrawn by syringe, filtered through 0.45-μm membrane filters, and injected (20 μL) into the chromatographs. Two types of column were used: the first, an Aminex HPX-87-P cation (Pb^{2+})-exchange column (Bio-Rad Laboratories) was heated to 60° and eluted with water at a constant flow-rate of 0.6 mL/min. The second, a Carbohydrate Analysis column (Millipore–Waters Chromatography Division) was kept at 15° and eluted with 83:17 acetonitrile–water at a flow rate of 2.0 mL/min. This column was used to quantitate mannose and fructose, as these two monosaccharides cannot be resolved satisfactorily on the first column, especially when there is a large excess of one of them (see Fig. 2). In both instances the flow rate was maintained by a Waters 501 pump attached to a Waters 410 Differential refractometer connected to a Hewlett–Packard Model 3392A integrator. The retention times and the areas of the peaks produced by known weights of standard sugars (including glyceraldehyde), were determined before the addition of alkali and used to identify and quantify the isomerization products. Fig. 1 was plotted using a Harvard Presentation Graphics software (Software Publishing Corporation) on an IBM XT computer.

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