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HYDROXIDE-CATALYZED ISOMERIZATION OF D-[1-^{13}C]MANNOSE: EVIDENCE FOR THE INVOLVEMENT OF 3,4-ENEDIOLS

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ABSTRACT

The KOH-catalyzed isomerization of D-[1-^{13}C]mannose under anaerobic conditions was studied by ^{13}C-n.m.r. spectroscopy. D-[1-^{13}C]Glucose and D-[1-^{13}C]fructose are generated during the reaction, as expected. In addition, however, [6-^{13}C]glucose, [6-^{13}C]mannose, and [6-^{13}C]fructose are produced in small proportions, possibly *via* symmetrical 3,4-enediol intermediates. The involvement of the latter structures in ^{13}C-label shifting is inferred from the observation of [1-^{13}C]sorbose and [6-^{13}C]sorbose in the reaction mixture.

INTRODUCTION

It is well recognized[1] that aldoses undergo four reactions in the presence of aqueous base: (*1*) anomerization; (*2*) aldose–ketose isomerization, known as the Lobry de Bruyn–Alberda van Ekenstein reaction[2]; (*3*) reverse aldol reaction and β-elimination, to produce α-dicarbonyl derivatives; and (*4*) benzylic acid-type rearrangements thereof, to give lactic and saccharinic acids. The extent to which these reactions occur depends on the solution pH, with reactions *1* and *2* occurring in mild base, and reactions *3* and *4* under more strongly alkaline conditions. Oxygen also affects the nature and distribution of degradation products[1a,3].

The isomerization of aldoses (reaction *2*), catalyzed by OH^-, pyridine[4], or enzymes[5], is known to involve 1,2-enediol intermediates (see Scheme 1)*. In enzyme-catalyzed isomerization [*e.g.*, with phosphoglucoisomerase (EC 5.3.1.9)], Rose and co-workers[5] showed from isotope studies that this intermediate has the *cis* configuration. In contrast, the configuration of the 1,2-enediol in base-catalyzed isomerization remains unknown, but it may depend on the type of cations present (*i.e.*, monovalent *vs.* divalent).

*Under alkaline conditions, the enediol intermediate may exist mainly in ionized form as an enediolate.

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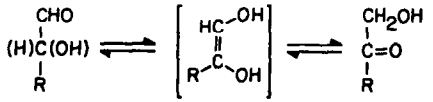


Scheme 1

We have been examining KOH-catalyzed isomerization as a simple and inexpensive means by which to convert D-[1-^{13}C]mannose into D-[1-^{13}C]fructose and D-[1-^{13}C]glucose. The *manno* compound is generated in preference to its more useful C-2 epimer during the chemical synthesis of labeled glucose[6]; previous attempts at epimerization with molybdic acid were unsuccessful, generating an equilibrium mixture of D-[1-^{13}C]mannose and D-[2-^{13}C]glucose[7]. The present report describes the fate of ^{13}C label during base-isomerization, which implicates 3,4-enediols in the reaction.

EXPERIMENTAL

Materials and methods. — D-[1-^{13}C]Mannose (99 atom-% ^{13}C) was prepared from D-arabinose and K^{13}CN, as described previously[6,8]. Base-isomerization reactions were performed as follows. Crystalline D-[1-^{13}C]mannose (10.1 g, 56 mmol) was dissolved in 560 mL of distilled water in a 1-L flask, the pH was adjusted to 11.5 with 2M KOH, and the solution was degassed with nitrogen for 30 min. The reaction vessel was immersed in a water-bath at 25°, and was fitted with a mineral-oil gas-bubbler for continuous N_2 aeration during the course of the reaction. At 24-h intervals, 3-mL samples were removed, made neutral with Dowex-50 X-8 (H^+) ion-exchange resin (20–50 mesh), and stored at 4° for subsequent analysis by ^{13}C-n.m.r. spectroscopy. Larger (250 mL) sample-volumes were also removed after 3 and 7 days, made neutral, concentrated at 30° *in vacuo*, and chromatographed on a column (15 × 100 cm)[9] of Dowex-50 X-8 (Ca^{2+}) ion-exchange resin (200–400 mesh). Fractions were assayed for reducing sugar with phenol–sulfuric acid[10]; labeled glucose was eluted first; mannose, second; and fructose, third. Fractions were pooled, and evaporated to syrups at 30° *in vacuo*. These labeled products crystallized from methanol, and were analyzed by ^{13}C-n.m.r. spectroscopy.

Reactions on a smaller scale (10 mmol of D-[1-^{13}C]mannose) were performed at pH 11.0, 11.5, and 12.0 under anaerobic conditions at 25°, in order to determine a pH at which aldose–ketose isomerization occurred at an appreciable rate with minimal generation of degradation products. From these experiments, pH 11.5 was found to be ideal.

Instrumentation. — ^{1}H-Decoupled, ^{13}C-n.m.r. spectra were recorded at 75 MHz with a Nicolet NT-300 Ft-n.m.r. spectrometer, using 10-mm sample-tubes and a deuterium (^2H_2O) lock. Fully relaxed spectra were collected at ~25°, and f.i.d.s. were processed with and without resolution enhancement, to facilitate identification of sites of [^{13}C]-enrichment.

RESULTS AND DISCUSSION

General aspects of D-[*1-*^{13}*C*]*mannose base isomerization.* — The reaction of D-[1-^{13}C]mannose (0.1M) in distilled water at pH 11.5 and 25° under anaerobic conditions can be monitored readily by ^{13}C-n.m.r. spectroscopy. During the reaction, the C-1 signals of D-[1-^{13}C]mannose (α-pyranose, 95.5 p.p.m.; β-pyranose, 95.2 p.p.m.) are converted into two new anomeric-carbon signals, at 93.6 and 97.4 p.p.m. (α- and β-D-[1-^{13}C]glucopyranoses, respectively) and to a group of signals (~65 p.p.m.) characteristic of hydroxymethyl carbon atoms of sugars (see Fig. 1)[11]. The C-1 resonances of D-[1-^{13}C]fructose contribute to the latter group, and are found at 65.4, 64.4, and 64.2 p.p.m. (β-pyranose, α-furanose, and β-furanose, respectively); the remaining resonances arise from various by-products (see later). From spectral integration (see Fig. 1B, 1C), the following product ratios in the reaction mixture (Man:Glc:Fru:by-products) were determined: 10.8:3.2:4.2:1.0 (3 d) and 3.0:2.4:2.3:1.0 (7 d). After 7 days, the reaction has not reached chemical equilibrium; using pyranose conformational interaction-energies[12] and equilibrium constants from reactions with D-xylose (D-glucose) isomerase (EC 5.3.1.5)[13], ΔG^0 values of -1.97 kJ.mol^{-1} and ~0

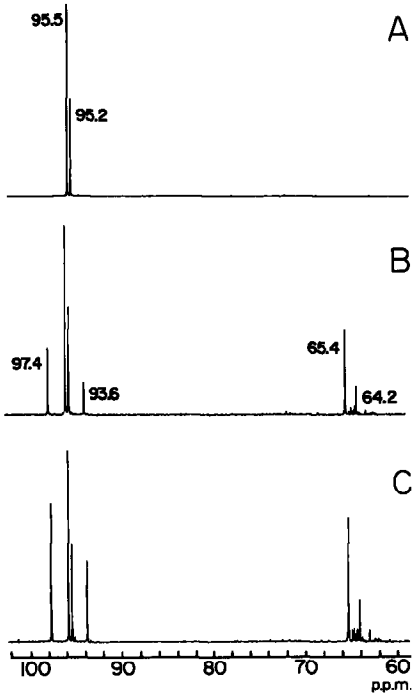


Fig. 1. (A) ^{1}H-Decoupled ^{13}C-n.m.r. spectrum (75 MHz) of D-[1-^{13}C]mannose, showing only the enriched carbon atoms: α-pyranose, 95.5 p.p.m.; β-pyranose, 95.2 p.p.m. (B and C) ^{13}C-Spectra of an isomerization reaction-mixture (pH 11.5, 25°, anaerobic) after 3 and 7 days, respectively, showing the production of D-[1-^{13}C]glucose (97.4 and 93.6 p.p.m.) and D-[1-^{13}C]fructose (65.4 and 64.2 p.p.m.).

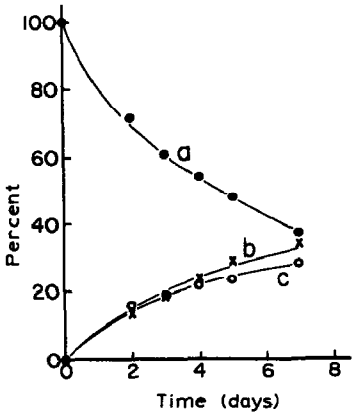


Fig. 2. Rate profile for D-[1-^{13}C]mannose isomerization (pH 11.5, 25°, anaerobic), showing loss of D-[1-^{13}C]mannose (●), and formation of D-[1-^{13}C]glucose (×) and D-[1-^{13}C]fructose (○). The percent reaction refers only to mannose, glucose, and fructose components (*i.e.*, percentages of by-products are not considered here).

J.mol^{-1} are estimated for mannose–glucose and fructose–glucose equilibria, from which the equilibrium condition of Man:Glc:Fru = 0.44:1.0:1.0 can be calculated. Interestingly, however, the glucose–fructose reaction is close to equilibrium after 7 days.

The rates at which D-[1-^{13}C]glucose and D-[1-^{13}C]fructose are generated from D-[1-^{13}C]mannose at pH 11.5 (25°, anaerobic) are illustrated in Fig. 2. From these data, it appears that mild-base isomerization might be a useful means by which to effect conversion of D-[1-^{13}C]mannose into D-[1-^{13}C]glucose and D-[1-^{13}C]fructose.

^{13}C-N.m.r. analysis of D-[*1-^{13}C*]*glucose and* D-[*1-^{13}C*]*fructose generated from* D-[*1-^{13}C*]*mannose isomerization.* — The ^{13}C-n.m.r. spectrum (unenriched region) of authentic D-[1-^{13}C]glucose (with carbon assignments) is shown in Fig. 3A. ^{13}C–^{13}C Couplings to C-1 are as follows: α-pyranose, $^{1}J_{C1,C2}$ 46.2, $^{2}J_{C1,C3}$ 0.0, $^{2}J_{C1,C5}$ 1.8, and $^{3}J_{C1,C6}$ 3.3 Hz; β-pyranose, $^{1}J_{C1,C2}$ 46.0, $^{2}J_{C1,C3}$ 4.5, $^{2}J_{C1,C5}$ 0.0, and $^{3}J_{C1,C6}$ 4.1 Hz. Comparison of this spectrum with those obtained for D-[1-^{13}C]glucose isolated from an isomerization reaction-mixture (pH 11.5, 25°) shows a significant change *only in the C-6 signals.* After 3 days (see Fig. 3B), the normal doublets for C-6 of both pyranoses have been converted into broad singlets; resolution-enhancement (see inset) shows each C-6 signal to be a triplet. In addition, the intensities (and areas) of these signals have increased relative to the remaining, unenriched signals. After 7 days (see Fig. 3C), the C-6 signals appear as singlets, and are stronger than those in the 3-day spectrum. This result suggests that a small proportion (2–3%) of [6-^{13}C]glucose had been generated during the reaction. The C-2 β-pyranose signal in the 7-day spectrum supports this conclusion by revealing a more-intense, center signal caused by C-6-labeled glucose in which the C-2 signal is no longer split by an enriched C-1 nucleus.

^{13}C-N.m.r. analysis of the D-[1-^{13}C]fructose generated by D-[1-^{13}C]mannose

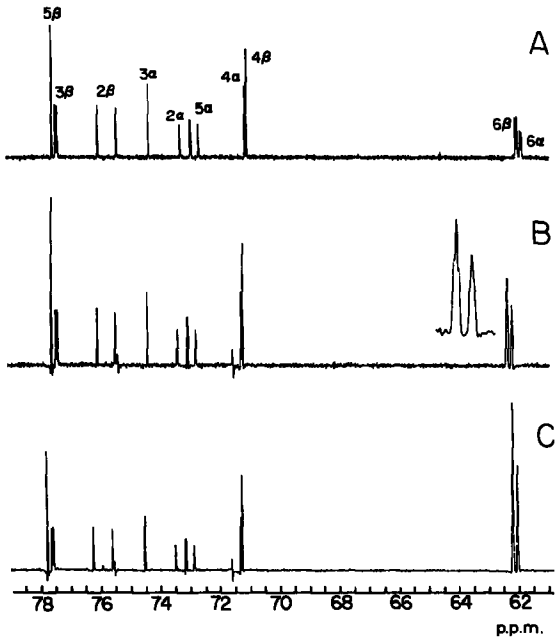


Fig. 3. (A) ^{1}H-Decoupled ^{13}C-n.m.r. spectrum of D-[1-^{13}C]glucose, showing only the natural-abundance carbon atoms, with assignments. (B and C) ^{13}C-Spectra of D-[1-^{13}C]glucose isolated after 3 and 7 days of reaction, respectively. Only C-6 appears to contain [^{13}C]-enrichment, which increases as the reaction proceeds. The out-of-phase signals are due to "fold-over" of the intense, enriched signals caused by quadrature detection. Spectra A–C are resolution-enhanced.

isomerization also reveals the presence of selective C-6-enrichment (see Fig. 4A–4C). The C-6 signals are close to those of the intense C-1 signals, but can nevertheless be observed to increase in intensity (and area), relative to the enriched signals, as the reaction proceeds. Likewise, selective C-6-enrichment is observed in D-[1-^{13}C]mannose recovered after 3 and 7 days of reaction (see Fig. 5A–5C).

Mechanistic interpretation of the observed label shifting. — The KOH-catalyzed isomerization of D-[1-^{13}C]mannose at pH 11.5 (anaerobic) results mainly in the production of D-[1-^{13}C]glucose and D-[1-^{13}C]fructose. A fraction of the ^{13}C label, however, is shifted, generating a small proportion of [6-^{13}C]glucose and [6-^{13}C]fructose. [6-^{13}C]Mannose also appears in the course of increasing reaction-times. From inspection of ^{13}C-n.m.r. spectra, no other carbon sites appear to contain [^{13}C]-enrichment.

Selective [^{13}C]-enrichment of C-6 is inconsistent with a fragmentation–racemization–recombination route. If D-[1-^{13}C]fructose is cleaved by dealdolization, to yield [1-^{13}C]dihydroxy-2-propanone and D-glyceraldehyde, it is expected that, with time, some [^{13}C]-enrichment would appear at C-3, C-4, and C-6 of D-[1-^{13}C]fructose, as well as in the mannose and glucose derived from it. [^{13}C]-Enrichment at C-3 would be most expected early in the reaction, because of

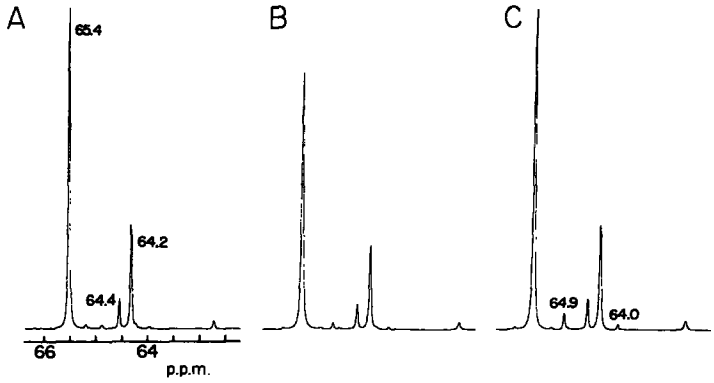


Fig. 4. (A) The ^{13}C-n.m.r. spectrum of D-[1-^{13}C]fructose (enriched carbon atoms), showing the presence of three major forms (see text). (B and C) ^{13}C-Spectra of D-[1-^{13}C]fructose isolated after 3 (B) and 7 (C) days of reaction, which show increasing signal intensities at 64.9 (C-5, β-pyranose) and 64.0 (C-6, β-furanose) p.p.m.

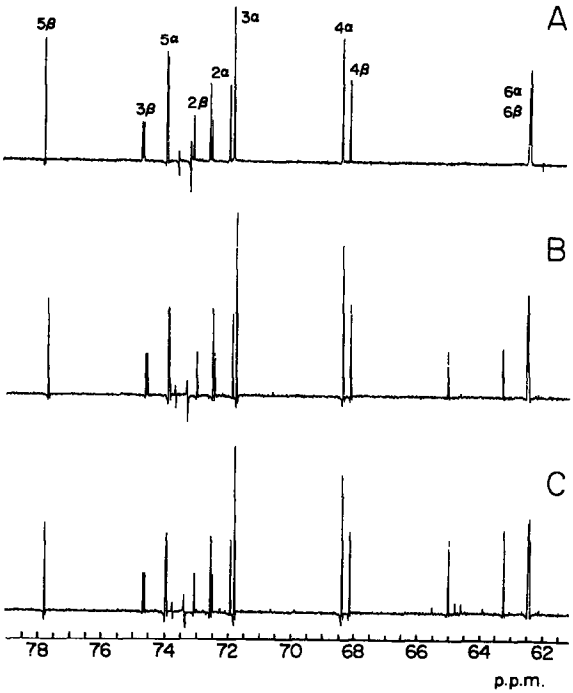
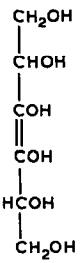


Fig. 5. (A) The ^{13}C-n.m.r. spectrum (resolution-enhanced) of D-[1-^{13}C]mannose (natural-abundance carbons), showing resonance assignments. Out-of-phase signals are an artifact (see Fig. 3). (B and C) Resolution-enhanced ^{1}H-decoupled, ^{13}C-n.m.r. spectra of D-[1-^{13}C]mannose isolated after 3 (B) and 7 (C) days of reaction. Only natural-abundance carbon atoms are shown. The loss in resolution of the C-6 signal (C-6 of both pyranoses are coincident) indicates ^{13}C enrichment at this site. The resonances at 65.0 and 63.3 p.p.m. are due to contaminating [1-^{13}C]- and [6-^{13}C]sorbose (see text).

the achiral structure of 1,3-dihydroxy-2-propanone. In the absence of fragmentation, the labeling pattern observed points to the generation of a *symmetrical* intermediate during the reaction.

A hint at what may be occurring is found in the ^{13}C-n.m.r. spectra of D-[1-^{13}C]mannose isolated after 3 and 7 days of reaction (see Fig. 5). The two signals at 65.0 and 63.3 p.p.m. are due to a [^{13}C]-enriched impurity that co-chromatographs and co-crystallizes with D-mannose. These signals correspond exactly with the C-1 (65.0 p.p.m.) and C-6 (63.3 p.p.m.) signals of authentic α-sorbopyranose, the preponderant (95%) form of sorbose in aqueous solution[14]. It may be noted that both carbon atoms appear to be [^{13}C]-enriched to a similar extent. Sowden and Thompson[15] reported the production of D-[^{14}C]sorbose during isomerization of D-[1-^{14}C]glucose at 50–60° by a strong-base resin. They found ^{14}C label mainly at C-1 and C-6 of D- and L-sorbose, respectively, and very little ^{14}C at internal carbon atoms. They explained this result by postulating the involvement, in the reaction, of 3-keto intermediates that undergo enolization to generate symmetrical 3,4-enediol structures **1**. The symmetry of these structures explains the C-1–C-6 labeling pattern of sorbose and the aforenoted configurational dependence of this labeling.



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Although we have not established the enantiomeric form of the ^{13}C-labeled sorboses, our ^{13}C-n.m.r.-spectral results are consistent with the results of Sowden and Thompson[15], and lend further support to the importance of the enolization–ketonization mechanism of rearrangement, even under mildly basic conditions. This mechanism predicts that the C-6-labeled compounds generated during the course of the reaction will have the L configuration, but this matter has not yet been examined.

CONCLUSIONS

This study has shown that, under mildly basic conditions, D-[1-^{13}C]mannose equilibrates with D-[1-^{13}C]fructose and D-[1-^{13}C]glucose, but that some selective [^{13}C]-enrichment of C-6 of these hexoses also occurs as the reaction proceeds. The latter observation implicates symmetrical 3,4-enediols in the reaction, a conclusion

supported experimentally by the presence of [1-^{13}C]- and [6-^{13}C]sorbose in the reaction mixture.

Because this minor pathway causes label shifting, the Lobry de Bruyn–Alberda van Ekenstein reaction cannot be used to convert D-[1-^{13}C]mannose into its more useful C-2-epimer having isotopic integrity.

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