Carbohydrate Research, 153 (1986) 263–270
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

PARAMOLYBDATE ANION-EXCHANGE RESIN, AN IMPROVED CATALYST FOR THE C-1–C-2 REARRANGEMENT AND 2-EPIMERIZATION OF ALDOSES*

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(Received August 19th, 1985; accepted for publication, November 25th, 1985)

ABSTRACT

Aqueous solutions of molybdate at 90° bring about the inversion of the C-1–C-2 fragment of aldoses having four or more carbon atoms, generating thermodynamically equilibrated mixtures of the starting aldose and its 2-epimer. In some cases, notably with the aldopentoses, substantial proportions of the 3-epimers are produced, as well as 2-epimers that have not undergone inversion of the C-1–C-2 fragment. These side-reactions can be controlled by using the paramolybdate form of an anion-exchange resin (AG MP-1) together with the formate form of the same resin. The latter acts to scavenge unbound molybdate and paramolybdate anions that appear to be responsible for the side reactions.

INTRODUCTION

In 1972, Bílik described a facile 2-epimerization reaction of aldoses[1]. The reaction is catalyzed by acidic solutions of molybdate (paramolybdate), and occurs with simple aldoses containing four or more carbon atoms[2–4]. Recently, we studied the mechanism of this reaction by using specifically ^{13}C- and ^{2}H-enriched aldoses[5], and showed that the epimerization is accomplished by a novel rearrangement of the carbon skeleton (see Scheme 1). Specifically, we showed that if a [1-^{13}C]aldose is incubated with molybdate, a thermodynamic equilibrium mixture of the starting aldose and its [2-^{13}C] 2-epimer is formed.

The rearrangement is highly stereospecific. The formation of a dimolybdate complex with the carbonyl-oxygen atom and the adjacent three hydroxylic oxygen atoms of the aldehyde form of aldoses leads to a transition state (see Scheme 1) in which C-1 and C-2 are enantiomeric[5]. Dissociation of the complex produces either the starting aldose or the 2-epimer generated by inversion of the C-1–C-2 fragment.

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*Supported, in part, by Grant GM-21731 from the National Institute of General Medical Sciences and by a program of the Stable Isotopes Resource of the Los Alamos National Laboratory, funded jointly by the U.S. department of Energy and the National Institutes of Health (RR-00962).

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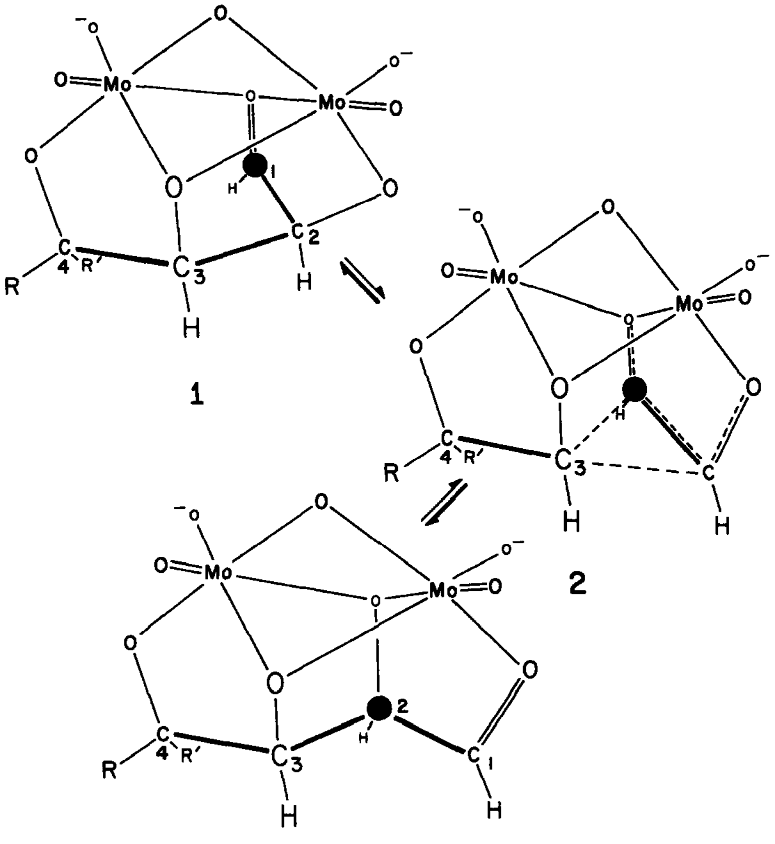
0008-6215/86/$ 03.50



Scheme 1. 3

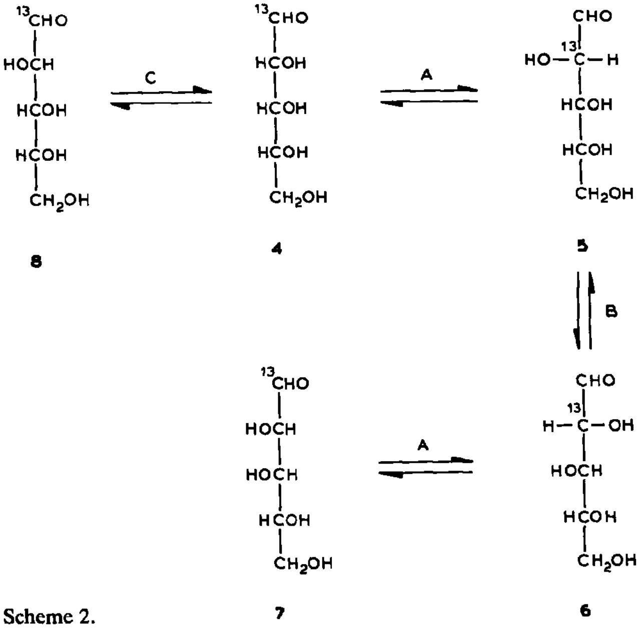
The reaction is a powerful adjunct to cyanohydrin methods for isotopic enrichment, as one less cyanide addition (with its attendant ~60% loss of label due to epimer and side-product formation) is needed for syntheses of aldoses enriched at internal carbon sites.

Although the two 2-epimers are the major products during prolonged incubations, secondary products are formed that are 3-epimers of the starting aldose and the primary product[3,5]. The secondary reaction is most pronounced with aldopentoses; indeed, incubation of any aldopentose with the stoichiometric proportion of molybdate leads to an equilibrium mixture of all four aldopentoses[3]. The presence of the 3-epimers complicates product-separation. In all cases, the 2-epimers can be readily separated by chromatography on columns of Dowex 50 (Ca^{2+}) resin by elution with water[6]. However, in a set of four C-2 and C-3 aldose epimers, there is generally one pair that is incompletely separated on the Ca^{2+} column, requiring other, more difficult, purification procedures. It is desirable then, with isotopically enriched compounds, to keep side-product formation to a minimum in order to facilitate purification, as well as to maximize yields.

We report here an improved procedure, as well as observations and cautions regarding the use of molybdate isomerizations, for the preparation of isotopically enriched carbohydrates.

RESULTS AND DISCUSSION

Reaction stereospecificity. — Paramolybdate-catalyzed rearrangements have been used extensively in this laboratory to prepare aldoses specifically enriched with isotopes of H, C, and, recently, O. As shown in Scheme 2, reaction A, the principal product of the epimerization of D-[1-^{13}C]ribose (**4**) is D-[2-^{13}C]arabinose (**5**). Using ^{13}C-enriched aldoses and n.m.r. spectroscopy, it is easy to identify and quantify the amounts of various products formed during the reaction.



Scheme 2.

In addition to the primary reaction that produces the 2-epimer, a secondary reaction (see Scheme 2, reaction B) produces small amounts of the 3-epimer (**6**) of the starting aldose, which in turn equilibrates with its 2-epimer (**7**). We have also observed, when using aldoses enriched with ^{13}C at C-1 or C-2, small amounts of a product (**8**) that is 2-epimeric to the starting aldose and has the same labelling pattern, rather than the pattern due to C-1–C-2 inversion. This product appears to arise from an alternative reaction-pathway involving epimerization without carbon-skeleton rearrangement (see Scheme 2, reaction C). For example, the major product isolated from the reaction of [1-^{13}C]mannose with molybdate is [2-^{13}C]glucose. Close examination of the glucose shows, however, that it contains ~0.2% of

[1-^{13}C]glucose, the isotopomer of the major product. Its presence is observable as an additional resonance at C-1 in the proton-decoupled, natural-abundance ^{13}C-n.m.r. spectrum (see Fig. 1). This low level of contamination can be estimated, because the sum of the two resonances due to C-1 of [2-^{13}C]glucose equals 1.1% of the total carbon of C-1, and serves as a convenient, internal standard. Reactions that require longer incubations to reach equilibrium, *e.g.*, talose to galactose, and ribose to arabinose, give greater proportions of side-products (see Table I). Although these proportions of contamination are acceptable for most applications of enriched compounds, unnecessarily long reaction-times or serial application of the reaction when the equilibrium is unfavorable increase the label scrambling, so that, eventually, a complex mixture of 1- and 2-labelled aldoses is formed.

It is obvious that label scrambling will appear to have occurred if the starting [1- or 2-^{13}C]aldose is contaminated with a small proportion of the 2-epimer labelled at the same carbon atom. Although the materials used in the experiments described here were carefully purified to avoid this possibility, the situation can occur when the ^{13}C-enriched aldose is a product of the cyanohydrin reaction, and the products are incompletely separated by chromatography on Dowex 50 (Ca^{2+}) resin.

Effect of pH on reaction products. The reactive, molybdate–sugar complexes that we have proposed involve the acyclic, aldehyde form of the aldose (see Scheme 1). Some aldoses also form molybdate complexes in their cyclic forms, *i.e.*, [pyranose–O_2Mo–O–MoO_2–pyranose]$^{2-}$. The two types of complex have different pH dependencies. The optimum pH for formation of pyranose–molybdate complexes lies[6] between 5.5 and 6.0, and, for acyclic polyol–molybdate complexes, between 2 and 3. If side reactions involve pyranose forms of the aldose, lower pH values may favor primary-product formation.

Bílik *et al.*[4] showed the marked influence of pH on the reaction rate, but they did not report its effect on the formation of other products. The pH optimum for the primary reaction is between 2.5 and 3.5. The rate decreases as much as twenty

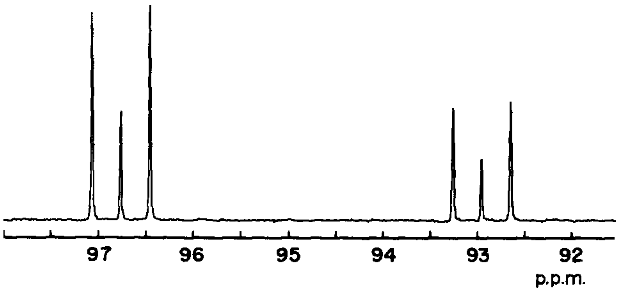


Fig. 1. The anomeric region of the 75-MHz, ^{13}C-n.m.r. spectrum of D-[2-^{13}C]glucose isolated from the MoO_3-catalyzed epimerization of D-[1-^{13}C]mannose. (The center line in each triplet is due to the presence of a small amount of D-[1-^{13}C]glucose produced by a secondary reaction in the epimerization. The outer lines in each triplet are due to 1-^{13}C at natural abundance, split by 2-^{13}C at 99% enrichment. Resonances in other parts of the spectrum confirm that the center line is not due to the presence of D-[2-^{12}C]glucose.)

TABLE I

THE MOLYBDATE-CATALYZED CONVERSION OF [1-^{13}C]RIBOSE INTO [2-^{13}C]ARABINOSE. EFFECT OF pH, APROTIC SOLVENT, AND RESIN CATALYSIS

Conditions	*Products (%)*				
	[1-^{13}C]-Ribose	*[2-^{13}C]-Arabinose*	*[2-^{13}C]-Xylose*	*[1-^{13}C]-Lyxose*	*[1-^{13}C]-Arabinose*
H_2O, pH 2.6, 6 h	31.7	59.2	4.6	3.5	1.0
H_2O, pH 4.5, 13 h	30.6	57.8	5.4	4.0	1.2
DMF, 50°, 48 h	62.2	36.5	0.6	0.4	0.2
$[Mo_7O_{24}^{6-}]$ resin, 6.5 h	34.7	63.5	0.8	0.6	0.3
$[Mo_7O_{24}^{6}]$–$[HCOO^-]$ resin, 6.5 h	34.5	65.1	0.16	0.12	0.20

fold at pH 5.9. We had previously examined the reaction mechanism at pH 4.5, the pH obtained by using 0.1 to 0.5M aldose and 1.43mM $(NH_4)_6Mo_7O_{24}$ or 10mM molybdic acid 85% (which is also ammonium molybdate)[5]. We have now compared the formation of side products at this pH with those obtained at pH 2.6, which is conveniently achieved by using 10mM molybdenum trioxide as the catalyst. The nearly insoluble MoO_3 dissolves rapidly in the presence of an excess of an aldose.

We chose ribose (**4**) for this study because it forms high levels of both the 3-epimers (**6** and **7**) and the isotopomer (**8**) of the major product (arabinose). Solutions of [1-^{13}C]ribose (0.5M) were incubated at 92 ±1° in the presence of 1.43mM $(NH_4)_6Mo_7O_{24}$ (pH 4.5) or 10mM MoO_3 (pH 2.6). The reactions were stopped when the concentrations of [2-^{13}C]arabinose were 97 ±1% of the equilibrium value, the endpoint having been determined in a separate experiment. After de-ionization, the amount and nature of the products were determined by ^{13}C-n.m.r. spectroscopy (see Table I). We found that the proportion of side products is only slightly lower using MoO_3, although the primary reaction-rate is approximately doubled. On this basis, aqueous epimerizations should be performed at pH 2.5 to 3.0 in those cases where small proportions of side products are acceptable.

Effect of aprotic solvent on reaction products. Abe *et al.*[7] reported that use of aprotic solvents and dioxobis(2,4-pentanedionato-O,O′)molybdenum(VI) as the catalyst allow lower reaction-temperatures (50° in *N,N*-dimethylformamide *vs.* 90° for aqueous reactions) and decrease the formation of secondary products. We have confirmed their findings. In addition, the amount of isotopomer is less than is obtained under aqueous conditions (see Table I). However, when these reaction conditions are used with aldoses that can form molybdate complexes in their cyclic form(s), such as ribose, talose, or lyxose, the reaction proceeds very slowly. With ribose, for example, equilibrium (5 half-lives) is reached only after 8 days. Raising the temperature of the reaction by 10° increases the primary reaction-rate, but also produces side products (presumably by dehydration reactions) as evidenced by formation of a dark-brown color. The procedure is not as convenient or as economical as Bílik's, as it requires extraction and lyophilization prior to purification of the products. Furthermore, we were interested in establishing improved aqueous conditions in order to use molybdate catalysis to incorporate isotopes of oxygen at O-2 of aldoses[8].

Resin-immobilized paramolybdate. The fact that the nonaqueous system gives rise to smaller amounts of side products suggests that H_2O may be involved in these reactions, possibly through a direct, second-order reaction, or by stabilizing the proposed hydride-shift intermediate. We reasoned that an aqueous system in which the catalyst was bound to an anion-exchange resin might have properties similar to those of the molybdate complex used in the nonaqueous system. A solid-phase catalyst would bind the aldose in a different geometry, perhaps limiting side-reactions. in addition, it would diminish the concentration of free molybdate that might be responsible for the side reactions through the formation of higher-order complexes with the reactive aldose–dimolybdate complex.

The rearrangement reaction occurs only below pH 6, the pH at which molybdate (MoO_4) begins to polymerize to paramolybdate ($Mo_7O_{24}^{6-}$). This differential reactivity is also shown by molybdate and paramolybdate anion-exchange resins. Resins were prepared by passing solutions of Na_2MoO_4 or $(NH_4)_6Mo_7O_{24}$ through AG MP-1, and used for the epimerization of mannose. The paramolybdate resin was effective in catalyzing the rearrangement, whereas the molybdate resin was completely ineffective.

The paramolybdate-resin-catalyzed reaction was examined for the formation of side products, again using [1-^{13}C]ribose as the starting material. As may be seen from Table I, the amounts of 3-epimers of the primary products were decreased by a factor of 6 over the aqueous conditions, whereas the amount of isotopomer formation was lessened by a factor of $\sim$5. It seemed possible that a further diminution of the side products might result if the reaction was carried out in the presence of additional AG MP-1 resin in the formate form, acting as a scavenger of soluble paramolybdate. To test this, a reaction was carried out using paramolybdate and formate-form AG MP-1 resins in the ratio of 2:1 (v/v). Preliminary experiments showed that, with the mixed resins, the rate of the primary reaction was decreased. To compensate for this, the proportion of paramolybdate resin was doubled, in comparison to the earlier resin experiments. Under these conditions, there is a further 5-fold diminution in the amounts of 3-epimers, while there is no significant decrease in the amount of isotopomer production. Fig. 2 shows the lessening in side-product formation achieved by using the mixed-resin catalyst, as compared to Bílik's original, aqueous conditions. There is a 29-fold lowering of the 3-epimers ([1-^{13}C]lyxose and [2-^{13}C]xylose) and a 5-fold diminution in the isotopomer ([1-^{13}C]arabinose).

The mixed-resin catalysis is clearly superior to the reaction in $HCONMe_2$ (DMF) in terms of decrease of 3-epimer, particularly when it is noted that the DMF reaction listed in Table I was only 56% complete, so that the amount of side products would be substantially greater if the reaction was allowed to reach equilibrium. On the other hand, the aprotic system is better than the aqueous, mixed-resin

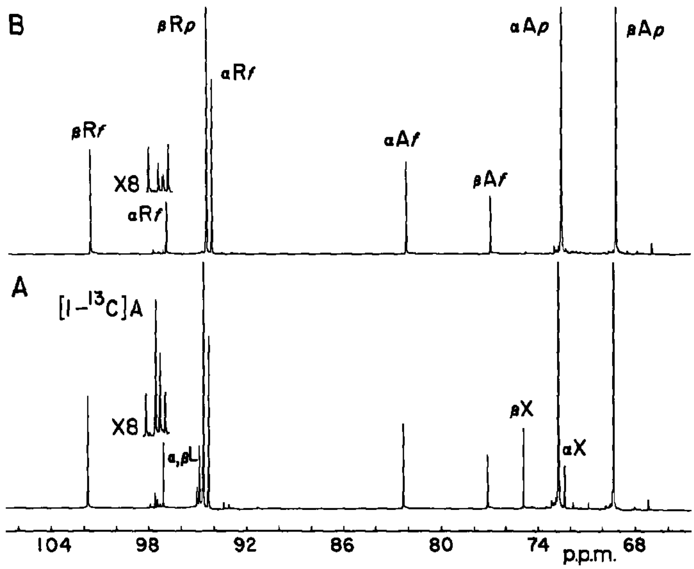


Fig. 2. 75-MHz, ^{13}C-n.m.r. spectra of the reaction mixture obtained from D-[1-^{13}C]ribose on treatment with A, 10mM MoO_3 (pH 2.6) for 6 h at 92°, and B, paramolybdate–formate AG MP-1 resin for 6.5 h at 92°. (A, R, L, and X identify arabinose, ribose, lyxose, and xylose. The X-8 expansions at 97.6 p.p.m. show the relative amounts of [1-^{13}C]arabinose formed in the two treatments.)

system in lessening the amount of epimerization not accompanied by C-1–C-2 inversion. There will be a few instances when this small level of contamination will be unacceptable, although the occurrence of these side-reactions points to the possibility that rearrangements equally as interesting as C-1–C-2 inversion remain to be elucidated.

EXPERIMENTAL

Materials. — $K^{13}CN$ with 99 atom% enrichment was purchased from Merck Sharp and Dohme. ^{13}C-Enriched carbohydrates were prepared as described previously[9]. Ammonium paramolybdate and sodium molybdate were obtained from Mallinckrodt. Dioxobis(2,4-pentanedionato-O,O′)molybdenum(VI) [$MoO_2(acac)_2$] was from Alfa Products. AG MP-1 (Cl^-) resin (200–400 mesh) was from Bio-Rad, as were the Dowex resins for de-ionization and chromatography.

Homogeneous catalysis. — Aqueous solvent. A 0.5M solution of aldose and either MoO_3 (10mM) or $(NH_4)_6Mo_7O_{24}$ (1.43mM) was stirred at 92 ±1°. At the end of the reaction, the solution was passed through a column of Dowex-2 X-8 (HCO_3^-) resin (200–400 mesh), and the effluent and wash were stirred with Dowex-50 X-8 (H^+) resin (200–400 mesh) for 10 min, and the suspension was filtered. The filtrate was dried by rotary evaporation prior to n.m.r. spectroscopy, or to separation of products by Ca^{2+} chromatography[6].

Aprotic solvent. — A 0.6M solution of aldose in *N,N*-dimethylformamide containing[7] 30mM $MoO_2(acac)_2$ was incubated for 5 to 48 h at 50°, and cooled to 5°; two volumes of H_2O were added, and the mixture was extracted twice with two volumes of CH_2Cl_2. The aqueous layer was de-ionized as just described.

Heterogeneous catalysis. — AG MP-1 (Cl^-) resin was converted into the paramolybdate form by passing a 0.1M solution of $(NH_4)_6Mo_7O_{24}$ through a column of the resin until no Cl^- ion was detectable in the effluent. The resin was then washed with at least 20 column-volumes of H_2O, and stored as an aqueous suspension. Molybdate resin was prepared in a similar manner, using 0.5M Na_2MoO_4.

To an aqueous solution of an aldose was added a measured, settled volume of AG MP-1 resin, in the selected form, from a calibrated pipet. The final concentration of aldose was in all cases 0.5M. Resin was added in the ratio of 0.05 mL of settled resin per mmol of aldose, for both the paramolybdate- and molybdate-form reactions. For the mixed-resin reactions, 0.1 mL of paramolybdate-form resin and 0.05 mL of formate-form resin were added per mmol of aldose. The reactions were carried out at 92 ±1° (oil bath) in vials sealed with Teflon-lined screw-caps. The vial contents were stirred with a magnetic stirrer, and the pressure in the vial was released several times during the first 10 min of incubation. At the end of the incubation, an equal volume of H_2O was added, and the pH of the reaction adjusted to 7.0 by addition of Dowex-2 X-8 (HCO_3^-) resin. The mixture was stirred for a few minutes, and filtered, and the filtrate was treated with Dowex 50 (H^+) resin as already described.

N.m.r. spectroscopy. — Carbon-13 n.m.r. spectra were recorded at 75 MHz with a Bruker WM-300 superconducting, F.t. spectrometer. Spectra were obtained at 35° with 60° pulse angles, a spectral width of 3.5 kHz, and 8 k real data points. A 0.2-Hz line-broadening, exponential filter was applied to the f.i.d. prior to a zero filling to 16 k real data points, giving a digital resolution of 0.21 Hz/pt.

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