C-2 Epimerization of Aldoses by Calcium Ion in Basic Solutions. A Simple System to Transform D-Glucose and D-Xylose into D-Mannose and D-Lyxose

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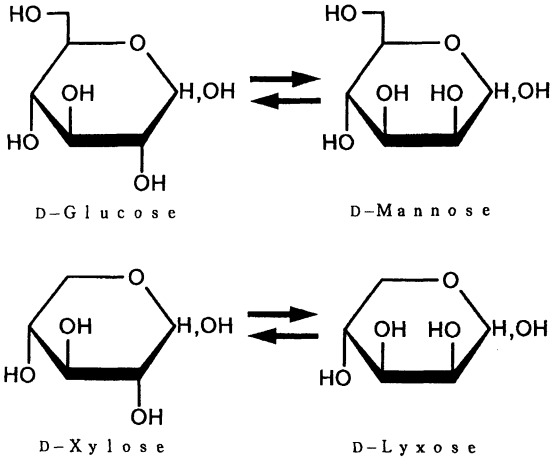
Aldoses were epimerized at C-2 by Ca^{2+} in aqueous or alcoholic basic solutions through a stereospecific [1-2] rearrangement of the carbon skeletons of the aldose. The skeletal rearrangement was confirmed by ^{13}C NMR analysis of the reaction products. The reaction of [1-^{13}C]- and [2-^{13}C]-D-glucose afforded [2-^{13}C]- and [1-^{13}C]-D-mannose, respectively, as major products. The effects of metal ions, bases, and solvents were examined, and it was found that a high concentration of Ca^{2+} and a base (>pH 12.3) were especially effective for the present rearrangement. Separation of the reaction products was also effected by using Ca^{2+} cation-exchange chromatography. Thus, under the optimized conditions, D-mannose and D-lyxose, which are rare in nature and expensive, were easily obtained in high isolated yields from D-glucose and D-xylose, respectively.

It was well known that carbohydrate shows remarkable and interesting interactions with Ca^{2+} in biological and artificial systems. For example, Ca^{2+} works as an effective catalyst in various reactions of carbohydrate, such as a formose reaction[1] and a C-2 epimerization of aldose.[2] Aldoses are epimerized under basic conditions; it has been said that epimerization proceeds according to a Lobry–Alberda (LA) rearrangement via the 1,2-enediol intermediate.[2]

In recent years, a novel epimerization system has been developed by us, the mechanism of which is quite distinct from that of the LA rearrangement (Scheme 1). It was revealed that the C-2 epimerization by a complex comprising Ni^{2+} and diamine proceeded through a stereospecific rearrangement of the carbon skeleton.[3—7] In this study aldoses were epimerized in a simple system containing Ca^{2+} and a base. The object of this paper is to clarify the mechanism of the C-2 epimerization under this condition. In addition, the influences of metal ions, bases and solvents were studied. Some sugars labeled with ^{13}C were examined in order to understand in detail the mechanism on the rearrangement.



Scheme 1.

Experimental

The abbreviations of the sugars involved are as follows: Glc, glucose; Man, mannose; Xyl, xylose; Fru, fructose, Xlu, xylulose.

Materials. All of the aldoses used in the present study were of commercial-grade reagents, and were used without further purification. [1-^{13}C]-D-Glc, [2-^{13}C]-D-Glc, and [1-^{13}C]-D-mannose were purchased from ISOTEC Inc. Calcium chloride dihydrate and all of the bases used were of commercial origin.

NMR Measurements. The 67.9 MHz ^{13}C NMR spectra of the products were obtained with a JEOL GSX-270 spectrometer in D_2O along with a small amount of methanol as an internal standard (49.8 ppm); the signals were assigned by comparisons with authentic data.[9—11]

General Epimerization Reaction. To an aqueous or alcoholic solution of calcium chloride dihydrate (1 mmol in 13 cm^3 of a solvent), a solution of sodium hydroxide (1 mmol in 2 cm^3 of a solvent) was added with vigorous stirring. An aldose (1 mmol) was then added to the solution, which was kept at 65 °C for 5 min (or 10 min) with stirring. The solution was then cooled in an ice bath, diluted with water (10 cm^3), and neutralized with hydrochloric acid (1 mol dm^{-3}). The mixture was deionized by an ion-exchange resin of Dowex 50 W×2 (100—200 mesh, H^+ form, 50 cm^3 in wet volume) and Dowex 2×8 (100—200 mesh, HCO_3^- form, 50 cm^3 in wet volume), then evaporated in vacuo. The products were separated by column chromatography on Dowex 50×8 (200—400 mesh, Ca^{2+} form, 120 cm^3 in wet volume) with water used as an eluent. Fractions (3 cm^3) were collected at a rate of 0.5 cm^3 min^{-1} and evaporated in vacuo to dryness. The resulting sugars were trimethylsilylated in the usual way, then qualitatively and quantitatively analyzed by GLC[8] with a glass capillary column (OV-1 Bonded; 0.25 mm i.d.×25 m), isothermally at 200 °C using a flame ionization detector.

On the other hand, the epimerization by metal hydroxide was carried out as follows. An aldose (1 mmol) was added to 15 ml of the metal hydroxide solution (66.7 mM, M=mol dm^{-3}) and kept at 65 °C for 5 min with stirring. Deionization and analyses of the resulting sugars were carried out in the same manner as described above.

The pH of the solutions was measured at 25 °C with a TOA HM-30S pH meter.

Results and Discussion

Mechanism of the Carbon Skeleton Rearrangement. To confirm the mechanism of this epimerization, the ^{13}C-enriched substrate ([1-^{13}C]-Glc, [2-^{13}C]-Glc, and [1-^{13}C]-Man) was studied. The reaction products were characterized by ^{13}CNMR. The spectra are shown in Fig. 1. In the reaction of [1-^{13}C]-Glc (Spectrum (a)) four remarkable new peaks appeared in addition to the peaks of α- and β-pyranose of the substrate. Peaks at 72.0 and 72.6 ppm were assigned as being those for α- and β-pyranose of [2-^{13}C]-Man.[11] It was noteworthy that no peaks due to [1-^{13}C]-Man and [2-^{13}C]-Glc were observed in spectrum (a) of the product. In addition, as can be seen from spectrum (b), it was confirmed that [1-^{13}C]-Man was prepared from [2-^{13}C]-Glc; from spectrum (c) it is certain that [2-^{13}C]-Glc was prepared from [1-^{13}C]-Man. These observations indicated that the epimerization proceeded not via an LA rearrangement, but via a stereospecific type in the reaction system introduced here. Fru prepared during the reaction was not subjected to a stereospecific skeletal interconversion.

Many of the previous reviews concerning epimerization have stated that epimerization by bases such as metal hydroxide proceeds via an LA rearrangement through a reversible enolization.[12,13] The skeletal rearrangement of sugars has scarcely been discussed. The results obtained in this study have shown that epimerization by calcium hydroxide proceeded by a mechanism which is different from that proposed by earlier investigators.

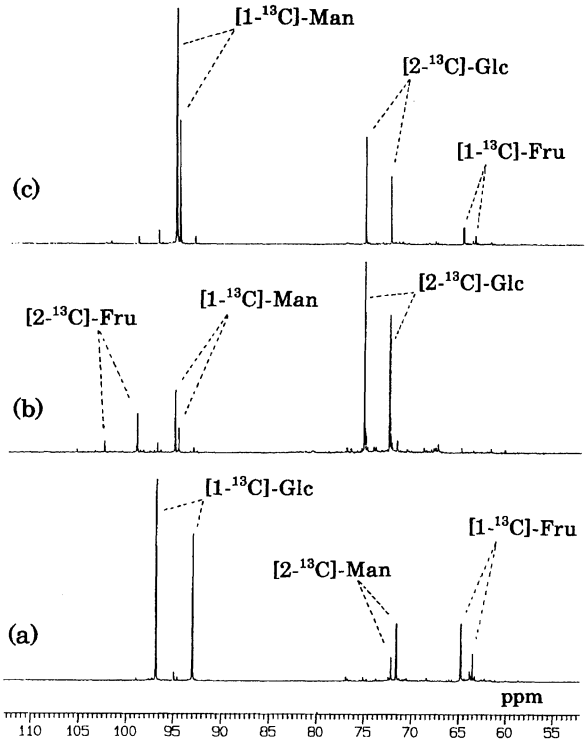


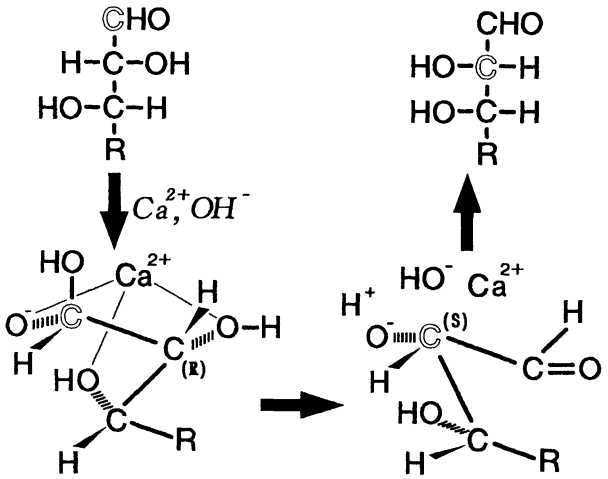
Fig. 1. ^{13}CNMR spectra of the reaction products obtained by treating [1-^{13}C]-Glc (a), [2-^{13}C]-Glc (b), and [1-^{13}C]-Man (c) with aqueous $Ca(OH)_2$ (66.7 mol m^{-3}) at 65 °C for 5 min.

The structure of the postulated intermediate glycoside complex is shown in Scheme 2. The rearrangement may take place via a Ca^{2+} -involved five-membered chelate intermediate in which the leaving OH group and the migrating C(2)–C(3) bond are arranged in an antiperiplaner relationship to create an *S*-configuration at C(2) by an inversion.

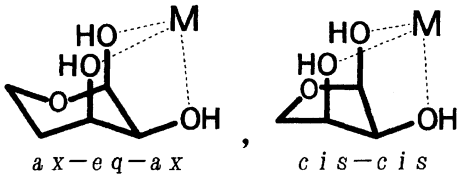
As mentioned above, the calcium–glycoside complex plays an important role during the epimerization. Here, two kinds of complexes must be condsidered in order to estimate the obtained results. One is the complex at which the sugar was coordinated in an acyclic form, as shown in Scheme 2. The other is a complex comprising Ca^{2+} and a cyclic-formed sugar as shown in Scheme 3.

According to Angyal, a linear polyol containing a *threo-threo* sequence at three consecutive hydroxyl groups shows a high affinity for metal ions.[14] The formation of an active acyclic sugar complex is promoted by Ca^{2+} when Glc and Xyl are subjected to epimerize, since they have a suitable configuration of hydroxyl groups for the complexation.

At the same time, Angyal has said that the stability of the Ca^{2+} complex which is coordinating cyclic aldose, such as Man and Lyx, is larger than that of Glc and Xyl, since the former two aldoses have an ability



Scheme 2.



Scheme 3.

to coordinate to Ca^{2+} using their *ax-eq-ax* sequences of three hydroxyl groups at C-1, C-2, and C-3 in a pyranose ring.[15] The cyclic sugar complex is no longer an active species for a skeletal rearrangement. The configuration of aldose provides important roles for the coordination of a sugar. Glc-type aldoses, whose two hydroxyl groups at C-2 and C-3 form a *threo* configuration, tend to prepare the acyclic sugar complex, whereas the Man-type aldoses, having *erythro* configurations at C-2 and C-3, can easily form a cyclic sugar complex. Therefore, Man and Lyx were abundantly obtained from Glc and Xyl in a system in which the acyclic sugar complex formation was promoted. Stating reversely, when Man or Lyx was applied the reaction was placed under non-suitable conditions for epimerization, since the strong affinity between Ca^{2+} and the substrate sugar would form a stable and inactive Ca^{2+} complex. It will prevent not only the C-2 epimerization by the skeletal rearrangement, but also an LA rearrangement. The fact that there is a greater formation of Fru from Glc than from Man could thus be reasonably interpreted.

Base and pH. Man and Fru were prepared from Glc in a basic aqueous solution containing a definite amount of Ca^{2+} and a base (Fig. 2). The concentrations of Man and Fru in the reaction mixture were plotted as a function of the pH of the solution, which was altered by the addition of different kinds of bases. Various kinds of inorganic and organic bases such as sodium hydroxide, amine and diamine were studied in order to clarify the influence of basicity in the reaction medium. The more Fru and Man were formed as pH of solution increased. When the pH of the solution was greater than 12.3, the yields of the C-2 epimer, Man, remarkably increased. The formation of Fru gradually increased as the solution became more basic.

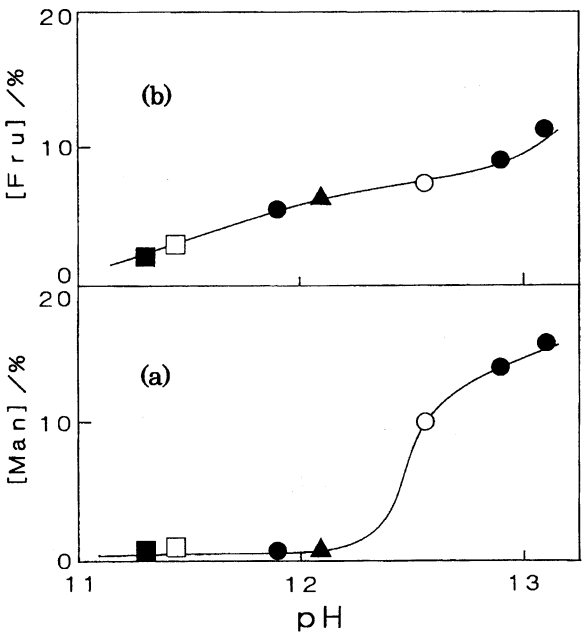


Fig. 2. Formation of Man (a) and Fru (b) from the reaction of Glc with $CaCl_2 \cdot 2H_2O$ (66.7 mol m^{-3}) and base; ●: NaOH (13.3 mol m^{-3}, 66.7 mol m^{-3}, 133 mol m^{-3}), ○: tetramethylammonium hydroxide (66.7 mol m^{-3}), ▲: triethylamine (66.7 mol m^{-3}), □: 2-diethylaminoethanol (66.7 mol m^{-3}), ■: 2-aminoethanol (66.7 mol m^{-3}).

Solvent. Epimerization was carried out in three kinds of solvents (water, ethanol, and methanol) in order to study the influence of the medium on the epimerization (Table 1 and Figs. 3 and 4).

The rate of epimerization depended upon the solvents. In methanol (Fig. 3) the final solution compositions were almost identical (equilibrium) when either Glc or Man was used as the starting sugar. Equilibrium was attained smoothly in about 10 min in methanol. However, when water or ethanol was used as the solvent, eqilibrium was not accomplished within even 30 min. An extended reaction (more than 20 min) changed the solution from colorless to dark brown. Many peaks

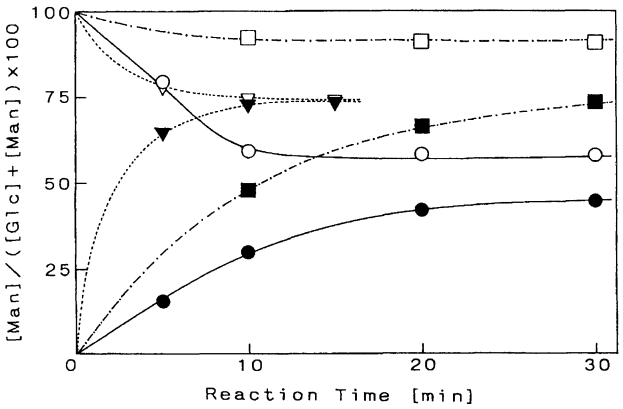


Fig. 3. Relation of epimerizations between Glc and Man to the solvents; ●, ▼, ■: from Glc; ○, ▽, □: from Man; ——: in water, ····: in methanol, –·–: in ethanol.

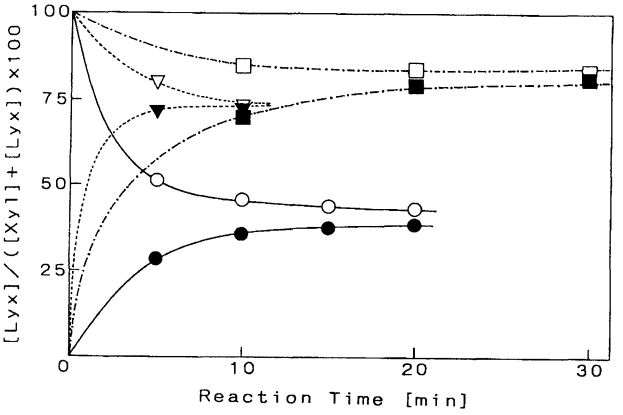


Fig. 4. Relation of epimerizations between Xyl and Lyx to the solvents; ●, ▼, ■: from Xyl; ○, ▽, □: from Lyx; ——: in water, ····: in methanol, –·–: in ethanol.

Table 1. The Effect of Solvent[a)]

Solvent (Volume ratio)	Composition of products/%											
	from Glc			from Man			from Xyl			from Lyx		
	Glc	Man	Fru	Glc	Man	Fru	Xyl	Lyx	Xlu	Xyl	Lyx	Xlu
H_2O	64	27	9	38	59	3	61	35	4	52	43	5
H_2O+CH_3OH (1 : 9)							44	51	5	43	52	5
H_2O+CH_3OH (1 : 24)							36	58	6	35	61	4
CH_3OH	18	44	38	18	52	30	27	67	6	27	68	5
CH_3OH+C_2H_5OH (1 : 2)	23	54	23				23	72	5	17	78	5
C_2H_5OH	47	45	8	9	88	3	27	64	9	13	83	4
C_2H_5OH[b)]	21	57	22	10	80	10	16	71	13	14	80	6
C_2H_5OH[b,c)]	5	61	34				16	76	8			

a) Concentration of aldose, calcium, and NaOH was 66.7 mol m^{-3}. Reaction time and temperature were 10 min and 65 °C, respectively. b) Reaction time and temperature were 30 min and 65 °C, respectively. c) Concentration of calcium was 267 mol m^{-3}.

due to unidentified compounds, accompanied by those of the substrate and epimer, were observed on a GLC. It was not recommended to wait for the achievement of equilibrium in water and ethanol. The equilibrium between two epimers shifted to the side of the mannose-type aldose, compared with those in the themodynamic equilibrium state, which was estimated from their free energies by Angyal[16)] and Yano.[4)] As the polarity of solvent decreased, the ratios of the C-2 epimers shifted to be more Man. The solubility of the Ca^{2+}-aldose complex in a solvent also affected the epimerization as well as did the polarity of the solvent. Since ethanol had only limited solubilities for the complexes, in their cases epimerization was deactivated. Although water showed excellent solubility, at the same time Ca^{2+} and aldose were so strongly hydrated in an aqueous solution, that the most important process, coordination of aldose to Ca^{2+}, was inhibited by the hydration effect.[16)] Similar results were observed in the epimerization of Xyl and Lyx. (Fig. 4).

Methanol was the most suitable solvent for epimerization among the solvents tested. These differences were probably due to both the ease of complexation and the stability of the complex formed. Under the present experimental conditions, thermodynamically unstable epimers were obtained from the naturally abundant epimer.

Concentration of Ca^{2+}. Table 2 shows the influence of the concentration of Ca^{2+} on the epimerization. As mentioned above, the mannose-type aldoses would make an inactive cyclic glycoside complex, even if they were subjected as a substrate or a epimerized product. As the concentration of Ca^{2+} increased, the yield of the epimers, Man and Lyx from Glc and Xyl, increased, whereas, the yield of the opposite epimer, Glc from Man, decreased. Fructose formation was also depressed under a high Ca^{2+} concentration medium. When no Ca^{2+} was present, the yield of Man was much lower than that of Fru.[17)] These observations indicated that both Ca^{2+} and the base were indispensable for efficient epimerization.

The epimerized Man from Glc was obtained in the highest yield by calcium chloride dihydrate and sodium hydroxide in methanol solution. The ^{13}C NMR spectrum of the product (Fig. 5) was similar to that shown in Fig. 1(a). The peak at 99.0 ppm was assigned for [2-^{13}C]-β-fructopyranose,[9)] which should be formed by an LA rearrangement of [2-^{13}C]-Man derived from [1-^{13}C]-Glc. A combination of calcium chloride and sodium hydroxide was more pertinent than calcium hydroxide alone for the epimerization of aldose.

Suitability of Metals. As shown in Table 3, among the metal hydroxides bases, only calcium hydroxide clearly showed the ability to epimerize Glc into Man by a skeletal rearrangement between C-1 and C-2. Alkaline metal hydroxides, such as lithium, sodium, and potassium hydroxides, gave the corresponding ketose and epimer; these results were in accord with the LA rearrangement theory.

During our study, the suitable metal ion for epimerization by a skeletal rearrangement was ascertained. The metal ion should have a definite affinity against the substrate sugar and should be bivalent. The ion radius should be close to 0.1 nm, a suitable size for complexation with aldoses and polyols.[18)] A definite solubility of the metal hydroxide in a solvent was required in or-

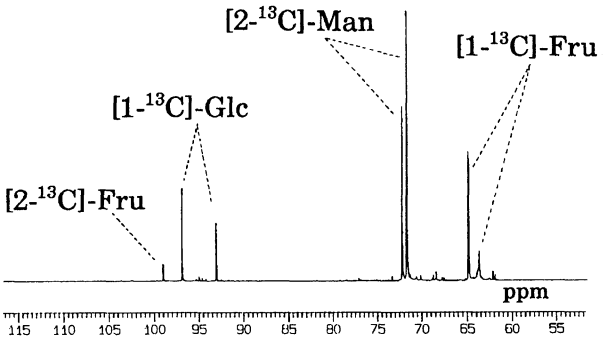


Fig. 5. ^{13}C NMR spectrum of the product mixture derived from [1-^{13}C]-Glc with $CaCl_2 \cdot 2H_2O$ and NaOH in methanol.

Table 2. The Effect of Concentration of Ca^{2+} in Methanol[a)]

$C_{Ca^{2+}}$	Composition of products/%								
	from Glc			from Man			from Xyl		
mol m^{-3}	Glc	Man	Fru	Glc	Man	Fru	Xyl	Lyx	Xlu
66.7	23	42	35	17	61	22	27	69	4
133	13	55	32	14	64	22			
267	15	62	23	13	75	12	20	78	2

a) Concentration of aldose and NaOH was 66.7 mol m^{-3}. Reaction time was 5 min.

Table 3. Reaction of [1-^{13}C]-D-Glucose with Various Metal Hydroxides in H_2O[a)]

Base	pH	Composition of products/%			^{13}C Position of Man[b)]	
		Glc	Man	Fru	C-1	C-2
LiOH	12.4	79	2	19	○	—
NaOH	12.8	74	3	23	○	—
KOH	12.8	71	3	26	○	—
$Mg(OH)_2$	9.1	99	0	1	—	—
$Ca(OH)_2$	12.4	75	17	8	△	○
$Sr(OH)_2$	12.9	79	4	17	○	△
$Ba(OH)_2$	13.0	65	5	30	○	—
$Al(OH)_3$	7.9	99	0	1	—	—
$Co(OH)_2$	7.3	99	0	1	—	—
$Ni(OH)_2$	7.1	100	0	0	—	—
$Cu(OH)_2$	8.1	99	0	1	—	—

a) Concentration of [1-^{13}C]-Glc and base was 66.7 mol m^{-3}, and calcium chloride was not used. Reaction time was 5 min. b) ○: Major products, △: minor products, —: not detected.

der for the epimerization to proceed. The last four hydroxides in Table 3 showed no activities due to their extremely low solubilities. The excellent epimerizing ability of Ca^{2+} seems to accommodate the conditions mentioned above.

Isolation of Sugars by the Ca^{2+} Chromatography.[19)] The reaction products were treated and separated as described in the experimental section. The obtained complex was characterized by its elution behavior in Ca^{2+} ion-exchange chromatography. The retention time of hexoses followed the sequence Glc<Man<Fru; for pentoses it was Xyl<Lyx<Xlu.

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