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COMPLEXES OF CARBOHYDRATES WITH ALUMINATE ION. ALDOSE–KETOSE INTERCONVERSION ON ANION-EXCHANGE RESIN (ALUMINATE AND HYDROXIDE FORMS)*

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ABSTRACT

Kinetic parameters for aldose and ketose transformations in the D-glucose–D-mannose–D-fructose system at 27° on aluminate resin and hydroxide resin were obtained. On both resins, hydroxide ion functions as the catalyst for isomerization. By forming a complex with D-fructose, resin-bound aluminate ion stabilizes the ketose and permits high yields (up to 72%) of D-fructose from D-glucose. The effect of temperature on D-glucose-to-D-fructose conversion was studied; lower temperatures give the higher maximum yields. Maltose is converted into maltulose in moderately high yield (63%) at 24° on aluminate resin; higher yields are not possible at this temperature because of a marked tendency for maltulose to undergo elimination of D-glucose at C-4.

INTRODUCTION

The alkali-catalyzed interconversion of aldoses and ketoses, an isomerization first reported by Lobry de Bruyn and Alberda van Ekenstein[1], has been intensively studied. The hexose system of D-glucose, D-fructose, and D-mannose has received the greatest attention[2–9b], although investigations of many other aldose–ketose isomerizations[2,6,10–14] have been reported. In the absence of strong complexing agents, yields of D-fructose from base-catalyzed isomerization of D-glucose are only about 20–30%. However, in alkaline solution containing appropriate amounts of certain complexing agents, much higher yields have been realized. Mendicino[6] reported that the presence of borate ion leads to yields of D-fructose as high as 80–85%; other investigators[7], unable to reproduce these high yields, have stated that a maximum of only 50% of D-fructose is attained by the borate method. Barker and co-workers[9],

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nevertheless, have shown that the use of arylboronates can provide yields as high as 81%. Haack and coworkers[7] claimed yields of about 70% in solutions containing aluminate ion. Parrish[15] has used columns of finely divided basic alumina to convert D-glucose, but yields of D-fructose were no higher than 45%.

D-Mannose has not been produced in yields greater than 5% from either D-glucose or D-fructose. Yet, in dilute aqueous sodium hydroxide at 35°, an apparent 30% conversion of D-fructose into D-glucose, 29% conversion of D-glucose into D-fructose, 21% conversion of D-mannose into D-fructose, and 13% conversion of D-mannose into D-glucose have been reported[16]. In the presence of benzeneboronate ion, D-mannose can be transformed[9] into D-fructose to the extent of 65%.

Strong-base catalyzed interconversion of D-glucose, D-mannose, and D-fructose is accompanied by side reactions that are often undesirable and which lead to various amounts of organic acids (such as metasaccharinic acid), colored substances of unknown structure, small amounts of reductones, and traces of sugars that arise either from carbonyl migration or from dealdolization with subsequent recombination of fragments[2].

Anion-exchange resins have been used as matrices for base-catalyzed aldose–ketose interconversions. Langlois and Larson[8] reported a maximum yield of about 32% for D-fructose from D-glucose on hydroxide resin. Barker and coworkers[9] reported a 57% yield from D-glucose in M sodium hydroxide solution in contact with poly(4-vinylbenzeneboronate) resin. Kinetic studies on anion-exchange resin were never made, although rate studies of the D-glucose–D-mannose–D-fructose system in aqueous sodium hydroxide[4,5,5a,5b] and aqueous sodium aluminate[9b] and the cellobiose–cellobiulose–4-*O*-β-D-glucosyl-D-mannose system in aqueous sodium hydroxide[17] have been published. After detecting isomerization in chromatographic separations of aldoses from ketoses on columns of aluminate anion-exchange resin[18], we sought to determine the practicality of converting aldose into ketose on aluminate resin and to establish, by means of kinetic data, a plausible mechanism for this transformation. D-Glucose, D-mannose, and maltose were the aldoses chosen for the study.

RESULTS AND DISCUSSION

The usual procedure for converting a sugar on aluminate or hydroxide resin consisted of pressing a small sample of aldose or ketose (generally 0.1–0.2 mmol) into a column of resin (3–4 mL) with several mL of water and then immersing the column in a constant-temperature bath for a chosen period of time. At intervals, each of a series of such columns would be quickly chilled in an ice–water bath and then eluted with water to remove the sugars. In general, oxygen was not excluded in these operations, because the presence of atmospheric oxygen did not significantly affect either the rate at which D-fructose was consumed or the rate at which D-glucose was formed when D-fructose was allowed to react on hydroxide resin. Any amber or brownish substances formed on the resins during reaction were not eluted. Color

was not observed in the eluates, even after removal of all solvent. Furthermore, soluble aluminum compounds could not be detected in the eluate, although trace amounts of finely divided aluminum oxide were occasionally present. These particles resulted from decomposition of aluminate ion on the resin and were readily removed. An aliquot sample of each eluate was analyzed quantitatively with $\pm 1\%$ accuracy for 2-ketose by a modified anthrone method[19]. The remainder of each eluate was subjected to quantitative g.l.c. analysis for sugars (estimated accuracy, $\pm 3\%$).

Preparation of aluminate resins. — When the formate or chloride form of a strongly basic anion-exchange resin is stirred with aqueous 1.3M sodium aluminate ($NaAlO_2$), the resin anions are partially replaced by aluminate and hydroxide ions[18]. Complete substitution by aluminate and hydroxide ion was not attempted. Although displacement by OH^- cannot be avoided, decreasing the alkalinity of an aluminate solution allows the preparation of an aluminate resin of lower-than-normal hydroxide content. A low hydroxide content is desirable because it minimizes loss of ketose through alkaline degradation.

The most common method for decreasing the alkalinity of sodium aluminate solution[18] involved lowering the pH of 1.3M $NaAlO_2$ from 13.14 to 12.2 by addition of the H^+ form of a cation-exchange resin. Another method occasionally employed involved adding D-glucitol to 1.3M $NaAlO_2$ until the pH dropped to 11.5. From a practical standpoint, this particular pH was perhaps too low, because it led to the formation of an aluminate resin of low aldose-binding capacity. The lower the OH^- content of a resin, the lower the capacity of the resin to bind D-glucose[18].

Aluminate resin of normal hydroxide content has a greater loading capacity than resin of low hydroxide content. However, because the maximum yield of D-fructose from D-glucose obtained on the latter resin is appreciably higher than that on the former (to be discussed later), we chose the low-hydroxide resin for most of our work. Unless otherwise noted, these low-hydroxide resins were prepared by stirring formate resin once with an aluminate solution whose pH had been decreased by the cation-exchange method. The loading capacity of such a resin is approximately 13 mg of D-glucose per mL. (Note: Multiple treatment of formate resin with aluminate solution imparts a loading capacity that is considerably greater than that obtained by a single treatment[18].)

Loading capacity decreases with continued use of an aluminate column. This decrease is probably caused by loss of both aluminate and hydroxide ion through alkaline degradation of reducing sugars. However, as many as five D-glucose-to-D-fructose conversions have been carried out on a single column with little or no change in maximum yield. Age *per se* has not been observed to be a factor affecting loading capacity.

Conversion of D-glucose to D-fructose. — Effect of time and temperature. The respective maximum yields of D-fructose from D-glucose on aluminate resin of low hydroxide content at 2, 13, 23, and 35° were 72, 68, 62, and 44%. Use of aluminate resin of normal OH^- content gave yields lower than those obtained with aluminate resin of low-hydroxide content (54% was the maximum yield at 2° on normal-

TABLE I

CONVERSION OF D-GLUCOSE ON ALUMINATE RESIN

t (°)	*Sample wt. (mg)*	*Hydroxide content of resin*[a]	*Time (h)*	*Eluate analysis*		
				A D-*Glucose* (%)	*B* D-*Fructose* (%)	*100 − (A + B) Other substances*
2	30	Low	66	80	16	4
2	30	Low	168	62	29	9
2	30	Low	476	33	50	17
2	30	Low	1007	10	72	18
2	30	Normal	164	58	38	4
2	30	Normal	330	29	53	18
2	30	Normal	670	22	54	24
2	30	Normal	1078	15	49	36
25	30	Low	4	65	28	7
25	30	Low	8	26	53	21
25	30	Low	15	15	58	27
25	30	Low	22	9	50	41
25	30	Low	31	7	45	48
25	30	Low	47	4	30	66
35	30	Low	1	62	30	8
35	30	Low	2	45	41	14
35	30	Low	3.25	35	44	21
35	30	Low	4.5	25	43	32

[a]The resin of normal hydroxide content was prepared by treating formate resin with 1.3M sodium aluminate. Resin of low hydroxide content was prepared by treating formate resin with 1.3M sodium aluminate that had been treated with cation-exchange resin (H^+). The volume of resin bed was 3.4 mL.

hydroxide resin). At 55°, rates of disappearance of D-glucose and D-fructose through degradation are so great that only a 1% yield of D-fructose and no D-glucose remained when D-glucose was allowed to react on aluminate resin for 4 h.

Table I presents typical quantitative data for reactions of D-glucose on aluminate resin at 2, 25, and 35°. Formation of D-mannose is very slow compared with formation of D-fructose. Yields of D-mannose are no greater than about 3%. G.l.c. has, thus far, provided no evidence for isomerization of D-glucose to sugars other than D-mannose and D-fructose. (Note: Psicose can be overlooked in g.l.c. because of convergence of its peaks with those of D-fructose.) The yields of "other substances" presented in Table I are based upon the assumption that the formation of sugars other than D-fructose is negligible. These "other substances" are probably largely acid anions that are firmly bound to the resin. We have made no attempt to isolate or identify them.

B. *Effect of resin type.* When the chloride form of an anion-exchange resin was treated with a mixture of sodium aluminate (1.3M) and D-glucitol, an aluminate resin

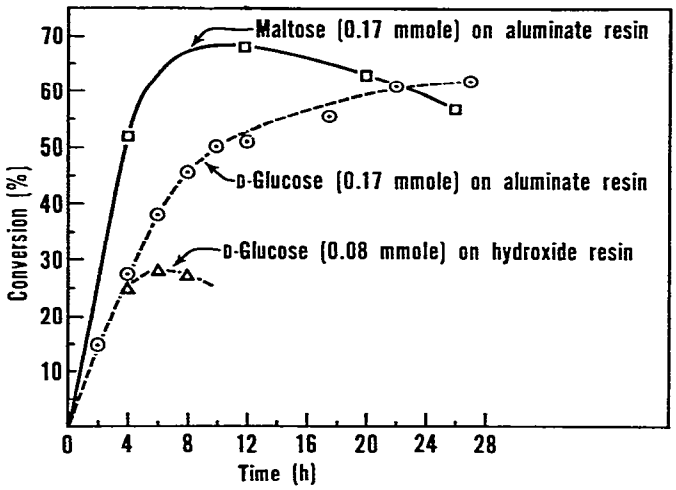


Fig. 1. Effect of time on conversion of aldose into ketose at 24–25°.

of low-hydroxide content was produced. On this resin, D-glucose was converted into D-fructose in yields as high as 66% at 27°. However, there were two disadvantages in using this particular resin over that prepared from formate resin: (*1*) the loading capacity was less and (*2*) the time required to reach maximum yield was approximately doubled. An advantage was that the resin was not discolored by pigments, whereas other resins prepared without D-glucitol became yellowish or light brown in those regions occupied by sugar. The low OH^- content (that is, OH^-/sugar <1) in the D-glucitol-treated resin is probably responsible not only for low loading-capacity and low rate of conversion into D-fructose but also for repression of pigment-forming reactions. Multiple treatment of chloride resin with sodium aluminate solution should increase the loading capacity of the resin; however, such a treatment has not been investigated.

The presence of a high concentration of hydroxide ions in an aluminate resin has a deleterious effect upon the yield of D-fructose. In an extreme situation where only OH^- and no aluminate ion are present, the maximum yield of the ketose is only 28% at 25°. Fig. 1 shows the variation in yield of D-fructose from D-glucose with time on both hydroxide resin and aluminate resin. Fig. 1 also shows that the initial rate of conversion of maltose on aluminate resin is approximately twice that of D-glucose. The greater reactivity of maltose might be caused by a higher rate of enolization (or carbanion formation) of maltose. Isbell and coworkers[20] have indicated that maltose enolizes 2.4 times faster than D-glucose.

C. *Comparison of conversion on aluminate resin with conversion in aluminate solution.* Yields of D-fructose from D-glucose on aluminate resin at 23–24° (~62% in 24–28 h) compare reasonably well with the yield reported by Haack and coworkers[7] for reaction in aqueous sodium aluminate at 30° (67% in 28 h). Yields closer to 70% are possible on resin, provided the reaction temperature is decreased to ~13° or below; however, decreasing the temperature causes an increase in the time required

TABLE II

CONVERSION OF D-MANNOSE ON ALUMINATE RESIN OF LOW HYDROXIDE CONTENT[a]

t (°)	*Sample wt. (mg)*	*Time (h)*	*Eluate analysis*			
			A D-*Mannose* (%)	*B* D-*Fructose* (%)	*C* D-*Glucose* (%)	*100 − (A + B + C)*
27	30	4	83	14	1	2
27	30	22	n.d.	38	n.d.	
27	30	31	24	45	8	23
27	30	35	n.d.	46	n.d.	
27	30	45	n.d.	37	n.d.	

[a]Same footnote as for Table I.

TABLE III

CONVERSION OF MALTOSE ON ALUMINATE RESIN[a] AT 24°

Time (h)	*Eluate analysis*				
	A Maltose (%)	*B Maltulose* (%)	*C* D-*Glucose* (%)	*D* D-*Fructose* (%)	*100 − (A + B + C + D)*
4.0	44	50	5	1	0
11.7	10	63	16	6	5
20.0	4	46	25	19	6
26.3	2	32	25	28	13
40.8	0	21	22	35	22
69.8	0	8	14	37	41

[a]Aluminate resin was of low hydroxide content prepared from formate resin and sodium aluminate solution treated with cation-exchange resin (H^+). Sample size, 0.167 mmol.

to reach maximum yield. An obvious advantage of the resin method is elimination of the aluminate-precipitation step. Aluminate ion remains on the resin during elution. Although large volumes of water are used for elution, the exceptional chromatographic properties of aluminate resin[18] allow, concurrent with elution, separation of D-fructose from unconverted D-glucose and any D-mannose that might be present. Retentivity of aluminate resin is very high for D-fructose, compared with that for D-glucose and D-mannose.

Conversion of D-*mannose into* D-*fructose.* — The rate of conversion of D-mannose into D-fructose is much lower than rate of D-glucose conversion under similar conditions. The lower conversion rate for D-mannose is at least partly related to the lower rate at which D-mannose forms a carbanion. Enolization studies by Isbell and coworkers[20] have indicated that D-mannose is only half as reactive as D-glucose. On an aluminate column at 27°, the maximum yield of D-fructose from D-

mannose was ~46% (see Table II). In these reactions, the small amounts of D-glucose formed arose from epimerization of D-mannose and isomerization of the product (D-fructose).

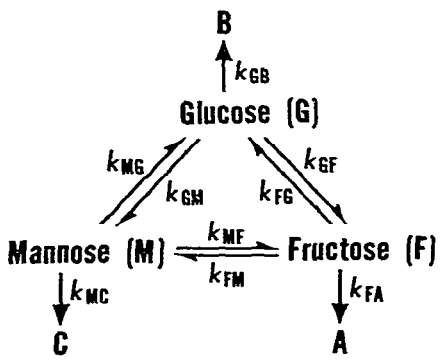
Conversion of maltose into maltulose. — On an aluminate column, maltose undergoes both conversion into maltulose and "peeling" (base-catalyzed elimination of the D-glucosyloxy group at C-4 of the reducing moiety) to give D-glucose which, in turn, isomerizes to D-fructose (see Fig. 1). The rate of isomerization to maltulose is much higher than the rate of peeling. A 63% maximum yield of maltulose was reached in about 8 h at 24°. Data on these reactions are presented in Table III. Epimerization of maltose to 4-*O*-α-D-glucopyranosyl-D-mannose was ignored, because of the high probability that the D-mannose derivative is produced in negligible amount. MacLaurin and Green[17] have reported that the extent to which cellobiose is converted to 4-*O*-β-D-glucopyranosyl-D-mannose in M sodium hydroxide at 22° is no greater than 2%; and, as we stated earlier, a similar low yield of D-mannose is produced from D-glucose on aluminate resin.

Our data leave no doubt that maltulose eliminates D-glucose. D-Glucose is also formed from maltose in alkali[21]. The slight time-lag in the generation of D-glucose from maltose on aluminate resin during the first 4 h should not be taken as evidence that maltose cannot be degraded to D-glucose without first undergoing conversion into a ketose intermediate.

Table III also shows that the pseudo-equilibrium between maltose and maltulose is greatly in favor of the latter: At the end of 41 h, a moderately large amount of maltulose and no maltose remain in the system.

In aluminate solution, the rate of peeling, relative to the rate of conversion into maltulose, appears to be much less than on resin[13].

Kinetics of the D-*glucose*–D-*mannose*–D-*fructose system on aluminate resin and hydroxide resin.* — The simplified reaction scheme shown (Scheme 1) was postulated by MacLaurin and Green[4] as a basis for calculating pseudo-first-order rate constants for reactions of D-glucose, D-mannose, and D-fructose in M sodium hydroxide. The symbols A, B, and C represent "other substances," such as saccharinic acids, produced by irreversible pathways.



Scheme 1. Simplified concept of the D-glucose–D-mannose–D-fructose system.

This same scheme was used in our own work to calculate pseudo-first-order rate constants for reactions of these sugars on aluminate and hydroxide resin. The kinetic studies have provided strong evidence (to be discussed later) that OH^- ion is solely responsible for sugar interconversions and degradations on aluminate resin, and that the aluminate ion functions only as a complexing agent for D-fructose. Complexation of D-fructose with aluminate ion increases the stability of the ketose in an alkaline environment.

Rates at which sugars G, F, and M appear or disappear may be expressed by equations *1*, *2*, and *3*.

$$d[G]/dt = k_{FG}[F] + k_{MG}[M] - (k_{GF} + k_{GM} + k_{GB})[G] \quad (1)$$

$$d[F]/dt = k_{GF}[G] + k_{MF}[M] - (k_{FG} + k_{FM} + k_{FA})[F] \quad (2)$$

$$d[M]/dt = k_{GM}[G] + k_{FM}[F] - (k_{MG} + k_{MF} + k_{MC})[M] \quad (3)$$

At the beginning of a reaction, the first two terms on the right side of each equation may be set equal to zero. The expressions then become:

$$d[G]/dt = - (k_{GF} + k_{GM} + k_{GB})[G] = - k_{-G}[G] \quad (4)$$

$$d[F]/dt = - (k_{FG} + k_{FM} + k_{FA})[F] = - k_{-F}[F] \quad (5)$$

$$d[M]/dt = - (k_{MG} + k_{MF} + k_{MC})[M] = - k_{-M}[M]. \quad (6)$$

Apparent k-values for the disappearance of starting sugar are calculated by means of equation *7*, in which a represents the percentage

$$k = \frac{2.303}{t} \log \frac{100}{a} \quad (7)$$

of starting sugar remaining at time t. Apparent k-values for the formation of a product are calculated with equation *8*, where p is the percentage

$$k = \frac{2.303}{t} \log \frac{100}{100 - p} \quad (8)$$

yield of product (molar basis).

Apparent values for k_{-G} (the rate constant for the disappearance of D-glucose), k_{GM}, and k_{GF} were determined experimentally by monitoring, respectively, the disappearance of D-glucose, the formation of D-mannose, and the formation of D-fructose during the reaction of D-glucose on aluminate or hydroxide resin. Values for k_{GB} were calculated from the expression $k_{GB} = k_{-G} - k_{GF} - k_{GM}$. By similar means, apparent values were obtained for k_{-M}, k_{MG}, k_{MF}, and k_{MC} in reactions of D-mannose and for k_{-F}, k_{FG}, k_{FM}, and k_{FA} in reactions of D-fructose.

Because of side reactions and the reversibility of aldose–ketose interconversions, apparent values of k often varied considerably during the course of reaction. True values for pseudo-first-order rate constants were therefore determined by plotting the apparent values against time and extrapolating the resulting curves to zero time. The values of $k_{initial}$ obtained in this manner should closely approximate true rate-constants. Error was minimized by using data from reactions in which sugar consump-

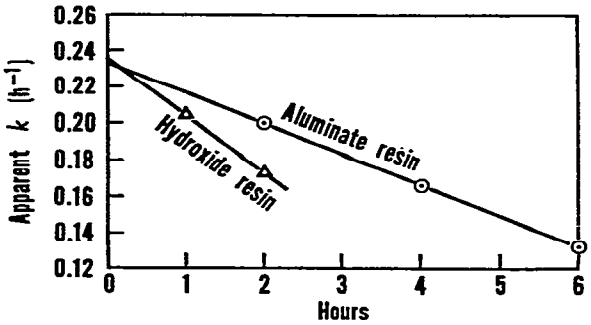


Fig. 2. Determination of $k_{initial}$ for the disappearance of D-glucose on aluminate resin and hydroxide resin at 27°.

TABLE IV

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE SYSTEM D-GLUCOSE-D-MANNOSE-D-FRUCTOSE

Pathway	*Symbol*	*Rate constant[a] (h^{-1})*			
		Hydroxide resin[b] (low OH^-) at 27°	*Hydroxide resin[b] (high OH^-) at 27°*	*Aluminate resin[b] (low OH^-) at 27°*	*1M NaOH solution at 22° (from MacLaurin and Green[4])*
G→F	k_{GF}		0.14	0.14	0.036
G→M	k_{GM}		0.0033[c]	0.0037	0.0005
G→B	k_{GB}		0.10	0.09	0.002
G→F + M + B	k_{-G}		0.24	0.23	
F→G	k_{FG}		0.096	0.0066	0.038
F→M	k_{FM}		0.018	0.0023	0.006
F→A	k_{FA}		0.101	0.0022	0.072
F→G + M + A	k_{-F}	0.214	0.215	0.0111	
M→G	k_{MG}		n.d.	0.0026[d]	0.0005
M→F	k_{MF}		0.047	0.042	0.011
M→C	k_{MC}		0.006[e]	0.005	0.002
M→G + F + C	k_{-M}		0.056	0.050	

[a]Constants for the reactions on resin are initial constants. [b]Hydroxide resins were prepared by treating formate resin with sodium hydroxide solution of appropriate concentration. Aluminate resin was prepared from formate resin and sodium aluminate solution treated with cation-exchange resin (H^-). See Experimental section for details. [c]Based solely upon a 2-h run in which the yield of D-mannose was 0.66%. [d]Estimated from the 1.05% yield of D-glucose obtained at the end of a 4-h reaction period. [e]Calculated with the assumption that $k_{MG} = 0.0026$.

tion was generally no greater than 15%. An example of such an extrapolation is shown in Fig. 2.

Evidence that reactions of reducing sugars on hydroxide or aluminate resin are pseudo-first order was provided by mathematical treatment of experimental data for reactions involving different initial weights of sugar sample. When the logarithm of

TABLE V

PRODUCT YIELDS FROM REACTIONS OF D-GLUCOSE AND D-FRUCTOSE ON ALUMINATE RESIN AND HYDROXIDE RESIN

Starting material	*Sample wt. (mg)*	*Time (h)*	*t* (°)	*Resin*[a]	*Eluate analysis*		
					D-*Glucose* (%)	D-*Mannose* (%)	D-*Fructose* (%)
D-Glucose	15	1	27	Hydroxide (high-OH^-)	81.5		11.3
D-Glucose	15	2	27	Hydroxide (high-OH^-)	70.7	0.66	17.0
D-Glucose	15	4	25	Hydroxide (high-OH^-)	64	1.2	25
D-Glucose	15	6	25	Hydroxide (high-OH^-)	53	1.9	28
D-Glucose	15	8	25	Hydroxide (high-OH^-)	43	2.3	27
D-Glucose	30	1	27	Aluminate (low-OH^-)			11.9
D-Glucose	30	2	27	Aluminate (low-OH^-)	67.0	0.70	22.1
D-Glucose	30	4	27	Aluminate (low-OH^-)	51.6	1.30	35.5
D-Glucose	30	6	27	Aluminate (low-OH^-)	45.2	1.8	42.6
D-Glucose	30	8	27	Aluminate (low-OH^-)	26	2.4	53.3
D-Fructose	30	0.67	27	Hydroxide (high-OH^-)	5.7	0.87	87.0
D-Fructose	30	1.25	27	Hydroxide (high-OH^-)	9.6	1.87	77.3
D-Fructose	30	2.5	26	Hydroxide (high-OH^-)	11	2.0	67.7
D-Fructose	30	4	26	Hydroxide (high-OH^-)	15	2.9	59.3
D-Fructose	30	7	26	Hydroxide (high-OH^-)	18	3.7	48.7
D-Fructose	30	4	27	Aluminate (low-OH^-)	2.3	0.73	96.8
D-Fructose	30	9	27	Aluminate (low-OH^-)	4.6	1.03	91.5
D-Fructose	30	13	27	Aluminate (low-OH^-)	6.3	0.99	88.6

[a]Resins were prepared as described in footnote *b* of Table IV.

TABLE VI

EFFECT OF SAMPLE WEIGHT AND HYDROXIDE CONTENT OF RESIN ON THE APPARENT PSEUDO-FIRST-ORDER RATE CONSTANT FOR DISAPPEARANCE OF STARTING SUGAR AT 27°

Starting material	*Sample wt. (mg)*	*Time (h)*	*Resin*[a]	*Eluate analysis*		*Apparent* k_{-sugar} (h^{-1})
				D-*Glucose* (%)	D-*Fructose* (%)	
D-Glucose	15	2	Hydroxide (low-OH^-)	74	16.2	0.15
D-Glucose	15	2	Hydroxide (high-OH^-)	71	17.0	0.17
D-Glucose	7.5	2	Hydroxide (high-OH^-)	69	18.7	0.19
D-Glucose	30	2	Aluminate (low-OH^-)	67	22.1	0.20
D-Glucose	15	2	Aluminate (low-OH^-)	68	22.0	0.19
D-Fructose	30	2.5	Hydroxide (high-OH^-)	15	58	0.22
D-Fructose	15	2.5	Hydroxide (high-OH^-)	—	59	0.21

[a]Resins were prepared as described in footnote *b* of Table IV.

the initial rate of disappearance of either D-glucose or D-fructose on hydroxide resin was plotted against initial weight of sugar starting material, a straight line was formed with slope = 1; a similar plot for D-glucose on aluminate resin also resulted in a slope of unity.

Table IV presents rate constants we obtained for the reaction pathways in Scheme 1 and includes rate constants k_{-G}, k_{-M}, and k_{-F} for the disappearance of D-glucose, D-mannose, and D-fructose. Table V contains some of the experimental data from which these constants were calculated. Although no attempt was made to determine k_{MG} for reaction on hydroxide resin, the value of this rate constant for reaction on hydroxide resin is probably identical with that for reaction on aluminate resin.

The reaction rates of D-fructose on aluminate resin vary with composition of the resin. On the one hand, slight differences in the preparation of this resin often resulted in substantial differences in reaction rate for D-fructose, but not for D-glucose or D-mannose. On the other hand, with hydroxide resin, reaction rates of all three sugars were unaffected by moderate change in OH^- content. Table VI contains rate data on reactions performed for short time-periods on hydroxide resins of different OH^- content. (Note: Comparison of runs is valid only where reaction times are short and identical. Actually, values for $k_{initial}$ would be preferred in a comparative study of this type.) On aluminate resin, the variable behavior of D-fructose is probably related to the ratio of aluminate to hydroxide ion in the resin; the higher the proportion of OH^-, the higher the reaction rate. Conceivably the D-fructose exists in equilibrium between two forms: a very stable aluminate complex and an alcoholate form (which may actually be a mixture of alcoholate and sugar-hydroxide adduct). The higher the proportion of OH^- in the resin, the larger would be the proportion of alcoholate, and the greater would be the probability that isomerization and degradation will occur.

In contrast to the behavior of D-fructose, neither D-glucose nor D-mannose appears to interact significantly with resin-bound aluminate ion to form an aluminate complex. Rate constants for D-glucose or D-mannose on aluminate resin are virtually identical with those on hydroxide resin (see Table IV). Furthermore, chromatography of sugars on aluminate-resin columns has provided additional evidence[18] that D-fructose, but not D-glucose or D-mannose, is strongly complexed to resin-bound aluminate: retentivity of D-fructose is very high, whereas retentivities of D-mannose and D-glucose are low and nearly the same.

A moderate change in either the weight of sugar sample or the hydroxide content of aluminate or hydroxide resin has little or no effect upon k-values for D-glucose. Likewise, rate constants for D-fructose on hydroxide resin are little influenced by sample weight. These observations (see Table VI) suggest that almost every molecule of sugar pressed into a column of hydroxide resin becomes bound to the resin either as the alcoholate anion or as sugar–hydroxide adduct. On aluminate resin, the same situation is expected for D-glucose and D-mannose. Interestingly, Garrett and Young[5] have shown that rate constants for each of these three sugars in aqueous sodium

hydroxide may vary greatly with OH^- concentration; these variations are probably caused largely by variation in the degree of alcoholate formation, which is a function of both OH^- and sugar concentration.

Comparison of k-values from studies on hydroxide resin with k-values from studies in aqueous sodium hydroxide. — The last column of Table IV lists the rate constants reported by MacLaurin and Green[4] for a D-glucose–D-mannose–D-fructose system at 22° that was initially 2mM in D-glucose and M in sodium hydroxide. Because of the 5° difference in temperature between our studies and theirs, the two sets of results are not strictly comparable. Comparability should be improved, however, by multiplying the constants of MacLaurin and Green by 1.5 in order to compensate for the temperature effect. In general, k-values for reaction on hydroxide resin are larger than those for reaction in solution; however, the relative magnitudes of k-values for reaction on resin are roughly similar to the relative magnitudes for reaction in solution, with the exception of k_{GB}. On both hydroxide resin and aluminate resin, the reaction G→B is much faster than in aqueous solution. The relatively large value for k_{GB} on aluminate resin would explain why we were unable to achieve yields of D-fructose greater than ~70%.

The pseudo-equilibrium constant K for the conversion of D-glucose into D-fructose may be calculated from known rate constants for the forward and reverse reactions. For reactions on hydroxide resin at 27°, $K = k_{GF}/k_{FG} = 1.4$. This value compares favorably with the average value of 1.3 (±0.2) for the reactions of Garrett and Young[5] in solution over a concentration range of 0.001–0.2M sodium hydroxide at 35°. At somewhat higher alkalinities (0.4–0.6M sodium hydroxide), the data of

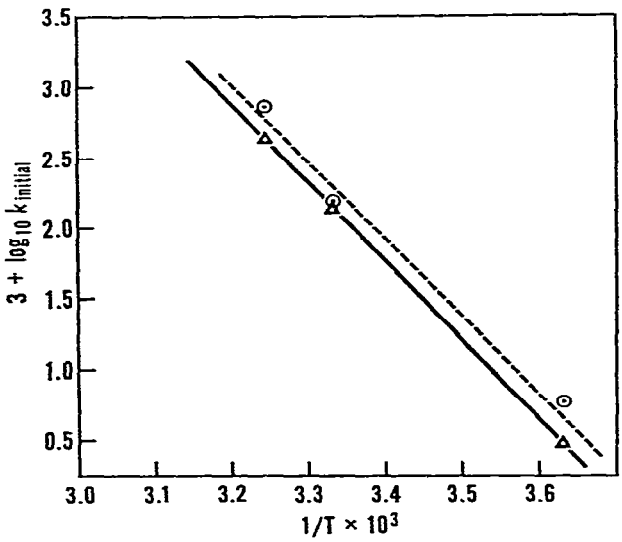


Fig. 3. Plot of $k_{initial}$ against the reciprocal of the absolute temperature for the reaction of D-glucose on aluminate resin. The solid line is for the pathway G→F. The dotted line is for the overall disappearance of D-glucose.

Garrett and Young indicate that K is slightly less than unity. The data of MacLaurin and Green[4] in M sodium hydroxide at 22° indicate a value of 0.95.

On aluminate resin, the value for K is larger than on hydroxide resin, because of the lower magnitude of k_{FG} on aluminate resin. Also, the value for K varies somewhat from one aluminate resin to another, because of the variability of k_{FG}. From the data in Table IV, $K = 21$.

Influence of temperature on rate of disappearance of D-*glucose and rate of G→F on aluminate resin.* — The energy of activation, ΔH_a, which is the energy that 1 mole of D-glucose must have in order to undergo transformation to an activated complex in the rate-determining step, was obtained by first plotting log $k_{initial}$ against the reciprocal of the absolute temperature (see Fig. 3) and then using the slope of the resulting straight line in the equation ΔH_a = slope × 2.303 × R, where R is the gas constant. For the disappearance of D-glucose, $\Delta H_a = 105$ kJ.mol^{-1}. For the conversion of D-glucose to D-fructose, $\Delta H_a = 108$ kJ.mol^{-1}, which compares reasonably well with the value of 101 kJ.mol^{-1} obtained by Garrett and Young[5] for the same reaction in sodium hydroxide solution. Shaw and Tsao[9b] obtained a value of 102 for conversion in sodium aluminate solution. The close similarity between ΔH_a for the reaction G→F and ΔH_a for the disappearance of D-glucose suggests that the same activated complex may be involved in each of the transformation pathways for D-glucose, G→F, G→M, and G→B. Kooyman and coworkers[5b] found an activation energy of ~121 kJ.mol^{-1} for both G→F and F→G in aqueous sodium hydroxide.

Mechanistic views on the alkali-catalyzed transformation of aldoses. — It is generally agreed that the first major step in aldose transformation is the formation

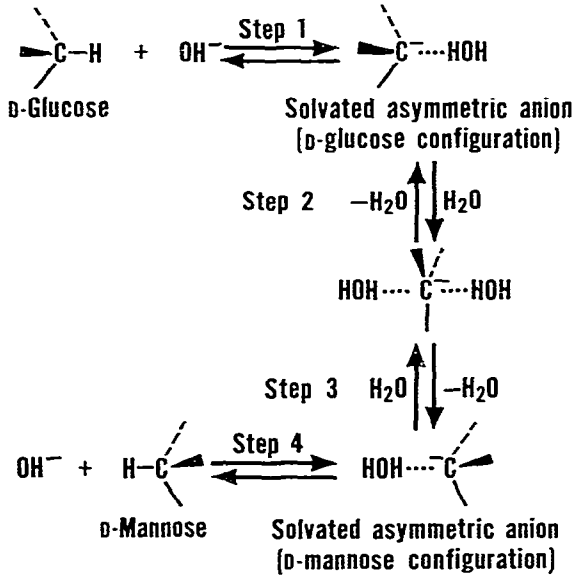


Fig. 4. Possible inversion mechanism for D-glucose and D-mannose. The carbon atom in the figures is C-1 of the acyclic aldehydo form of the aldose.

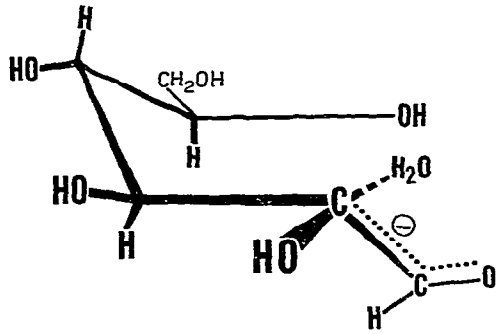


Fig. 5. Possible structure of the α-D-glucose carbanion, showing delocalization of the unshared pair of electrons.

of a carbanion intermediate through abstraction by OH^- of H-2 of the acyclic aldehydo form of the sugar. Rate of formation of carbanion possibly determines the rate of disappearance of aldose. If this is true, then subsequent transformation of the carbanion into products should be much faster than the rate at which the carbanion was formed; and the relative proportions of the various possible products would be determined by the relative magnitudes of the rate constants for the various pathways leading from carbanion to products. The extreme slowness with which D-glucose and D-mannose are epimerized has perplexed investigators for many years and deserves special comment. The carbanion formed directly from D-glucose is not identical with the one formed from D-mannose. However, in the light of current views on carbanion chemistry[22], it is reasonable to assume that the two different carbanions are interconvertible through an inversion of the type shown in Fig. 4. An assumption is made that the charged carbon atom has pyramidal geometry stabilized by solvation. The extraordinarily low yields of D-mannose from D-glucose and of D-glucose from D-mannose in alkaline solution or on aluminate and hydroxide resins suggest strongly that rate of inversion (Steps 2 and 3) is extremely low compared with the rate at which a solvated asymmetric anion is formed from its parent sugar (Steps 1 or 4). In fact, yields are so small that the question might be raised as to whether any significant amount of epimer is at all produced by this inversion process. It is conceivable that some of the epimer, if not all, is produced via the enediol through abstraction of the 1-OH proton by hydroxide ion. (The enediol is formed by rapid interaction of a water molecule with O-1 of the hybrid carbanion. This oxygen atom is the one that bears part of the delocalized negative charge as shown in Fig. 5.) Abstraction of the 2-OH proton would lead to D-fructose through an intermediate carbanion whose charge is delocalized between O-2 and C-1.

In the alkaline transformation of D-fructose into D-glucose and D-mannose, only a route involving an enediol intermediate seems plausible. The higher rate at which D-glucose is formed, relative to the rate at which D-mannose is produced (see Table IV), cannot be explained readily on the basis of either carbanion stability, carbanion inversion, or differences in reactivity between the *cis*- and *trans*-enediols

postulated by Isbell and coworkers[20]. An explanation for this difference in rate possibly lies in the difference in ease with which the acyclic forms of these two sugars cyclize. There are more nonbonded interactions in cyclic D-mannose than there are in cyclic D-glucose.

Recently, Shaw and Tsao[9a,9b] presented their views on a possible mechanism for conversion of D-glucose into D-fructose in sodium aluminate solution. These investigators looked upon the reaction as a transformation of a D-glucose–aluminate complex into a D-fructose–aluminate complex by way of an intermediate 1,2-enediol–aluminate complex. Such a mechanistic route is not applicable to transformation of D-glucose on aluminate resin, because D-glucose complexes only very weakly, if at all, with aluminate counterions.

EXPERIMENTAL

Materials and pH measurements have been described earlier[18]. Maltose was prepared free of D-glucose and oligosaccharide impurities.

Quantitative analyses of effluents. — A. *From reactions of* D-*glucose,* D-*mannose, and* D-*fructose.* D-Fructose was determined in the presence of D-glucose and D-mannose by a modified anthrone method[19], the precision of which was ±1%. To determine D-glucose and, occasionally, D-mannose, the effluent was first evaporated to dryness in a rotary evaporator (bath temperature, 50°). The residue was then dissolved in a measured volume of water (5–10 mL). A portion of this solution was analyzed for D-glucose either by means of D-glucose oxidase[23] or by g.l.c. of the *O*-trimethylsilyl derivative[18]. For the g.l.c. method, a measured amount of internal standard (methyl α-D-mannopyranoside or sucrose) was added to an aliquot of the concentrated effluent. Because of the problem of peak overlap, only the chromatographic peak for the β anomer was used in determining the content of D-glucose (estimated accuracy and precision, ±3%).

D-Mannose was determined by g.l.c. of the peracetylated D-mannononitrile. An aliquot of the concentrated mixture together with a measured amount of internal standard was evaporated. The dry residue was then treated with the proper reagents to convert the internal standard into a peracetylated derivative and to convert aldoses into peracetylated aldononitriles[24]. G.l.c. was performed isothermally at 185° on a 1.83-m × 0.32-cm stainless-steel column packed with Chromosorb W support (60–80 mesh) with 3% 3BP (neopentyl glycol succinate) as the liquid phase.

B. *From reactions of maltose.* D-Fructose, D-glucose, and combined maltose and maltulose were determined by the g.l.c. method described in Part A. Combined maltulose and D-fructose was determined by the modified anthrone method, also mentioned earlier. By difference, the amounts of maltulose and unconverted maltose were obtained. Formation of D-mannose was ignored.

Reaction apparatus. — The column for the resin was a simple, heavy-walled glass tube (23 × 0.6 cm i.d.) equipped with a stopcock at the lower end and a 500-mL (or larger) glass reservoir at the upper end. A small plug of glass wool supported

the resin bed. A ground-glass mouth at the top of the reservoir permitted the application of sample to the resin bed by means of a long-stem pipette and allowed the introduction of eluant. The volume of resin used was 3.4 mL for the aluminate-resin experiments and 4.0 mL for the hydroxide-resin experiments.

Preparation of aluminate and hydroxide resins from the formate form of anion-exchange resin. — The general preparative procedure was to stir 20 mL of wet Bio-Rad AG1-X8 strongly basic anion-exchange resin (formate form; 200–400 mesh; 3.2 mequiv per g, dry weight; 1.1 mequiv per mL, wet volume) for 5 min in 30 mL of the appropriate sodium aluminate or sodium hydroxide solution. After filtration with a fritted-disc funnel, the resin was washed repeatedly with distilled water until the washings were neutral to wide-range indicator paper.

A. *Aluminate resin of normal hydroxide content.* Formate resin (20 mL) was treated with 1.3M sodium aluminate (30 mL).

B. *Aluminate resin of low hydroxide content.* Formate resin (20 mL) was treated with 1.3M sodium aluminate solution (30 mL), the pH of which had been lowered to 12.2 by means of cation-exchange resin (H^+ form)[18]. Analysis of the filtrate for formate by a thiobarbituric acid method[25] indicated that 20% of the formate ions on the resin had been displaced by this operation.

C. *Hydroxide resins.* Formate resin (20 mL) was treated with 0.65M sodium hydroxide (20 mL) to afford a resin of moderate hydroxide content (20% substitution of formate ions by hydroxide ions, as determined by acid titration); 0.15M sodium hydroxide was used to prepare a resin of lower hydroxide content (7% substitution).

Preparation of aluminate resin of low hydroxide content from the chloride form of anion-exchange resin (D-glucitol method). — D-Glucitol (20 g) was added to 1.3M sodium aluminate (35 mL) in order to decrease the pH from ~13.2 to 11.5. The final volume of the solution was 49 mL. Into 30 mL of this solution was stirred 20 mL of Bio-Rad AG1-X10 (200–400 mesh) strongly basic anion-exchange resin (Cl^-). After 5 min, the mixture was filtered in the usual manner and then washed with a continuous flow of distilled water until essentially no trace of D-glucitol remained (the effluent was tested periodically with periodic acid–silver nitrate reagent). Incomplete removal of D-glucitol was readily detected by g.l.c. analysis of a sample of effluent. Titration of an aliquot of the combined filtrate and washings with silver nitrate showed that 13% of the Cl^- ions on the original resin had been displaced during treatment of that resin with aluminate–D-glucitol solution.

General reaction procedure. — Each sample of sugar was applied to the top of a resin bed as an aqueous solution (0.2–0.5 mL) and pressed into the column with 1–3 mL of distilled water. This operation was often accelerated by application of compressed nitrogen. (The total volume of water used in introducing a sample was occasionally governed by the loading capacity of the resin. The smallest amounts of water were used with those columns of aluminate resin prepared by treatment of Cl^- resin with D-glucitol–sodium aluminate mixture.) Immediately after the introduction of a sample, the column was immersed in a water bath maintained at the desired reaction-temperature. At intervals of time, reactions were arrested by quickly chilling

the columns in an ice–water bath. Cold water applied at 2° under pressure (provided by compressed nitrogen) produced rapid elution. Volumes of eluate from hydroxide and aluminate columns measured 500 and 1000 mL, respectively.

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