Alkaline Transformations Among Glucose, Fructose, and Mannose

EDWARD R. GARRETT AND JOHN F. YOUNG

College of Pharmacy, University of Florida, Gainesville, Florida 32601

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The kinetic parameters of the aldose–ketose transformation in dilute alkali are obtained by measuring the ultraviolet absorption of the acid derived (1.0 *M* HCl, 80.0°, 10.0 hr) hydroxymethylfurfural (HMF, λ_{max} 283 nm) which is proportional to the concentration of fructose in alkali with time when the original solution contained only the single hexoses, glucose, mannose, or fructose. An analog computer is utilized to evaluate a proposed kinetic model for the aldose–ketose transformation. The rate constants obtained are used to calculate the apparent heats of activation, entropy factors, bimolecular rate constants, and the hexose pK_a's. Ultraviolet chromophores were observed at 310 and 270 nm in the alkaline solution. These chromophores were dependent on the hexose concentration, the alkaline concentration, and the atmosphere (air, nitrogen, or oxygen) under which the reactions were conducted.

The Lobry de Bruyn–Alberda van Ekenstein alkaline interconversion among aldoses and ketoses[1,2] has been extensively studied in the hexose system of fructose, glucose, and mannose with regard to possible mechanisms and intermediates.[3] However, kinetic data were not obtained with the exception of the very recent paper by MacLaurin and Green[4] who used one set of conditions. Our present study evaluates the kinetic parameters of the interconversions among these hexoses for a series of alkaline concentrations and temperatures.

A fraction of the glucose in alkaline solution should be converted to fructose and mannose to achieve the equilibrium, and conversely.[1,2] Since an accurate method was available to measure fructose by acidic transformation (1.0 *M* HCl, 80.0°, 10.0 hr) to hydroxymethylfurfural (HMF),[5] the conversion of glucose and mannose to fructose and the loss of fructose was measured under varying conditions of temperature and alkali concentration. Haworth and Jones[6] had used essentially the same techniques of sequential alkali and acid treatment to convert glucose to HMF, but they did not attempt to quantify or study the kinetics of the alkaline conversion.

Experimental Section

Materials and Equipment.—Glucose, fructose, and mannose were obtained from Distillation Products Industries. All other chemicals were of analytical reagent grade.

A Beckman Model DU spectrophotometer, slit width 0.1 mm, was used to measure the absorbance of HMF at 283 nm. Absorbance spectra were obtained from a Cary Model 15 recording spectrophotometer. The kinetic data from the three hexoses were analyzed by the use of an EAI Model TR-48 analog computer.

Fructose Analysis.—Fructose was analyzed by the method of Garrett and Blanch.[5] A 5.0-ml aliquot of an alkaline hexose solution (0.003–0.030 *M*) was acidified to 1.0 *M* HCl and maintained for 10.0 hr at 80.0°. An aliquot was cooled and appropriately diluted. The absorbance of HMF was measured at 283 nm against a water blank.

Kinetic Procedures.—Alkaline solutions (235.0 ml) were prepared so that the desired alkaline strength (0.001, 0.002, 0.004, 0.007, 0.01, 0.02, 0.10, 0.20. 0.40, or 0.60 *M* NaOH) would be obtained after dilution to 250.0 ml. These solutions were preheated to the desired temperatures (25.0, 35.0, 40.0, 50.0, or 95°) in a constant temperature water bath. Aliquots (15.0 ml) of the 0.10 *M* hexose solutions (glucose, fructose, or mannose) were added to the preheated alkaline solutions and shaken; 5.0-ml samples were immediately and subsequently taken. These alkaline samples were acidified (20.0 ml of 1.30 *M* HCl) and stored (5°) until convenient to place in the 80.0° temperature bath for 10.0 hr. On removal from the 80.0° bath, the samples were cooled and appropriately diluted, and the absorbance read at 283 nm against a water blank. The above procedure was conducted under ambient atmospheric conditions.

In addition some of the alkaline solutions were purged for 15 min with oxygen-free nitrogen before the addition of the hexose and purged for 5 min after each sample was taken. Care was taken to tighten the glass stoppers after each purging sequence to minimize the amount of air in contact with the solutions. These nitrogen purged solutions were analytically monitored in parallel with the solutions under ambient atmospheric conditions.

Glucose–Fructose Alkaline Equilibrium Mixtures.—Mixtures of glucose and fructose (Table I) were prepared which were representative of the equilibrium established between the two hexoses at specific alkaline concentrations (0.20, 0.40, and 0.60 *M* NaOH) at 35.0°. A 15.0-ml aliquot from each of the appropriately prepared standard mixtures was put into 235.0 ml of the desired preheated NaOH solution. Initially and periodically thereafter, 5.0-ml aliquots from each of the three alkaline solutions were added to 20.0 ml of 1.30 *M* HCl. Each acidic solution was then heated at 80.0° for 10.0 hr, sampled, cooled and appropriately diluted, and the HMF absorbance read at 283 nm against a water blank.

Chromophores from Hexoses in Alkali.—The cell compartment of a Cary Model 15 spectrophotometer with a Cary automatic sample changer attachment was equilibrated at 40.0°. The cell compartment was continuously purged with oxygen-free nitrogen or oxygen as desired.

Alkaline solutions (80.0 ml) were prepared so that the desired alkaline strength (0.20, 0.40, or 0.60 *M* NaOH) would be obtained after dilution to 100.0 ml. These alkaline solutions were preheated to 40.0°. Glucose or fructose solutions (20.0 ml) were prepared so that the desired hexose concentration (0.002, 0.004, 0.006, or 0.008 *M*) would be obtained after addition to the 80.0 ml of preheated alkaline solution. On addition of the hexose solution to the alkaline solution, these solutions were shaken, immediately sampled, and placed in one of the Cary cells. Four of the five cells were used for these hexose samples; the fifth contained a blank of the specific alkaline solution being tested without hexose.

Results and Discussion

Kinetics.—The Lobry de Bruyn–Alberda van Ekenstein transformation of glucose (Figure 1), mannose (Figure 2), and fructose (Figure 3) was studied at various alkali concentrations at 35.0°. Periodic samples were taken from these alkaline reaction solutions and analyzed for the amount of HMF derived from the fructose present by acidifying (1.0 *M* HCl, 80.0°, 10.0 hr) and measuring the absorbance of the HMF produced.

The effects of temperature on acid-derived HMF at a constant alkaline concentration for glucose (Figure 4), mannose (Figure 5), and fructose (Figure 6) were also

(1) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Recl. Trav. Chim. Pays-Bas*, **14**, 203 (1895).

(2) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *ibid.*, **16**, 262 (1897).

(3) J. C. Speck, Jr., *Advan. Carbohyd. Chem.*, **13**, 63 (1958).

(4) D. J. MacLaurin and J. W. Green, *Can. J. Chem.*, **47** (21), 3947 (1969).

(5) E. R. Garrett and J. Blanch, *Anal. Chem.*, **39**, 1109 (1967).

(6) W. H. Haworth and W. G. M. Jones, *J. Chem. Soc.*, 667 (1944).

TABLE I

EXPERIMENTAL AND CALCULATED APPARENT FIRST-ORDER RATE CONSTANTS FOR THE LOSS OF HMF ABSORBANCE DERIVED FROM THE ALKALINE DECOMPOSITION OF AN EQUILIBRIUM MIXTURE[a] OF GLUCOSE, G, AND FRUCTOSE, F, WITH TIME AT 35.0°

			10^4k_1 (sec^{-1})[b]			k_{app}	
10^4[G][a]	10^4[F][a]	[NaOH], M	k_1	k_2	k_3	Calcd[c]	Expt[d]
5.29	6.71	0.20	0.542	0.692	0.186	0.104	0.100
6.20	5.80	0.40	0.711	0.658	0.303	0.146	0.155
6.70	5.30	0.60	0.844	0.669	0.436	0.193	0.180

[a] The specified mixtures of G and F were those ratios of G:F that would exist at equilibrium. [b] These are the rate constants obtained from HMF yields with time under similar conditions on the assumption of the model

$$G \underset{k_1}{\overset{k_2}{\rightleftharpoons}} F \xrightarrow{k_3} P$$

where P represents degradation products. [c] The apparent rate constant is calculated from $k_{app} = k_3/(1 + k_1/k_2)$. [d] The apparent rate constant is determined experimentally from the slope of plots of log [HMF] *vs.* time.

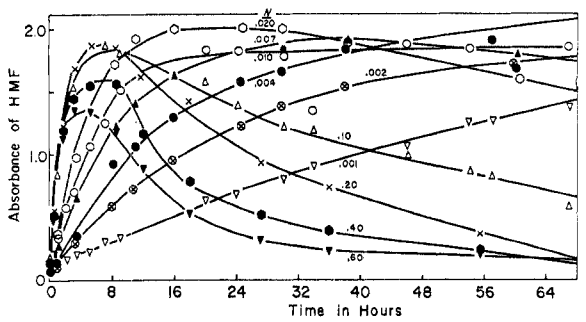


Figure 1.—Effects of NaOH concentration on the HMF absorbance derived (1.0 M HCl, 80.0°, 10.0 hr) from 0.006 M glucose at 35.0° as a function of time.

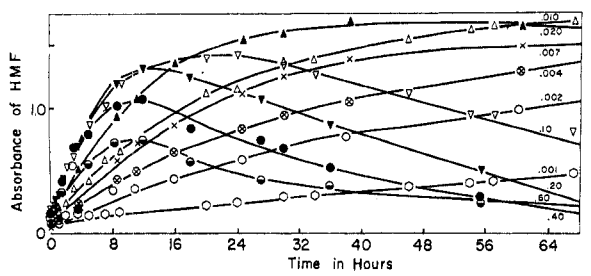
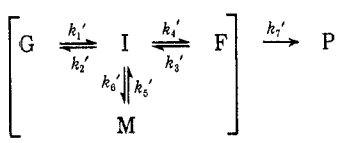


Figure 2.—Effects of NaOH concentration on the HMF absorbance derived (1.0 M HCl, 80.0°, 10.0 hr) from 0.006 M mannose at 35.0° as a function of time.

studied. The degradation of the hexoses was too fast at 95° to monitor the conversion among the hexoses.

The generally accepted theory for the Lobry de Bruyn–Alberda van Ekenstein transformation implicates an enediol intermediate. A theoretically acceptable model[7] would be the following



(1)

where glucose (G), mannose (M), and fructose (F) are in equilibrium with the intermediate (I) and where one or all species can degrade to other products (P). This intermediate has never been isolated[3] and may be assumed to be present only in small quantities. Under

(7) E. A. Davidson, "Carbohydrate Chemistry," Holt, Rinehart and Winston, New York, N. Y., 1967, pp 185–191.

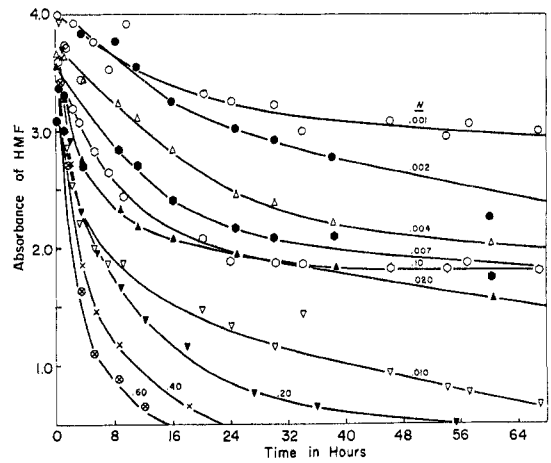
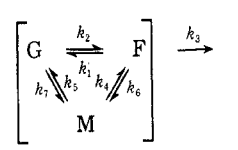


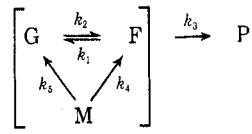
Figure 3.—Effects of NaOH concentration on the HMF absorbance derived (1.0 M HCl, 80.0°, 10.0 hr) from 0.006 M fructose at 35.0° as a function of time.

these conditions $k_2' > k_1'$, $k_4' > k_3'$, $k_6' > k_5'$ and a kinetically equivalent model of eq 1 would be



(2)

This is essentially the model used by MacLaurin and Green.[4] Wolfrom and Lewis[8] stated that only small amounts of mannose (about 2%) could be recovered from an equilibrium mixture when starting with either glucose or fructose. Miyada[9] stated that he was unable to detect any mannose when starting with glucose or fructose in alkaline solutions. This would suggest that eq 2 could be simplified to the following



(3)

If it is postulated that only the fructose degrades to other products or if the achievement of the glucose–fructose equilibrium and the loss of mannose is fast

(8) M. L. Wolfrom and W. L. Lewis, *J. Amer. Chem. Soc.*, **50**, 837 (1928).

(9) D. S. Miyada, The Lobry de Bruyn Transformation of D-Glucose and 3,4,6-Trimethyl-D-Fructose, Ph.D. Dissertation, Michigan State University, East Lansing, Mich., 1953.

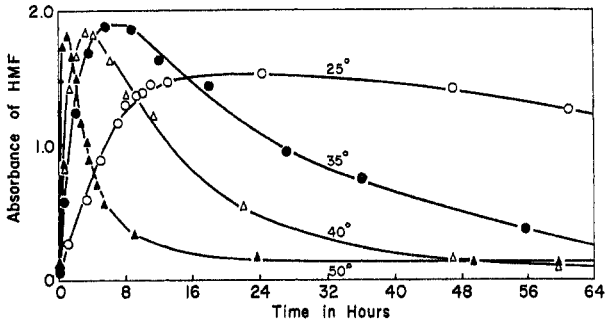


Figure 4.—Effects of temperature on the HMF absorbance derived (1.0 *M* HCl, 80.0°, 10.0 hr) from 0.006 *M* glucose solution in 0.20 *M* NaOH as a function of time.

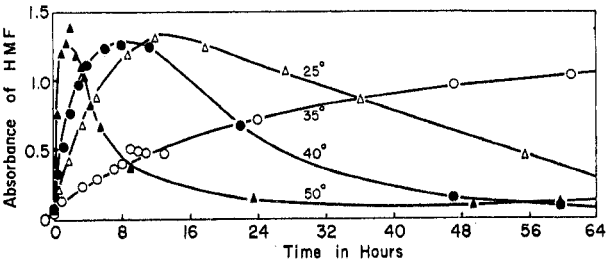
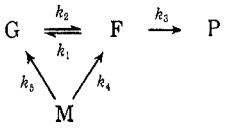


Figure 5.—Effects of temperature on the HMF absorbance derived (1.0 *M* HCl, 80.0°, 10.0 hr) from 0.006 *M* mannose solution in 0.20 *M* NaOH as a function of time.

compared to the production of other products, the model of eq 3 can be written



(4)

MacLaurin and Green[4] estimated rate constants for the degradation of mannose and glucose to other products but they were 36 times smaller in magnitude than that for the conversion of fructose to other products.

The analog computer was programmed to test the consistency of the data with eq 4. The differential equations based on eq 4 are

$$-\mathrm{d[G]}/dt = k_2[\mathrm{G}] - k_1[\mathrm{F}] - k_5[\mathrm{M}] \quad (5)$$

$$-\mathrm{d[M]}/dt = (k_4 + k_5)[\mathrm{M}] \quad (6)$$

$$\mathrm{d[F]}/dt = k_2[\mathrm{G}] + k_4[\mathrm{M}] - k_1[\mathrm{F}] - k_3[\mathrm{F}] \quad (7)$$

$$\mathrm{d[P]}/dt = k_3[\mathrm{F}] \quad (8)$$

The HMF yields were proportional to the fructose present. These yields were determined as a function of time for the same concentrations of glucose (G) and fructose (F) at a given alkali concentration and temperature. The curves were fitted by the programmed analog computer based on the model of eq 4. Appropriate values for the k_1, k_2, and k_3 constants were chosen for this fitting. These constants were maintained in the analog computer program and the k_4 and k_5 values were adjusted to fit the HMF data obtained from the reaction of the same concentration of mannose (M) under the same conditions as a function of time. This did not assume a finite amount of mannose when equilibrium was achieved.

Typical plots of the fitting of such data obtained at

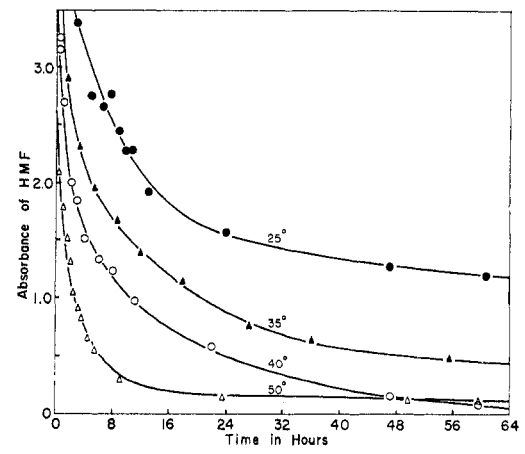


Figure 6.—Effects of temperature on the HMF absorbance derived (1.0 *M* HCl, 80.0°, 10.0 hr) from 0.006 *M* fructose solution in 0.20 *M* NaOH as a function of time.

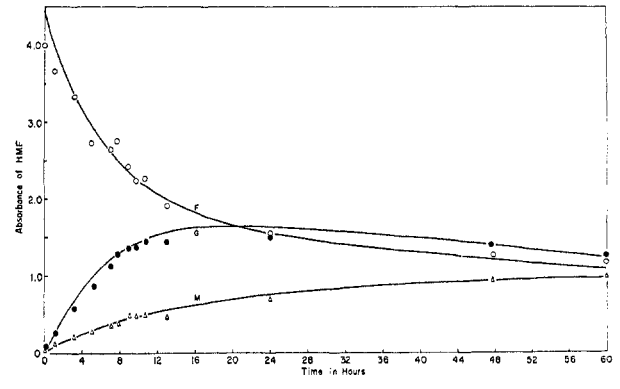
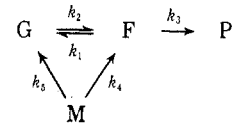


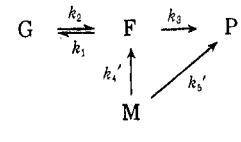
Figure 7.—Absorbance of HMF derived (1.0 *M* HCl, 80.0°, 10.0 hr) from 0.006 *M* hexose (glucose, G, fructose, F, or mannose, M) in 0.20 *M* NaOH at 25.0° as a function of time. The drawn curves were generated from the analog computer program for



the same temperature and alkaline concentration are given in Figure 7. The obtained rate constants are summarized in Table II.

The rate constants for the interconversions were all greater than that for the degradation of fructose in the cases studied in contrast to the one set of high alkaline conditions studied by MacLaurin and Green.[4]

The rate constants, k_3 and k_4 (Table II), obtained by analog computer fitting of the data are approximately equal for the higher concentrations at 35.0°, and at 40.0 and 50.0°. Although this is inconsistent with the assumption that the loss of mannose is fast compared to the production of other products (eq 4), the model of eq 4 is still valid if the major route of sugar degradation is *via* fructose. If mannose is assumed to degrade directly to other products, eq 4 may be rewritten



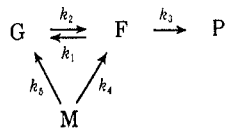
(9)

TABLE II

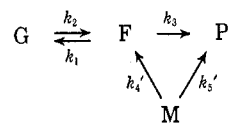
APPARENT FIRST-ORDER RATE CONSTANTS OBTAINED FROM THE ANALOG COMPUTER FITTING OF HYDROXYMETHYLFURFURAL ABSORBANCE (1.0 *M* HCl, 80.0°, 10 HR) DERIVED FROM ANALYSES OF THE REACTION OF ALKALINE SOLUTIONS OF 0.006 *M* GLUCOSE, FRUCTOSE, AND MANNOSE WITH TIME[a]

			$10^4 k_i$ (sec^{-1})						
°C	[NaOH]	pH[b]	k_1	k_2	k_3	k_4	k_5	k_4'[c]	k_5'[c]
25.0	0.20	13.16	0.194	0.186	0.0528	0.0472			
35.0	0.001	10.68	0.0169	0.0206		0.00472			
35.0	0.002	10.99	0.0478	0.0466	0.00175	0.0189			
35.0	0.004	11.24	0.0658	0.0808	0.00908	0.0329			
35.0	0.007	11.48	0.0933	0.138	0.0132	0.0591			
35.0	0.01	11.66	0.176	0.198	0.0107	0.0763	0.0151		
35.0	0.02	11.95	0.174	0.308	0.0261	0.119			
35.0	0.10	12.56	0.479	0.588	0.102	0.193	0.0283		
35.0	0.20	12.86	0.542	0.692	0.186	0.189	0.0794	0.247	0.00847
35.0	0.40	13.13	0.711	0.658	0.303	0.200	0.108	0.272	0.00333
35.0	0.60	13.30	0.844	0.669	0.436	0.167	0.0444	0.206	0.0272
40.0	0.20	12.70	1.278	1.517	0.303	0.472	0.0583	0.532	0.0150
50.0	0.20	12.42	4.222	4.389	1.489	2.083	0.542	2.272	0.0316

[a] Fitted in accordance with the model



[b] The pH was calculated from $pH = pK_w + \log [a_{OH^-}]$. [c] Alternate fittings in accordance with the model



This did not change significantly the fit of the mannose data and may be considered as a kinetically equivalent model. A typical curve is given in Figure 8 and the data is presented in Table II.

The validity of eq 4 was reinforced by following the HMF production from a mixture of glucose and fructose in the proportions anticipated at equilibrium in a given concentration of alkali at a given temperature; *i.e.*, $[F]:[G] = k_2:k_1$ (Table I). It was anticipated that a typical first-order plot should be obtained for the production of HMF as a function of time in alkali. In this case, eq 4 has reduced to

$$G \underset{k_1}{\overset{k_2}{\rightleftharpoons}} F \xrightarrow{k_3} P \qquad (10)$$

The total hexose concentration of the equilibrium mixture would be

$$[H] = [G] + [F] \qquad (11)$$

At equilibrium

$$k_1[F] = k_2[G] \qquad (12)$$

The differential equation for the disappearance of the total hexose is

$$-d[H]/dt = k_3[F] \qquad (13)$$

Equations 11 and 12 may be substituted into eq 13 and

$$-d\{[F] + (k_1/k_2)[F]\}/dt = k_3[F] \qquad (14)$$

On rearrangement of eq 14

$$-d[F]/dt = [k_3/(1 + k_1/k_2)][F] = k_{app}[F] \qquad (15)$$

On integration

$$\ln [F] = \ln [F_0] - [k_3/(1 + k_1/k_2)]t = \ln [F_0] - k_{app}t \qquad (16)$$

Since the derived HMF absorbance is proportional to the fructose (F) present, the plot of ln [HMF absorbance] *vs.* time for the reaction of this equilibrium mixture should give a straight line with a slope equal to $[k_3/(1 + k_1/k_2)]$ (eq 16). A good correlation (Table I) between the slopes using the k_i values obtained from the analog computer data of Table II for the specific equilibrium conditions tested supports the validity of eq 10.

The apparent first-order rate constants (Table II) in the Lobry de Bruyn–Alberda van Ekenstein transformation were functions of hydroxide ion activity, a_{OH^-}. In all cases but that of k_3, the slopes of such plots decreased with increasing a_{OH^-}. The hydroxide ion activity was calculated from

$$a_{OH^-} = \gamma[NaOH] \qquad (17)$$

where the NaOH values were experimental and the activity coefficients, γ, were obtained from the literature[10] and interpolated from the constructed curve of γ *vs.* [NaOH] for 35.0°.

The dependence of the apparent first-order rate constant (k_i) on the hydrogen ion activity may be given by

$$k_i = (k_{OH^-})(a_{OH^-})(f) \qquad (18)$$

(10) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold, New York, N. Y., 1958, p 729.

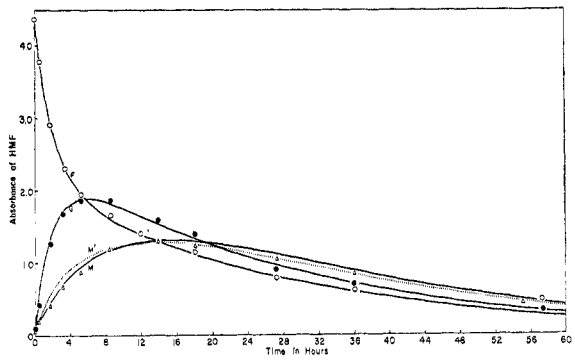
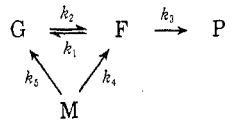
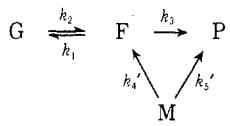


Figure 8.—Absorbance of HMF derived (1.0 *M* HCl, 80.0°, 10.0 hr) from 0.006 *M* hexose (glucose, G, fructose, F, mannose, M) in 0.20 *M* NaOH at 35.0° as a function of time. The solid lines were generated from the analog computer program



and the dotted line was generated from a similar program



where k_{OH^-} is the bimolecular rate constant and f is the fraction of the hexose in the undissociated form. The logarithm of eq 18 is

$$\log k_i = \log k_{OH^-} + \log a_{OH^-} + \log f \quad (19)$$

Since

$$\log a_{OH^-} = -pOH \quad (20)$$

and

$$-pOH = pH - pK_w \quad (21)$$

eq 19 becomes

$$\log k_i = \log k_{OH^-} + pH - pK_w + \log f \quad (22)$$

When only the uncharged species is present in a pH range, the plot of log k_i *vs.* pH should be a straight line with a slope of unity within that range. This is the case with k_3 for the entire pH range studied (Figure 9).

However, the slope is unity for k_1, k_2, and k_4 (Figure 9) only during the lower pH regions and the curves bend over in the higher pH regions. This is indicative of hydroxyl ion attack on two different species and eq 18 must be modified to

$$k_i = (k_{OH^-})(a_{OH^-})(f_U) + (k_{OH^-})(a_{OH^-})(f_D) \quad (23)$$

where f_U is the fraction undissociated and f_D is the fraction dissociated. The hydroxyl ion attack on the undissociated species is kinetically equivalent to water attack on the anion.[11] The possibility of a further hydroxyl ion attack on the dissociated species might be seen from the deviation from a subsequent plateau at the higher pH values of curve k_1 in Figure 9. The k_{OH^-}' can be roughly estimated from these two points as 2.29×10^{-4} l./mol sec (Table III, footnote *c*).

(11) E. R. Garrett, *J. Amer. Chem. Soc.*, **79**, 3401 (1957).

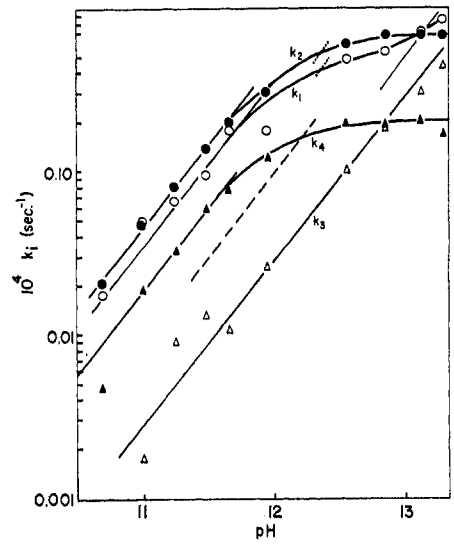


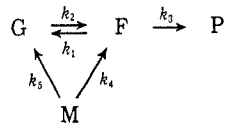
Figure 9.—Semilogarithmic plots of the apparent rate constants, k_i, at 35.0° obtained from the analog computer program for the aldose–ketose transformation against pH. The dashed line, drawn parallel to and at half the nonlogarithmic values of the ordinates of the log *k* −pH line of unit slope, intersects the log *k* − pH profile at the pK_a for that hexose.

TABLE III

THERMODYNAMIC AND KINETIC CONSTANTS FOR THE APPARENT FIRST-ORDER RATE CONSTANTS FITTED BY THE ANALOG COMPUTER PROGRAM[a]

k_i	ΔH_a[b]	log P[b]	$10^4 k_{OH^-}$[c]	ΔS^*[d]
k_1	24.1	14.9	16.1	4.75
k_2	24.2	15.0	20.7	5.67
k_3	25.4	15.3	1.34	4.15
k_4	28.6	17.6	8.44	18.1

[a] Based on the model



The individual values of the k_i are given in Table I. [b] Calculated from the slope and intercept of Arrhenius plots in accordance with log k_i = log P − $(\Delta H_a/2.303R)(1/T)$ where ΔH_a is in kcal/mol. [c] Calculated from the intercepts of log k_i *vs.* pH at 35.0° from the pH range where the plots are of unit slope (Figure 9) and conform to the expression log k_i = log k_{OH^-} − pK_w + pH where k_{OH^-} is in l./mol sec. A k_{OH^-}' can be estimated for hydroxide ion attack on the ionized sugar for the k_1 case on the premise that $k_1 = k_{OH^-}a_{OH^-}f_U + k'_{OH^-}a_{OH^-} - f_D$ where f_U and f_D are the undissociated and dissociated fractions, respectively. This can be estimated from the approach to unit slope at the higher pH values for the log k_1 *vs.* pH plot (Figure 9) and k_{OH}' is 2.29×10^{-4} l./mol sec. [d] Calculated from $\Delta S^* = 2.3R$ [log k_{OH^-} − log (kT/h) + $(\Delta H_a - RT)(2.3\,RT)$], with units of eu.

However, in light of the data at 1.0 *N* NaOH on this system by MacLaurin and Green,[4] the likelihood of OH^- attack on the ionized species is small and the deviation may be assigned to a slight aberration in the data. Thus it is most probable that $k_{OH}' \sim 0$ and eq 18, 19, and 22 are valid.

In the region where the slope of the curve from the log *k* *vs.* pH plot approaches unity, the hexose exists mainly as the uncharged species and $f_U = 1$. Thus the log k_{OH^-} values (Table III) can be calculated from corresponding pH and log k_i values on the straight line of

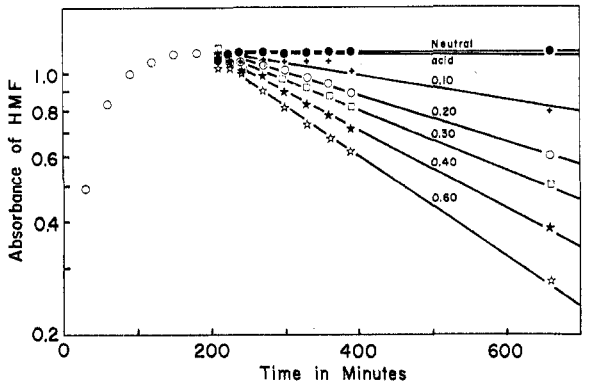


Figure 10.—Semilogarithmic plots of the absorbance of HMF derived (1.0 *M* HCl, 80.0°, 10.0 hr) from a 0.004 *M* glucose solution in 0.20 *M* NaOH at 40.0° in which the alkaline concentration was varied after 3.5 hr against time. The curves are labeled as to [NaOH].

unit slope (eq 22). The pK_w values at 35.0° were obtained from the literature.[10] A line may be drawn parallel to, and at half the nonlogarithmic values of the ordinates of the log k — pH line of unit slope. The intersection of this line and the log k — pH profile occurs at the apparent pK_a for that hexose. An example (dashed line) is presented in Figure 9 for k_4.

At a specific pH, the hydroxyl ion activity and the fractional amount of the hexose as an ionic species are fixed and the k_i for the pH can be calculated from eq 22. These calculated k_i values will coincide with the data when the pK_a has been properly designated. The curves (Figure 9) that are drawn are calculated from eq 22 and their agreement with the experimental values is apparent.

The determined kinetic pK_a values at 35.0° are consistent with the values of Izatt and coworkers[12] (Table IV).

TABLE IV
CALCULATED AND LITERATURE pK_a VALUES FOR THE TESTED HEXOSES

Hexose	Calcd pK_a	Lit. pK_a[a]
Glucose	12.36	12.46
Mannose	12.23	12.08
Fructose	12.44	12.27

[a] Reference 12.

The dependencies of rate constants on the absolute temperature were determined from the appropriate plot of data obtained at four temperatures (Table II) for 0.006 *M* hexose in 0.20 *M* NaOH in accordance with the Arrhenius' equation

$$\log k_i = -(\Delta H_a/2.303R)(1/T) + \log P \quad (24)$$

where the apparent first-order rate constants, k_i, are in sec^{-1}. The Arrhenius energy of activation, ΔH_a (Table III), was calculated from the slopes of these curves. From the absolute rate equation[13]

$$k_{OH^-} = (kT/h)e^{(\Delta S^*/R)}e^{-(\Delta H_a - RT)/RT} \quad (25)$$

or

$$\Delta S^* = 2.303R[\log k_{OH^-} - \log (kT/h) + (\Delta H_a - RT)/(2.303RT)] \quad (26)$$

(12) R. M. Izatt, J. H. Rytting, L. D. Hansen, and J. J. Christensen, *J. Amer. Chem. Soc.*, **88**, 2641 (1966).

(13) W. J. Moore, "Physical Chemistry," 3rd ed, Prentice-Hall, Englewood Cliffs, N. J., 1963, p 297.

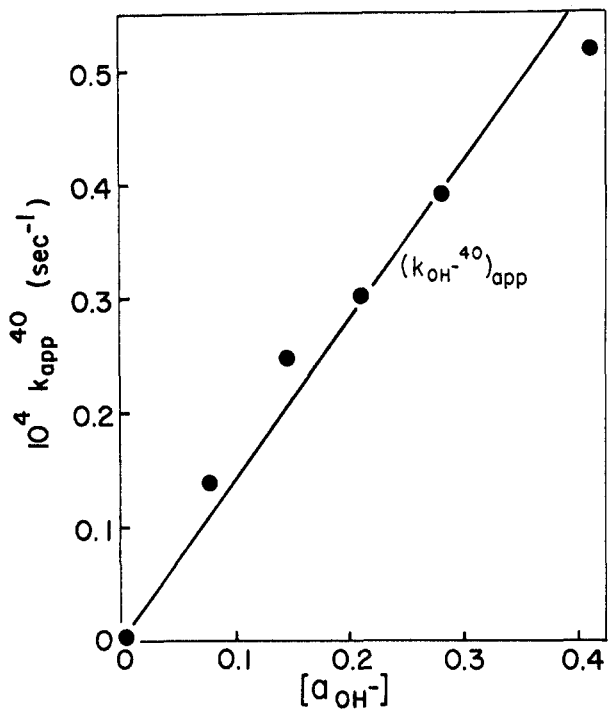


Figure 11.—Plot of the apparent rate constants, k_{app}^{40}, for the loss of HMF derived from fructose equilibrated with glucose in 0.20 *M* NaOH at 40.0° against the hydroxyl ion activity, a_{OH^-}.

the entropy of activation, ΔS^*, was obtained (Table III) where k = Boltzmann constant, h = Planck constant, R = gas constant in cal/mol °K, and T = temperature in °K.

The small positive entropy values for k_1, k_2, and k_3 indicate that the transition states have degrees of randomness similar to the original hexose. However, the high positive entropy value for k_4 is indicative of a less ordered transition state.[14]

The maximum conversion to fructose at 40.0° in 0.20 *M* NaOH occurs when glucose is allowed to react for 3.5 hr. The alkalinity of this reacting solution was varied at this time. In addition, the production of acid-derived HMF (1.0 *M* HCl, 80.0°, 10.0 hr) was followed with time for the reaction at 40.0° of one aliquot neutralized and one aliquot acidified to 0.20 *M* HCl. The first-order plots of these HMF absorbances after 3.5 hr were linear (Figure 10) and the apparent first-order rate constants, k_{app}^{40}, were a linear function of the alkaline concentration (Figure 11). The apparent bimolecular rate constant, $(k_{OH^-}{}^{40})_{app}$, was obtained from the slope of the curve in Figure 11 and was 1.40×10^{-4} l./mol sec at 40.0°.

Since the yield of HMF was invariant with time in the neutral and acid solutions (Figure 10), the established equilibrium among the hexoses must be stabilized under these nonalkaline conditions The $(k_{OH^-}{}^{40})_{app}$ value was converted to 35.0°, $(k_{OH^-}{}^{35})_{app} = 0.744 \times 10^{-4}$ l./mol sec, by a variation of eq 24; *i.e.*,

$$\log (k_{OH^-}{}^{35})_{app} = \log (k_{OH^-}{}^{40})_{app} - (\Delta H_a/2.303R)(1/T^{35} - 1/T^{40}) \quad (27)$$

where the superscripts refer to the temperatures involved and the ΔH_a (Table III) is 25.4 kcal/mol. This

(14) A. N. Martin, "Physical Pharmacy," Lea and Febiger, Philadelphia, Pa., 1964, pp 652–655.

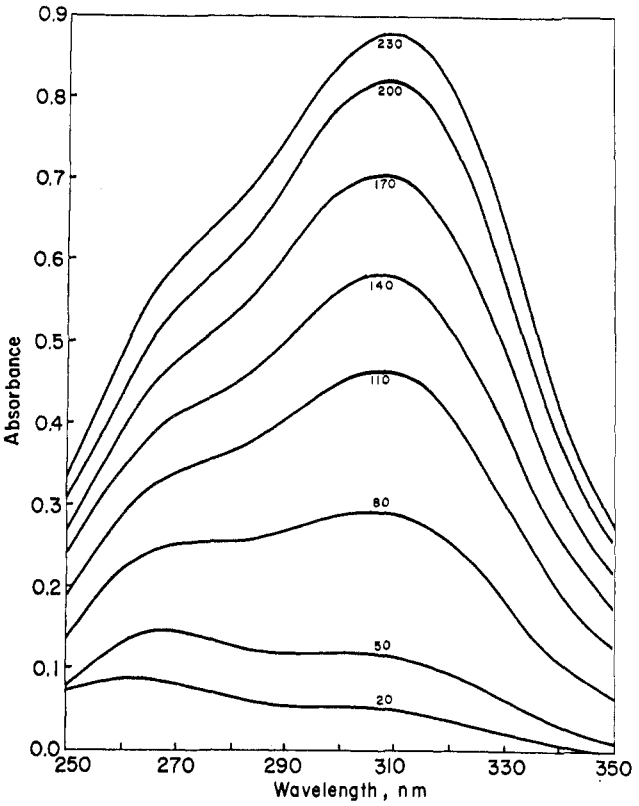


Figure 12.—Absorbance spectra of 0.008 M glucose in 0.20 M NaOH at 40.0°. The curves are labeled in minutes from time of mixing of the NaOH and glucose.

value compared favorably to the $(k_{OH^-}{}^{35})_{app}$ of 0.754 × 10^{-4} l./mol sec calculated from eq 15

$$(k_{OH^-}{}^{35})_{app} = k_{OH_3^-}/(1 + k_{OH_1^-}/k_{OH_2^-}) \quad (28)$$

where the $k_{OH_i^-}$ values were obtained from Figure 9 and listed in Table III.

Alkaline Chromophores—Two ultraviolet absorbance maxima (λ_{max} at 270 and 310 nm) appeared when the hexoses (glucose or fructose) were treated in alkaline solution. The relative absorbances of these two chromophores appeared to be concentration dependent in the early stages of the reaction. In 0.20 M NaOH at 40.0° the absorbance of the 270-nm chromophore was predominant in a 0.002 M glucose solution. The absorbances of the two chromophores were about equal in a 0.004 M glucose solution, and the absorbance of the 310-nm chromophore was predominant in a 0.008 M glucose solution (Figure 12). However, with time the 270-nm chromophore became predominant in all three cases as the 310-nm chromophore disappeared.

An explanation for the hexose concentration effect on the chromophoric response was that the 310-nm chromophoric species was being formed by a first-order process from the hexose and subsequently was converted by a zero-order process to the 270-nm chromophoric species. This may be represented by

$$G \xrightarrow{k_1} A_{310} \xrightarrow{k_0} A_{270} \quad (29)$$

where A_{310} and A_{270} represent the species that have their λ_{max} at 310 and 270 nm, respectively.

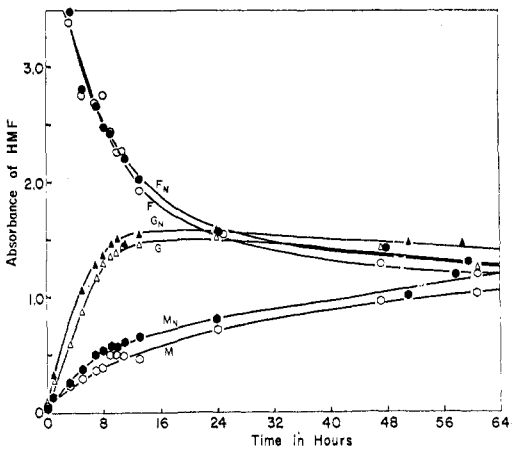


Figure 13.—Comparisons of the derived HMF absorbance between nitrogen purged (H_N) and nonpurged (H) solutions of 0.006 M hexose (glucose, G, fructose, F, or mannose, M) in 0.20 M NaOH at 25.0° as a function of time.

The pH of the hexose solution also affected the magnitude of the absorbance of the chromophores. The absorbance spectra when monitored at several pH values after a 0.004 M glucose solution was reacted in 0.20 M NaOH at 40.0° for 3.5 hr showed a decrease in absorbance of the 270-nm chromophore with lower pH.

The atmospheric conditions under which the alkaline hexose solutions were reacted drastically affected the pattern of chromophoric response. For a 0.004 M fructose solution in 0.20 M NaOH at 40.0°, the absorbance of the two chromophores was about equal under normal atmospheric conditions. However, under nitrogen atmosphere or when the fructose solution has been purged with nitrogen as well as reacted under nitrogen atmosphere, the absorbance of the 310-nm chromophore was predominant to such an extent that the absorbance contributed by the 270-nm chromophore was negligible. In contrast, when the fructose solution was purged with oxygen and reacted under oxygen atmosphere, the absorbance of the 270-nm chromophore was predominant and the absorbance contribution of the 310-nm chromophore was negligible.

An explanation for the atmospheric influence on the chromophoric response could be that the conversion of the 310-nm to the 270-nm chromophoric species was an oxidative step. This can be represented by

$$G \longrightarrow A_{310} \xrightarrow{[O]} A_{270} \quad (30)$$

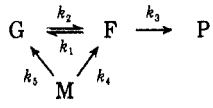
Tremendous differences were noticed in the absorbance of the alkaline chromophores with the atmosphere (nitrogen purged or unpurged) of the solution. However, the amount of fructose produced from glucose or mannose or remaining from fructose was seemingly unaffected by a difference in nitrogen or air atmosphere (Figure 13 for 25.0°) when the fructose was monitored by the acid derived absorbance of HMF. This would indicate that the alkaline chromophores were not representative of an intermediate in the aldose–ketose transformation but were the result of a degradative reaction of the hexose.

The time of maximum achievement of the 310-nm chromophore was about 6 hr, whereas, under the same conditions (0.20 M NaOH, 40.0°), the production of

fructose from glucose was 3.5 hr as monitored by the formation of HMF (1.0 *M* HCl, 80.0°, 10.0 hr) from the fructose produced.

Conclusions

The progress of this aldose–ketose transformation can be followed by monitoring the amount of fructose (1.0 *M* HCl, 80.0°, 10.0 hr) in solution with time as a function of temperature and alkaline concentration. The solutions initially contained either glucose, fructose, or mannose and the yield of fructose can be monitored from each. From the three curves generated, the analog computer can be utilized to simultaneously analyze the data according to the model



(4)

This model assumes that any intermediate in the aldose–ketose transformation is short lived and immediately forms one of the hexoses. Another assumption is that the glucose–fructose equilibrium is fast compared to the degradation to other products. A third assumption is that negligible amounts of mannose are formed from either glucose or fructose. From the rate constants obtained from the analog computer plots, the thermodynamic and kinetic parameters can be defined.

If these curves from glucose, fructose, and mannose are generated under oxygen free conditions, very little or no differences in the amount of fructose present with time is observed. This would indicate that the aldose–ketose transformation is not dependent on oxidation.

The alkaline hexose solution gives rise to two ultraviolet chromophore maxima, one at 270 nm and the other at 310 nm. The relative magnitudes of these two chromophores are affected by the concentration of hexose, concentration of alkali, and absence or presence of oxygen. The 270-nm chromophore is predominant in the lower hexose concentration range (0.002 *M* hexose) and the 310-nm chromophore is predominant in the higher range (0.008 *M* hexose). Under nitrogen atmosphere (oxygen free), the 270-mn chromophore is nonexistent while the 310-nm chromophore is greatly exaggerated. Under oxygen atmosphere, the 270-nm chromophore is predominant with the 310-nm chromophore appearing only briefly and to a limited extent. In normal atmosphere (air), there appears to be an isosbestic point around 284 nm which indicates a one to one transfer between the absorbing species. Under normal conditions the 310-nm chromophore begins to recede after about 6 hr and the 270 nm begins to dominate. Compilation of the existing data suggests the following model

$$\text{hexose} \longrightarrow A_{310} \xrightarrow{[O]} A_{270} \longrightarrow X$$

where A_{310} and A_{270} represent the species that have their λ_{max} at 310 and 270 nm, respectively.

The alkaline chromophores may not be involved in the Lobry de Bruyn–Alberda van Ekenstein transformation. Several factors may support this view. First, the time of maximum production of fructose from glucose is 3.5 hr in 0.20 *M* NaOH at 40.0°, whereas the maximum absorbance of the 310-nm chromophore is not achieved until about 6 hr under the same conditions. Secondly, nitrogen purging has little effect on the production of fructose from glucose, whereas the alkaline chromophores are drastically affected by the absence or presence of oxygen.

Registry No.—Glucose, 50-99-7; fructose, 57-48-7; mannose, 3458-28-4.

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