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Rates of Reaction of Nitrogen Bases with Sugars. I. Studies of Aldose Oxime, Semicarbazone and Hydrazone Formation[1]

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Rates of oxime, semicarbazone and hydrazone formation have been measured for a series of aldoses over the *p*H range 2.5 to 7.0. Under certain conditions anomeric rate differences were noted. The results are discussed in terms of a mechanism involving the formation of a carbonyl-containing sugar modification followed by addition of nitrogen compound to form the carbinolamine which is subsequently dehydrated to the oxime, semicarbazone or hydrazone.

The derivatives obtained by condensing sugars with compounds of the general formula $R-NH_2$, long investigated for purposes of identification and synthesis, have been of increasing biochemical interest; however, little detailed mechanistic information is available. Previous studies of aldose hydrazone[3] and glycosylamine[4] formation suggest that the reaction pathway is similar to that for other carbonyl compounds. Jencks and co-workers have recently established the *p*H dependence of the rate-limiting step for oxime,[5] semicarbazone[6] and Schiff base[7] formation. At low *p*H the rate step involves attack of free nitrogen base on the carbonyl compound. However, as *p*H is raised to neutrality the acid-catalyzed dehydration of the carbinolamine addition product becomes rate determining. The analogous reactions with sugars are complicated by the fact that aldoses in aqueous solution can exist in a number of ring modifications interrelated through an aldehydo open-chain form.[8] Ultraviolet[9] and polarographic[10] data indicate that the concentration of the latter form is very small (0.0029% for glucose[10]).

In this paper we report the detailed results for the reaction of three aldohexoses and the four aldopentoses with hydroxylamine, semicarbazide and hydrazine.

Experimental

Materials.—The "Mann Assayed" sugars: α-D-glucose, β-D-glucose, α-D-galactose, D-mannose, D-xylose, D-arabinose, D-lyxose and D-ribose were recrystallized three times from alcohol, vacuum dried at 60° for 4 hours and stored in a vacuum desiccator over Drierite. β-D-galactose was prepared by the method of Hudson and Yanovsky.[11] Sugar purity was established by optical rotation measurements. The oxime and semicarbazone derivatives were prepared as described previously.[12] The hydrochlorides of hydroxylamine, semicarbazide and hydrazine were recrystallized from aqueous ethanol and dried *in vacuo* over Drierite. The purified materials indicated acidimetric titers within two parts per 1,000 of the iodometric titers. Solutions of the nitrogen reagents were made up from the solid and, following addition of the requisite buffering materials, were neutralized to the desired *p*H with NaOH or HCl. The ionic strength was adjusted to 0.50 with NaCl. All other chemicals were reagent grade, used without further purification.

Apparatus.—Polarographic data were obtained with a Sargent model XII polarograph connected to a Fisher laboratory recorder. The conventional dropping mercury electrode had values of $m^{2/3}t^{1/6}$ of 1.65 mg.$^{2/3}$ sec.$^{-1/2}$ at −1.30 volt. The cell was a Sargent S29390 electrolysis vessel. *p*H measurements were made with a Beckman Zeromatic *p*H meter. Optical rotations were obtained with a Hilgar polarimeter. A thermostated Beckman model DU spectrophotometer fitted with matched 1-cm. silica cells was employed for spectral measurements.

Analytical Procedure.—Procedures were devised to measure the concentration of nitrogen reagent and sugar derivative. Iodometric analysis for hydroxylamine,[13] semicarbazide[14] and hydrazine[15] followed literature methods. Aliquot samples of *p*H <7.0 were buffered to *p*H 7.0 before analysis. Error due to iodine oxidation of sugar or nitrogenous derivative was negligible if analysis was completed within 2 minutes.

The spectrophotometric method was employed for oxime estimation at *p*H 7.0; >C=N—absorption was measured at 250 mμ *vs.* a blank containing buffer and hydroxylamine. It was confirmed that Beer's law is obeyed over the concentration range used in the kinetic measurements and that no other reactant absorbed at the wave length chosen for kinetic measurement.

The polarographic characteristics of aldose oximes,[12] semicarbazones[12] and hydrazones[16] have been described by Haas and co-workers. Oximes and semicarbazones may be determined from *p*H 2 to 4.6 and hydrazones from *p*H 2 to 7. The method involved setting the voltage at a point following the wave maximum (from −1.2 to −1.5 v. depending on the *p*H) and recording current as a function of time.[17] Preliminary experiments indicated that wave height is directly proportional to the concentration of nitrogenous derivative and that the sugars and nitrogen reagents do not interfere. The mercury in the polarographic cell did not appear to influence the reactions as rates obtained for systems maintained in the cell were identical with those kept in the cell only for the time interval required for intermittent current measurement. Systems requiring more than 4 hours to achieve equilibrium were routinely maintained apart from the polarographic cell except when measurements were being made.

Rate Determination.—For most experiments aqueous sugar solutions were allowed to stand overnight to achieve anomeric equilibrium. Flasks containing the nitrogen reagent and equilibrated sugar solution were placed in the thermostat and brought to temperature. Oxygen was removed by bubbling with purified nitrogen for 0.5 hr.

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before the reaction was begun. The reaction was initiated by pipetting a desired amount of one component into the other and mixing vigorously. The time of initiation of the reaction was taken as that when half the volume of pipetted solution had been delivered. A portion of the reaction mixture was then added to the thermostated polarographic or spectrophotometric cell and readings begun or aliquot portions removed periodically for iodometric analysis. For studies involving the relative reaction rates of the α- and β-anomers the weighed solid was added to the thermostated, gased nitrogen reagent solution contained in the polarographic cell, mixed with nitrogen and current measurement begun. The reaction solutions were maintained at 25 ± 0.05°.

Equilibrium conditions were considered achieved when invariant concentration was observed over a reasonably long period of time. The effectiveness of the buffer in maintaining consistent acidity was established by measuring *p*H at 0 and ∞ time for each kinetic run.

Results

A careful study of the validity of the analytical procedures by replicate analysis of known solutions, comparison of rate constants obtained with varying initial reactant concentrations and consistency of rate constants for a given run indicate that polarographic data are precise within 1.5%, the titrimetric and spectrophotometric data within 2%.

Equilibrium Constants.—Equilibrium constants for oxime, semicarbazone and hydrazone formation using the basis $K_{over\text{-}all}$ = [>C=N—R]/[a dose][R—NH] are listed in Tables I, II and III. $K_{over\text{-}all}$ values refer to total concentrations of all reactants at a given *p*H. The values are the average of at least three separate experiments employing different concentrations of starting material. Oxime formation is essentially complete at *p*H 7 as solutions containing the oximes remained unchanged over a period of several weeks.

TABLE I

EQUILIBRIUM CONSTANTS, $K_{over\text{-}all}$,[a] FOR ALDOSE OXIME FORMATION AT 25°

	*p*H		
Sugar	2.5[b]	3.6[c]	4.6[d]
Glucose	0.96	8.68	70.5
Galactose	3.46	33.3	210
Mannose	3.93	39.8	324
Xylose	5.06	51.7	403
Arabinose	5.33	50.9	411
Ribose	13.1	138	1140
Lyxose	12.0	118	1040

[a] $K_{over\text{-}all}$ = [oxime]/[sugar][NH_2OH]. [b] 0.25 *M* phosphate buffer. [c] 0.25 *M* formate buffer. [d] 0.25 *M* acetate buffer.

TABLE II

EQUILIBRIUM CONSTANTS, $K_{over\text{-}all}$,[a] FOR ALDOSE SEMICARBAZONE FORMATION AT 25°

	*p*H[b]	
Sugar	2.5	4.6
Glucose	0.97	16.1
Xylose	5.26	80.8
Arabinose	5.27	82.5
Ribose	12.9	191
Lyxose	10.2	180

[a] $K_{over\text{-}all}$ = [semicarbazone]/[sugar][$H_2NNHCONH_2$]. [b] Same reaction media as Table I.

Kinetic Studies.—Rate studies were designed to see whether sugars exhibit the same kinetic pattern as other carbonyl compounds and to evaluate the effect of sugar conformation. Rate constants were calculated from the appropriate first- or second-order equation as the average of 8 to 10 points during the reaction. The reactions were followed to about 80% of completion. All kinetic runs were made at least in duplicate. Least squares analysis indicated no drift in the values of the specific rate constants during any single kinetic experiment where equilibrated sugar solution was employed. However, runs involving the solid anomers of glucose and galactose indicated that initially the α-anomer reacts faster and the β-form slower than the equilibrium mixture (Fig. 1). However, as the reaction proceeds the rates for both anomers approach that for equilibrated sugar solution. This effect (observed with all nitrogen bases) was most pronounced at *p*H 4.6. The rates of disappearance of nitrogen base and appearance of oxime, semicarbazone or hydrazone were identical in the *p*H interval 2.5 to 7. In Fig. 2 the *p*H–rate profile for xylose oxime formation is illustrated. Tables IV, V and VI illustrate comparative kinetic data for a number of sugars and nitrogen reagents at various *p*H values. At neutral *p*H the reaction rates were affected by the buffer concentration. At *p*H values lower than

TABLE III

EQUILIBRIUM CONSTANTS, $K_{over\text{-}all}$,[a] FOR ALDOSE HYDRAZONE FORMATION AT 25°

	*p*H[b]		
Sugar	3.6	4.6	7.0
Glucose		0.451	
Xylose	0.456	1.62	29.8
Arabinose	0.464	1.91	31.0
Ribose	2.78	5.80	

[a] $K_{over\text{-}all}$ = [hydrazone]/[sugar][H_2NNH_2]. [b] At *p*H 3.6 and 4.6 reaction media follow Table I; at *p*H 7.0, 0.05 *M* phosphate buffer.

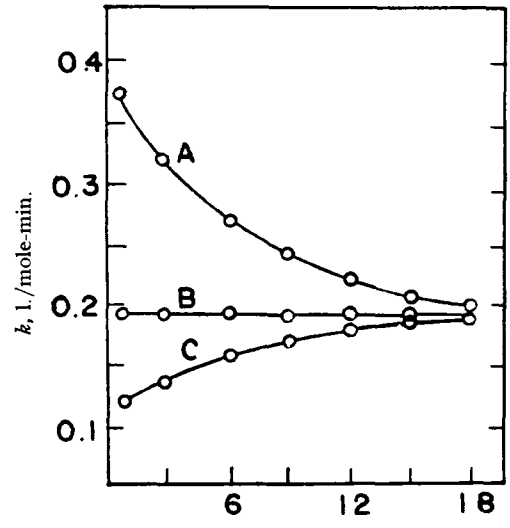


Fig. 1.—The rate of oxime formation for (A) α-D-galactose, (B) equilibrated galactose solution and (C) β-D-galactose at 25°: 1 × 10^{-3} *M* D-galactose, 1 × 10^{-3} *M* hydroxylamine; *p*H 4.6, 0.25 *M* acetate buffer.

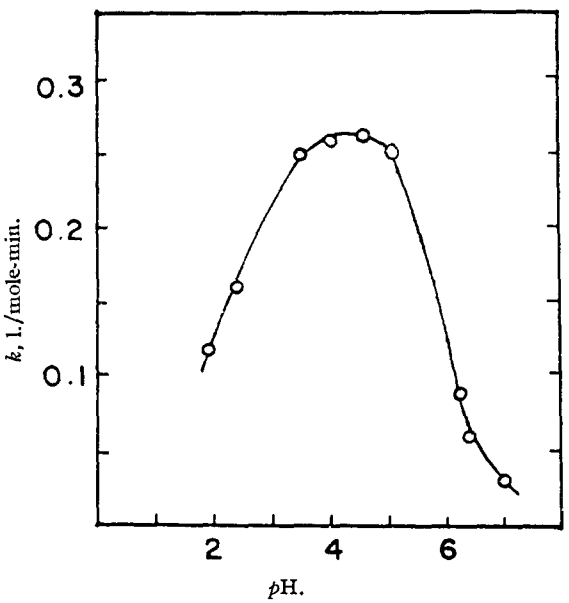


Fig. 2.—The rate of formation of xylose oxime as a function of *p*H at 25°: 1×10^{-3} *M* D-xylose, 1×10^{-3} *M* hydroxylamine; 0.25 *M* phosphate *p*H 2-3 and 6-7, 0.25 *M* formate *p*H 3-4, 0.25 *M* acetate *p*H 4-5, rates extrapolated to zero buffer concentration.

4.6 this effect was found only for semicarbazone formation. Under the same experimental conditions the reaction rates for the sugars increased in the order glucose < galactose < mannose < xylose < arabinose < ribose < lyxose.

TABLE IV

SECOND-ORDER RATE CONSTANTS (L./MOLE-MIN.) FOR OXIME FORMATION AT 25°

Sugar	*p*H[a] 2.5	3.6	4.6	7.0
Glucose	0.0210	0.0412	0.0430	0.078
Galactose	.088	.182	.192	
Mannose	.118	.197	.242	
Xylose	.145	.248	.259	0.139
Arabinose	.216	.439	.475	.206
Ribose	.327	.601	.679	.347
Lyxose	.360	.700	.838	.431

[a] Same reaction media as Tables I and III.

TABLE V

SECOND-ORDER RATE CONSTANTS (L./MOLE-MIN.) FOR SEMICARBAZONE FORMATION AT 25°

Sugar	*p*H[a] 2.5	4.6
Glucose		0.107
Xylose	0.140	.404
Arabinose	.233	.622
Ribose	.331	.910
Lyxose	.375	

[a] Same reaction media as Table I.

TABLE VI

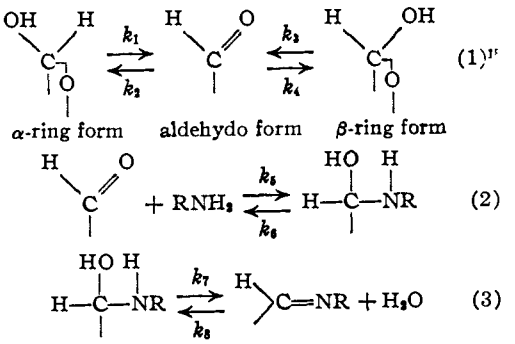
SECOND-ORDER RATE CONSTANTS (L./MOLE-MIN.) FOR HYDRAZONE FORMATION AT 25°

Sugar	*p*H[a] 4.6	7.0
Glucose	0.077	
Xylose	.281	0.072
Arabinose	.462	0.096

[a] Same reaction media as Table IV.

Discussion

The data are consistent with a reaction pathway involving formation of a reactive sugar modification followed by reaction with nitrogen base to form the carbinolamine and subsequent dehydration.



Consider the equilibria relating the aldehydo (>C=O) and ring sugar modifications in aqueous media

$$K_{1,2} = [>C{=}O]/[\alpha],\ K_{3,4} = [>C{=}O]/[\beta] \quad (1a,b)$$

At equilibrium (evidenced by attainment of constant optical activity)

$$K_{1,2}[\alpha] = K_{3,4}[\beta] \quad (1c)$$

and

$$K_{1,2}/K_{3,4} = [\beta]/[\alpha] = K_{\alpha,\beta} \quad (1d)$$

Literature data[19] for a number of aldoses indicate that $K_{\alpha,\beta} \neq 1$ because the structural differences between the α- and β-ring forms result in different degrees of steric strain. Since the amount of aldehydo form in equilibrium with cyclic modification is different for each anomer, the addition of a given anomer to solution will result in time-dependent aldehydo concentration until equilibrium among all species is achieved. >C=O concentration will decrease to equilibrium for the less stable anomer and increase with time in the case of the more stable modification.

If the aldehydo form is the reactive sugar species, three kinetic patterns could appear in the formation of nitrogen derivatives: Case 1. If attainment of anomeric sugar equilibrium (constant >C=O) is rapid compared to reaction with R–NH_2 the over-all rate constant for derivative formation (k_{obs}) will be identical for both anomers and time invariant.

Case 2. Where mutarotation rate is similar to that for reaction with R–NH_2, k_{obs} will be affected by the change in aldehydo concentration during the course of reaction depending on the sugar anomer—increasing or decreasing with time to a constant value similar to that for a sugar solution equilibrated prior to reaction with R–NH_2.

Case 3. If reaction with R–NH_2 is rapid compared with mutarotation, conversion of sugar anomer to >C=O is rate determining. Consequently k_{obs} will depend on the anomer employed and will not vary with time. The *p*H–rate pattern

(18) For purposes of simplicity only the two pyranose ring modifications are considered at this point.

(19) Reference 8, p. 52.

should follow that observed for mutarotation, *e.g.*, minimum rate at *p*H ~4.5 which increases rapidly on either side of this value.[20]

At *p*H 4.6 the kinetics follow the lines suggested by case 2. The less stable sugar anomer is initially more reactive than the more stable form (Fig. 1). In time the reaction rates increase or decrease to a value similar to that obtained for a previously equilibrated sugar solution. This effect is most apparent at *p*H 4.6 where minimum rate of mutarotation[20] and maximum rate of reaction with R-NH_2[5-7] are observed. Change in *p*H from 4.6 accelerates mutarotation and retards reaction with nitrogen base with the result that an anomeric steady state is reached early in the reaction and kinetics consistent with Case 1 are observed. Sugars which mutarotate rapidly at all *p*H values (lyxose, ribose) do not exhibit anomeric rate variation. Anomeric rate differences have been observed for a number of sugar reactions including oxidation[21] and addition of HCN.[22]

Under conditions where mutarotation is not rate determining the kinetic features suggest that reaction steps following formation of reactive sugar species are similar to those observed for other carbonyl compounds.[5-7] At neutral *p*H the rate-determining step involves dehydration of the carbinolamine addition product (eq. 3) as the reaction is subject to both general and specific acid catalysis. The rate increases to a maximum at *p*H 4 to 5, decreasing rapidly upon further increase in acidity (Fig. 2). This is consistent with a transition in rate step to rate-determining attack of R-NH_2 (eq. 2). The *p*H–rate profile (Fig. 2) is complicated by the observation that ring opening is rate determining in the vicinity of *p*H 4.6 and thus this portion of the curve is not comparable to situations in which the rate step involves the nitrogen base. Bimolecular kinetics are observed at all *p*H values. At low *p*H this is consistent for a rate step involving reaction of nitrogen base and carbonyl. At neutral *p*H first-order kinetics would be expected if most of the carbonyl compound were present as the addition compound. However, for the sugars the relative amount of carbonyl form available for formation of carbinolamine addition product is extremely small compared with the over-all sugar concentration and second-order kinetics are observed.

The similarity in constitution of the isomeric aldoses seems to leave little scope for explanation of rate differences based solely on the structure of the reactive (aldehydo) modification. We suggest that rates are related to the concentration of this species in equilibrated sugar solution. Although the concentration of >C=O has been evaluated only for glucose,[10] it is reasonable to expect that it would be a function of the stability of the ring forms. Theoretical studies of pyranoside conformation have led to the assignment of instability ratings based on structural features.[23] An anomer is assumed to be found in the conformation with the lowest instability rating. For a given sugar the anomer with the lower instability rating would be expected to predominate at equilibrium. This has been verified for a number of aldoses.[23a] For the aldohexoses reported herein instability ratings increase in the order glucose < galactose < mannose suggesting that >C=O (and rate constant) should follow the same sequence. This is borne out experimentally (Tables IV–VI). Instability ratings for the aldopentose pyranosides follow the order xylose < arabinose = ribose < lyxose. Rate constants are observed to follow this sequence with the exception that ribose is considerably more reactive than arabinose (Tables IV and V). Although the number of stability factors is the same for ribose and arabinose, the effect occurs at a different carbon on the two rings and thus can provide a further stability variable. A further complication arises from the observation that although ribose and lyxose are found in the pyranose form at the moment of dissolution,[24] mutarotation[19] data and proton magnetic resonance spectra[25] indicate the presence of considerable amounts of furanose ring modifications upon equilibration. Thus a more complete interpretation of the effect of sugar structure on reaction rate must await additional information concerning aldehydo concentration and the size and conformation of the ring forms.

The contention that reactivity is a function of carbonyl concentration is supported by the results of a study of hydrazone formation for related galactose acetates.[3c] The rate of hydrazone formation increased in the order tetra-O-acetylgalactopyranose <<< tetra-O-acetylgalactofuranose < aldehydo-galactose pentaacetate. Here reaction rate is consistent with the ability of an acetate to provide aldehydo intermediate in that the less stable furanose acetate would yield more >C=O than the pyranose derivative but less than the aldehydo pentaacetate.

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