Sodium *N*-Chlorobenzenesulfonamide as a Selective Oxidant for Hexosamines in Alkaline Medium: A Kinetic and Mechanistic Study

Kanchugarakoppal S. Rangappa,* Manikanahally P. Raghavendra,
Dandinasivara S. Mahadevappa, and Doddegowda Channegowda[†]

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore - 570 006, India

Oxidation of D-mannosamine (**1**), D-glucosamine (**2**), and D-galctosamine (**3**) by sodium *N*-chlorobenzenesulfonamide or chloramine-B (CAB) at 313 K is followed by a shortening of carbon chain and obeys the rate law, rate = k[CAB][sugar][HO$^-$]^x, where x is less than unity. The products are arabinonic acid, ribonic acid, and erythronic acid for **1** and **2** with smaller amounts of glyceric and hexonic acids, while lyxonic and threonic acids are predominant in the oxidation of **3** with smaller amounts of glyceric and hexonic acids. Proton inventory studies made in a H$_2$O−D$_2$O mixture point toward a single transition state. In the proposed mechanism the alkoxy anion (S$^-$) of the hexosamine formed in a base-catalyzed reaction at C-1 carbon is subjected to an electrophilic rate-limiting attack by Cl$^+$ of the oxidant. The hexonic acid formed is decarboxylated with loss of ammonia to form the respective pentose, which is further converted into the corresponding pentonic acid. The breaking of the bond between C-1 and C-2 carbons in pentose yields tetronic acids. The thermodynamic parameters for sugar alkoxy anion formation and activation parameters for the rate-limiting step have been evaluated.

Introduction

The sodium salts of *N*-arylhalosulfonamides generally known as organic haloamines are strong electrolytes[1−3] in aqueous solution and behave as sources of halonium cations. They are capable of effecting an array of molecular transformations, including limited oxidation of specific groups. The benzene analogue, chloramine-B (C$_6$H$_5$SO$_2$NClNa·1.5H$_2$O or CAB) is easy to prepare, and it has been employed for the oxidation of diverse substrates. An important reaction that has been well documented is the decarboxylation and deamination of amino acids.[4] A survey of literature indicates limited information[5] on the mechanistic aspects of oxidation of hexosamines by halogens. The hexosamines are oxidized by NaOCl to the respective pentoses, thus decreasing the carbon chain.[6] Herbst[7] reports the oxidation of hexosamines by chloramine-T to the corresponding pentoses, but no mechanistic details are available. As a part of our broad program on the oxidtion of monosaccharides by the *N*-haloamines,[5] we herein report the kinetic and mechanistic aspects of the oxidation of three hexosamines, D-mannosamine, D-glucosamine, and D-galactosamine, by CAB in alkaline medium at 40 °C. The results of this study suggest that the oxidation occurs through the cleavage of both C−C and C−H bonds to form a mixture of aldonic acids from these hexosamines. On the basis of these data, a novel pathway for the oxidation of hexosamines by CAB is proposed. It was interesting to note that the oxidation products analyzed by HPLC and GC-MS gave products which indicated that oxidation went beyond the pentose stage. The products were found to be the identical under both stoichiometric and kinetic conditions.

Results and Discussion

The kinetics of oxidation of hexosamines by CAB were investigated at several concentrations of the reactants. With hexosamine (S) in excess, plots of log [CAB] vs time were linear ($r > 0.9992$, $s \le 0.01$) indicating a first-order dependence on [CAB]$_o$. The pseudo-first-order rate constants k_{obs} calculated from these plots are given in Table 1. The values decrease slightly with varying [CAB]$_o$ possibly owing to a side reaction[8] involving the formation of NaClO$_3$ (3NaOCl → NaClO$_3$ + 2NaCl). The rate increases with increase in [S]$_o$ (Table 1) and a plot of log k_{obs} vs log[S]$_o$ was found to be linear ($r > 0.9990$, $s \le 0.01$) with unit slope, indicating a first-order dependence on hexosamine as well. Further, a plot of k_{obs} vs [S]$_o$ was linear ($r > 0.9980$, $s \le 0.03$) and passed through the origin, showing that the sugar−oxidant complex has only a transient existence. The rate of reaction shows a fractional order dependence on [HO$^-$] (Table 2), as plots of log k_{obs} vs log[HO$^-$]$_{eff}$ were linear ($r > 0.9988$, $s \le 0.02$) with slopes less than unity.

Addition of the reaction products, benzenesulfonamide (BSA) and Cl$^-$ ion did not alter the rate of reaction,

---

* Author for correspondence.
† Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC 20007-2197.
⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1997.
(1) Campbell, M. M.; Johnson, G. *Chem. Rev.* **1978**, *78*, 65.
(2) Bishop, E.; Jennings, V. J. *Talanta* **1958**, *1*, 197.
(3) Mahadevappa, D. S.; Ananda, S.; Murthy, A. S. A.; Rangappa, K. S. *Tetrahedron* **1984**, *10*, 1673.
(4) Gowda, B. T.; Mahadevappa, D. S. *J. Chem. Soc., Perkin Trans. 2* **1983**, 323.
(5) Iyengar, T. A.; Puttaswamy; Mahadevappa, D. S. *Carbohydr. Res.* **1990**, *197*, 119; **1990**, *204*, 197.
(6) Matsushima, Y. *Sci. Pap. Osaka Univ.* **1951**, No. 31, 1 through *Chem. Abstr.* **1952**, *46*, 7052.
(7) Herbst, R. M. *J. Biol. Chem.* **1937**, *119*, 85.

(8) Agarwal, M. C.; Mushran, S. P. *J. Chem. Soc., Perkin Trans. 2* **1973**, 762.

Table 1. Effect of Reactant Concentrations on the Rate of Oxidation of Hexosamines by CAB at 40 °C[a]

$10^3[CAB]_o$, (M)	$10^2[S]_o$, (M)	$10^4 k_{obs}$ (s^{-1})		
		D-mannos-amine	D-glucos-amine	D-galactos-amine
1.0	2.0	3.40	5.22	18.80
1.5	2.0	3.29	5.16	18.66
2.0	2.0	3.20	5.15	18.50
2.5	2.0	3.16	5.10	18.56
3.0	2.0	3.10	5.15	18.53
3.5	2.0	3.14	5.10	18.45
4.0	2.0	3.05	4.98	18.20
2.0	1.0	1.52	2.50	8.95
2.0	3.0	4.68	7.50	27.45
2.0	4.0	6.30	10.00	36.12
2.0	5.0	7.60	12.70	46.20
2.0	6.0	9.20	14.84	57.57

[a] $[HO^-]_{eff} = 30.0 \times 10^{-2}$ M, $I = 0.6$ M.

Table 2. Effect of Varying [HO$^-$] on the Reaction Rate at 40 °C[a]

$10^2[HO^-]_{eff}$[b] (M)	D-mannosamine, $10^4 k_{obs}$ (s^{-1})[c]	D-glucosamine, $10^4 k_{obs}$ (s^{-1})[c]	D-galactosamine, $10^4 k_{obs}$ (s^{-1})[c]
5.0	1.29 (1.17)	2.15 (1.92)	7.50 (7.40)
10.0	1.90 (1.88)	3.10 (3.13)	10.94 (11.60)
20.0	2.60 (2.69)	4.20 (4.55)	15.05 (15.10)
30.0	3.20 (3.15)	5.15 (5.36)	18.50 (18.58)
40.0	3.70 (3.64)	5.95 (5.89)	20.80 (20.70)
50.0	4.25 (4.04)	6.66 (6.26)	22.30 (22.13)

[a] $[CAB]_o = 2.0 \times 10^{-3}$ M, $[S]_o = 2.0 \times 10^{-2}$ M, $I = 0.6$ M. [b] $[HO^-]_{eff}$ indicates that its neutralization by the hydrochloride of hexosamine has been taken into account. [c] Values in parentheses refer to rate constants calculated from eq 9.

indicating the absence of these compounds in a preequilibrium, to the rate-limiting step.

The effect of varying ionic strength (I) of the medium, maintained by the addition of NaClO$_4$, was checked. The rate increased with increase in ionic strength and a plot of log k_{obs} vs $(I)^{1/2}$ gave straight lines ($r > 0.9991$, $s \leq 0.001$) with slopes of 0.6–0.8 (Figure 1).

The solvent composition of the medium was varied by adding methanol (0–40% v/v). The rate decreased with increase in methanol content. Plots of log k_{obs} vs $1/D$, where D is the dielectric constant of the medium, were linear ($r > 0.9983$, $s \leq 0.02$) with negative slopes (Figure 2).

The reaction was studied over a range of temperatures and the Arrhenius plot, log k_{obs} vs $1/T$ was linear ($r > 0.9978$, $s \leq 0.03$). The activation energy, E_a, for the composite reaction was calculated from this plot. The other parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, $\Delta G^{\ddagger}$ and the Arrhenius parameter A (in terms of log A) were computed from the E_a values (Table 3).

The rate increased in D$_2$O medium, and proton inventory studies were made in H$_2$O–D$_2$O mixtures of varying deuterium content. Values of the solvent isotope effect, $k(H_2O)/k(D_2O) = 0.50$–0.60 for the three hexosamines.

Addition of reaction mixture to acrylamide solutions did not initiate polymerization of the latter, indicating the absence of free radical species in the mixture.

A comparison of the HPLC and GC retention times of the reaction products with those of the standards indicated that arabinonic, ribonic, erythronic, and glyceric acids are the oxidation products for both D-glucosamine and D-mannosamine. However, with D-galactosamine, lyxonic, threonic, and glyceric acids were found to be the products of oxidation. Besides these, small quantities of hexosaminic acids were observed (Figure 3 and Table 4)
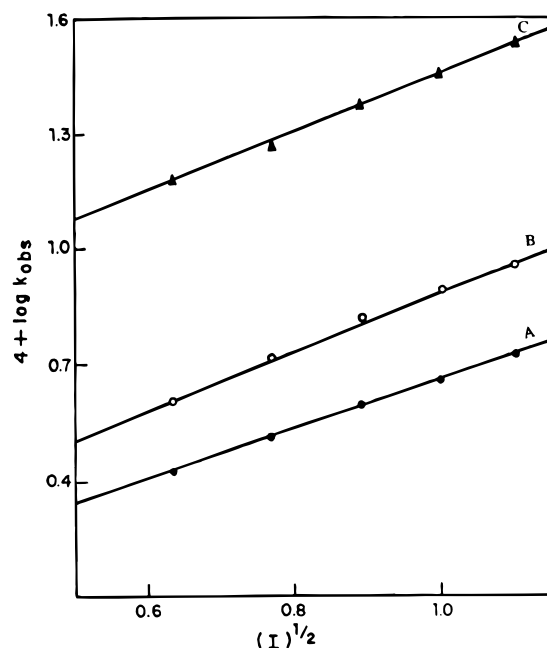

Figure 1. Plots of log k_{obs} vs $I^{1/2}$: (A) D-mannosamine, (B) D-glucosamine, (C) D-galactosamine. $[CAB]_o = 2.0 \times 10^{-3}$ M; $[S]_o = 2.0 \times 10^{-2}$ M; $[HO^-] = 30.0 \times 10^{-2}$ M; temperature = 40 °C.
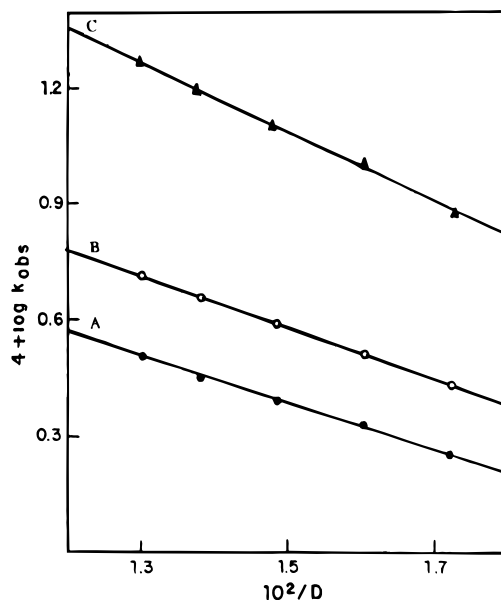
Figure 2. Plots of log k_{obs} vs $1/D$: (A) D-mannosamine, (B) D-glucosamine, (C) D-galactosamine. $[CAB]_o = 2.0 \times 10^{-3}$ M; $[S]_o = 2.0 \times 10^{-2}$ M; $[HO^-] = 30.0 \times 10^{-2}$ M; $I = 0.6$ M; temperature = 40 °C.

in the oxidation of the hexosamines. The oxidation products of hexosamines were also analyzed at 0.5, 1, 2, 4, 8, 16, and 24 h. The relative proportions of various aldonic acids formed (Figure 3 and Table 4) were similar at all time points analyzed (but the formation of six-carbon aldonic acids were observed only after 4 h), revealing that the lower-carbon aldonic acids were not derived from the initially formed six-carbon aldonic acids. The identities of all the oxidation products were confirmed from their mass fragmentation patterns.

The sodium salts of *N*-aryl-*N*-halosulfonamides behave like strong electrolytes in aqueous solution. Bishop and

Table 3. Kinetic Data for the Oxidation of Hexosamines by CAB in Presence of NaOH

hexosamine	temp (K)	$10^4 k_{obs}$ (s^{-1})	E_a (kJ mol^{-1})	$\Delta H^\ddagger$ (kJ mol^{-1})	$\Delta G^\ddagger$ (kJ mol^{-1})	$\Delta S^\ddagger$ (J K^{-1} mol^{-1})	log A
D-mannosamine	308	1.75	105.0	102.4	97.8	14.7	16.0
	313	3.20					
	318	6.20					
	323	11.52					
D-glucosamine	308	2.89	97.9	95.3	96.6	−3.9	15.0
	313	5.15					
	318	9.33					
	323	16.50					
D-galactosamine	308	11.30	81.1	78.5	93.2	−46.9	12.8
	313	18.50					
	318	30.00					
	323	48.50					

Table 4. HPLC Analysis of the Products Formed by the Oxidation of Hexosamines by CAB in Alkaline Medium

hexosamine	products (mol %)[a]						
	arabinonic acid	ribonic acid	erythronic acid	lyxonic acid	threonic acid	glyceric acid	hexonic acid
D-mannosamine	36	24	32	–	–	5	3
D-glucosamine	37	20	33	–	–	7	3
D-galactosamine	–	–	–	33	52	10	5

[a] Based on the peak areas normalized using response factors obtained by analyzing standard aldonic acid solution. The mole proportions of products formed at 0.5, 1, 2, 4, 8, and 16 h were similar to those observed at 24 h, except that the presence of six-carbon aldonic acids was evident only after 4 h. Similar product profiles were observed even when the reactions were carried out under kinetic conditions.
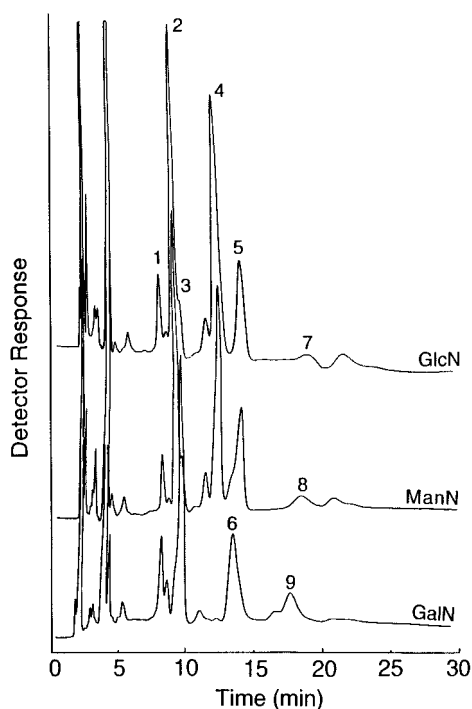

Figure 3. HPLC analysis of the products formed by the oxidation of hexosamines by CAB in presence of alkali at 40 °C. (1) Glyceric acid; (2) erythronic acid; (3) threonic acid; (4) arabinonic acid; (5) ribonic acid; (6) lyxonic acid; (7, 8, and 9) hexonic acids Glc N, Man N, and Gal N, respectively, represent the oxidation products of D-glucosamine, D-mannosamine, and D-galactosamine.

Jennings,[2] Morris and co-workers,[9] Hardy and Johnston,[10] and Higuchi and Hussain[11] have explained the several types of equilibria present in acid and alkaline solutions of these compounds. Thus chloramine-B (RNClNa, where

R = $C_6H_5SO_2^-$) ionizes in aqueous solution (eq 1) and furnishes different oxidant species. The anion is protonated in acid solutions (eq 2):

$$RNClNa \rightleftharpoons RNCl^- + Na^+ \qquad (1)$$

$$RNCl^- + H^+ \underset{K_a}{\rightleftharpoons} RNClH \qquad (2)$$
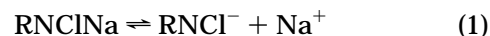
$$K_a = 1.5 \times 10^{-5} \text{ at } 25 \text{ °C}$$

Although the free acid has not been isolated, there is sufficient experimental evidence for its formation in solution.[12] It can further undergo disproportionation/hydrolysis (eqs 3 and 4, respectively) giving the sulfonamide RNH$_2$, the dichloramine RNCl$_2$, and HClO:

$$2RNClH \rightleftharpoons RNH_2 + RNCl_2 \qquad (3)$$

$$RNClH + H_2O \rightleftharpoons RNH_2 + HClO \qquad (4)$$
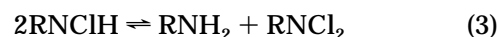
In alkaline solutions, the following equilibria are reported.

$$RNCl^- + H_2O \rightleftharpoons RNH_2 + ClO^- \qquad (5)$$

$$RNCl^- + H_2O \rightleftharpoons RNClH + HO^- \qquad (6)$$

$$RNClH + HO^- \rightleftharpoons RNH_2 + ClO^- \qquad (7)$$

Equations 5 and 7 suggest a retardation of rate with the addition of the reaction product RNH$_2$, while eq 6 predicts a decrease in rate by hydroxyl ions. Since neither of these have been observed in the present set of experiments, the most likely oxidizing species for hexosamines is the anion RNCl$^-$ itself.

(9) Morris, J. C.; Salazar, J. R.; Winemann, M. A. *J. Am. Chem. Soc.* **1948**, *70*, 2036.
(10) Hardy, F. F.; Johnston, J. P. *J. Chem. Soc., Perkin Trans. 2* **1973**, 642.
(11) Higuchi, T.; Hussain, A. *J. Chem. Soc.* **1967**, 549.
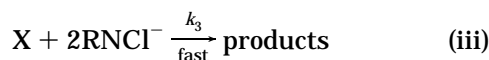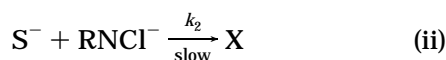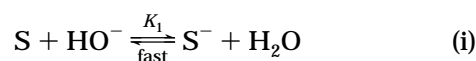
(12) Mahadevappa, D. S.; Rangaswamy, *Rev. Roum. Chim.* **1977**, *22*, 1233.

Table 5. Thermodynamic Quantities for Alkoxy Anion Formation and Activation Parameters for the Rate-Limiting Step and for the Oxidation of Hexosamines by CAB

temp (K)	D-mannosamine		D-glucosamine		D-galactosamine	
	K_1', dm^3 mol^{-1}	$10^2 k_2$, dm^3 mol^{-1} s^{-1}	K_1', dm^3 mol^{-1}	$10^2 k_2$, dm^3 mol^{-1} s^{-1}	K_1', dm^3 mol^{-1}	$10^2 k_2$, dm^3 mol^{-1} s^{-1}
308	5.78	1.43	4.80	2.27	5.14	7.69
313	6.50	2.38	6.00	4.17	7.72	13.33
318	8.31	4.20	8.00	7.10	9.50	22.22
323	9.55	7.40	10.29	12.50	13.13	38.20
ΔH (kJ mol^{-1})		27.9		42.7		35.9
ΔS (J K^{-1} mol^{-1})		40.9		−94.0		−113.9
ΔG (kJ mol^{-1})		72.0		−72.4		71.9
E_a (kJ mol^{-1})		88.3		95.8		88.9
$\Delta H^{\ddagger}$ (kJ mol^{-1})		85.7		93.2		86.3
$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})		−2.6		25.5		13.2
$\Delta G^{\ddagger}$ (kJ mol^{-1})		86.6		85.1		82.1
log A		15.1		16.5		15.9

In alkaline solutions, sugars undergo enolization to form the alkoxy anions.[13] In the absence of other reactants, these anions undergo epimerization and isomerization (Lobry de Bruyn-Alberda van Ekenstein transformation) to form a mixture of aldoses and ketoses.[13] However, in the presence of CAB, the alkoxy anion (S$^-$) reacts with RNCl$^-$ to form an intermediate, which in turn undergoes cleavage to form the products (Table 4).

In view of the observed first-order dependence of rate on [CAB]$_o$ and [S]$_o$ and fractional order dependence on [HO$^-$], the following reaction sequence (Scheme 1) is proposed for the oxidation of hexosamines by CAB in alkaline solutions:

Scheme 1

$$S + HO^- \underset{\text{fast}}{\overset{K_1}{\rightleftharpoons}} S^- + H_2O \qquad (i)$$

$$S^- + RNCl^- \xrightarrow[\text{slow}]{k_2} X \qquad (ii)$$

$$X + 2RNCl^- \xrightarrow[\text{fast}]{k_3} \text{products} \qquad (iii)$$

If [S]$_t$ represents the total hexosamine concentration, then [S]$_t$ = [S] + [S$^-$], from which

$$[S]_t = [S^-]\left[\frac{[H_2O] + K_1[HO^-]}{K_1[HO^-]}\right]$$

Therefore

$$[S^-] = \frac{K_1[S]_t[HO^-]}{[H_2O] + K_1[HO^-]}$$

Since rate = k_2[S$^-$][CAB], substituting for [S$^-$], rate law 8 can be derived as

$$-d[CAB]/dt = \frac{k_2 K_1[CAB][S]_t[HO^-]}{[H_2O] + K_1[HO^-]} \quad \text{or}$$

$$-d[CAB]/dt = \frac{k_2 K_1'[CAB][S]_t[HO^-]}{1 + K_1'[HO^-]} \quad (8)$$

where $K_1' = K_1/[H_2O]$.

From eq 8,

$$k_{obs} = \frac{k_2 K_1'[S]_t[HO^-]}{1 + K_1'[HO^-]} \quad \text{or}$$

$$1/k_{obs} = 1/k_2 K_1'[S]_t[HO^-] + 1/k_2[S]_t \quad (9)$$

Values of formation constant K_1' of alkoxy anion and its decomposition rate constant k_2 were evaluated from the double reciprocal plots of $1/k_{obs}$ vs $1/[HO^-]$. Using the values of K_1' and k_2 obtained at 40 °C (Table 5), k_{obs} values (in Table 2) were checked using eq 9. It is seen that there is good agreement between the calculated and experimental sets of values (Table 2), thus supporting the proposed mechanism.

The thermodynamic parameters for the equilibrium step i and activation parameters for the rate-limiting step ii in Scheme 1 could be evaluated as follows: Hydroxyl ion concentration (as in Table 2) was varied at several temperatures, and values of K_1' and k_2 were determined at each temperature. A van't Hoff plot was made for the variation of K_1' with temperature (i.e, log K_1' vs $1/T$), and values of the enthalpy of reaction ΔH, entropy of reaction ΔS, and free energy of reaction ΔG were calculated. An Arrhenius plot of log k_2 vs $1/T$ yielded the activation parameters for the rate-limiting step in Scheme 1. These are shown in Table 5. A comparison of the latter values with those obtained for the composite reaction shows that these values mainly refer to the rate-limiting step, supporting the fact that reaction before the rate-determining step is fairly rapid, involving little activation energy.

Scheme 1 and rate law 8 can explain the observed experimental facts. The increase of rate in D$_2$O medium supports a fast preequilibrium hydroxyl ion transfer.[14-16] A comparison of the proton inventory plots with standard curves[17] implies the involvement of a single proton in the transition state. A primary salt effect is observed as the rate increases with increase in ionic strength of medium,[18] thus supporting the involvement of two negative ions in the rate-limiting step. The Debye−Huckel plot

(13) Pigman, W.; Annet, E. F. L. J. *The Carbohydrates: Chemistry and Biochemistry*, 2nd ed.; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1972; Vol. 1A, p 165.
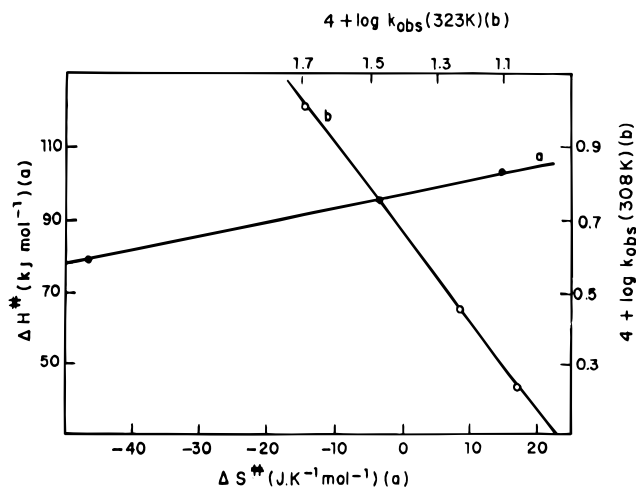
(14) Collins, C. J.; Bowman, N. S. *Isotope Effects in Chemical Reactions*; Van-Nostrand-Reinhold: New York, 1970; p 267.

(15) Albery, W. J.; Davies, M. H. *J. Chem. Soc., Faraday Trans.* **1972**, *68*, 167.

(16) Gopalakrishnan, G.; Hogg, J. L. *J. Org. Chem.* **1985**, *50*, 1206.

(17) Isaacs, N. S. *Physical Organic Chemistry*; Longman: New York, 1987; p 273.

(18) Laidler, K. J. *Chemical Kinetics*, 3rd ed.; Harper and Row: New York, 1987; p 200.

Figure 4. Isokinetic plot of (a) $\Delta H^{\ddagger}$ vs $\Delta S^{\ddagger}$ and (b) log k_{obs} (308 K) vs log k_{obs} (323 K).

has a slope of 0.6–0.8 (Figure 1) and the expected slope of unity has not been realized. This may be due to the fact that the ionic strength employed is well above the formal Debye–Huckel range. Alternatively there could be Bjerrum ion pair formation.[18]

The rate decreased with decrease in the dielectric constant (*D*) of the medium. The effect of composition of the solvent on rate for a reaction involving two negative ions of charges $Z_A e$ and $Z_B e$ is given[19] by the Scatchard equation 10,

$$\log k = \log k_o - Z_A Z_B e^2 / D k T d_{AB} \qquad (10)$$

where k_o is the rate constant in a medium of infinite dielectric constant, d_{AB} refers to the size of activated complex, and k and T are the Boltzmann constant and absolute temperature, respectively. Figure 2 shows that the plot of log k_{obs} vs $1/D$ is linear and from the slope, d_{AB} is computed as 3.90, 3.40, and 2.50 Å for D-mannosamine, D-glucosamine, and D-galactosamine, respectively. The values are found to be reasonable in comparison with those of other reactions of similar nature.[20]

An isokinetic relation was noted when plots of enthalpy and entropy of activation were found to be linear (Figure 4, *r* = 0.9996, *s* = 0.01). The value of isokinetic temperature from this plot was 409 K, which is greater than the experimental temperature (313 K), indicating that the reaction is enthalpy controlled. The relationship was found to be genuine through the Exner criterion by plotting log k_{obs} (308 K) vs log k_{obs} (323 K) (*r* = 0.9989, *s* = 0.02, Figure 4). The value of β calculated from the relation, $\beta = T_1(1 - q)/(T_1/T_2) - q$, where *q* is the slope of the Exner plot,[21] was found to be 407 K in agreement with the isokinetic point (409 K) calculated from the enthalpy–entropy relation. Since the β-value is higher than the experimental temperature (313 K), it indicates an enthalpy-controlled reaction, which is further supported by the fact that E_a values (Table 3) are the least for the fastest reaction and highest for the slowest reaction.

(19) Laidler, K. J. *Chemical Kinetics*, 3rd ed.; Harper and Row: New York, 1987; p 193.
(20) Laidler, K. J. *Chemical Kinetics*, 2nd ed.; Tata-Mcgraw Hill: Bombay, 1965; p 214.
(21) Exner, O. *Collect. Czech. Chem. Commun.* **1964**, *29*, 1094.

Scheme 2
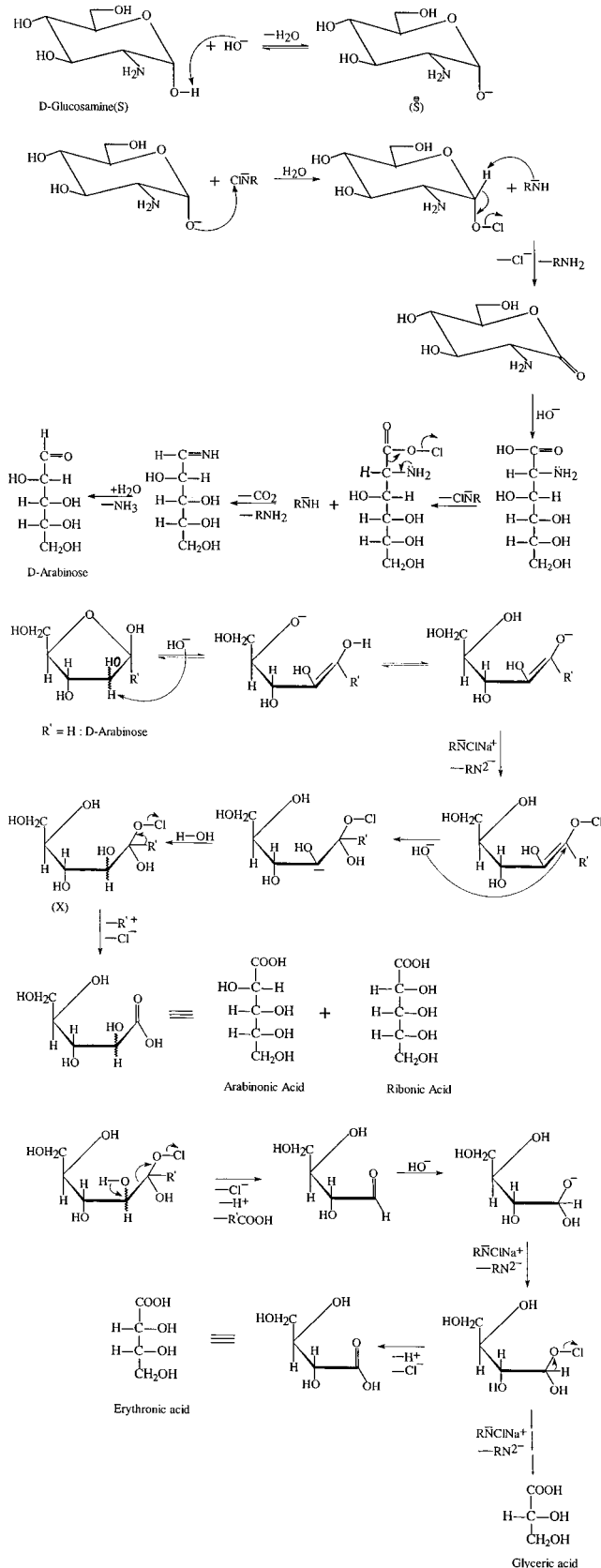
The rate-determining step (Scheme 1) involves an interaction between similarly charged ions which would require a high activation energy. It is found to be so, as the composite activation energy is around 90 kJ mol^{-1} for the reactions. The near constancy of $\Delta G^{\ddagger}$ values for

all the three hexosamines indicates that a similar mechanism is operative in the oxidation of hexosamines. The rate of oxidation of D-galactosamine is faster than both D-glucosamine and D-mannosamine, possibly because of the fact that the former has a higher percentage of the reactive β-anomer.[22] This is also clearly reflected in the values of k_2, the rate constant for the rate-limiting step.

A possible mode of oxidation of D-glucosamine (or D-mannosamine) by CAB in alkaline medium is shown in Scheme 2. The hexosamine reacts with the anion of oxidant in presence of alkali to form 2-aminogluconic acid, which reacts with the oxidant to form D-arabinose through decarboxylation followed by deamination in the form of NH_3 via hydrolysis of the intermediate imine. The pentose is further oxidized to arabinonic acid and its epimer ribonic acid. The former predominates over its epimer. This is possibly due to stabilization of transition state, as the preceeding intermediate has hydrogen bonding involving hydroxyl hydrogen on C-5 with oxygen on C-2. The C−C bond scisson between C-1 and C-2 on pentose yields the tetronic acids. The H and OH groups on C-3 are not significantly isomerized. This explains the formation of erythronic acid. Further bond breaking between C-2 and C-3 results in the formation of glyceric acid.

A similar mechanism can be drawn for the oxidation of D-galactosamine by CAB into a mixture of D-lyxonic acid, D-threonic acid, and D-glyceric acids in alkaline medium.

Experimental Section

Materials. D-Mannosamine hydrochloride, D-glucosamine hydrochloride, D-galactosamine hydrochloride, and D-ribono-1,4-lactone were from Sigma Chemicals. D-Arabino-1,4-lactone from Pfansfiehl Labs (Waukegan, IL) and analytical reagent grade chemicals were used. Fresh aqueous solutions of hexosamines were prepared using triply distilled water.

Chloramine-B, was prepared[23] by passing chlorine through a solution of benzenesulfonamide in 4.0 M NaOH for 1 h at 343 K. The product was collected, dried, and recrystallized from water (mp 170 °C dec). Its purity was checked by its ^{1}H and ^{13}C NMR spectra and also by iodometric assay of active chlorine in its aqueous solutions. Solutions of CAB were preserved in brown bottles to arrest its photochemical deterioration. Sodium perchlorate was used for maintaining a constant high ionic strength "to swamp" the reaction. It was found that the sugars were unaffected by the presence of this compound in high concentrations. Heavy water (99.4% D) for solvent isotope studies was supplied by the Bhabha Atomic

Research Centre, Trombay, India. The dielectric constant of the reaction medium was altered by the addition of methanol in varying proportions (% v/v), and values of permittivity for methanol−water mixtures reported[24] in literature were employed. Blank experiments performed showed that methanol was not oxidized by CAB under the experimental conditions.

Stoichiometry. The stoichiometry run of oxidation of hexosamines with excess CAB solution over that of sugar indicated that three moles of oxidant were consumed per mole of hexosamine, to form the aldonic acids.

Product Analysis. The oxidation products were analyzed by Dionx HPLC with pulsed amperometric detection using a CarboPac PA1 high pH anion exchange column (4 × 250 mm).[25] An isocratic elution with 0.2 M NaOH was used. The products were identified by comparison of their HPLC retention times with retention times of the standard aldonic acids and by GC-MS (see below).

For GC-MS characterization, the reaction mixture was extracted with diethyl ether to remove benzenesulfonamide and then passed through Ag 50W-X12(H^{+}) and Ag 4-x4(base) resins. The Ag 4-x4 resins were eluted with 1 M pyridine/1 M acetic acid, pH 5.2, and lyophilized. The products were converted into their trimethylsilyl drivatives and then analyzed by GC-MS.

Kinetic Measurements. Pseudo-first-order conditions were maintained for the kinetic runs ([S]$_o$ ≫ [CAB]$_o$). The oxidant and the requisite amounts of hexosamines, alkali, NaClO$_4$ solutions, and H$_2$O (for constant total volume) were thermostated for 30 min at 40 °C. The reaction was initiated by the rapid addition of CAB to the mixture, and its progress was monitored by iodometric estimation of unconsumed CAB in known aliquots of the reaction mixture at regular intervals of time. The reaction was studied for more than two half-lives. Pseudo-first-order rate constants calculated from log [CAB] vs time plots were reproducible within ±5%. Allowance was made in adjusting the alkali concentration [NaOH]$_{eff}$ for neutralizing the hydrochloride of substrate during the kinetic runs.

Regression analysis of experimental data, to obtain regression coefficient r, and s, the standard deviation of points from the regression line, was performed with an EC-72 statistical calculator.

(22) Angyal, S. J. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 15.
(23) Chrzaszewska, A. *Bull. Soc. Sci. Lett. Lodz, Cl. 3* **1952**, *16*, 5 through *Chem. Abstr.* **1955**, *49*, 212.

(24) Akerloff, G. *J. Am. Chem. Soc.* **1932**, *54*, 4125.
(25) Hardy, M. R.; Townsend, R. R. *Methods Enzymol.* **1994**, *230*, 208.