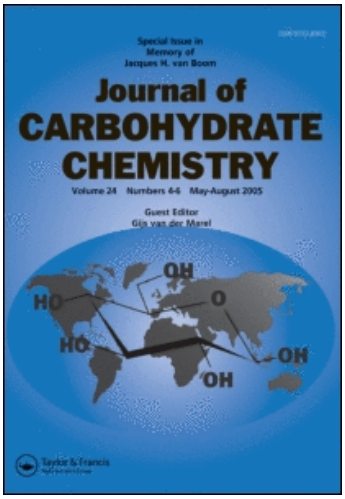


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# Mechanistic Investigations of Oxidation of Amino Sugars by Sodium *N*-Chloro-*P*-Toluenesulfonamide in Alkaline Medium

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# MECHANISTIC INVESTIGATIONS OF OXIDATION OF AMINO SUGARS BY SODIUM *N*-CHLORO-*p*-TOLUENESULFONAMIDE IN ALKALINE MEDIUM

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## ABSTRACT

The kinetics of oxidation of D-glucosamine and D-galactosamine (S) to the respective aldonic acids by sodium *N*-chloro-*p*-toluenesulfonamide or chloramine-T (CAT) in the presence of alkali at 40 °C follows the rate law, -d [CAT] / dt = $k$ [CAT] [S] $[HO^-]^x$, where x <1. The products, *p*-toluenesulfonamide and $Cl^-$ ions had no influence on rate. Increase of ionic strength increased the rate and the Debye-Hückel plot had a slope of 0.6. The rate decreased when the dielectric constant of medium was decreased and values of $d_{AB}$, the size of activated complex, were calculated by using the Scatchard equation. The rate increased in $D_2O$ solution and the inverse solvent isotope effect $k(D_2O)$ / $k(H_2O)$ was 1.94 and 1.53, respectively, for the two amines. Proton inventory was studied in $H_2O$-$D_2O$ mixtures. Composite activation parameters for the reaction were determined from Arrhenius plots. The mechanism assumes the formation of sugar alkoxy anion, a rate limiting complexation with oxidant, formation of the respective pentose through decarboxylation and deamination, and further oxidation to D-arabonic and D-lyxonic acids, respectively. The thermodynamic parameters for sugar-alkoxy anion formation and activation parameters for the rate limiting step were evaluated from double reciprocal plots of $1/k_{obs}$ vs.1/ $[HO^-]$.

## INTRODUCTION

The sodium salts of *N*-arylhalosulfonamides generally known as organic haloamines are strong electrolytes[1] 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 prominent member of this group is chloramine-T ($p$-$CH_3$-$C_6H_4SO_2NClNa$. $3H_2O$, hereinafter abbreviated as TsNClNa or CAT) and many of its oxidation reactions have been kinetically investigated.[2,3] An important CAT 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 amino sugars by halogens. The amino sugars are oxidised by NaOCl to the respective pentoses, thus reducing the carbon chain.[6] Herbst[7] reports the oxidation of amino sugars by CAT to the corresponding pentoses, but no mechanistic details are available. As a part of our broad programme on the oxidation of sugars by the *N*-haloamines,[5] we herein report the kinetic aspects of the oxidation of two amino sugars, D-glucosamine and D-galactosamine by CAT in alkaline medium at 40 °C. It was interesting to note that the oxidation of amino sugar went beyond the pentose stage, resulting in the formation of the respective aldonic acid.



## RESULTS AND DISCUSSION

The kinetics of oxidation of amino sugars by CAT were investigated at several concentrations of the reactants. With substrate in excess, plots of log [CAT] vs. time were linear ($r > 0.9992$, $s \leq 0.01$) indicating a first order dependence on $[CAT]_o$. The pseudo first order rate constants $k_{obs}$ calculated from these plots are given in Table 1. The values decrease slightly with varying $[CAT]_o$ possibly owing to a side reaction[8] involving the formation of $NaClO_3$. 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 ($r > 0.9955$ $s \leq 0.03$) with unit slope, indicating a first order dependence on amino sugar as well. Further, a plot of $k_{obs}$ vs. $[S]_o$ was linear ($r > 0.9910$, $s \leq 0.04$) (Figure 1) and passed through the origin, showing that the sugar-oxidant complex has only 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 ($r > 0.9985$, $s \leq 0.01$) with slopes < 1. Addition of the reaction products $p$-toluenesulfonamide (TSA) and $Cl^-$ ion did

**Table 1.** Effect of varying $[CAT]_0$ and $[amino\ sugar]_0$ on the rate of oxidation of amino sugars at 40 °C.

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

| $[CAT]_0$ x $10^3$ M | $[S]_0$ x $10^2$ M | $10^4\ k_{obs}(s^{-1})$ | |
|---|---|---|---|
| | | D-Glc amine | D-Gal amine |
| 0.9 | 2.0 | 3.98 | 15.90 |
| 1.0 | 2.0 | 3.87 | 15.86 |
| 1.5 | 2.0 | 3.75 | 15.15 |
| 2.0 | 2.0 | 3.71 | 15.05 |
| 2.5 | 2.0 | 3.56 | 15.00 |
| 3.0 | 2.0 | 3.69 | 15.06 |
| 3.5 | 2.0 | 3.45 | 14.90 |
| 4.0 | 2.0 | 3.29 | 14.75 |
| 4.5 | 2.0 | 3.07 | 13.90 |
| 2.0 | 1.0 | 1.78 | 7.50 |
| 2.0 | 3.0 | 5.68 | 21.93 |
| 2.0 | 4.0 | 7.47 | 31.35 |
| 2.0 | 5.0 | 8.83 | 37.78 |
| 2.0 | 6.0 | 12.50 | 45.50 |



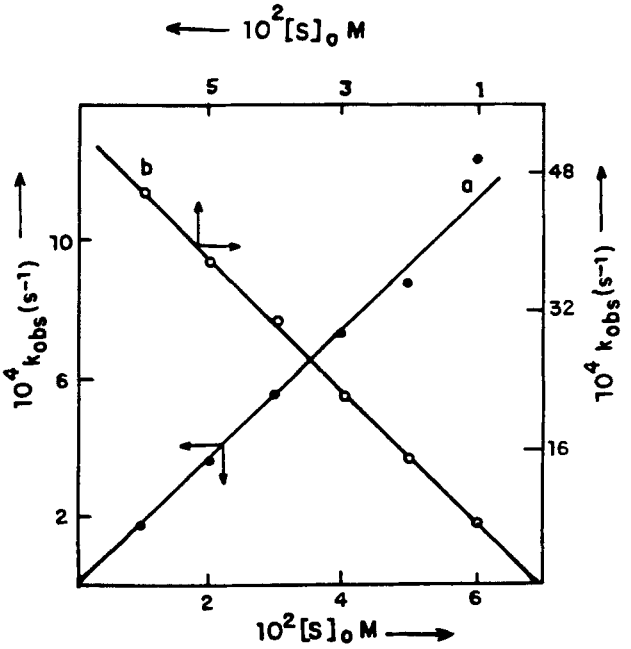



**Figure 1.** Plots of $k_{obs}$ vs. $[S]_0$: $[CAT]_0$ = 2.0 x $10^{-3}$ M, $[HO^-]_{eff}$ = 30.0 x $10^{-2}$ M, I = 0.6 M temp = 40 °C; a = D-Glucosamine, b = D-Galactosamine.

**Table 2.** Effect of varying $[HO^-]_o$ on the reaction rate at 40 °C. $[CAT]_o$ = 2.0 x $10^{-3}$ M, $[S]_o$ = 2.0 x $10^{-2}$ M, I = 0.6 M,

| $[HO^-]_{eff}$ x $10^2$ [a] (M) | D-Glc amine $10^4$ $k_{obs}$ $[s^{-1}]$[b] | D-Gal amine $10^4$ $k_{obs}$ $[s^{-1}]$[b] |
|---|---|---|
| 5.0 | 1.60 (1.25) | 05.80 (05.96) |
| 10.0 | 2.36 (2.10) | 09.50 (09.52) |
| 20.0 | 3.07 (3.20) | 13.40 (13.55) |
| 30.0 | 3.71 (3.86) | 15.05 (15.78) |
| 40.0 | 4.22 (4.32) | 17.06 (17.20) |
| 50.0 | 4.86 (4.64) | 18.39 (18.18) |

a. $[HO^-]_{eff}$ indicates that its neutralization by the hydrochloride of amino sugar has been taken into account. b. Values in parentheses refer to rate constants calculated from equation (13).



not alter the rate of reaction, indicating the absence of these compounds in a pre-equilibrium 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 ($r>0.9975$, $s\leq0.02$) with a slope 0.6 (Figure 2). 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. $1/\varepsilon$ where $\varepsilon$ is the relative permittivity of the medium were linear ($r>0.9969$, $s\leq 0.03$) with a negative slope (Figure 3). The reaction was studied over a range of temperatures (Table 3) and the Arrhenius plot, log $k_{obs}$ vs. 1/T was linear ($r > 0.9981$, $s \leq 0.02$). The activation energy, $E_a$, was calculated from this plot. The other parameters $\Delta H^{\#}$, $\Delta S^{\#}$, $\Delta G^{\#}$, and the Arrhenius parameter '$A$' (in terms of log $A$) were calculated from the following equations:

$$\Delta H^{\#} = E_a - RT \quad ...(1)$$

$$\Delta S^{\#} = \Delta H^{\#} / T - 4.576 \log T / k_{obs} - 47.22 \quad ...(2)$$

where $k_{obs}$ is in $s^{-1}$

$$\Delta G^{\#} = \Delta H^{\#} - T \Delta S^{\#} \quad ...(3)$$

$$\log A = \log RT / Nh + \Delta S^{\#} / 2.303 R \quad ...(4)$$

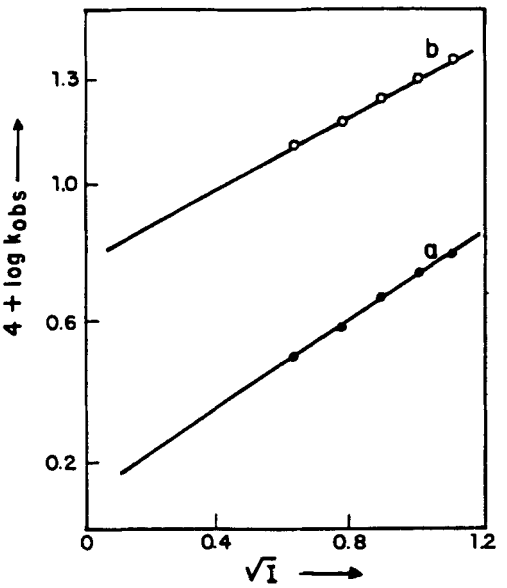



**Figure 2.** Plots of log $k_{obs}$ vs. $(I)^{1/2}$: $[CAT]_0$= 2.0 x $10^{-3}$ M, $[S]_0$ = 2.0 x $10^{-2}$ M $[HO^-]_{eff}$ = 30.0 x $10^{-2}$ M, temp = 40 °C ; a = D-Glucosamine, b = D-Galactosamine.

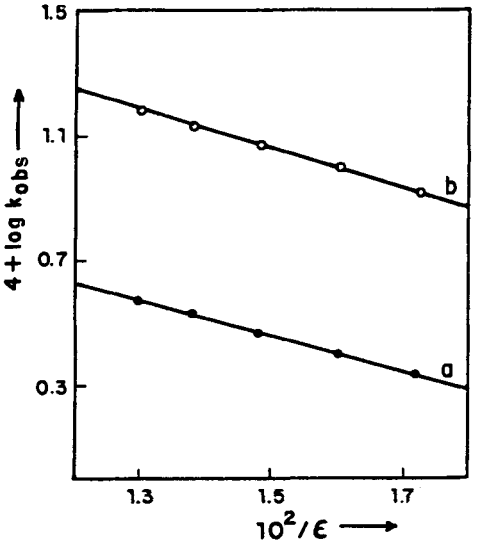



**Figure 3.** Plots of log $k_{obs}$ vs. 1/ ε : $[CAT]_0$ = 2.0 x $10^{-3}$ M, $[S]_0$ = 2.0 x $10^{-2}$ M, $[HO^-]_{eff}$=30.0 x $10^{-2}$ M, I=0.6 M, temp=40 °C: a = D-Glucosamine, b = D-Galactosamine.

**Table 3.** Effect of temperature on the rate of oxidation by CAT

$[CAT]_0$ = 2.0 x $10^{-3}$ M, $[S]_0$ = 2.0 x $10^{-2}$ M, $[HO^-]_{eff}$ = 30.0 x $10^{-2}$ M, I = 0.6 M.

| Temp (K) | $10^4 k_{obs}$ ($s^{-1}$) | |
|---|---|---|
| | D-Glc amine | D-Gal amine |
| 308 | 1.62 | 7.96 |
| 313 | 3.71 | 15.05 |
| 318 | 6.52 | 25.69 |
| 323 | 12.40 | 41.41 |

**Table 4.** Proton inventory studies for oxidation of amino sugars by CAT in $H_2O$ - $D_2O$ mixtures at 40 °C.

$[CAT]_0$ = 2.0 x $10^{-3}$ M, $[S]_0$ = 2.0 x $10^{-2}$ M, $[HO^-]_{eff}$ = 30.0 x $10^{-2}$ M, I = 0.6 M

| Atom Fraction of Deuterium (n) | $10^4 k^n_{obs}$ ($s^{-1}$) | |
|---|---|---|
| | D-Glc amine | D-Gal amine |
| 0.000 | 3.71 | 15.05 |
| 0.250 | 3.98 | 16.02 |
| 0.500 | 4.61 | 17.50 |
| 0.750 | 5.75 | 20.45 |
| 0.904 | 7.20 | 23.03 |

where "*R*" is the gas constant, "*N*" is the Avogadro number and "*h*" is the Planck constant.

| | $E_a$ kJmol$^{-1}$ | $\Delta H^{\#}$ kJmol$^{-1}$ | $\Delta S^{\#}$ JK$^{-1}$mol$^{-1}$ | $\Delta G^{\#}$ kJmol$^{-1}$ | log *A* |
|---|---|---|---|---|---|
| D-Glucosamine | 110.1 | 107.5 | 31.6 | 97.6 | 16.8 |
| D-Galactosamine | 92.0 | 89.3 | -14.4 | 93.9 | 14.5 |

The rate increased in $D_2O$ medium and proton inventory studies were made in $H_2O$-$D_2O$ mixtures of varying "D" content. The results are given in Table 4. Values

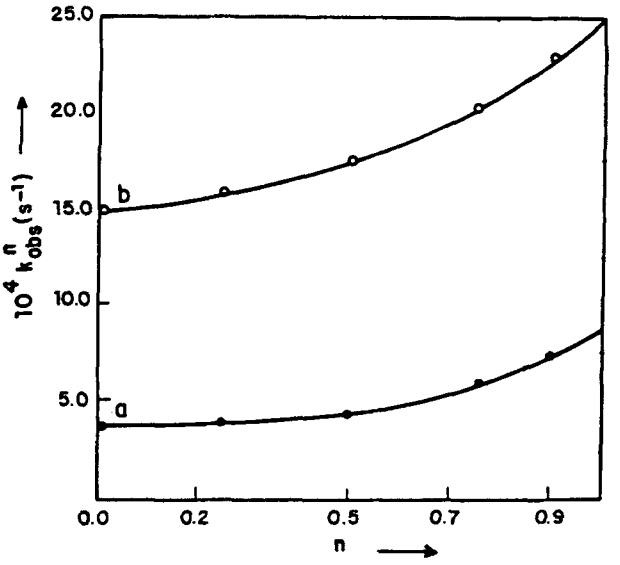



**Figure 4.** Proton inventory plot for the oxidation of D-glucosamine (a) and D-galactosamine (b) by CAT in $H_2O$-$D_2O$ mixtures of 313 K: Experimental conditions are as in Table 4. $k^n_{obs}$ refers to the observed rate constant in mixtures of $H_2O$-$D_2O$ containing atom fraction 'n' of deuterium.



of the solvent isotope effect, $k$ ($H_2O$) / $k$ ($D_2O$) are thus 0.52 and 0.65, respectively for the oxidation of D-glucosamine and D-galactosamine by CAT. Proton inventory plot relating the rate constant $k_{obs}$ with the deuterium atom fraction 'n' in the solvent mixture is shown in Figure 4.

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

CAT ionizes in aqueous solution ( eq. 5 ) and the anion picks up a proton in acid solution to give the free acid,[1] monochloramine-T, TsNClH (*eq.6*):

$$TsNClNa \rightleftharpoons TsNCl^- + Na^+ \quad ...(5)$$

$$TsNCl^- + H^+ \underset{K_a}{\rightleftharpoons} TsNClH \quad ...(6)$$

$K_a$ = 2.82 x $10^{-5}$ at 18 °C.

Although the free acid has not been isolated, there is sufficient experimental evidence for its formation in solution.[9] It can further undergo disproportion / hydrolysis (eq. 7 and 8 respectively) giving $TsNH_2$, the dichloramine-T $TsNCl_2$, and HOCl:

$$2TsNClH \underset{}{\overset{K_d}{\rightleftharpoons}} TsNH_2 + Ts\,NCl_2 \qquad ...(7)$$

$K_d = 6.1 \times 10^{-2}$ at 25 °C.

$$TsNClH + H_2O \overset{K_h}{\rightleftharpoons} TsNH_2 + HClO \qquad ...(8)$$

$K_h = 4.88 \times 10^{-8}$ at 25 °C.

In alkaline solutions of CAT, the following equilibria are reported.

$$TsNCl^- + H_2O \rightleftharpoons TsNH_2 + ClO^- \qquad ...(9)$$

$$TsNCl^- + H_2O \rightleftharpoons TsNClH + HO^- \qquad ...(10)$$

$$TsNClH + HO^- \rightleftharpoons TsNH_2 + ClO^- \qquad ...(11)$$

Equations 9 and 11 suggest a retardation of rate with the addition of the reaction product, $TsNH_2$, while equation 10 predicts a decrease in rate by $HO^-$ ions. Since neither of these have been observed in the present set of experiments, the most likely oxidising species for amino sugars is the anion $TsNCl^-$ itself. Hence scheme 1 is tentatively suggested for the oxidation of the substrate (S) by CAT, where the substrate is first converted to the alkoxy anion [$S^-$] which subsequently reacts with the oxidant in a rate limiting step.

If $[S]_t$ represents the total substrate concentration, then $[S]_t = [S] + [S^-]$ from which $[S]_t = [S^-]\{[H_2O] + K_1 [HO^-]\}/K_1 [HO^-]$ and $[S^-] = K_1 [HO^-] [S]_t / [H_2O] + K_1 [HO^-]$

Since rate $= k_2 [S^-] [TsNCl^-]$

substituting for $[S^-]$, a rate law (12) can be derived as

$$-d\,[CAT] / dt = \frac{k_2 K'_1 [CAT] [S]_t [HO^-]}{1 + K'_1 [HO^-]} \qquad ... (12)$$

Where $K'_1 = K_1 / [H_2O]$

From equation (12)

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

$$S^- + TsNCl^- \xrightarrow[\text{slow}]{k_2} X + TsNH_2 + Cl^- \qquad ...(ii)$$

$$X + HO^- \xrightarrow[\text{fast}]{} X' \qquad ...(iii)$$

$$X' + 2TsNCl^- \xrightarrow[\text{fast}]{k_3} \text{Products} \qquad ...(iv)$$

**Scheme 1**

$$k_{obs} = \frac{k_2 K'_1 [S]_t [HO^-]}{1 + K'_1 [HO^-]}$$

**or** $$1 / k_{obs} = 1 / k_2 K'_1 [S]_t [HO^-] + 1 / k_2 [S]_t \qquad ... (13)$$

A double reciprocal plot $1/k_{obs}$ vs. $1/[HO^-]_{eff}$ is linear ($r > 0.9921$, $S \leq 0.03$) (Figure 5) and from the slope and intercept of this line, values of the formation constant $K'_1$ of alkoxy anion and its decomposition rate constant $k_2$ through its reaction with the oxidant have been calculated.

At 40 °C the values are $K'_1 = 4.6$ dm$^3$ mol$^{-1}$ and $k_2 = 3.33 \times 10^{-2}$ dm$^3$ mol$^{-1}$ s$^{-1}$ for D-glucosamine, the corresponding values for D-galactosamine being 6.8 dm$^3$ mol$^{-1}$ and $11.76 \times 10^{-2}$ dm$^3$ mol$^{-1}$ s$^{-1}$. Using the values of $K'_1$ and $k_2$, values of $k_{obs}$ were calculated from equation (13) and these are shown in Table 2. It is seen that there is good agreement between the calculated and experimental sets of values, thus supporting the proposed scheme. The thermodynamic parameters for the equilibrium step and activation parameters for the rate limiting step in scheme 1 were evaluated as follows: Hydroxyl ion concentration (as in Table 2) was varied at several termperatures and values of $K'_1$ and $k_2$ were determined at each temperature. A van't

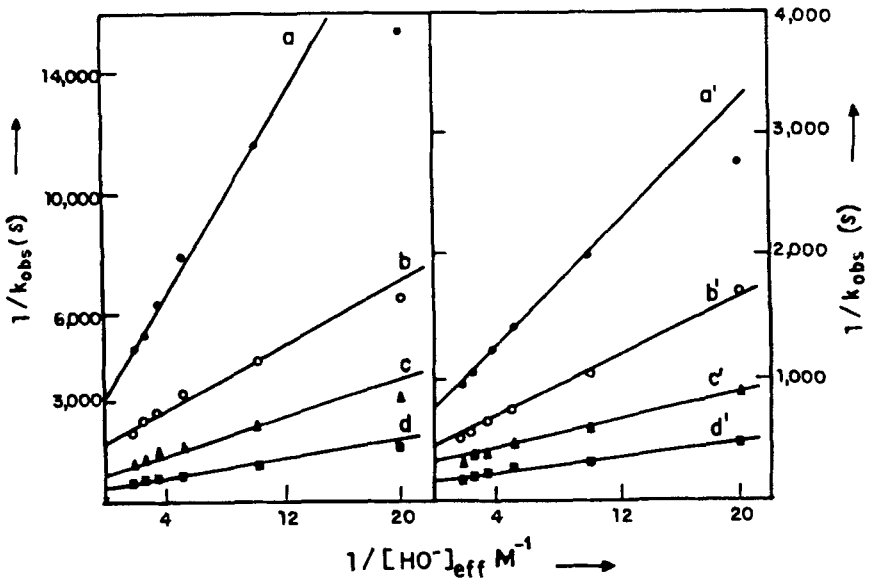



**Figure 5.** Plots of $1 / k_{obs}$ vs. $1 / [HO^-]_{eff}$ at different temperatures for the oxidation of D-glucosamine and D-galactosamine by CAT: (a, a' ) 308 K, (b, b') 313 K, (c, c') 318 K, (d d') 323 K; Lines a b c and d. refer to D-glucosamine and a', b', c', and d' refer to D-galactosamine.



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 $\Delta H$, entropy of reaction $\Delta S$, and free energy of reaction $\Delta G$ 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 values are shown in Table 5. A comparison with the activation parameters obtained for the composite reaction shows that the values mainly refer to the rate limiting step, supporting the fact that reaction before the rate determining step is fast, involving little activation energy.

Scheme 1 and rate law (12) can explain the observed experimental facts.

(i) The proposed mechanism is supported by the increase in rate in $D_2O$ medium. Since $DO^-$ ion is a stronger base than $HO^-$ by a factor of 2, we expect an approximate doubling of rate in heavy water, for a reaction involving a preequilibrium proton or hydroxyl ion transfer.[10] The values of inverse solvent sotope effect, $k(D_2O) / k (H_2O)$ are found to be 1.94 and 1.53 respectively for D-glucosamine and

**Table 5.** Thermodynamic quantities for alkoxy anion formation and activation parameters for the rate limiting step, for the oxidation of amino sugars by CAT.

| Temp (K) | D-Glc amine | | D-Gal amine | |
|---|---|---|---|---|
| | $K'_1$ $dm^3\ mol^{-1}$ | $10^2\ k_2$ $dm^3\ mol^{-1}\ s^{-1}$ | $K'_1$ $dm^3\ mol^{-1}$ | $10^2\ k_2$ $dm^3\ mol^{-1}\ s^{-1}$ |
| 308 | 3.82 | 1.67 | 6.00 | 6.67 |
| 313 | 4.60 | 3.33 | 6.80 | 11.76 |
| 318 | 5.12 | 6.25 | 9.94 | 16.66 |
| 323 | 6.55 | 12.50 | 10.91 | 33.33 |
| $\Delta H$ | = 33.0 kJ $mol^{-1}$ | | $\Delta H$ | = 33.3 kJ $mol^{-1}$ |
| $\Delta S$ | = -127.6 $JK^{-1}$ $mol^{-1}$ | | $\Delta S$ | = -122.5 $JK^{-1}$ $mol^{-1}$ |
| $\Delta G$ | = 73.3 kJ $mol^{-1}$ | | $\Delta G$ | = 72.0 kJ $mol^{-1}$ |
| $Ea$ | = 114.9 kJ $mol^{-1}$ | | $Ea$ | = 96.5 kJ $mol^{-1}$ |
| $\Delta H^{\#}$ | = 112.3 kJ $mol^{-1}$ | | $\Delta H^{\#}$ | = 93.9 KJ $mol^{-1}$ |
| $\Delta S^{\#}$ | = 84.9 $JK^{-1}$ $mol^{-1}$ | | $\Delta S^{\#}$ | = 36.2 $JK^{-1}$ $mol^{-1}$ |
| $\Delta G^{\#}$ | = 85.5 kJ $mol^{-1}$ | | $\Delta G^{\#}$ | = 82.5 kJ $mol^{-1}$ |
| log $A$ | = 19.6 | | log $A$ | = 17.1 |



D-galactosamine, thus justifying our expectations. Proton inventory plots (Figure 4) could throw some light on the nature of the transition state. The dependence of rate constant $k^n_{obs}$ on n, the atom fraction of deuterium, in a solvent mixture containing $H_2O$ and $D_2O$ is given[11,12] by the Gross-Butler Equation (equation 14).

$$(k^0_{obs}) / (k^n_{obs}) = \frac{\prod^{TS} (1 - n + n\ \Phi_i)}{\prod^{RS} (1 - n + n\ \Phi_j)} \qquad ...(14)$$

Where $\Phi_i$ and $\Phi_j$ are the isotopic fractionation factors (equilibrium constants for the H-D exchange) for isotopically exchangeable hydrogen sites in the transition state (TS) and reactant states (RS), respectively. A knowledge of isotopic fractionation factors of reactants would enable us to calculate the fraction factors of transition state.

However, from a qualitative point of view, the curvature of proton inventory plots can be compared with those of standard curves available in literature,[13] and it may be concluded that $HO^-$ ion is involved in the formation of the transition state.

(ii) A primary salt effect is observed as the rate increases with increase in ionic strength of medium[14] thus supporting the involvement of two negative ions in the rate limiting step. The Debye-Hückel plot has a slope of 0.6 (Figure 2) and the expected slope of unity has not been realised. This may be due to the fact that the ionic strength employed is well above the formal Debye-Hückel range. Alternatively there could be Bjerrum ion pair formation[14] or it is probable that there is charge delocalization in the sugar or oxidant molecule shown in scheme 1.

(iii) The rate decreased with decrease in the relative permittivity (ε) 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[15] by the Scatchard equation (15).

$$\log k = \log k_o - Z_A Z_B e^2 / \varepsilon k T d_{AB} \quad \text{....(15)}$$

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. From the slope of log $k_{obs}$ vs. $1/\varepsilon$ plot (Figure. 3), $d_{AB}$ is computed as 4.25 Å and 3.72 Å for D-glucosamine and D-galactosamine respectively. The values are found to be reasonable in comparision with those of other reactions of similar nature.[16]

(iv) 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 100 kJ/mol for the reactions. The near constancy of $\Delta G^{\#}$ values for both amines indicates that a similar mechanism is operative in the oxidation of amino sugars. The entropy of activation is negative for D-galactosamine, but is positive (31.6 $JK^{-1}mol^{-1}$) for D-glucosamine, indicating that the transition state of the latter is more disordered. This is also reflected in the size of the activated complex ($d_{AB}$) determined from equation (16). The rate of oxidation of D-galactosamine is faster than D-glucosamine, possibly because of the fact that the former has a higher percentage of the reactive β-anomer.[17] This is also clearly reflected in the values of $k_3$, the rate constant for the rate limiting step.

A probable mode of oxidation of D-glucosamine to D-arabonic acid is shown in Scheme 2. The amino sugar reacts with $TsNCl^-$ ion in the presence of alkali to form 2-aminogluconic acid (**5**) which reacts with a second oxidant molecule to form D-arabinose (**8**) through decarboxylation followed by deamination in the form of $NH_3$ via hydrolysis of the intermediate imine (**7**). A third molecule of oxidant converts the pentose into D-arabonic acid (**9**) and its epimer, D-ribonic acid as final products.

A similar scheme can be drawn for the oxidation of D-galactosamine to D-lyxonic acid by CAT in alkaline medium.

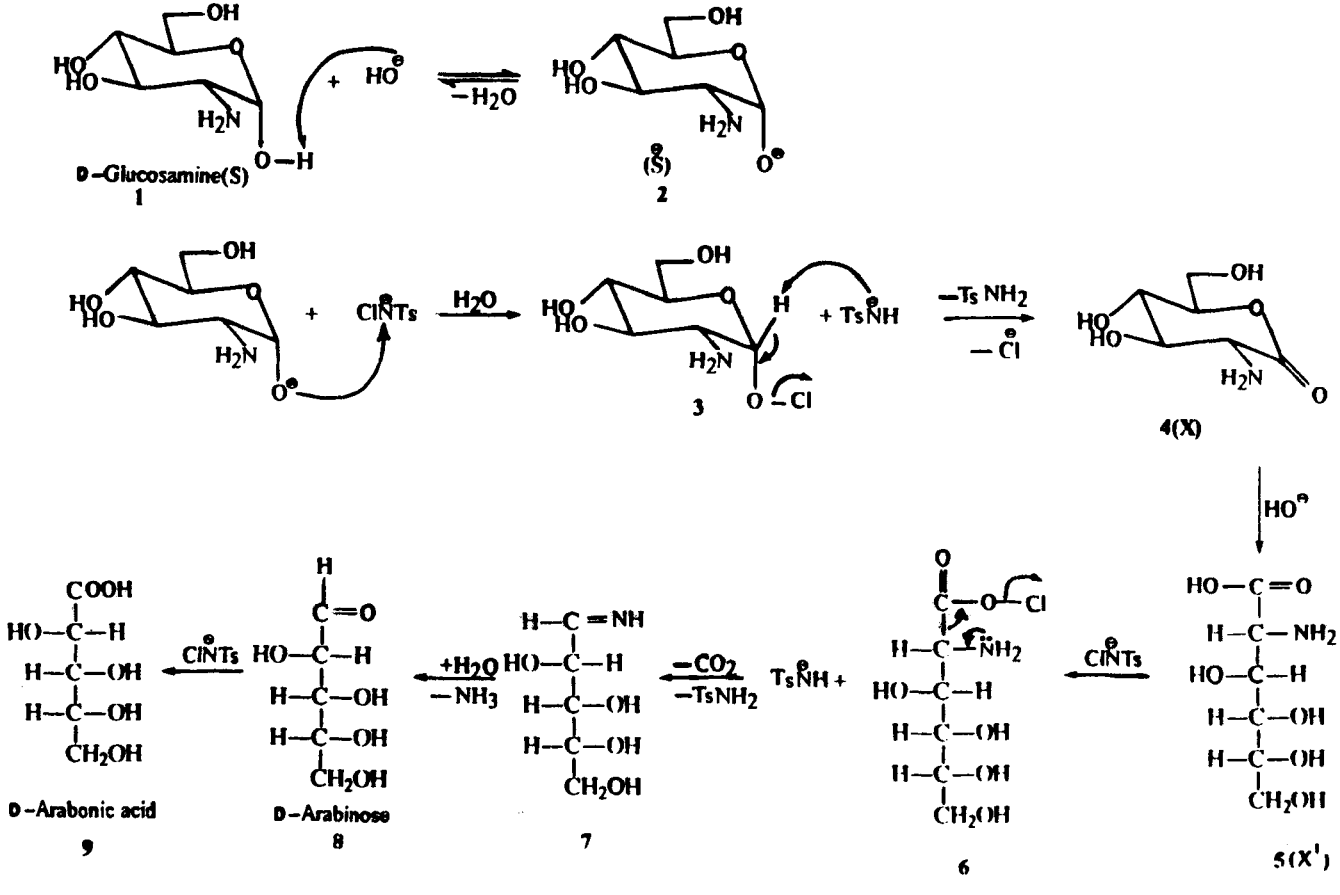
## EXPERIMENTAL

### Materials and Methods

Pure samples of D-glucosamine hydrochloride and D-galactosamine hydrochloride (both Sigma) and analytical reagent grade chemicals were used. Fresh aqueous solutions of amino sugars were prepared using triply distilled water. Chloramine-T (Loba chemie) was purified from its dichloro contaminant by washing with carbon tetrachloride. The purity of compound was ascertained from its $^1H$ and $^{13}C$ NMR spectra and also by the iodometric assay of active chlorine in its aqueous solutions. Solutions of CAT 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. The compound was neutral towards the sugar. Heavy water (99.4% D) for solvent isotope studies was supplied by the Bhabha Atomic Research Centre, Trombay, India. Permittivity 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[18,19] in literature were employed. The reaction products were analyzed by Dionex BioLC HPLC coupled to pulsed amperometric detection using a carbo Pac PA1 high pH anion-Exchange column (4 x 250 mm).[20]

### Stoichiometry

The stoichiometry run of oxidation of amino sugar with excess CAT solution over the sugar $[S]_o$ indicated that three moles of oxidant were consumed per mole of sugar, to form the corresponding aldonic acid. The change can be represented by equation (16):




Scheme 2

$$C_6H_{13}O_5N + 3\ TsNClNa + 3\ H_2O \longrightarrow C_5H_{10}O_6 + CO_2 + NH_3 + TsNH_2 + 3Na^+ + Cl^- \quad ....(16)$$

The reaction product, *p*-toluenesulfonamide was detected by paper chromatography, benzyl alcohol saturated with water was used as the solvent with 0.5 % vanillin in 1 % HCl in ethanol as spray reagent ($R_f$ = 0.905). From an aliquot of the reaction mixture, toluenesulfonamide was extracted into ether and the residual solution was then successively passed through Amberlite IR-120 ($H^+$) and Dowex-2 ($HO^-$) columns for removing $Na^+$ and $Cl^-$ ions. The final eluate was then concentrated to 30 % and analysed by paper chromatography (1-butanol-pyridine-water, 5:3:2 v/v/v) and detected with *p*-anisidine hydrochloride ($R_f$ = 0.57 and 0.54 for D-arabonic acid and D-lyxonic acid respectively). Further, the products were identified by comparison of their HPLC retention times with retention times of the standard aldonic acids.

Identification of reaction products was also carried out under kinetic conditions ($[substrate]_o >> [oxidant]_o$) which yielded the same products.



**Kinetic Measurements**

The reactions were carried out in glass stoppered pyrex boiling tubes coated black on the outside. Pseudo-first order conditions were maintained for the kinetic runs ($[sugar]_o >> [oxidant]_o$). The oxidant and the requisite amounts of amino sugar, alkali, $NaClO_4$ solutions and $H_2O$ (for constant total volume) taken in separate boiling tubes, were thermostated for 30 min at 40 °C. The reaction was initiated by the rapid addition of CAT to the mixture and its progress was monitored by iodometric estimation of unconsumed CAT 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 [CAT] vs. time plots were reproducible within ± 3 %. Allowance was made in adjusting the alkali concentration $[NaOH]_{eff}$ for neutralising hydrochloride of the 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.

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