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Mechanistic investigation of oxidation of 6-deoxyhexoses by sodium *N*-chloro-*p*-toluenesulfonamide in alkaline medium

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The kinetics and mechanism of oxidation of L-fucose and L-rhamnose by chloramine-T (CAT) in alkaline medium has been investigated at 40°C. The rate of the reaction is first order with respect to [sugar][CAT] and [hydroxide ion]. The reaction rate is influenced by a change in ionic strength of the medium and the dielectric effect has been found to be negative. Proton inventory studies have been made in H_2O–D_2O mixtures. Activation parameters have been determined from the Arrhenius plots. HPLC and GLC-MS analyses of the products indicate that 6-deoxyhexoses are oxidized to a mixture of aldonic acids. The proposed mechanism is consistent with the observed results.

We recently reported the kinetics of oxidation of erythrose- and threose-series namely, pentoses and hexoses with chloramine-T/chloramine-B in alkaline medium[1-4]. Each sugar is oxidized to mixture of several aldonic acids. Whereas pentoses gave mainly pentonic acids as major products as expected, surprisingly hexoses also formed pentonic acids as major products. In both the cases in addition to pentonic acid, significant amounts of 4-carbon and 3-carbon aldonic acids were also formed. Furthermore, interestingly aminosugars such as 2-amino-2-deoxy-hexoses also formed products analogous to those formed from the corresponding hexoses. Based on these data and on the basis of results of kinetics, stoichiometrics and thermodynamic studies, we proposed a novel pathway for the oxidation of sugars[1-4]. In the present study we investigated for the first time the kinetics and mechanism of two naturally occurring deoxy sugars, L-fucose and L-rhamnose with chloramine-T in an alkaline medium. The results of these studies are presented in this paper.

Materials

L-Fucose (Sigma), L-rhamnose (Sigma) were used without further purification. Chloramine-T was from Aldrich Chemical Co (Milwaukee WI) and its purity was checked by iodometric assay of its active chlorine content and also by its ^1H and ^{13}C NMR spectra. The aqueous solution of CAT was standardized iodometrically and stored in brown bottles to minimize its photochemical deterioration. A concentrated aqueous $NaClO_4$ was used to maintain a high ionic strength to 'swamp' the reaction mixture. D_2O (99.4%) was obtained from Bhabha Atomic Research Centre (Trombay, India). All other reagents were of analytical grade. Triply distilled water was used in the preparation of aqueous solutions. Dielectric constant of the reaction medium was altered by the addition of methanol in varying proportions (v/v) and values of dielectric constant for methanol-water mixtures reported[5,6] in the literature were employed.

Kinetic Measurements

Pseudo-first order conditions were maintained for the kinetic runs ($[S]_0$>>$[oxidant]_0$). The oxidant and the requisite amounts of 6-deoxyhexoses, alkali $NaClO_4$ solutions and H_2O (for constant total volume) 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 ±5%.

Stoichiometry and Product Analysis

The reaction mixture containing 6-deoxyhexoses, alkali with an excess of CAT were kept for 24 hr at 40°C. Iodometric determination of the unconsumed CAT indicated that three moles of CAT were

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consumed per mole of the substrate to give the mixture of aldonic acids.

The oxidation products were analyzed by Dionex HPLC with pulsed amperometic detection using a CarboPac PA1 high-*p*H anion-exchange column (4×250 mm)[7]. An isocratic elution with 0.2*M* NaOH was used. The products were identified by comparison of their HPLC retention periods with retention periods of the standard aldonic acids and by GLC-MS (see below).

For GLC-MS characterization, the reaction mixture was extracted with diethyl ether to remove toluenesulfonamide and then passed through AG 50W-×12 (H+) and Ag4-×4 (base) resins. The Ag 4-×4 resins were eluted with 1*M* pyridine/1*M* acetic acid, *p*H 5.2 and lyophilized. The products were converted into their trimethylsilyl derivatives and then analyzed by GLC-MS.

Results

With pseudo-first order conditions under which $[S]_0 >> [CAT]_0$ and at constant $[OH^-]$, plots of log [CAT] vs time were linear for each $[CAT]_0$ ($r > 0.9990$, $s < 0.01$). Values of k_{obs} obtained from these plots were constant for the different initial concentrations of CAT employed, thus indicating a first order dependence of rate on $[CAT]_0$ (**Table I**). This was further confirmed from the unit slope obtained from the linear plots of log $[-dc/dt]_0$ vs log $[CAT]_0$ using the initial velocity method. The rate increased with the increase in $[S]_0$ and the plots of log k_{obs} vs log $[S]_0$ were linear ($r > 0.9976$, $s < 0.02$) with unit slopes. The second order rate constants ($k_2 = k_{obs}/[S]_0$) were constant within the experimental error (**Table I**), demonstrating a first order dependence of rate on [S]. The lines in the plots of k_{obs} vs $[S]_0$ passed through the origin ($r > 0.9997$, $s < 0.01$) indicating that the intermediates formed with the oxidant have only transient existence.

The rate of reaction also increased with the increase in alkali concentration (**Table II**) and plots of log k_{obs} vs log $[OH^-]$ were linear with unit slope ($r > 0.9994$, $s < 0.01$) thus indicating a first order dependence on $[OH^-]$. Further, a plot of k_{obs} vs $[OH^-]$ passes through the origin ruling out an alkali independent path for the reaction.

Addition of the reduced products *p*-toluenesulfonamide and Cl^- ion did not alter the rate of reaction, indicating the absence of these compounds in a pre-equilibrium to the rate-limiting step.

The ionic strength (I) of medium was varied by adding a concentrated solution of $NaClO_4$. The rate increased with the increase in ionic strength. A conventional Debye-Huckel plot of logk_{obs} vs $(I)^{1/2}$ resulted in a linear plot ($r > 0.99855$, $s < 0.02$) with slopes of about 0.5.

The solvent composition of the reaction medium was varied by the addition of methanol (0-40%). The rate decreased with the increase in methanol content. Plots of log k_{obs} vs 1/D where 'D' is the dielectric constant of the medium, were linear ($r > 0.9972$. $s < 0.02$) with a negative slope.

The reaction was studied at different temperatures (308-323K). From the Arrhenius plots of log k_{obs} vs 1/T which were linear ($r > 0.9969$, $s < 0.03$), the activation energy E_a was calculated from the slope. Values of the other kinetic parameters, $\Delta H^{\#}$, $\Delta S^{\#}$. $\Delta G^{\#}$ and log A were computed from the measured E_a values (**Table III**).

The reactions were performed in pure D_2O. The solvent-isotope effects $k(H_2O)/k(D_2O)$ were between 0.50 and 0.60 (**Table IV**). Proton inventory studies were made in H_2O–D_2O mixtures.

Addition of an aqueous solution of acrylamide to the reaction mixture did not cause polymerization.

Table I — Effect of [reactant] on the rate of oxidation of 6-deoxyhexoses by CAT at 40°C
{$[OH^-]$=0.2*M*; I=0.5*M*}

$10^3[CAT]_0$ (*M*)	$10^2[S]_0$ (*M*)	$10^4 k_{obs}$ (s^{-1})	
		L-Fucose	L-Rhamnose
1.0	2.0	11.30	3.30
1.5	2.0	11.80	3.38
2.0	2.0	12.00	3.50
2.5	2.0	12.14	3.55
3.0	2.0	12.31	3.50
3.5	2.0	12.62	3.60
4.0	2.0	13.00	3.75
2.0	1.0	5.95	1.90
2.0	3.0	18.68	5.98
2.0	4.0	24.70	7.77
2.0	5.0	30.78	9.60
2.0	6.0	37.38	11.25

Table II — Effect of [NaOH] on the rate of oxidation of 6-deoxyhexoses by CAT at 40°C
{$[CAT]_0$=0.002*M*; $[S]_0$=0.02*M*; I=0.5*M*}

10^2 [NaOH] (*M*)	$10^4 k_{obs}$ (s^{-1})	
	L-Fucose	L-Rhamnose
10.0	5.60	2.05
20.0	12.00	3.50
30.0	17.70	6.30
40.0	23.60	8.30
50.0	30.00	10.20
60.0	36.10	11.98

Table III — Thermodynamic parameters for the oxidation of 6-deoxyhexoses by CAT in the presence of [NaOH]

6-deoxyhexoses	E_a kJ mol^{-1}	$\Delta H^{\#}$ kJ mol^{-1}	$\Delta G^{\#}$ kJ mol^{-1}	$\Delta S^{\#}$ JK^{-1} mol^{-1}	Log A
L-Fucose	89.3	86.7	94.4	25.0	14.4
L-Rhamnose	130.7	128.0	97.3	97.5	20.7

Table IV — Proton inventory studies for the oxidation of 6-deoxyhexoses by CAT in H_2O–D_2O mixtures at 40°C

{$[CAT]_0$=0.002*M*, $[S]_0$=0.02*M*, $[OH^-]$=0.2*M*, I=0.5*M*}

Atom fraction of deuterium (n)	$10^4 k_{obs}$ (s^{-1})	
	L-Fucose	L-Rhamnose
0.00	12.00	3.50
0.25	13.50	3.98
0.50	15.30	4.60
0.75	17.70	5.50
0.92	21.00	6.40

Table V — HPLC analysis of the products formed by the oxidation of 6-deoxyhexoses by CAT in presence of NaOH

Products (mole percent)[a]	6-Deoxyhexoses	
	L-Fucose	L-Rhamnose
5-Deoxylyxonic acid	33	—
5-Deoxyxylonic acid	32	—
5-Deoxy threonic acid	26	—
5-Deoxyerythronic acid	—	45
5-Dexoyarabinonic acid	—	25
5-Deoxyribonic acid	—	15
5-Deoxypropanoic acid	6	5
5-Deoxyhexonic acid	3	10

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

This suggests the absence of free radical involvement during the oxidation.

A comparison of the HPLC and GLC retention periods of the reaction products with those of the standards, indicated that 4-deoxythreonic acid, 5-deoxyxylonic acid, 5-deoxylyxonic acid and 2-hydroxypropanoic acid and small amounts of 6-deoxygalactonic acid are the oxidation products of L-fucose. But for L-rhamnose, the oxidation products are found to be 5-deoxyarabinonic acid, 5-deoxyribonic acid, 4-deoxyerythronic acid, 6-deoxymannonic acid and small amounts of 2-hydroxypropanoic acid (**Figure 1** and **Table V**). The identities of all the oxidation products were confirmed from their mass fragmentation patterns. The oxidation products of 6-deoxyhexoses were also analyzed at 0.5, 1, 2, 4, 8, 16 and 24 hr. The relative propositions of various aldonic acids formed (**Figure 1** and **Table V**) were similar at all time points analyzed but the formation of six-carbon aldonic acids were observed only after 4 hr, revealing that the lower-carbon aldonic acids were not derived from the initially formed six-carbon aldonic acids. The results of the study suggest that the oxidation occurs through the cleavage of C–C and C–H bonds to form a mixture of aldonic acids in both 6-deoxyhexoses. It is interesting to note that the oxidation went beyond the pentose stage.

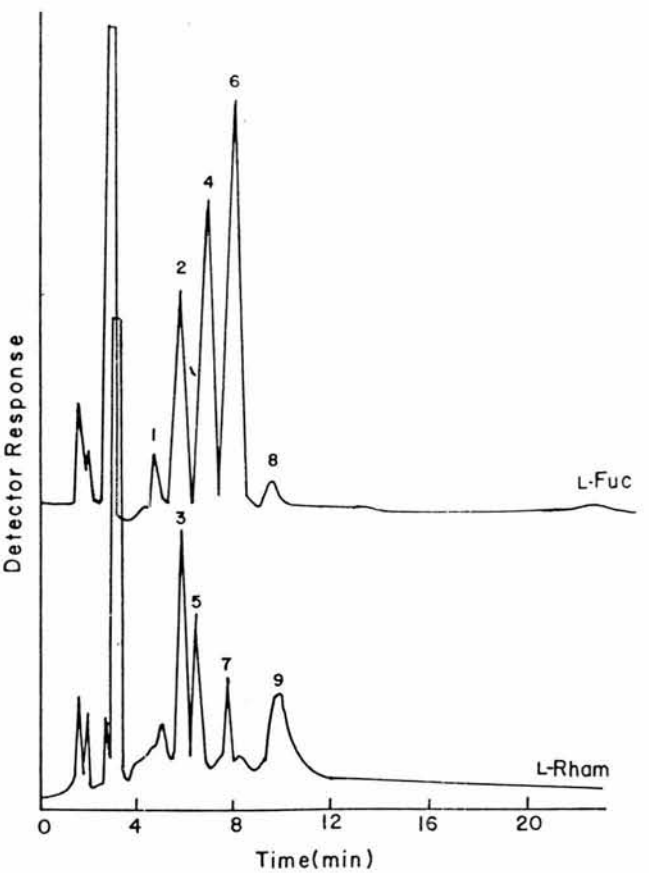


Figure 1 — HPLC analysis of the products formed by the oxidation of 6-deoxyhexoses by CAT in presence of NaOH at 40°C. (1) 2-Hydroxypropanoic acid; (2) 4-Deoxythreonic acid; (3) 4-Deoxyerythronic acid; (4) 5-Deoxylyxonic acid; (5) 5-Deoxyarabonic acid; (6) 5-Deoxyxylonic acid; (7) 5-Deoxyribonic acid; (8) 6-Deoxygalactonic acid.

Discussion

N-Metallo-*N*-haloarylsulphonamides acts as an oxidizing agents in both acidic and alkaline media. The oxidation potentials of oxidant RNH_2 couple is *p*H dependent and decreases with the increase in *p*H

of the medium. Depending on the *p*H of the medium, RNClNa furnishes different types of reactive species[8] in solution as RNHCl, RNH_2, HOCl and possibly H_2O^+Cl. The results obtained under the present investigation show that RNHCl is the active oxidant.

In alkaline solutions, sugars undergo enolization to form enediol anions[9]. 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. However, in the presence of CAT, the enediol anions (S^-) reacts with $RNCl^-$ ion to form intermediates, which in turn undergo cleavage to form the products (**Table V**).

In view of the observed first order dependence of rate on $[CAT]_0$, $[S]_0$ and $[OH^-]$ the following reaction sequence as shown in **Scheme I** is proposed for the oxidation of 6-deoxyhexoses by CAT in alkaline solutions.

Applying steady-state conditions to $[S^-]$, the rate of disappearance of oxidant is given by the Eq. (1).

$$\text{Rate} = -\frac{d[\text{CAT}]}{dt} = \frac{k_1 k_2 [\text{CAT}][\text{S}][\text{OH}^-]}{k_{-1}[\text{H}_2\text{O}] + k_2[\text{CAT}]} \quad \ldots(1)$$

$$\text{S} + \text{OH}^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{S}^- + \text{H}_2\text{O} \quad \ldots(\text{i})$$

$$\text{S}^- + \text{RNCl}^- \xrightarrow[\text{slow}]{k_2} \text{X} \quad \text{r.d.s} \quad \ldots.(\text{ii})$$

$$\text{X} \xrightarrow[\text{fast}]{k_3} \text{Products} \quad \ldots(\text{iii})$$

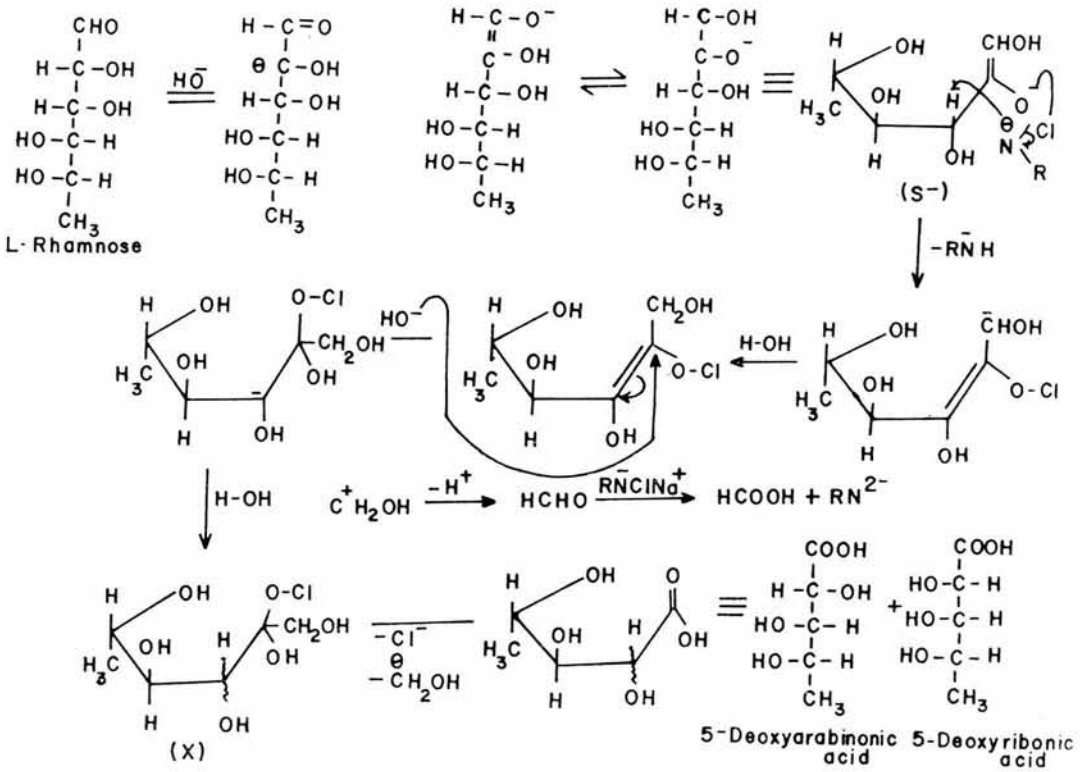
Scheme I

Since k_{-1} is fairly large and k_2 is small, being the rate constant for the rate-limiting step, the approximation $k_{-1}[H_2O] >> k_2[CAT]$ is valid and rate law (1) is reduced to Equation (2) in agreement with experimental results.

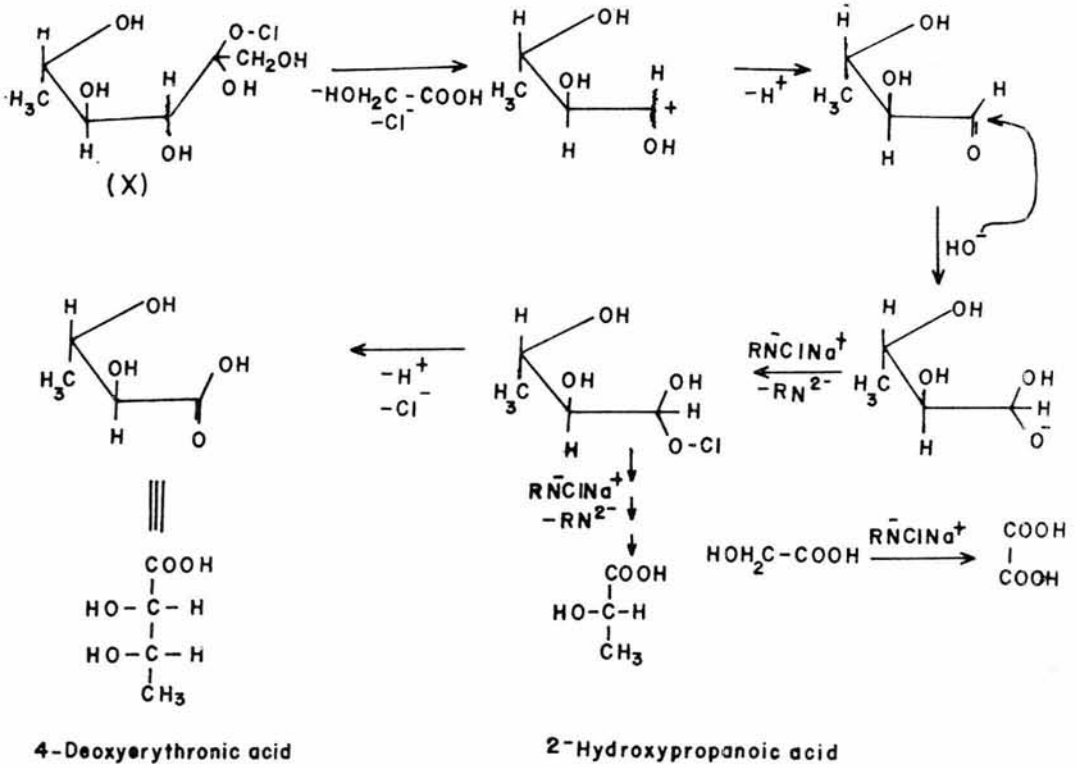
$$\text{Rate} = -\frac{d[\text{CAT}]}{dt} = \frac{k_1 k_2 [\text{CAT}][\text{S}][\text{OH}^-]}{k_{-1}[\text{H}_2\text{O}]} \quad \ldots(2)$$

which agrees with the observed rate $= k'[CAT][S][OH^-]$.

In **Scheme IIa(i)**, a plausible mechanism for the oxidation of L-rhamnose by CAT is proposed. The anion (E^-) intermediates are predominantly the keto-enolic forms react with CAT forming the intermediates X1 and X2. In case of E^-, the loss of hydrogen can occur at either C1 or C3 to form C1-C2 or C2-C3 enediols containing chloroxyl group at C2.



Scheme IIa(i)



Scheme IIa(ii)

Since epimerization at C3 was limited as evidenced by the formation of only very minor proportions of epimeric pentonic acids, it can be concluded that the cleavage of C3-H bond to form C1-C2 enediols. The enediols thus formed, contain polarized double bonds to which hydroxide ion can add at C2 to form intermediates X1 (major) and X2 (minor). X1 and X2 then can undergo cleavage of C–C bonds between C1 and C2, the former giving 5-deoxyarabinonic acid and the latter forming a mixture of 5-deoxyarabinonic acid and deoxyribonic acids. The cleavage of C–C bonds between C2 and C3 in X1 and X2 yield aldo tetrose without epimerization at C4. The aldo tetrose further oxidizes to yield 5-deoxyerythronic acid (**Table V**). The reaction can proceed further, with the cleavage of C–C bonds between C1 and C2 from the keto-enolic form of tetrose forming 2-hydroxypropanoic acid as a minor product (**Scheme II(a)**(*ii*).

A similar mechanism can be drawn for the oxidation of L-fucose by CAT into a mixture of 5-deoxyxylonic acid, 4-deoxythreonic acid and 2-hydroxypropanoic acid in alkaline medium.

The following results support the above conclusion.

(i) The proposed mechanism is supported by the increase in rate in D_2O medium. Since DO^- ion is a stronger base than OH^- by a factor of 2, we expect an approximate doubling of rate in heavy water medium[10]. The value of inverse solvent isotope effect, k_{obs} (D_2O)/k_{obs} (H_2O)≈2.0 (**Table IV**), thus justifying our expectations and also substantiating a pre-equilibrium hydroxyl ion transfer. Proton inventory plots could throw some light on the nature of the transition state[11,12]. 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 by the Gross Butler equation[13] (Equation 3),

$$\frac{k^0_{obs}}{k^n_{obs}} = \frac{\pi^{TS(1-n+n\phi i)}}{\pi^{RS(1-n+n\phi j)}} \qquad \ldots(3)$$

where ϕ_i and φ_j are the isotopic fractionation factors (equilibrium constants for the H-D exchange) for isotopically exchangeable hydrogen sites in the transition state (TS) and rectant state (RS), respectively. A qualitative examination of the proton inventory plots by comparing their curvature with standard curves available in literature[12], shows that there is a single transition state and one HO^- ion is involved in its formation.

(ii) A primary salt effect is observed as the rate

increases with the increase in ionic strength of medium and this is indicative that the involvement of two negative ions in the rate-limiting step. The Debye-Huckel plot has a slope of 0.5-0.6 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-Huckel range. Alternatively, there could be Bjerrum ion pair formation[13] or it is probable that there is charge delocalization in the substrate or oxidant molecule as shown in **Scheme I**.

(iii) The rate decreased with the decrease in the dielectric constant (D) of the medium. The effects of comparison of the solvent on the rate for a reaction involving two negative ions of charges Z_Ae and Z_Be is given[13] by the Scatchard Equation (4),

$$\log k = \log k_0 - Z_AZ_Be^2/DkT\, d_{AB} \quad \ldots(4)$$

where k_0 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/D plot, d_{AB} is computed as 4.40 A and 4.29 A for L-fucose and L-rhamnose, respectively. The values are found to be reasonable in comparison with those of other reactions of similar nature[14].

(iv) The rate determining step (**Scheme I**) involves an interaction between similarly charged ions which would require a very high activation energy. The observed activation energies are fairly high and agree with this prediction. Also the data in **Table III** show that the energy of activation is highest for the slowest reaction, indicating that the reaction is enthalpy controlled. Near constant $\Delta G^{\#}$ values (**Table III**) suggest that a common mechanism is operative for the oxidation of 6-deoxyhexoses.

(v) A comparison of rates of oxidation of the 6-deoxyhexoses is desirable (**Table I**). It is seen that the rate of L-fucose>L-rhamnose. The β-anomer (equatorial-OH) in general is oxidised more rapidly than the corresponding α-anomer (axial-OH). Between L-fucose and L-rhamnose, L-fucose has Δ2 instability[15] condition (The –OH on C-2 is axial while the OH on C-1 is equatorial), while L-rhamnose has a more stable C1 configuration. In L-fucose C-1 OH is axial but there is more of β-isomer than that in L-rhamnose, which accounts for the higher rate of oxidation of the former.

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