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Kinetics and mechanism of oxidation of *erythro*-series pentoses and hexoses by *N*-chloro-*p*-toluenesulfonamide

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Abstract

The kinetics and mechanism of oxidation of D-glucose, D-mannose, D-fructose, D-arabinose, and D-ribose with chloramine-T in alkaline medium were studied. The rate law, rate = k [Chloramine-T] [Sugar] $[HO^-]^2$, was observed. The rate of the reaction was influenced by a change in ionic strength of the medium, and the dielectric effect was found to be negative. The latter enabled the computation of d_{AB}, the size of the activated complex. The reaction rate was almost doubled in deuterium oxide. Activation energies were calculated from the Arrhenius plots. HPLC and GLC–MS analyses of the products indicated that the sugars were oxidized to a mixture of aldonic acids, consisting of arabinonic, ribonic, erythronic, and glyceric acids. Based on these data, a plausible mechanism involving the *aldo*-enolic anions of pentoses and *keto*-enolic anions of hexoses is suggested. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Erythro*-series sugars; Oxidation with chloramine-T; Kinetics and mechanism

1. Introduction

Aromatic *N*-halosulfonamides, a group of mild oxidizing agents, have been extensively used for the oxidation of a variety of organic compounds, including aldehydes, amines, and amino acids [1–3]. These oxidants contain strongly polarized *N*-linked halogens which are in the +1 state. They undergo two-electron change to form halide ions and corresponding sulfonamides. The prominent member of this class of oxidants is *N*-chloro-*p*-toluenesulfonamide (chloramine-T, abbreviated as CAT or RNClNa).

Abbreviations: CAT, RNClNa or chloramine-T, sodium salt of *N*-chloro-*p*-toluenesulfonamide; S, sugar; D, dielectric constant; T, absolute temperature; E_a, activation energy; n, atom fraction of deuterium; TS, transition state; RS, reactant site; I, ionic strength; d_{AB}, size of the activated complex; k, Boltzmann constant; HPLC, high performance liquid chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry

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Previously, it was reported that oxidation of sugars with *N*-chlorobenzenesulfonamide and *N*-bromo-*p*-toluenesulfonamide in alkaline solutions involves a sugar to oxidant stoichiometry of 1:1 for aldoses and 1:2 for fructose [4–6]. By paper chromatography, the products were identified as the corresponding aldonic acids for aldoses, and arabinonic acid for fructose [4–6]. However, the present study gave a stoichiometry of 2–3 moles of CAT per mole of sugar. HPLC and GLC–MS analyses of the oxidation products gave different product profiles than previously reported [4–6]. The results reported here demonstrate that *erythro*-series sugars, both pentoses and hexoses, undergo oxidation to a mixture of aldonic acids consisting of arabinonic, ribonic, erythronic, and glyceric acids. In the case of hexoses, oxidation occurs mainly with the cleavage of the C-1–C-2 and C-2–C-3 bonds, pentoses oxidize mainly with the cleavage of the C-1–H and C-1–C-2 bonds. Based on kinetic and thermodynamic parameters, and on product profiles, a mechanism for the oxidation of sugars by CAT is proposed.

2. Experimental

Materials.—D-Glucose, D-mannose, D-fructose, D-arabinose, D-ribose, D-gluconic acid, D-galactono-1,4-lactone, D-ribono-1,4-lactone, and 2-acetamido-2-deoxy-D-glucose were purchased from Sigma Chemical (St. Louis, MO). D-Manno-1,4-Lactone, D-xylo-1,4-lactone, D-arabino-1,4-lactone, and 2-deoxy-D-glucose were from Pfanstiehl (Waukegan, IL). Deuterium oxide (99.4%) was from Bhabha Atomic Research Center (Bombay, India).

General procedures.—The aqueous solution of CAT was standardized iodometrically, and sodium perchlorate was used ‘to swamp’ the reaction. The solvent isotopic studies were performed with deuterium oxide.

Kinetic measurements.—In all kinetic studies, pseudo-first order conditions were maintained with respect to the CAT concentration. Stock solutions of alkali, CAT, sugars, and sodium perchlorate were maintained at 35 °C. From these stock solutions, a mixture of sugars, alkali, and sodium perchlorate were prepared. The reaction was initiated by the addition of CAT and monitored for two half-lives by iodometric determination of unconsumed CAT at various time periods. First-order rate constants (k_{obs}) were calculated from the plots of log $[CAT]_0$ vs. time, and these were within ±5%.

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

Stoichiometry and product analysis.—The reaction mixtures containing sugar (0.01 M), alkali (0.1 M), and CAT (0.05 M) were kept for 24 h at 35 °C. The unconsumed CAT was determined iodometrically. From these data, the amount of the oxidant consumed per mole of sugar was calculated.

The oxidation products were analyzed by Dionex HPLC with pulsed amperometric detection using a CarboPac PA1 high-pH anion-exchange column (4 × 250 mm) [7]. An isocratic elution with 0.2 M NaOH

Table 1
Effect of reactant concentrations on the rate of oxidation of sugars by CAT at 35 °C

$10^3\,[CAT]_0$ (M)	$10^2\,[S]_0$ (M)	$10^4\,k_{obs}$ (s^{-1})				
		D-Mannose	D-Glucose	D-Fructose	D-Arabinose	D-Ribose
1.5	5.0	1.7	3.6	15.8	16.1	24.5
2.0	5.0	1.8	3.7	15.5	15.8	24.6
2.5	5.0	1.7	3.6	15.9	15.7	24.7
3.0	5.0	1.8	3.6	15.3	15.4	24.6
3.5	5.0	1.8	3.7	14.9	15.2	24.6
4.0	5.0	1.7	3.7	14.12	15.2	24.7
2.0	0.8	0.3	0.6	2.6	2.5	3.8
2.0	1.0	0.4	0.8	3.2	3.1	4.7
2.0	2.0	0.7	1.5	6.3	6.2	9.5
2.0	3.0	1.1	2.3	9.7	9.4	14.2
2.0	4.0	1.4	3.0	13.1	11.8	18.9
2.0	5.0	1.8	3.7	15.5	15.8	24.6
2.0	6.0	2.4	4.4	19.4	19.2	27.9

$[HO^-] = 0.1$ M, and $[I] = 0.4$ M.

was used. The products were identified by comparison of the HPLC retention times with retention times 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 *p*-toluenesulfonamide and then passed through AG 50 W-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 derivatives and then analyzed by GLC–MS (performed by Dr. Roberta Merkle, Complex Carbohydrate Research Center, University of Georgia, GA, and Dr. Lewis Pannell, Mass Spectrometry Facility, NIDDK, NIH, MD).

3. Results

Reactant concentrations.—The reactions were carried out with varying concentrations of CAT using constant [HO^-] and [S] (where S = sugar). The plots of log [CAT] vs. time were linear ($r > 0.9985$, $s < 0.04$), indicating a first-order dependence of reaction rate on $[CAT]_0$ (not shown). The pseudo-first order rate constants (k_{obs}) obtained with different $[CAT]_0$ were similar, confirming the first-order dependence of the rate on $[CAT]_0$. The k_{obs} values increased with increase in $[S]_0$ (Table 1). The plots of log k_{obs} vs. log $[S]_0$ were linear ($r > 0.9939$, $s < 0.05$) with unit slopes. The second-order rate constants ($k_2 = k_{obs}/[S]_0$) were constant within the experimental error (Table 1), demonstrating a first-order dependence of rates on $[S]_0$. The lines in the plots of k_{obs} vs. $[S]_0$ passed through the origin ($r > 0.9896$, $s < 0.06$), indicating that the intermediates formed with the oxidant have only transient existence.

Concentration of alkali.—At constant $[CAT]_0$ and $[S]_0$, values of k_{obs} increased with an increase in [NaOH] (Table 2). The order of reaction in hydroxide ions was calculated from the slope of plots of log k_{obs} vs. log [HO^-] ($r > 0.9974$, $s < 0.04$). These data demonstrated that the reactions follow second-order dependence on [HO^-].

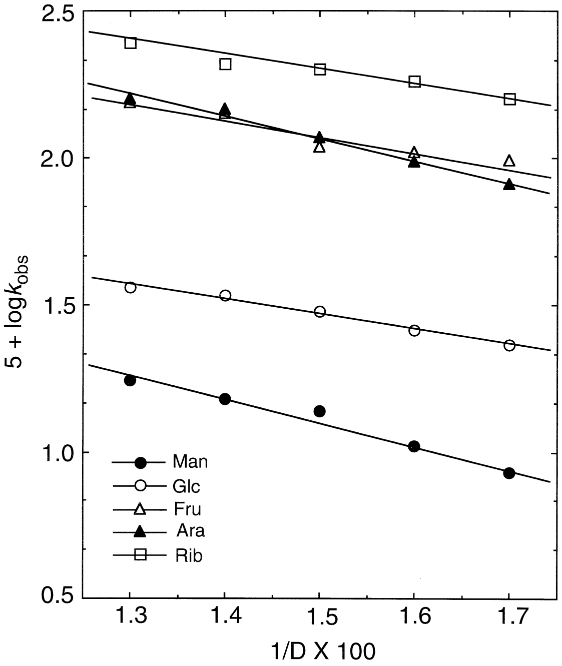


Fig. 1. Plots of log k_{obs} vs. $1/D$. $[CAT]_0 = 0.002$ M, $[S]_0 = 0.05$ M, [OH^-] = 0.1 M, [I] = 0.4 M; Temperature = 35 °C.

Effect of p*-toluenesulfonamide and chloride ions.*—Addition of *p*-toluenesulfonamide (0 to 0.008 M) to the reaction mixtures had no significant effect on the rates (data not shown). This indicates that the product, *p*-toluenesulfonamide, was not involved in pre-equilibrium with the oxidant. Addition of NaCl (0 to 0.02 M) to the reaction mixtures had no effect on the rates (data not shown), suggesting that the free chloride ion was not formed before the rate limiting step.

Table 2
Effect of [NaOH] on the rate of oxidation of sugars by CAT at 35 °C

10^2 [NaOH] (M)	10^4 k_{obs} (s^{-1})				
	D-Mannose	D-Glucose	D-Fructose	D-Arabinose	D-Ribose
2.0	0.1	0.2	0.7	0.6	1.0
4.0	0.3	0.6	2.9	2.4	3.9
6.0	0.6	1.3	6.3	5.4	9.1
8.0	1.2	2.5	11.3	9.8	16.2
10.0	1.8	3.7	15.5	15.8	24.6
20.0	6.8	15.3	56.3	57.5	68.8

$[CAT]_0 = 0.002$ M, $[S]_0 = 0.05$ M, and [I] = 0.4 M.

Table 3
Thermodynamic parameters for the oxidation of sugars by CAT at 35 °C

Sugars	E_a 10^4 (kJ mol^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta G^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (Jk^{-1} mol^{-1})	Log A
D-Mannose	133	131	98	107	22
D-Glucose	111	108	96	42	19
D-Fructose	75	73	92	−64	13
D-Arabinose	107	105	92	41	19
D-Ribose	90	88	102	−11	16

$[\mathrm{CAT}]_0 = 0.002$ M, $[\mathrm{HO}^-] = 0.1$ M, $[\mathrm{S}]_0 = 0.05$ M, and $[I] = 0.4$ M.

Effect of ionic strength.—Addition of sodium perchlorate (0 to 0.8 M) increased the rate of reaction. The plots of log k_{obs} vs. $I^{1/2}$ ($I =$ the ionic strength of medium) were linear with fractional slopes of 0.5–0.8 (data not shown).

Effect of solvent composition.—The solvent composition of the medium was varied by adding methanol up to 40%. The rate decreased with an increase in methanol content. The plots of log k_{obs} vs. $1/D$ ($D =$ dielectric constant of the medium) were linear with negative slopes (Fig. 1).

Activation parameters.—The reactions were studied at four different temperatures (303, 308, 313, and 318 K). Studies at temperatures higher than 318 K were not carried out due to the possible thermal decomposition of CAT. The Arrhenius plots of log k_{obs} vs. $1/T$ ($T =$ absolute temperature) were found to be linear ($r > 0.9991$, $s < 0.01$) (not shown). The activation energies (E_a, Table 3) were calculated from the slopes of the plots. From these values, the thermodynamic parameters $\Delta H^{\#}$, $\Delta S^{\#}$, and $\Delta G^{\#}$ (Table 3) were evaluated. The activation energy for fructose and ribose are considerably lower than other sugars. Furthermore, interestingly, both fructose and ribose showed negative enthalpy changes. These data suggest that the structures of fructose and ribose are favorably disposed for oxidation by CAT. This conclusion is consistent with the prediction that the enolate anion of fructose is the readily reacting structure for hexoses and the enolate form of ribose is that for pentoses (see below).

Solvent–Isotope studies.—The reactions were performed in pure deuterium oxide. The solvent–isotope effects, k_{obs}(water)/k_{obs}(deuterium oxide), were between 0.50 and 0.58 for all sugars. Proton-inventory studies were made in water–deuterium oxide mixtures (Table 4 and Fig. 2).

Analysis of oxidation products.—Product analysis indicated that arabinonic, ribonic, erythronic, and glyceric acids are the major oxidation products for all the *erythro*-series sugars studied; in the case of hexoses, besides these acids, small proportions of hexonic acids were formed (Fig. 3 and Table 5). Incubation of sugars with alkali alone, under the reaction conditions, did not degrade the sugars to significant extents.

The oxidation products of sugars were also analyzed at 0.5, 1, 2, 4, 8, 16, and 24 h. The relative proportions of various aldonic acids formed (see Fig. 3 and Table 5) were similar at all time points analyzed (except, in the case of hexoses, the formation of six-carbon aldonic acids was observed only after 4 h), revealing that the lower-carbon aldonic acids were not derived from the initially formed six-carbon aldonic acids. Consistent with these data, D-gluconic, D-mannonic, D-galactonic, D-ribonic and D-arabinonic acids were not oxidized by CAT.

HPLC analysis of reaction mixtures at various time

Table 4
Proton inventory studies for the oxidation of sugars by CAT in water–deuterium oxide mixtures at 35 °C

Atom fraction of deuterium (n)	10^4 k_{obs} (s^{-1})				
	D-Mannose	D-Glucose	D-Fructose	D-Arabinose	D-Ribose
0.00	1.8	3.7	15.5	15.8	24.6
0.25	1.9	4.2	16.9	17.0	27.5
0.50	2.2	5.0	19.9	20.5	31.8
0.75	2.8	6.3	24.1	24.3	37.0
0.93	3.2	7.5	28.4	31.5	42.3

$[\mathrm{CAT}]_0 = 0.002$ M, $[\mathrm{HO}^-] = 0.1$ M, $[\mathrm{S}]_0 = 0.05$ M, and $[I] = 0.4$ M.

Table 5
HPLC analysis of the products formed by the oxidation of sugars by CAT in alkaline medium

Sugar	Mole of CAT consumed per mole of sugar	Products (mole %)[a]				
		Arabinonic acid	Ribonic acid	Erythronic acid and threonic acid[b]	Glyceric acid	Hexonic acid
D-Mannose	2.4	36	19	35	4	6
D-Glucose	2.8	35	21	36	3	5
D-Fructose	2.8	30	20	40	8	4
D-Arabinose	2.2	28	8	49	14	–
D-Ribose	1.7	30	8	48	14	–

[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 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 with 0.01 M sugars and 0.01 M CAT.

[b] The shoulder at the tailing edge of the peak 2 in Fig. 3 represents threonic acid (2–4% of total aldonic acids formed).

points further indicated that, whereas 15–20% of glucose was isomerized into fructose within 30 min, less than 5% of mannose was isomerized to fructose at 30 min. Under similar conditions, 10–15% of fructose was isomerized to glucose. Parallel to this observation, a significant amount of mannose (retention time 4.25 min, Fig. 3) was not oxidized by CAT, even though under similar conditions, glucose and fructose (retention times 4.5 and 5.0 min, respectively) were almost completely oxidized. Furthermore, although a large proportion of mannose remained in the reaction mixture even after 24 h of incubation at 35 °C, only a small proportion of mannonic acid was formed (see Fig. 3 and Table 5). Thus, the ease of aldose–ketose tautomerization, the relative rates of oxidation, and the product profile suggest that hexoses undergo oxidation by CAT in the *keto*(*fructo*)-enolic form but not in the *aldo*-enolic form. Furthermore, 2-deoxy-D-*arabino*-hexose and 2-acetamido-2-deoxy-D-glucose were resistant to oxidation by CAT. This is likely due to the inability of these sugars to isomerize to a keto form, and the results agree with the conclusion that aldohexoses react with CAT in the keto form.

In the case of pentoses, the peaks with retention times 3.8 min and 5.1 min (see Fig. 3) corresponded, respectively, to arabinose and ribose. Whereas only a small proportion of arabinose was epimerized to ribose, a significant amount of ribose was epimerized to arabinose. Parallel to this observation, ribose was found to be oxidized by CAT faster than arabinose (Table 1). *erythro*-Pentulose was not detectable in the reaction mixture of arabinose or ribose at any of the all time points analyzed; this was the case even in the absence of CAT. Thus, pentoses react with CAT in the *aldo*-enolic form. This conclusion agrees with the formation of significant amounts of arabinonic and ribonic acids from arabinose and ribose.

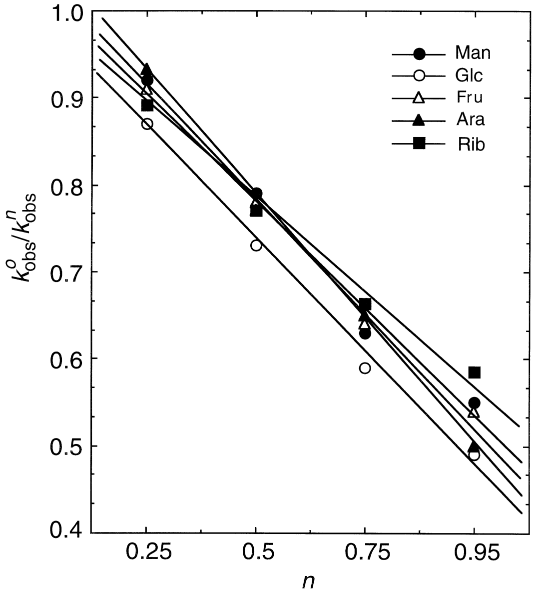


Fig. 2. Plots of $k_{\mathrm{obs}} / k_{\mathrm{obs}}$ vs. n in water–deuterium oxide mixtures. $[\mathrm{CAT}]_0 = 0.002$ M, $[\mathrm{S}]_0 = 0.05$ M, $[\mathrm{OH}^-] = 0.1$ M, $[I] = 0.4$ M; Temperature = 35 °C.

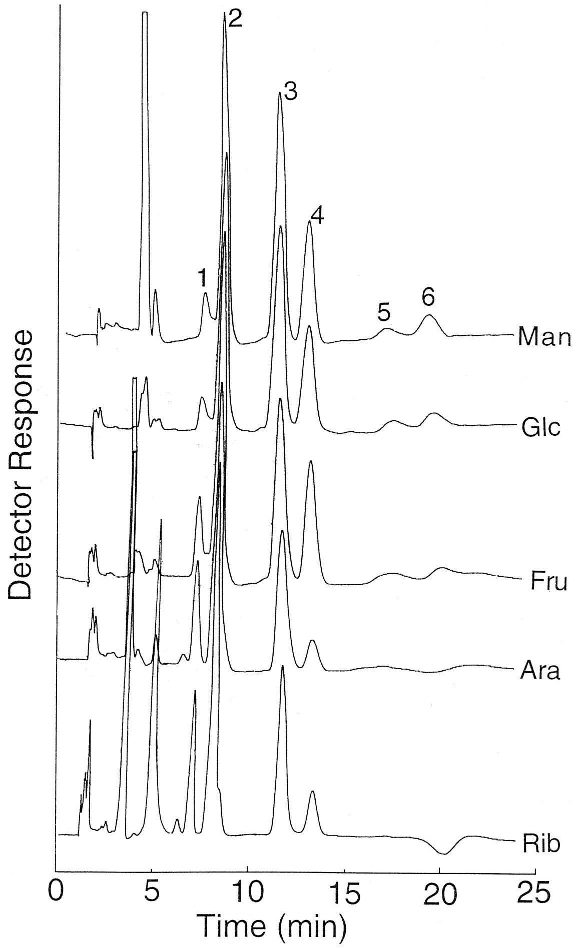
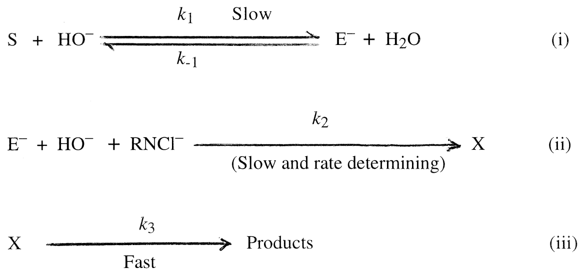


Fig. 3. HPLC analysis of the products formed by the oxidation of sugars (0.01 M) by CAT (0.05 M) in presence of alkali (0.1 M) at 35 °C. 1, glyceric acid; 2, erythronic acid; 3, arabinonic acid; 4, ribonic acid; 5 and 6, hexonic acids. Man, Glc, Fru, Ara and Rib, respectively, represent reaction of CAT with D-mannose, D-glucose, D-fructose, D-arabinose and D-ribose. The skewed shoulder on the tailing edge of the peak 2 represents a small amount (2–4%) of threonic acid.

4. Discussion

The identical orders with respect to both CAT and sugars suggest a common mechanism for the oxida-



Scheme 1.

tion of sugars by CAT. The organic haloamines behave as strong electrolytes in aqueous solutions, and the several equilibria present are predominantly pH dependent [7–10]. Although the oxidizing species in acidic solutions of CAT are RNClH, $RNCl^2$, and hypochlorous acid, it has been established that in alkaline medium, $RNCl^-$ is the active oxidant [8–12].

In alkaline solutions, sugars undergo enolization to form enediolate 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 isomeric aldoses and ketoses [13]. However, in the presence of CAT, the enediolate anions (E^-), react with $RNCl^-$ to form intermediates (X), which in turn undergo cleavage to form products (Table 5).

In view of the observed first-order dependence of rate on $[CAT]_0$ and $[S]_0$ and second-order dependence on $[HO^-]$, the following reaction sequence (Scheme 1) is proposed for the oxidation of sugars by CAT in alkaline solutions. Assuming steady state conditions for E^-, rate law (Eq. (1)) can be derived:

$$\text{Rate} = -\frac{d[CAT]}{dt} = \frac{k_2 k_1 [S][HO^-]^2 [CAT]}{k_{-1}[H_2O] + k_2 [HO^-][CAT]} \qquad (1)$$

Since there is a second-order dependence of rate on $[OH^-]$, the assumption that $k_{-1}[H_2O] > k_2[HO^-][CAT]$, where k_2 represents the specific reaction rate constant for the rate limiting step, is valid. Thus, rate law (Eq. (1)) is reduced to

$$\text{Rate} = -\frac{d[CAT]}{dt} = \frac{k_2 k_1 [S][HO^-]^2 [CAT]}{k_{-1}[H_2O]} \qquad (2)$$

which agrees with the observed rate = $k_{obs}[S][OH^-]^2[CAT]$.

The following results support the above conclusion.

(i) For reactions involving a fast pre-equilibrium H^+ or HO^- ion- transfer, the rate increases in deuterium oxide medium because D_3O^+ is a stronger acid, and DO^- is a stronger base. Therefore, the observed increase of oxidation rate in deuterium oxide agrees with the fast pre-equilibrium transfer of HO^- ion [14] (Eq. i). The dependence of rate constant (k^n_{obs}) on n ($n =$ the atom fraction of deuterium oxide in a solvent mixture of water and deuterium oxide) [15,16] is given by the Gross–Butler Eq. (3).

$$k^0_{obs}/k^n_{obs} = \frac{\dfrac{TS}{II}(1-n+n\phi_i)}{\dfrac{RS}{II}(1-n+n\phi_j)} \qquad (3)$$

where ϕ_i and ϕ_j are isotopic fractionation factors for the isotopically exchangeable hydrogen sites in the transition state (TS) and reactant site (RS), respectively. If the reaction proceeds through a single transition state, then Eq. (3) becomes Eq. (4):

$$\left(k^0_{obs}/k^n_{obs}\right) = 1 + n(\phi j - 1) \qquad (4)$$

A comparison of the plots of k_{obs} vs. n (not shown) with the standard curves [17], suggested a single proton exchange in the transition state. Furthermore, the plots of k^0_{obs}/k^n_{obs} vs. n (Fig. 2) were linear with slopes ($\phi_j - 1$). The ϕ_j for the oxidation of sugars by CAT is about 0.6. This value closely resembles the fractionation factor of HO^- ion.

(ii) The rate determining step in Scheme 1 involves three negative ions. Therefore, the reaction rate is expected to be increased with an increase in the ionic strength (I) of reaction medium. The plots of log k_{obs} vs. $I^{1/2}$ were linear with slopes between 0.5 and 0.8 (data not shown), even though the ionic strengths employed were beyond the Debye–Huckel range. The theoretical slope of unity has not been realized, and this is possibly due to the formation of Bjerrum ion pairs in concentrated solutions.

(iii) Addition of methanol to the reaction medium decreased the rates. The plots of log k_{obs} vs. $1/D$ ($D =$ dielectric constant) were linear with negative slopes (Fig. 1). Assuming a double sphere model [15] for the reaction, the effect of solvent composition on the rate of a reaction involving two negative ions is given by Eq. (5):

$$\log k = \log k_0 - Z_A Z_B e^2 / DkTd_{AB} \qquad (5)$$

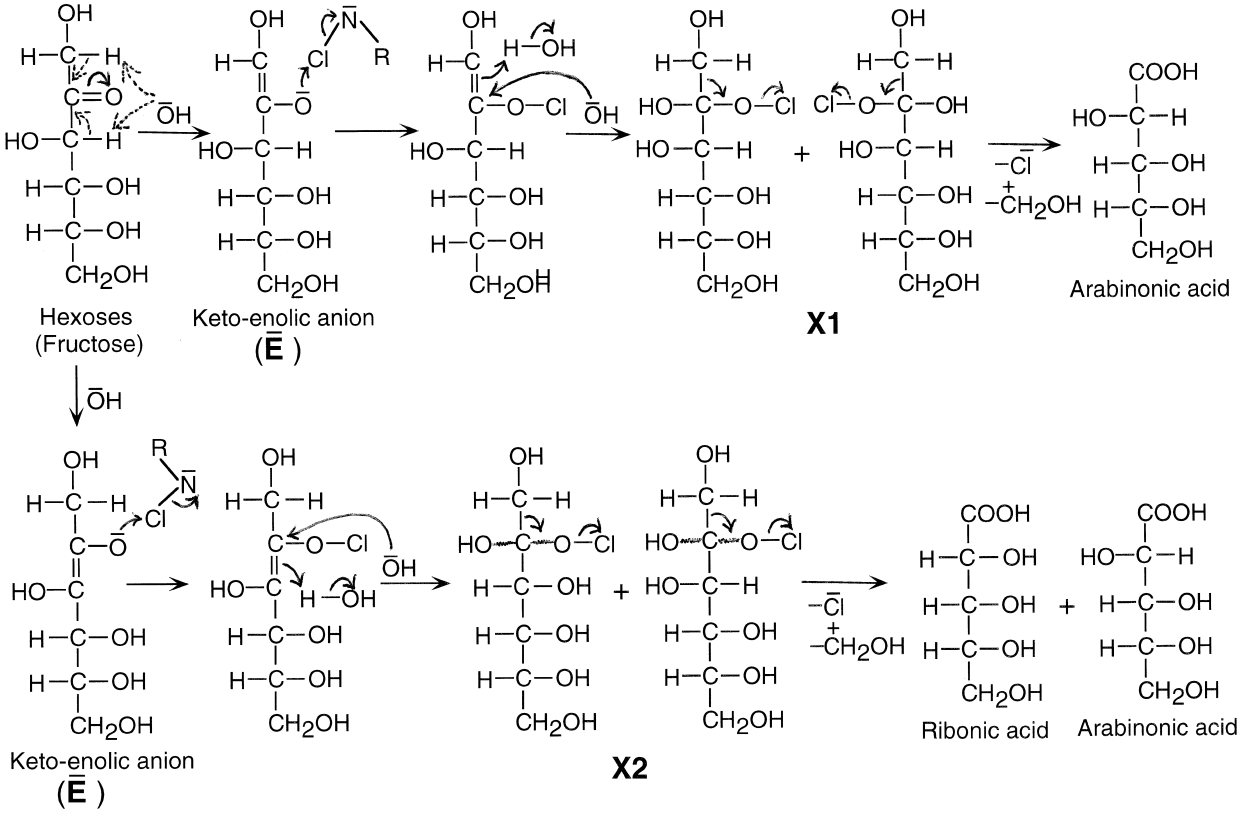
where k_0 is the rate constant in a medium of infinite dielectric constant, $Z_A e$, and $Z_B e$ are the charges on ions, d_{AB} is the size of the activated complex, k is the Boltzmann constant, and T is the absolute temperature. From the slopes of the straight lines in Fig. 1 (slope $= -Z_A Z_B e^2/kTd_{AB}$), d_{AB} was calculated. The derived d_{AB} values for D-glucose, D-mannose, D-fructose, D-arabinose, and D-ribose are 4.86 Å, 3.26 Å, 5.26 Å, 5.28 Å, and 5.18 Å, respectively. These values are comparable with those obtained for similar reactions [18].

(iv) Scheme 1 shows that the rate determining step involves interactions among three similarly charged ions (Eq. ii), which would require a very high activation energy. The observed high activation energies agree with this prediction.

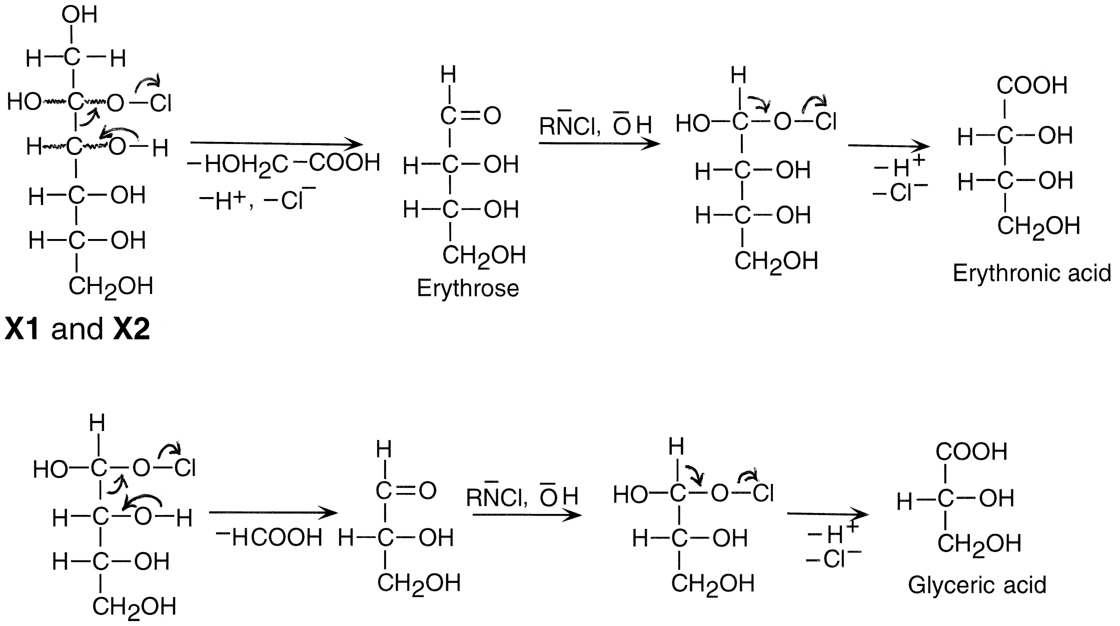
The formation of pentonic acids and erythronic acid by the loss of one and two carbon atoms, respectively, and minor proportions of hexonic acids from hexoses suggests that these sugars react with CAT mainly through *keto*-enolic anion intermediates. The formation of only minor amounts of hexonic acids further suggest that hexoses react extremely slowly with CAT in the *aldo*-enolic forms. This conclusion agrees with the formation of only a minor amount of mannonic acid despite the existence of a significant amount of unreacted mannose in the aldose form even after 24 h of incubation with CAT (see Fig. 3). The resistance of 2-deoxy-D-*arabino*-hexose and 2-acetamido-2-deoxy-glucose (although these hexoses can enolize, they can not isomerize to *keto*-forms) to oxidation by CAT further support this prediction.

In contrast to hexoses, pentoses gave significant amounts of corresponding aldonic acids (pentonic acids). From the product profiles, clearly, the major products are formed by the cleavage of C-1–H and C-1–C-2 bonds. Furthermore, since *erythro*-pentulose was not formed to a noticeable extent, by the alkali-catalyzed tautomerization of arabinose or ribose, this result together with the above data suggest that pentoses undergo oxidation by CAT through *aldo*-enolic intermediates.

The negative enthalpy change for fructose but not for glucose and mannose (Table 3) demonstrates that fructose has more orderly structure, and thus, it is sterically favored for oxidation by CAT. HPLC of the reaction mixture at various time points demonstrate that, in the case of aldohexoses, the fructose isomer formed by the alkali-catalyzed isomerization is the species that reacts with CAT. The observed higher reaction rate and lower activation energy for fructose (Tables 1 and 3) compared with glucose and mannose agree with the foregoing conclusion. In the case of

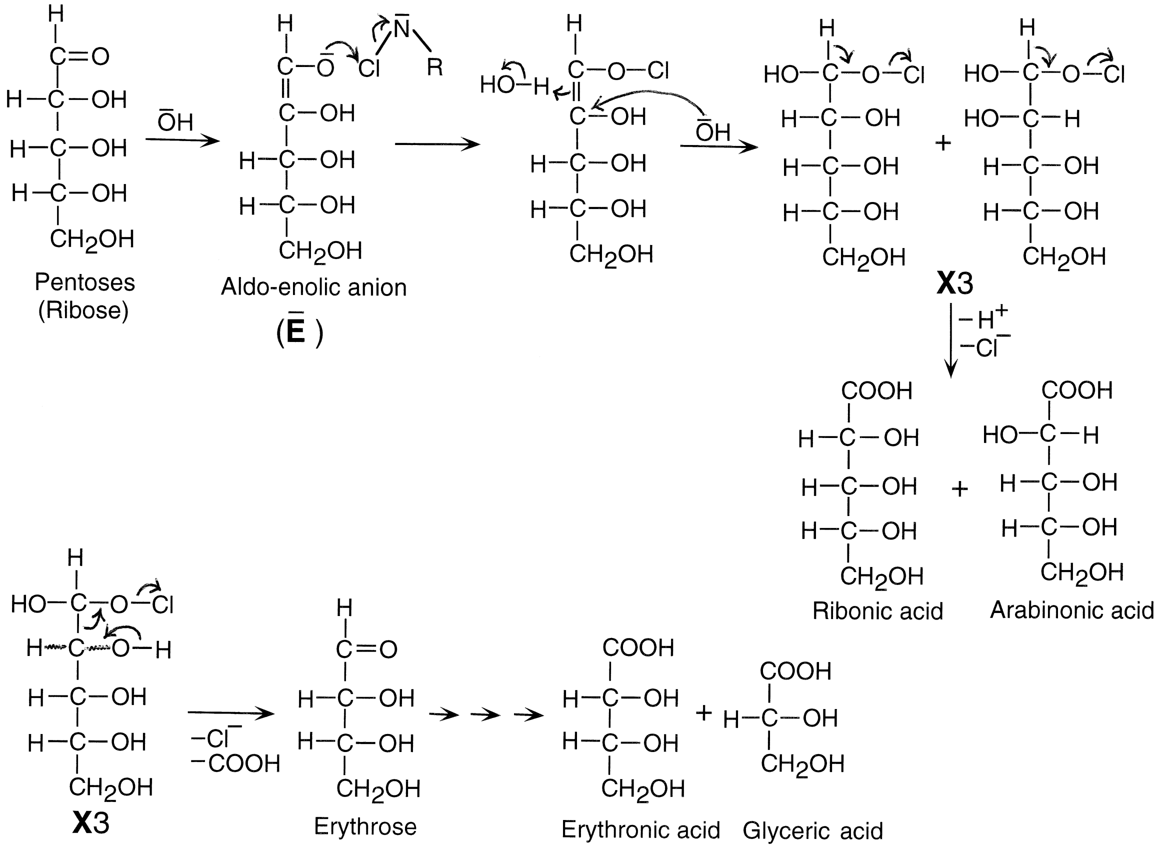


Scheme 2.



Scheme 2 (continued).

pentoses, the lower activation energy and faster reaction rate for ribose compared with arabinose, and negative enthalpy change for ribose support the conclusion that ribose is more easily oxidized than arabinose. Based on these considerations, a plausible mechanism for the oxidation of sugars by CAT is



Scheme 2 (continued).

proposed in Scheme 2. This mechanism accounts for the observed kinetics and products of oxidation.

In the proposed mechanism (Scheme 2), the anions (E^-) of sugars (*keto*-isomer in the case of hexoses and *aldo*-isomer in the case of pentoses) react with CAT to form intermediates X1–X3. In the case of anions (E^-) from hexoses, the loss of hydrogen can occur at either C-1 or C-3 to form C-1–C-2 or C-2–C-3 enediols containing chloroxyl group at C-2. Since these enediols contain polarized double bonds, hydroxide ion can add at C-2 to form intermediates X1 and X2; the formation of X2 accompanies epimerization at C-2 and C-3. X1 and X2 then can undergo cleavage of C–C bonds between C-1 and C-2, the former giving arabinonic acid and the latter forming a mixture of arabinonic and ribonic acids. In the case of E^- from pentoses, hydrogen can be removed only from C-2 to form the C-1–C-2 enediolate anion, which in the presence of CAT and alkali forms intermediate X3 with epimerization at C-2. The cleavage of C-1–H bonds from X3 gives a mixture of arabinonic and ribonic acids. The cleavage of C–C bonds between C-2 and C-3 in X1 and X2, and the breaking of C–C bonds between C-1 and C-2 in X3 yield *aldo*-tetrose without epimerization at C-4 (hexoses) or at C-3 (pentoses). The aldotetrose further oxidizes to yield erythronic acid and a minor proportion of threonic acid (Table 5). The reaction can proceed further, with the cleavage of C–C bonds between C-3 and C-4 of hexoses and the breaking of C–C bonds between C-2 and C-3 of pentoses, to form glyceric acid.

Although in the proposed mechanism, CAT is shown to react with preformed enediol-anion intermediate, it is possible that the ring opening of sugars is assisted by CAT. Since the furanosidic form of sugars is expected to be less energetic compared with the pyranosidic form, CAT may preferentially react with the furanosidic form rather than pyranosidic form. Consistent with this suggestion, it is known that a significant proportion of fructose exists in the furanose configuration, whereas glucose and mannose exist almost exclusively in the pyranose configuration [13]. Similarly, a higher proportion of ribose, compared with arabinose, is known to exist in the furanosidic form, 20% vs. 4.5% [13].

Previous studies, using oxidants that are closely related to CAT, reported a sugar to oxidant stoichiometry of 1:1 [4–6]. The reported lower stoichiometry was due to the premature (within 6 h after incubation) determination of CAT in the reaction mixture. Furthermore, in previous studies, paper chromatography was used for the identification of the oxidation products [4–6]. Because of poor resolution under the conditions employed, the product identification was not definitive. In the present study, the stoichiometry was estimated after an incubation period of 24 h, and the products were rigorously analyzed using HPLC and GLC–MS. Thus, the results of the present study suggest a novel pathway (Scheme 2) for the oxidation of sugars by CAT.

5. Graphic summary

N-Chloro-*p*-toluenesulfonamide oxidizes *erythro*-series pentoses and hexoses to a mixture of arabinonic, ribonic, erythronic, and glyceric acids through enediol-anion intermediates. Based on kinetics studies and on product profiles, a plausible mechanism is suggested.

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