

Kinetics and Mechanism of Oxidation of D-Cycloserine by Sodium N-Bromo-p- Toluenesulfonamide in Acid Medium

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ABSTRACT

The kinetics of oxidation of D-cycloserine (CS) by sodium-N-bromo-p-toluenesulfonamide or bromamine-T (BAT) in the presence of HCl at 313 K follows the rate law, $-d[BAT]/dt = k[BAT][CS]^x[HCl]^y$, where x and y are less than unity. The decrease in dielectric constant of the medium increases the rate. The variation of ionic strength or the addition of the reaction product, p-toluenesulfonamide, has no effect on the rate. The rate increases in D₂O medium and the inverse solvent-isotope effect is $k'_{D_2O} / k'_{H_2O} = 1.60$. Proton inventory studies in H₂O-D₂O mixtures have been used to calculate the isotopic fractionation factors. Composite activation parameters for the reaction have been determined from Arrhenius and Eyring plots. Michaelis-Menten type of kinetics is observed and activation parameters for the rate-determining step have been computed. The proposed mechanism assumes the simultaneous catalysis by H⁺ and Cl⁻ ions and is consistent with the observed kinetic data. Products of oxidation were identified.

INTRODUCTION

The organic N-haloamines are capable of producing halonium cations in aqueous solution. The important chlorine compound of this class is chloramine-T (CAT), which is a bi-product in saccharin manufacture and is a well-known analytical reagent. Mechanistic aspects of many of its reactions have been documented [1,2]. The bromine analogue sodium N-bromo-p-toluenesulfonamide (p-CH₃C₆H₄SO₂NBrNa.3H₂O, bromamine-T or BAT) is found to be a better oxidizing agent than the chloro derivate. However, there is less information in the literature [3,4] on BAT, particularly with respect to the oxidation kinetics of antibiotics. The drug, D-cycloserine (CS), finds applications in the pharma-

ceutical industry as an antibiotic. The compound and its derivatives have been oxidized anodically using glassy carbon electrodes [5] and by CAT [6] and chloramine-B [7]. The information about its kinetic and mechanistic aspects is still scanty. Hence, it is worthwhile to study the oxidation of CS by BAT in acidic medium.

EXPERIMENTAL

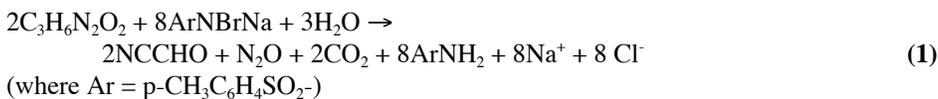
Bromamine-T was obtained by partial debromination of dibromamine-T (DBT) in 4M NaOH [8]. The purity of BAT obtained was checked iodometrically and through its mass, UV, IR, and ^1H and ^{13}C NMR spectral data. An aqueous solution of BAT was prepared afresh, standardized by the iodometric method, and preserved in brown bottles to prevent its photochemical deterioration. D-cycloserine (Sigma Chem. Co.) was used without further purification. Aqueous solutions of desired strength were prepared prior to use. All other chemicals were of Analar grade. The ionic strength of the medium was maintained at a constant high value ($\mu = 0.80 \text{ M}$) using a concentrated solution of sodium perchlorate. Solvent isotope studies were made with D_2O (99.4%) supplied by the Bhabha Atomic Research Centre, Bombay, India. Permittivity of the reaction medium was changed by the addition of MeOH in varying proportions (v/v) and the values of permittivity for MeOH-water mixtures reported in the literature were employed [9]. Triple-distilled water was used throughout.

Kinetic Measurement

Reactions were carried out in glass stoppered Pyrex boiling tubes coated black on the outside to eliminate photochemical effects. Pseudo-first-order conditions were maintained for the kinetic runs ($[\text{substrate}]_0 \gg [\text{oxidant}]_0$). The oxidant and the requisite amounts of CS, HCl, and NaClO_4 solutions and water (for constant total volume) taken in separate boiling tubes were thermostated for 30 min at 313 K. The reaction was initiated by the rapid addition of a measured amount of BAT to the mixture and was shaken intermittently for uniform concentration. The progress of the reaction was monitored by iodometric titration of unconsumed BAT in known aliquots of the reaction mixture withdrawn at regular intervals of time. The reaction was studied for more than two-half lives. Pseudo-first-order rate constants (k') were reproducible within $\pm 4\%$. Regression analysis of the experimental data to obtain regression coefficient "r" was performed with an EC-72 statistical calculator.

Stoichiometry and Product Analysis

Varying ratios of the oxidant-to-substrate (CS \ll BAT) in the presence of 0.20 M HCl were equilibrated at 313 K for 48 hr. The determination of the unconsumed BAT in the reaction mixture showed that one mole of CS consumed four moles of BAT:



The reduction product of BAT, p-toluenesulfonamide or ArNH_2 , among the reaction products was identified by paper chromatography [10] using benzyl alcohol saturated with water as the solvent system with ascending irrigation and using 0.5% vanillin in 1% HCl in EtOH as the spray reagent ($R_f = 0.905$). The presence of one of the oxidation

products, nitrile, was identified by its specific color reaction with hydroxylamine and ferric chloride [11]. The evolving CO₂ gas was detected by the lime water test.

RESULTS

The kinetics of oxidation of cycloserine by BAT was investigated at several initial concentrations of the reactants in HCl medium.

At constant [HCl] and [CS]₀, where ([CS]₀ >> [BAT]₀), plots of log [BAT] vs. time were linear ($r > 0.9908$) indicating a first-order dependence on the rate of [BAT]. The rate constant, k' , was not affected by a change in [BAT]₀ (average $10^4 k' = 3.60 \pm 0.10 \text{ s}^{-1}$). Values of k' increased with increase in [CS]₀. A plot of log k' vs. log [CS]₀ was linear ($r = 0.9935$), with a slope of 0.40, thus indicating a fractional-order dependence on [CS]. Furthermore, a plot of k' vs. [CS]₀ was also linear ($r = 0.993$) with a y-intercept, confirming the fractional-order dependence on [CS].

The rate of reaction increased with increase in [HCl] (Table 1) and a plot of log k' vs. log [HCl] was linear with a fractional slope of 0.66. Keeping the total chloride ion concentration in the reaction mixture constant at 0.60 M with NaCl, [H⁺] was varied using HCl. The rate increased (Table 1) with increase in [H⁺] and a plot of log k' vs. log [H⁺] was a straight line ($r = 0.9984$) with a fractional slope of 0.41. At constant [H⁺] = 0.20 M, maintained with HCl, the rate increased with the addition of NaCl (Table 1). A plot of log k' vs. log [Cl⁻] was linear ($r = 0.992$) with a slope of 0.25. Furthermore, plots of k' vs. [H⁺] and k' vs. [Cl⁻] were linear ($r > 0.992$) with y-intercepts.

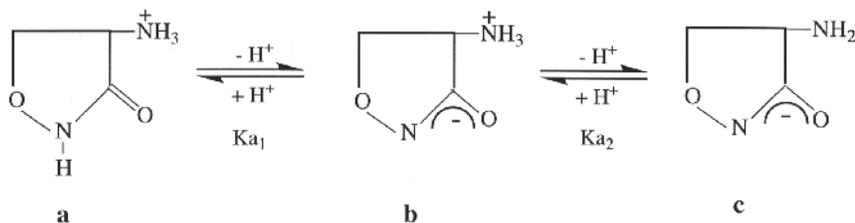
Addition of the reaction product, p-toluenesulfonamide (5.0×10^{-4} - 5.0×10^{-3} M) or the variation of ionic strength of the medium by adding NaClO₄ (0.2-1.0 M) did not affect the rate. Similarly, addition of Br⁻ ions in the form of NaBr (5.0×10^{-4} - 5.0×10^{-3} M) did not influence the rate. The dielectric constant of the medium was varied by addition of MeOH (0 - 40% v/v) to the reaction mixture. The rate increased with the increase in MeOH content. A plot of log k' vs. 1/D, where 'D' is the relative permittivity of the medium, was linear ($r = 0.9948$) with a positive slope. Control runs indicated that MeOH was very slightly oxidized (<2%) by BAT under the experimental conditions. This was taken into account in the calculation of the net rate constant for the oxidation of cycloserine each time.

The reaction was studied at different temperatures (308-323 K) and from the linear Arrhenius and Eyring plots ($r > 0.992$) of log k' vs. 1/T and log k'/T vs. 1/T, respectively, values of activation parameters for the overall reaction were computed (Table 2). Rate studies in D₂O medium showed that the reaction is faster in heavy water, with a formal solvent isotope effect $k'_{\text{H}_2\text{O}} / k'_{\text{D}_2\text{O}} = 0.63$. The reaction was studied in H₂O-D₂O mixtures containing varying deuterium atom fractions "n" (Table 3) and proton inventory plots relating the rate constant $k'^n_{\text{H}_2\text{O}}$ with 'n' are shown in Figure 1.

Olefinic monomers such as acrylonitrile and freshly prepared 10% acrylamide solution, under nitrogen atmosphere, were added to the reaction mixture to initiate polymerization by free radicals formed *in situ*. The lack of polymerization indicated the absence of free radicals in the reaction mixture. Proper control experiments were also conducted.

DISCUSSION

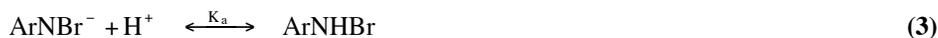
Cycloserine, having Zwitter ionic properties similar to amino acids [12], possesses two ionizable groups with $pK_{a1} = 4.5$ and $pK_{a2} = 7.4$ (Scheme I).



Scheme I

The Zwitter ion 'b' is the predominant species in moderately acidic solution. The anion 'c' and the cation 'a' are in excess in basic and highly acidic media, respectively.

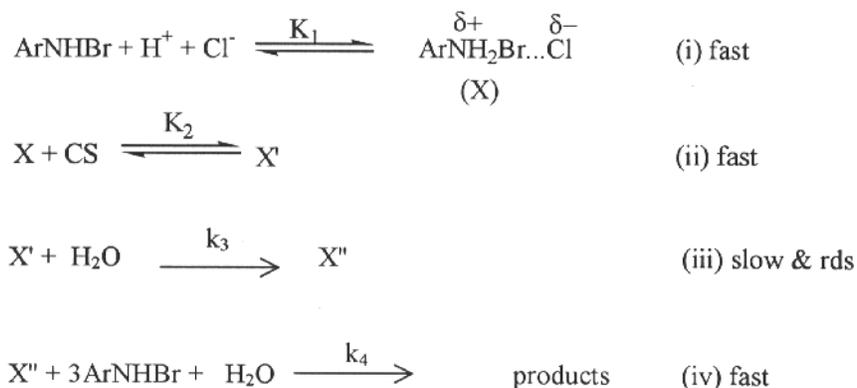
As organic halamines have similar chemical properties, it is expected that similar equilibria exist in solutions of these compounds. Bromamine-T acts as an oxidizing agent in both acidic and alkaline media. In general, BAT undergoes a two-electron change in its reactions. The oxidation potential of $BAT/ArHN_2$ is pH dependent and decreases with increase in the pH of the medium (values are 1.14 V at pH 0.65 and 0.50 V at pH 12.0). Depending on the pH of the medium, BAT furnishes different types of reactive species in solutions (eqs. 2-5) such as $ArNHBr$, $ArNBr_2$, and $HOBr$ in acid solutions [13-16].



Therefore, the possible oxidizing species in acidified BAT solutions are $ArNHBr$, $ArNBr_2$, and $HOBr$. If $ArNBr_2$ were the relative species, then the rate law would predict a second-order dependence of the rate on $[BAT]$ and retardation of the rate by the added $ArNH_2$ (eq. 4). Similarly, if $HOBr$ were the reactive species, a first-order retardation by $ArNH_2$ would be expected (eq. 5). Since these were not experimentally observed, the likely oxidizing species for CS is the free acid, $ArNHBr$ (eq. 3). Calculations of Hardy and Johnston [14] on aqueous bromamine-B and observations of Bishop and Jennings

[15] and Morris et al. [16] on CAT solutions have clearly shown that the conjugate acid (ArNHBr) is the predominant species in acid solutions of haloamines.

In the present investigations, oxidation of CS by BAT shows a first-order dependence on [BAT] and fractional-orders in [acid] and [substrate]. Non-involvement of the sulfonamide in pre-equilibrium with the oxidant and the absence of any ionic strength effect suggest that neutral species are taking part in the rate-determining step. Based on the above observations, the mechanism of the reaction is best explained by the following Scheme II with simultaneous catalysis by H^+ and Cl^- ions:



Where X, X' and X'' represent the intermediates

Scheme II

Step (iii) determines the overall rate,

$$\text{Rate} = \frac{-d[\text{BAT}]}{dt} = k_3[\text{X}'] \quad (6)$$

In eq. (6), $[\text{H}_2\text{O}]$ is omitted as it is constant in aqueous medium and included in the rate constant.

If $[\text{BAT}]_t$ represents the total concentration of the oxidant,

$$[\text{BAT}]_t = [\text{ArNHBr}] + [\text{X}] + [\text{X}'] \quad (7)$$

Solving for $[X']$ and substituting in eq. (6), the following rate law [eq. (8)] can be derived:

$$\text{Rate} = \frac{-d[\text{BAT}]}{dt} = \frac{K_1 K_2 k_3 [\text{BAT}]_t [\text{CS}] [\text{H}^+] [\text{Cl}^-]}{1 + K_1 [\text{H}^+] [\text{Cl}^-] + K_1 K_2 [\text{CS}] [\text{H}^+] [\text{Cl}^-]} \quad (8)$$

or

$$\text{rate} = \frac{K_1 K_2 k_3 [\text{BAT}]_t [\text{CS}] [\text{HCl}]}{1 + K_1 [\text{HCl}] + K_1 K_2 [\text{CS}] [\text{KCl}]}$$

The rate law is in agreement with experimental results. Eq. (8) can be transformed as,

$$\frac{1}{k'} = \frac{1}{K_2 k_3 [\text{CS}]} \left\{ \frac{1}{K_1 [\text{HCl}]} + 1 \right\} + \frac{1}{k_3} \quad (9)$$

From the intercepts of the linear double reciprocal plots of $1/k'$ vs. $1/[\text{CS}]_0$ at constant $[\text{HCl}]$ and $1/k'$ vs. $1/[\text{HCl}]$ at constant $[\text{CS}]_0$, the values of the rate constant, k_3 , for the rate-determining step found were, 7.40×10^{-4} and $1.03 \times 10^{-3} \text{ s}^{-1}$, respectively, which are comparable.

Since the rate was fractional in $[\text{CS}]_0$, Michaelis-Menten kinetics [17] were adopted to study the effect of $[\text{CS}]_0$ on the rate at different temperatures (308-323 K). From the plots of $1/k'$ vs. $1/[\text{CS}]_0$ (Figure 2), the values of k_3 obtained were ($10^4/\text{s}^{-1}$): 4.50 (308 K), 7.45 (313 K), 10.8 (318 K) and 17.7 (323 K). Activation parameters (Table 2) for the rate-determining step were computed from the Arrhenius and Eyring plots of $\log k_3$ vs. $1/T$ and $\log k_3/T$ vs. $1/T$ ($r > 0.9898$), respectively [17].

The rate increases with decrease in dielectric constant of the medium indicating a charge dispersal in the transition state, which is less polar than the reactants. Solvent isotope studies have shown that the rate of reaction is higher in D_2O medium. For a pre-equilibrium proton transfer [18], generally the inverse solvent isotope effect, $k'_{\text{D}_2\text{O}} / k'_{\text{H}_2\text{O}} = 2-3$, but the value of 1.60 obtained in the present investigation is a reflection of the fractional-order dependence of the rate on $[\text{H}^+]$. Since a part of the reaction takes place by chloride ion catalysis and through an acid independent pathway, the proton inventory studies in H_2O - D_2O mixtures could throw light on the nature of the transition state. The dependence of the rate constant (k_0^n) on n , the atom fraction of deuterium in a solvent mixture of D_2O and H_2O , is given by a form of the Gross-Butler equation [19, 20].

$$k_0^0 / k_0^n = \prod^{\text{TS}} (1 - n + n\phi_i) / \prod^{\text{RS}} (1 - n + \phi_j) \quad (10)$$

Where ϕ_i and ϕ_j are the isotopic fractionation factors for isotopically exchangeable hydrogen sites in the transition state (TS) and in the reactant state (RS), respectively. Eq. (10) allows the calculation of the fractionation factor of TS, if the reactant fractionation factor is known. However, the curvature of the proton inventory plot could reflect the number of exchangeable protons in the reaction [19, 21]. A plot of k_0^n vs. n (Figure 1, Table 3), in the present case, is more or less a straight line with the least convature.

Comparison with the standard curves [21] clearly indicates the involvement of a single proton or H-D exchange during the reaction sequence. Hence, the participation of H⁺ ion in the formation of transition state is inferred. Furthermore, if it is assumed that the reaction proceeds through a single transition state, eq. (10) takes the forms shown by eqs. (11) and (12).

$$k_0^n = k_0^o (1 - n + n\phi_j)^{-2} \quad (11)$$

$$(k_0^o / k_0^n)^{1/2} = [1 + n(\phi_j - 1)] \quad (12)$$

Eq. (12) indicates a linear relation between $(k_0^o / k_0^n)^{1/2}$ and n which is shown in Figure 1 ($r = 0.9905$). The slope $(\phi_j - 1) = -0.23$, from which the fractionation factor n is found to be 0.77. Kresge and Allred [22] have obtained a value of 0.67 for n of HClO₄ in H₂O-D₂O mixtures for which Gold [23] reported a value of 0.69. Considering the diversity of the procedures employed, it may be assumed that there is an agreement between the n values obtained for HClO₄.

The proposed mechanism is also supported by the moderate values of energy of activation. The fairly high positive values of the free energy of activation and the enthalpy of activation indicate that the transition state is highly solvated, while high negative entropy of activation suggests the formation of the compact activated complex. The constancy of rate constant on addition of neutral salts or the reaction product (ArNH₂) also supports the proposed mechanism.

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Table 1. Effect of varying [HCl], [H⁺], and [Cl⁻] on the rate^a,

[HCl]/M	k' x 10 ⁴ /s ⁻¹	*[H ⁺]/M	k' x 10 ⁴ /s-1	**[Cl ⁻]/M	k' x 10 ⁴ /s ⁻¹
0.100	2.30	0.100	3.72	0.200	3.68
0.200	3.60	0.200	4.70	0.300	4.20
0.300	2.82	0.300	5.58	0.400	4.65
0.400	5.68	0.400	6.43	0.600	4.98
0.500	6.70	0.500	6.95	0.800	5.30
0.600	7.56	0.600	7.40	-----	-----

^a [BAT]₀ = 1.00 x 10⁻³ M; [CS]₀ = 3.00 x 10⁻² M; μ = 0.80 M; and Temp = 313 K

* Experiments performed at constant [Cl⁻] = 0.600 M

** Experiments performed at constant [H⁺] = 0.200 M

Table 2. Activation parameters for the oxidation of cycloserine by BAT in HCl medium^a

Activation parameters	E _a (kJ mol ⁻¹)	ΔH [‡] (kJ mol ⁻¹)	ΔS [‡] (JK ⁻¹ mol ⁻¹)	ΔG [‡] (kJ mol ⁻¹)
Composite reaction ^b	68.5	65.7	-101	97.5
Rate-determining step ^c	71.5	68.8	-84.5	95.6

^a Conditions, except temp., are the same as listed in Table 3.

^b based on k'

^c based on k₃

Table 3. Proton inventory studies for cycloserine-BAT reactions in H₂O-D₂O mixtures^a

Atom fraction of deuterium (n)	k _o ⁿ x 10 ⁴ /s ⁻¹	(k _o ⁰ /k _o ⁿ) ^{1/2}
0.000	3.60	1.00
0.248	4.05	0.940
0.496	4.52	0.890
0.744	5.05	0.840
0.992	5.75	0.790

^a [BAT]₀ = 1.00 x 10⁻³ M; [CS]₀ = 3.00 x 10⁻² M; [HCl] = 0.200 M
μ = 0.80 M; and Temp. = 313 K

Figure 1. Proton inventory plots for the oxidation of D-cycloserine by BAT in H₂O-D₂O mixtures: Experimental conditions are as in Table 3.

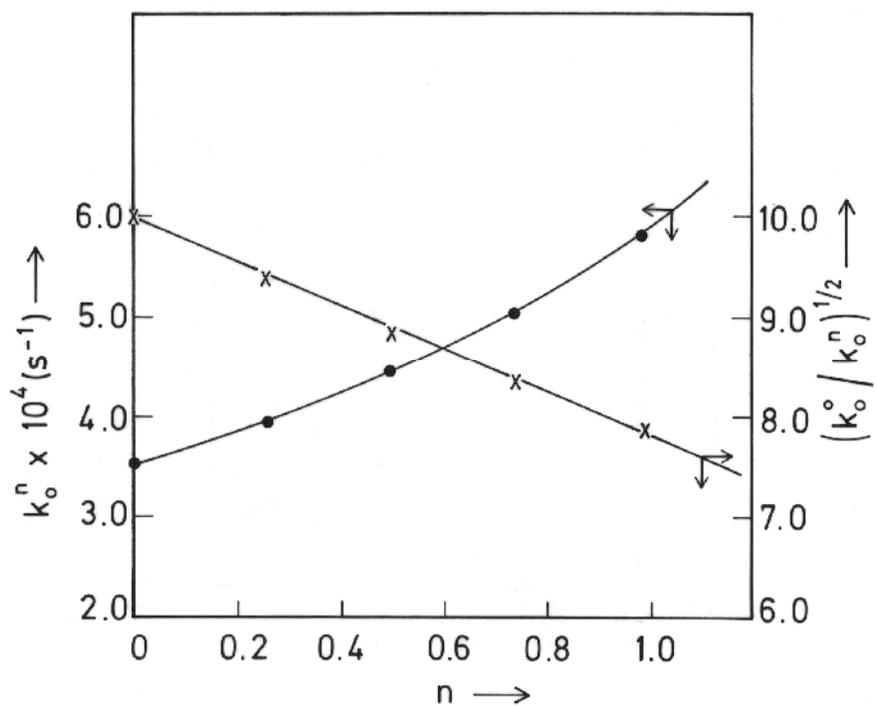


Figure 2. Double reciprocal plots of $1/k'$ vs. $1/[CS]_0$ at different temperatures: $[BAT]_0 = 1.00 \times 10^{-3} \text{ M}$; $[HCl] = 0.200 \text{ M}$; and $\mu = 0.80 \text{ M}$.

