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Kinetics and mechanism of oxidation of some basic amino acids with bromamine-T

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Abstract. Kinetics of oxidative decarboxylation of arginine, glutamine, histidine and lysine by bromamine-T (BAT) was investigated in acid and alkaline media at 30° and 20° respectively. The form of the rate law at low concentrations of $HClO_4$ has been worked out. Proton inventory studies in H_2O-D_2O mixtures with Arg as a probe have been made. The rate increases in the order: His > Lys > Arg > Glu – NH_2. In alkaline media, the rate shows a first order dependence on $[BAT]_0$ and is fractional in [S] and [OH^-]. *p*-Toluene sulphonamide retards the rate. Mechanisms proposed are consistent with the experimental rate laws.

Keywords. Bromamine-T; basic aminoacids; kinetics; acid and alkaline media.

1. Introduction

The oxidation of amino acids is of utmost importance from the chemical point of view and from its bearing on the mechanism of amino acid metabolism. Only the amino and carboxyl functional groups in $R'CH(NH_2)COOH$ generally undergo chemical transformations while the hydrocarbon moiety remains intact. This property is attributed to the higher reactivity of the former compared to R'. As a part of our broad programme (Mahadevappa *et al* 1984, 1985; Mahadevappa and Puttaswamy 1987) on the mechanistic aspects of oxidation of α-amino acids with N-metallo-N-bromoaryl sulphonamides, we report the oxidation of basic amino acids, arginine (Arg), glutamine (Glu-NH_2), histidine (His) and lysine (Lys) by sodium N-bromo-*p*-toluene sulphonamide (*p*-CH_3-$C_6H_4SO_2NBr$ Na, $3H_2O$ or bromamine-T, hereinafter abbreviated as BAT) in acidic and alkaline media.

2. Experimental

Bromamine-T was prepared by the reported procedure (Nair and Indrasenan 1976) and its purity was checked by iodometry and through its mass spectrum, UV, IR, ^1H and ^{13}C-spectral data (Rangappa *et al* 1981). An aqueous solution of BAT was prepared, standardized by the iodometric method and preserved in brown bottles.

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L-arginine hydrochloride, L-glutamine, L-lysine hydrochloride and L-histidine hydrochloride (Sisco Research Labs, India) were found to be chromatographically pure and were further assayed by the acetous-perchloric method (Vogel 1958). Aqueous solutions of amino acids were prepared. All other reagents were of analytical grade. Triply distilled water was used for preparing all aqueous solutions. A concentrated solution of $NaClO_4$ was used for 'swamping' the ionic strength of reaction mixtures. Heavy water (D_2O, 99.2%) used for solvent isotope studies was supplied by the Bhabha Atomic Research Centre, Trombay.

Regression analysis of experimental data (including determination of regression coefficient r and standard deviation of the estimate, S) was carried out on a TDC-316 (16 bits) computer supplied by Trombay Electronics, India.

Kinetic measurements

The reaction was carried out in glass-stoppered Pyrex boiling tubes whose outer surface was coated black to eliminate photochemical effects. Appropriate amounts of the amino acid, acid or alkali and enough water to keep the total volume constant for all runs were taken in the tube and thermostated (at 30° in acid medium and 20° in presence of alkali). A measured amount of BAT solution also thermostatted at the same temperature was rapidly added to the mixture in the tube. The progress of reaction was monitored by iodometric determination of unreacted BAT in an aliquot of the reaction mixture at different intervals of time. The course of reaction was studied for two half-lives. The pseudo first-order rate constants k' calculated were reproducible within ± 3%.

Stoichiometry

Varying ratios of BAT to amino acid were equilibrated in presence of $HClO_4$ (0.08 mol dm^{-3}) or NaOH (0.002 mol dm^{-3}) for 24 hours at 30° (20° for alkaline medium). The following stoichiometry was noted in the two media respectively:

$$R'CH(NH_2)COOH + 2\ TsNBr\ Na + 2\ H_2O \longrightarrow R'COOH + NH_3 + CO_2 + 2\ TsNH_2 + 2\ Na^+ + 2\ Br^-, \quad (1a)$$

$$R'CH(NH_2)COOH + TsNBr\ Na + H_2O \longrightarrow R'CHO + NH_3 + CO_2 + TsNH_2 + Na^+ + Br^-. \quad (1b)$$

Here Ts = p-CH_3-$C_6H_4SO_2$- and R' = $\begin{matrix} NH \\ \diagup\!\diagdown \\ NH_2 \end{matrix}$ C–$NH(CH_2)_3$ for arginine, $N \begin{matrix} \diagup\ CH=CCH_2\text{-} \\ \diagdown\ CH-NH \end{matrix}$ for histidine, $NH_2CO(CH_2)_2$- for glutamine and $NH_2(CH_2)_4$- for lysine.

The sulphonamide ($TsNH_2$) among the products was detected by paper chromatography. Benzyl alcohol saturated with water was used as the solvent with 0·5% vanillin in 1% HCl solution in ethanol as spray reagent (R_F = 0·905). Ammonia was quantitatively estimated by the microkjeldahl procedure (Vogel 1978). The aldehydes were detected by spot tests and estimated as their 2,4-DNP derivatives (Vogel 1973) after extraction into ether layer. The carboxylic acids identified by spot tests (Feigl 1956) were estimated by titrimetry. The results are shown in table 1.

Table 1. Estimation of ammonia, carboxylic acid and aldehyde in the oxidation of basic amino acids by BAT in presence of $HClO_4$ and NaOH.

	Acid medium		Alkaline medium	
Compound	Theoretical (found) NH_3, *m* mole	Theoretical (found) carboxylic acid, *m* mole	Theoretical (found) NH_3, *m* mole	Theoretical (found) aldehyde mg
Arginine	0·20 (0·20)	1·0 (0·90)	0·050(0·045)	133(119)
Arginine	0·80 (0·79)	2·0 (1·82)	0·450(0·443)	289(271)
Lysine	0·20 (0·20)	1·0 (0·91)	0·050(0·048)	125(116)
Lysine	0·80 (0·79)	2·0 (1·85)	0·450(0·442)	297(274)
Glutamine	0·20 (0·20)	1·0 (0·92)	0·050(0·045)	110(098)
Glutamine	0·80 (0·79)	2·0 (1·92)	0·450(0·445)	275(240)
Histidine	0·00045(0·00040)	0·030(0·025)	0·050(0·048)	120(106)
Histidine	0·00180(0·00170)	0·090(0·084)	0·450(0·441)	280(250)

3. Results

3.1 *Acid medium*

At constant $[HClO_4]$ and $[AA]_0$, plots of $\log[BAT]_0/[BAT]$ versus time were found to be linear after a short induction period ($r > 0{\cdot}9903$; $S \leq 0{\cdot}019$, figure 1) indicating a first-order dependence of rate on $[BAT]_0$. Values of k' are given in table 2. Increase of $[AA]_0$ increases the rate (table 2). A plot of log k' versus $\log[AA]_0$ gives a straight line ($r > 0{\cdot}9970$; $S \leq 0{\cdot}010$), with unit slope in the case of Arg and Lys and is fractional (0·4 and 0·6) in Glu-NH_2 and His respectively. The rate decreases with increase in $[HClO_4]$ but no simple relationship existed between k' and gross $[HClO_4]$. However, from pH measurements on experimental solutions, the free acid concentration $[H^+]_{ex}$ was calculated (table 3) and plots of log k' versus $\log[H^+]_{ex}$ were then found to be linear ($r > 0{\cdot}9970$; $S \leq 0{\cdot}020$) with a negative slope. The slope was -1 for Arg, His and Lys and $-0{\cdot}31$ for Glu-NH_2. Further a plot of k' versus $1/[H^+]_{ex}$ gave a straight line passing through the origin for Arg, His and Lys, while a *y*-intercept was obtained for Glu-NH_2. At higher acidities, the rate levelled off indicating a zero-order dependence on $[H^+]_{ex}$ (table 3). Under these conditions, the substrate showed a fractional order dependence on rate for Arg (0·44) and Lys (0·33), while Glu-NH_2 and His indicated zero order (table 4).

Addition of halide ions in the form of NaCl or NaBr ($1 \times 10^{-3} - 4 \times 10^{-3}$ mol dm^{-3}) or the reaction product, $TsNH_2$ had no effect on the rate. Similarly, variation of ionic strength of medium from 0·08 to 0·7 mol dm^{-3} and 1·0 to 1·8 mol dm^{-3} by adding $NaClO_4$ (in the case of histidine) did not affect the rate of reaction.

The solvent composition was varied by adding methanol (0–40% v/v) to the reaction mixture. The rate increased with decrease in dielectric constant (D) of the medium and a plot of log k' versus $1/D$ gave a straight line ($r > 0{\cdot}9934$; $S \leq 0{\cdot}015$) with a positive slope. Blank experiments performed showed that the oxidation of methanol by BAT during the period of study was negligible.

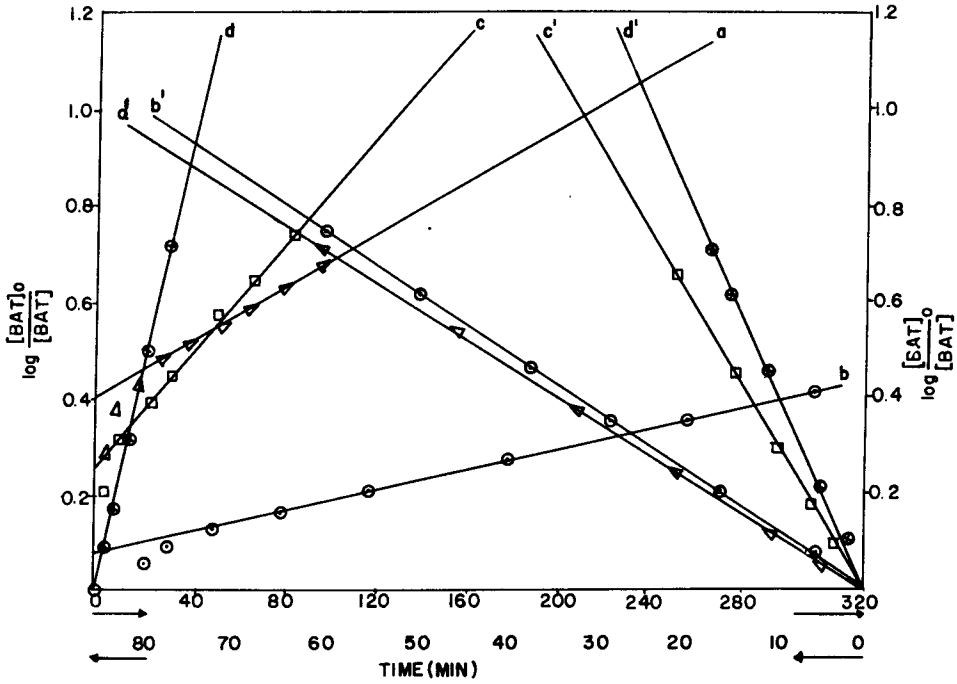


Figure 1. First-order plots for the oxidation of arginine (a, a'), glutamine (b, b'), lysine (c, c') and histidine (d, d'), where a, b, c, d refer to acid conditions with [AA] = 0·02 mol dm^{-3}, $[HClO_4]$ = 0·08 mol dm^{-3} and for histidine, [AA] = 0·0003 mol dm^{-3} and $[HClO_4]$ = 1·0 mol dm^{-3}, temp. 30°C. Conditions in alkaline medium (a'b'c'd') are [AA] = 0·01 mol dm^{-3}, $[OH^-]_R$ = 0·002 mol dm^{-3}; temp: 20°C.

Table 2. Effect of reactant concentrations on the rate of oxidation of amino acids by bromamine-T in acid medium at 30°C.
$[HClO_4]_0$ = 0·08 mol dm^{-3}, μ = 0·5 mol dm^{-3}

$10^3[BAT]_0$ (mol dm^{-3})	$10^2[AA]$ (mol dm^{-3})	$10^5k'/s^{-1}$			
		Lys	Arg	Glu-NH_2	His
1·0(0·10)	2·0(0·03)	21·8	10·7	3·80	87·0
2·0(0·15)	2·0(0·03)	22·1	10·9	3·68	85·5
3·0(0·20)	2·0(0·03)	21·6	10·9	3·76	87·0
4·0(0·25)	2·0(0·03)	21·1	10·6	3·88	84·4
5·0(0·30)	2·0(0·03)	21·8	10·1	3·75	85·3
6·0(–)	2·0(–)	21·6	10·4	3·84	–
3·0(0·30)	1·0(0·020)	10·6	5·54	2·90	67·5
3·0(0·30)	1·5(0·025)	15·7	8·53	–	80·6
3·0(0·30)	2·0(0·030)	21·6	10·9	3·76	85·3
3·0(0·30)	2·5(0·035)	27·5	13·8	–	96·0
3·0(0·30)	3·0(0·040)	33·6	16·5	4·40	103
3·0(0·30)	3·5(0·050)	–	19·2	–	118
3·0(0·30)	4·0(0·060)	–	–	4·96	133
3·0(0·30)	5·0(0·070)	–	–	5·45	143
3·0(–)	7·0(–)	–	–	6·40	–

Values in parentheses refer to histidine with $[HClO_4]_0$ = 1·0 mol dm^{-3} and μ = 1·5 mol dm^{-3}

Table 3. Effect of varying $[H^+]_{ex}$ on oxidation of amino acids by bromamine-T at 30°C.
$[BAT]_0 = 3{\cdot}0 \times 10^{-3}$ mol dm^{-3}; $[AA]_0 = 2{\cdot}0 \times 10^{-2}$ mol dm^{-3}; μ = 0·5 mol dm^{-3}
$[BAT]_0^* = 3{\cdot}0 \times 10^{-4}$ mol dm^{-3}; $[AA]^* = 3{\cdot}0 \times 10^{-4}$ mol dm^{-3}; μ^* = 1·5 mol dm^{-3}

$10^2[H^+]_{ex}$ (mol dm^{-3})	$10^5k'/S^{-1}$			
	His	Lys	Arg	Glu-NH_2
0·26	–	–	–	8·32
0·66	–	–	–	6·22
1·10	–	–	–	5·22
1·99	–	38·0	29·4	4·50
3·02	–	25·0	20·2	3·91
5·49	–	13·8	10·9	3·25
7·08	–	10·5	8·77	3·00
7·94	–	9·3	7·66	2·90
9·55	–	7·8	6·58	2·78
10·72	–	7·56	5·76	2·75
13·49	195	7·68	4·80	2·68
17·38	148	7·52	4·72	2·70
22·91	110	7·36	4·80	2·82
38·90	64.0	7·29	4·71	2·80
50·12	49.0	7·30	4·96	2·70
70·79	37.0	7·32	4·66	2·70
80·62	39.8	–	–	–
88·60	40.0	–	–	–
95·88	39.7	–	–	–

* Starred values refer to histidine

Table 4. Effect of varying $[\text{amino acid}]_0$ on oxidation of amino acids by bromamine-T at higher $[H^+]$ at 30°C.
$[BAT]_0 = 3{\cdot}0 \times 10^{-3}$ mol dm^{-3}; $[HClO_4] = 0{\cdot}70$ mol dm^{-3}; μ = 1·0 mol dm^{-3}
$[BAT]_0^* = 3{\cdot}0 \times 10^{-4}$ mol dm^{-3}; $[HClO_4]^* = 3{\cdot}5$ mol dm^{-3}; μ^* = 4·0 mol dm^{-3}

$10^2[AA]$ (mol dm^{-3})	$10^5k'/s^{-1}$			
	Lys	Arg	Glu-NH_2	His
(0·02) 1·00	5·76	3·68	2·70	38·0
(0·03) 1·50	6·50	4·32	–	39·2
(0·04) 2·00	7·29	4·96	2·80	38·2
(0·06) 2·50	–	5·46	–	37·0
(0·08) 3·00	8·22	6·05	–	39·8
(0·10) 4·00	9·12	–	2·74	39·0
(–) 7·00	–	–	2·88	–

* Starred values in parenthesis refer to histidine

Solvent isotope studies were made in D_2O medium. For Arg, value of k' in D_2O was $7{\cdot}68 \times 10^{-5}$ s^{-1} while the corresponding value in H_2O was $11{\cdot}13 \times 10^{-5}$ s^{-1} leading to a solvent isotope effect, $k'H_2O/k'D_2O = 1{\cdot}45$, with other conditions as in table 2. Proton inventory studies were made by carrying out the reaction with

arginine as the probe, in H_2O-D_2O mixtures and these results are shown in table 5. The corresponding proton inventory plot (Albery and Davies 1972; Gopalakrishnan and Hogg 1985) is shown in figure 2. The reaction was studied at different temperatures (25 – 45°) and from the Arrhenius plots of log k' versus $1/T$ ($r > 0.9968$; $S \leq 0.012$), the energy of activation E_a and other activation parameters were calculated.

Addition of reaction mixture to acrylamide did not initiate polymerization, showing the absence of free radicals.

3.2 *Alkaline medium*

The rate was found to be faster than in an acid medium and hence the reaction was studied at 20°C. The rate was first order with respect to [oxidant], since plots of

Table 5. Proton inventory studies for arginine in H_2O–D_2O mixtures at 30°C
$[BAT]_0 = 3.0 \times 10^{-3}$ mol dm^{-3}; [AA] = 2.0×10^{-2} mol dm^{-3}; $[HClO_4] = 0.08$ mol dm^{-3}; $[\mu] = 0.5$ mol dm^{-3}

Atom fraction of deuterium (n)	$10^5 k_n$/s^{-1}
0·000	11·1
0·248	10·8
0·496	10·6
0·744	9·77
0·992	7·68

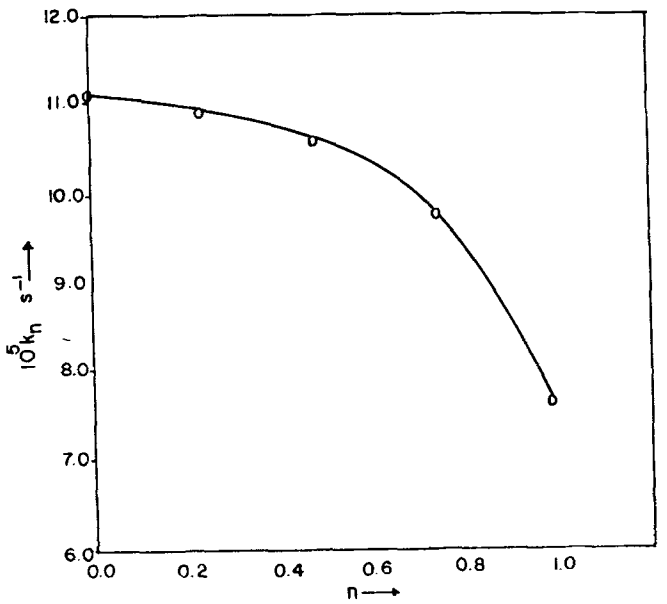


Figure 2. Proton inventory plot for the oxidation of arginine by BAT at 30°C. $[BAT]_0 = 0.003$ mol dm^{-3}; [AA] = 0·02 mol dm^{-3}; $[HClO_4] = 0.08$ mol dm^{-3}; $\mu = 0.5$ mol dm^{-3}.

$\log[BAT]_0/[BAT]$ versus time were found to be linear ($r > 0.9899$; $S \leq 0.016$, figure 1, table 6) at constant $[AA]_0$ and $[OH^-]$. The rate increased with increase in $[AA]_0$ (table 6) and plots of $\log k'$ versus $\log[AA]_0$ were linear ($r > 0.9964$; $S \leq 0.021$) with fractional slopes of 0·22, 0·22, 0·81 and 0·20 respectively for Arg, Glu-NH_2, His and Lys. Further plots of k' versus $[AA]_0$ were linear with an intercept indicating a rate law of the type rate $= a + b[AA]_0$. The reaction was catalysed by OH^- ions (table 7) and from the linear plots of $\log k'$ versus $\log[OH^-]$, fractional orders were obtained for Arg (0·50), Glu-NH_2 (0·41), His (0·43) and Lys (0·22). Also, plots of k' versus $[OH^-]$ were straight lines with intercepts, showing that the dependence is of the type, rate $= a' + b'[OH^-]$. The rate of reaction was not affected by the addition of Cl^- or Br^- ion, but addition of

Table 6. Effect of reactant concentrations on the rate of oxidation of amino acids by bromamine-T in alkaline medium at 20°C.
$[OH^-]_R = 2.0 \times 10^{-3}$ mol dm^{-3}, $\mu = 0.5$ mol dm^{-3}

$10^4[BAT]_0$ (mol dm^{-3})	$10^2[AA]$ (mol dm^{-3})	$10^4k'/s^{-1}$			
		His	Lys	Glu-NH_2	Arg
6·0	1·0	16·0	12·3	4·69	4·75
7·0	1·0	15·9	12·5	4·56	4·65
8·0	1·0	16·0	11·8	4·49	4·62
9·0	1·0	16·0	12·0	4·69	4·58
10·0	1·0	16·0	11·8	4·52	4·43
11·0	1·0	16·2	12·0	4·75	4·58
9·0	0·5	9·78	10·9	4·11	3·84
9·0	1·0	16·0	12·0	4·69	4·58
9·0	1·5	23·4	–	–	–
9·0	2·0	27·2	13·9	5·44	5·23
9·0	3·0	40·4	15·1	5·83	5·68
9·0	4·0	52·2	16·1	6·34	6·04
9·0	6·0	–	17·6	7·02	6·61

Table 7. Effect of $[OH^-]_R$ on the oxidation of amino acids by BAT at 20°C.
$[BAT]_0 = 9.0 \times 10^{-4}$ mol dm^{-3}; $[AA] = 1.0 \times 10^{-2}$ mol dm^{-3}; $\mu = 0.5$ mol dm^{-3}

$10^3[OH^-]_R$ (mol dm^{-3})	$10^4k'/s^{-1}$			
	His	Lys	Glu-NH_2	Arg
2·0	16·0	12·0	4·69	4·58
6·0	24·8	–	7·05	7·78
10·0	30·7	17·2	9·36	10·1
15·0	38·4	–	–	12·7
20·0	43·9	20·1	11·8	13·9
25·0	–	–	13·3	16·8
30·0	50·5	–	–	–
35·0	–	–	15·2	19·2
40·0	–	23·5	–	–
60·0	–	25·8	–	–

Table 8. Effect of reaction product, *p*-toluene sulphonamide on the rate of oxidation of amino acids by bromamine-T in alkaline medium. $[BAT]_0 = 9{\cdot}0 \times 10^{-4}$ mol dm^{-3}; $[AA]_0 = 1{\cdot}0 \times 10^{-2}$ mol dm^{-3}; $[OH^-]_R = 2{\cdot}0 \times 10^{-3}$ mol dm^{-3}; $\mu = 0{\cdot}5$ mol dm^{-3}; temp. = 20°C

$10^4[TsNH_2]$ (mol dm^{-3})	$10^5k'/s^{-1}$			
	Lys	Arg	Glu-NH_2	His
0·0	120	45·8	46·9	160
5·0	110	41·7	41·7	158
9·0	106	41·1	38·9	149
15·0	102	40·0	35·6	143

$TsNH_2$ retarded the rate (table 8). Upon varying the solvent composition by adding methanol (0–40% v/v), the rate increased with decrease in dielectric constant of medium and plots of log k' versus $1/D$ were linear with positive slopes.

Solvent isotope studies in D_2O medium for Arg gave $k'D_2O = 4{\cdot}94 \times 10^{-4}$ s^{-1}, while the corresponding $k'H_2O$ was $4{\cdot}29 \times 10^{-4}$ s^{-1}, leading to a solvent isotope effect, $k'H_2O/k'D_2O = 0{\cdot}89$.

Kinetic and thermodynamic parameters were calculated by studying the reaction at different temperatures (10–35°) and fitting the results to Arrhenius plots which were linear ($r > 0{\cdot}9991$; $S \leq 0{\cdot}014$).

Absence of free radicals in the reaction mixture was demonstrated, when it failed to initiate polymerization of acrylamide.

4. Discussion

The following equations represent the experimental rate laws in acid and alkaline media:

$$-\frac{d\log[BAT]}{dt} = k[S][H^+]^{-1}, \text{ at low } [H^+], \tag{2}$$

$$-\frac{d\log[BAT]}{dt} = k[S]^x, \text{ at high } [H^+], \tag{3}$$

$$-\frac{d\log[BAT]}{dt} = k[S]^y[OH^-]^z[TsNH_2]^{-c} \text{ in alkaline medium.} \tag{4}$$

Here x, y, z and c are fractions. Glutamine shows an inverse fractional order dependence on $[H^+]$ even at low acidities. These rate laws indicate the involvement of different oxidant and/or substrate species, but retardation of rate by H^+ or its enhancement by OH^- ion point towards a general pattern of oxidation of substrates by BAT in these media.

Amino acids are known to exist as neutral molecules (S), zwitterions (S°), anions (S^-) and cations (SH^+) in aqueous solutions and dissociation depends upon the pH of the medium:

$$\underset{\substack{S^-\\ \text{anion}}}{R'CH(NH_2)COO^-} \rightleftharpoons \underset{\substack{S^0\\ \text{dipolar zwitterion}}}{R'CH(NH_3^+)COO^-} \rightleftharpoons \underset{\substack{SH^+\\ \text{cation}\\ \text{(strongly acidic)}}}{R'CH(NH_3^+)COOH} \tag{5}$$

4.1 *Mechanism of oxidation in acid medium*

Bromamine-T is similar to chloramine-T (Morris *et al* 1948; Bishop and Jennings 1958) and similar equilibria exist in aqueous solutions. The possible oxidizing species in acidified BAT solutions are TsNHBr, $TsNBr_2$ and HOBr. If $TsNBr_2$ were to be the reactive species, the rate law predicts a second-order dependence of rate on $[BAT]_0$, which is contrary to experimental observations. Further, the hydrolysis of TsNHBr is slight (Pryde and Soper 1926, 1931) and if HOBr is primarily involved, a retardation of rate by the added *p*-toluene sulphonamide is expected. However, no such effect was noticed. Hardy and Johnston's (1973) calculations on aqueous bromamine-B solutions in the pH range 7–13 have shown that the concentration of the conjugate acid TsNHBr is higher when compared with the other species. At pH 7, $[TsNHBr] \approx 4{\cdot}1 \times 10^{-5}$ mol dm^{-3}, while $[HOBr] \approx 6{\cdot}0 \times 10^{-6}$ mol dm^{-3} and $[OBr^-] \approx 10^{-8}$ mol dm^{-3}. If BAT is assumed to be similar to bromamine-B, then TsNHBr is the likely species to react with the substrate. Morris *et al* (1948) have determined the pK_a of TsNHCl as 4·56 (at 25°) and if near value is assumed for the bromine analogue, then at the experimental conditions of acidity, BAT would be present at the free acid TsNHBr, and this would be the oxidizing species.

Rate law (2) indicates that unprotonated amino acid molecule is participating in the rate limiting step (RLS). Scheme 1 is employed to interpret the experimental observations:

$$SH^+ \overset{K_1}{\rightleftharpoons} S^0 + H^+ \qquad \text{(i) fast}$$

$$S^0 + TsNHBr \overset{K_2}{\rightleftharpoons} X \qquad \text{(ii) slow}$$

$$X \xrightarrow{k_3} X' + HBr + CO_2 \qquad \text{(iii) slow and RLS}$$

$$X' \xrightarrow{H_2O} R'CHO + NH_4^+ \qquad \text{(iv) fast}$$

$$R'CHO + TsNHBr \xrightarrow{k_4} \text{products} \qquad \text{(v) fast}$$

Scheme 1

Scheme 1 leads to rate law (6) which is in agreement with experimental results.

$$\text{Rate} = \frac{K_1 K_2 k_3 [BAT]_t [SH^+]}{[H^+] + K_1 K_2 [SH^+]}. \tag{6}$$

If K_2 is a small equilibrium constant, $K_1 K_2 [SH^+] < [H^+]$ and this gives rate law (7):

$$\text{Rate} = \frac{K_1 K_2 k_3 [BAT]_t [SH^+]}{[H^+]}. \tag{7}$$

In the other limiting situation, when $K_1 K_2 [SH^+] > [H^+]$, rate law (8) is obtained:

$$\text{Rate} = K_1 k_3 [BAT]_t. \tag{8}$$

Under intermediate conditions, fractional orders are observed in $[H^+]$ and [S]. Gowda and Mahadevappa (1983) have noted similar results during the oxidation of a number of amino acids by CAT. At higher $[H^+]$, the rate levels off and rate law (3) is obeyed. The substrate species SH^+ would then directly interact with TsNHBr (scheme 2), resulting in rate law (9):

$$SH^+ + TsNHBr \overset{K_4}{\rightleftharpoons} X'' \qquad \text{(i) slow}$$

$$X'' \xrightarrow{k_5} X''' \qquad \text{(ii) slow and RLS}$$

$$X''' + TsNHBr \xrightarrow{k_6} \text{products} \qquad \text{(iii) fast}$$

Scheme 2

$$-\frac{d[BAT]}{dt} = \frac{k_5 K_4 [BAT]_t [SH^+]}{1 + K_4[SH^+]} \cdot \tag{9}$$

Under the inequality, $1 < K_4[SH^+]$, the rate becomes independent of $[substrate]_0$ as was observed with glutamine and histidine. Equation (9) would be transformed into (10):

$$\frac{1}{k'} = \frac{1}{K_4 k_5 [SH^+]} + \frac{1}{k_5} \cdot \tag{10}$$

From the slope and intercept of double reciprocal plots ($r > 0{\cdot}9880$; $S \leq 0{\cdot}020$), values of K_4 and k_5 (formation and decomposition constants of X″) were calculated for Arg and Lys.

	Arg	*Lys*
$K_4(dm^3\ mol^{-1})$	78·40	137·00
$10^5 k_5(s^{-1})$	8·33	10·00

Addition of halide ions had no effect on the rate indicating that no interhalogen or free bromine was formed and the free acid TsNHBr interacts directly with the substrate species. The reaction product *p*-toluene sulphonamide ($TsNH_2$) does not influence the rate, showing that it is not involved in a pre-equilibrium. Variation of the ionic strength of the medium does not alter the rate indicating that non-ionic species are involved in the rate controlling step.

The dielectric effect was found to be positive, with the rate of reaction increasing in solvent mixtures of lower polarity than water. Hence the transition state formed is less polar and there is charge dispersal under these conditions.

Solvent isotope studies in D_2O medium show a retardation of rate as expected, since D_3O^+ is a stronger acid than the hydronium ion (Collins and Bowman 1970). The proton inventory plot (figure 2) is expected to throw light on the nature of transition state (Albery and Davies 1972; Gopalakrishnan and Hogg 1985). If the reaction proceeds through a single transition state, the dependence of rate constant

k_n, on the atom fraction of deuterium n, in mixture of H_2O and D_2O is given by (11).

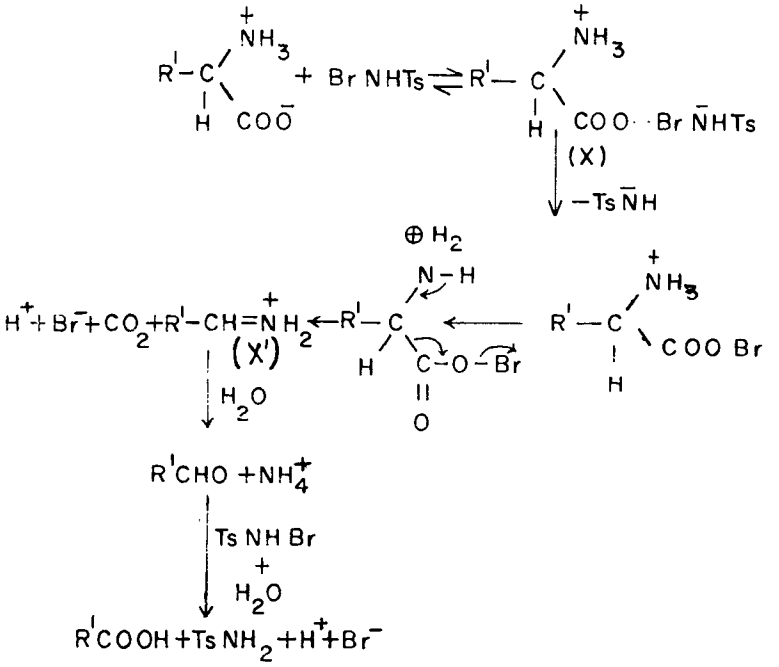
$$k_n = k_0(1 - n + n\phi_j)^{-2}, \tag{11}$$

where ϕ_j is the isotopic fractionation factor for isotopically exchangeable hydrogenic site in the reactant with that in the transition state (ϕ_i). Equation (11) can be transformed into (12).

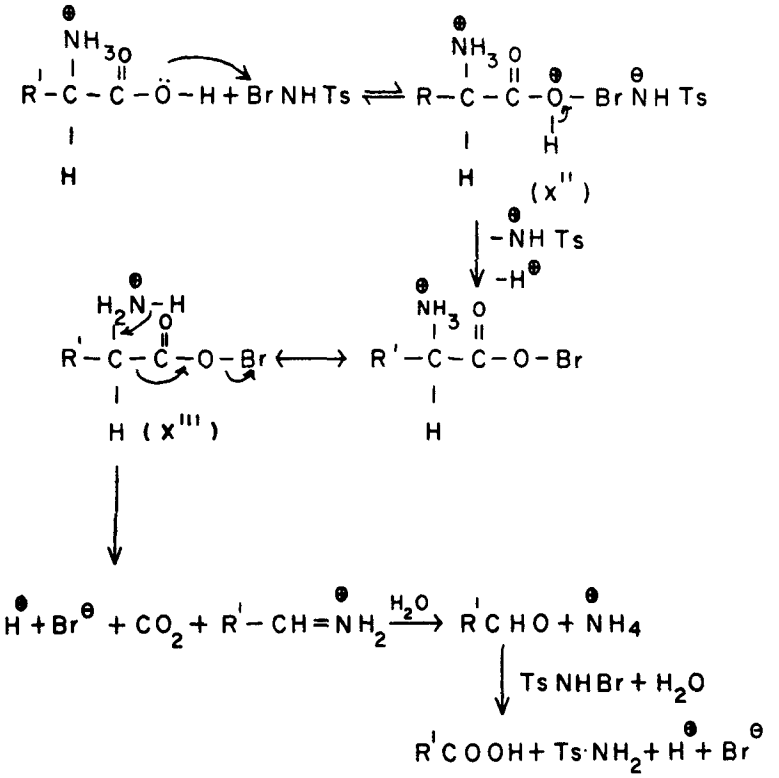
$$\left(\frac{k_0}{k_n}\right)^{\frac{1}{2}} = [1 + n(\phi_j - 1)]. \tag{12}$$

From (12) a plot of $(k_0/k_n)^{1/2}$ versus n should be linear. It is seen from table 5 that such a plot is approximately linear (r = 0·9688) with a slope of $(\phi_j - 1)$ = 0·07 giving a value of 1·07 for the fractionation factor. Gold and Lowe (1968) measured the fractionation factor of acetic acid in solution and have shown it to be nearly unity. It is likely that this ϕ_j value refers to TsNBrH which has a pK_a value almost equal to that of acetic acid and the former is involved in the formation of transition state.

Under acid conditions, the N atom attached to the α-carbon of amino acid is fully shared and it is likely that the electrophilic halogen attack takes place at the carboxyl group (schemes 3a and 3b) forming the hypobromoester, which breaks down in a rate limiting step. The rate of oxidation increases in the order: His > Lys > Arg > Glu-NH_2. This could probably be correlated with + I effect of methylene groups in the compounds. The fact that His is rapidly oxidized by BAT



Scheme 3 (a)



Scheme 3(b)

under these conditions shows that the heterocycle ring moiety in the latter exerts a considerable inductive effect during the reaction. The electron releasing effect should favour the cleavage mode in scheme 3, by stabilizing any partial positive charge that may develop on the α-carbon in the transition state due to the non-concertedness of bond making and bond breaking. The behaviour has to be attributed to the positive charge on the N atom which allows C–C bond cleavage to precede C–N bond formation, which substantiates the fact that the attack is at the carboxylate group rather than at the amino group of the substrate.

4.2 *Mechanism of oxidation in alkaline medium*

The oxidation potential haloamine/sulphonamide system is pH dependent (Murthy and Rao 1952) and decreases with increase in pH. The prominent species present in alkaline haloamine solutions are TsNHX, HOX and OX^- ion where X = Cl or Br. Hardy and Johnston's (1973) calculations on alkaline bromamine-B solutions have indicated that there could be considerable concentration of TsNHBr even in alkaline medium. In the present investigations, a fractional order dependence on $[OH^-]$ and $[S^-]$ and the observed retardation of rate by the reaction product, $TsNH_2$ can be explained by the two pathway mechanism shown in schemes 4 and 5, in which the amino acid reacts through the anionic form:

$$RNHBr + H_2O \xrightarrow{k_7} RNH_2 + HOBr \qquad \text{(i) slow}$$

$$HOBr + S^- \xrightarrow{k_8} Y \qquad \text{(ii) fast}$$

$$Y \xrightarrow{k_9} \text{products.} \qquad \text{(iii) fast}$$

Scheme 4

$$RNHBr + OH^- \underset{k_{-10}}{\overset{k_{10}}{\rightleftharpoons}} RNH_2 + OBr^- \qquad \text{(i) slow}$$

$$OBr^- + S^- \xrightarrow{k_{11}} Y' \qquad \text{(ii) slowest and RLS}$$

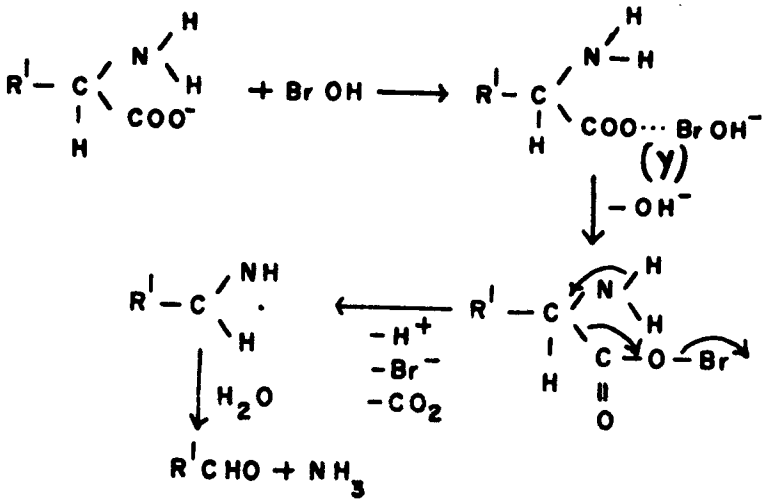
$$Y' \xrightarrow{\cdot\, k_{12}} \text{products.} \qquad \text{(iii) fast}$$

Scheme 5

Schemes 4 and 5 lead to the combined rate law (13), in agreement with experimental results:

$$-\frac{d[BAT]}{dt} = \frac{k_{10}k_{11}[BAT][OH^-][S^-]}{k_{-10}[TsNH_2] + k_{11}[S^-]} + k_7[BAT][H_2O]. \qquad (13)$$

Scheme 6 indicates the electron flow during the oxidation of amino acids in alkaline medium. A similar scheme can be written with BrO^- as oxidant. The dielectric effect points towards a spreading of charge in the transition state. Solvent isotope studies have shown that $k'_{H_2O}/k'_{D_2O} < 1$. This is generally correlated with the higher



Scheme 6

basicity of OD^- ion in comparison to OH^- ion (Collins and Bowman 1970), but the magnitude of solvent isotope effect is low in view of the rate limiting hydrolysis step (scheme 4), where $k'H/k'D > 1$.

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