Oxidation of Methionine by Sodium *N*-Chlorotoluene-*p*-sulphonamide in Aqueous Solution: a Kinetic Study

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Kinetics of oxidation of DL-methionine (Mt) in the presence of HCl, $HClO_4$, H_2SO_4, and NaOH by sodium *N*-chlorotoluene-*p*-sulphonamide (CAT) have been investigated at 30 °C (and 35 °C in alkaline medium) and the results are compared with those obtained with HOCl as the oxidant, in acid medium. The reactions follow identical kinetics, being first order each in [CAT] and $[H^+]$ and zero order in [substrate]. In alkaline medium the rate shows first-order dependence each on [CAT] and [Mt]. An inverse fractional order is noted in $[OH^-]$. Studies of solvent isotope effects using D_2O showed a retardation of rate both in acid and alkaline media. The derived rate laws are consistent with the observed kinetics.

The chemistry of aromatic sulphonyl halogenoamines in general and of the sodium salt of *N*-chlorotoluene-*p*-sulphonamide (chloramine-T) (CAT or $ArSO_2NClNa,3H_2O$, where Ar = p-$CH_3C_6H_4$) in particular has received considerable attention and the existing literature has been reviewed.[1]

The oxidation of amino acids is of the utmost importance, both from a chemical point of view and from its bearing on the mechanism of amino acid metabolism. As a part of our broad programme on the mechanistic aspects of the oxidation of amino acids,[2] we have now studied the kinetics of oxidation of methionine (Mt), a sulphur-bearing amino acid, with CAT in acid and alkaline media. Further, the results are compared with those obtained for the oxidation of Mt by HOCl in acid media. Methionine, an optically active essential amino acid, has been oxidized by iodine,[3] bromine,[4] and $KBrO_3$ and TCl[5] to the sulphoxide, while $NaClO_2$,[6] H_2O_2,[7] and bromate mixtures[4] convert it into the sulphone. However, except for the work of Gensch and Higuchi[3] and Young and Hsieh,[8] no kinetic data are available for the oxidation of Mt with any of these reagents. A nearer example is the study of the Mann–Pope reaction, *i.e.* conversion of sulphides with *N*-halogenoamines in buffered water–ethanol mixtures, by Ruff and Kucsman[9,10] where the formation of a halogenosulphonium–sulphonamide tight ion-pair intermediate is assumed, leading to sulphimides and sulphoxides. The sulphoxides are the major products (*ca.* 80%) with all the oxidants.

Results

The kinetics of oxidation of methionine by CAT and HOCl was investigated at several initial concentrations of the reactants, in acid and alkaline media.

(1) *Acid Medium.—Effect of varying reactant concentrations.* At constant $[H^+]$, with the substrate in stoicheiometric excess, plots of log (a—x) *versus* time were linear ($r > 0.9970$), indicating a first-order dependence of rate on [oxidant]. Values of the pseudo-first-order rate constants, k', are given in Table 1. The values of k' were unaffected with increase in $[Mt]_0$, indicating that the rate was independent of [amino acid]$_0$ (Table 2). However, a slight falling off of k' values at higher $[Mt]_0$ could probably be due to a dielectric effect.

Effect of varying $[H^+]$. The rate increased with increase in $[H^+]$ (Table 3) and a plot of log k' *versus* log $[H^+]$ was linear in all three acid media with slopes of unity ($r > 0.9980$).

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Table 1. Effect of reactant concentrations on the rate of oxidation of methionine by chloramine-T and hypochlorous acid in different acid media at 30 °C. [HCl] = $[HClO_4]$ = 0.02M; $[H_2SO_4]$ = 0.01M; $[Mt]_0$ = 0.01M; μ = 0.5M

$10^3[CAT]_0$ or $10^3[HOCl]_0$/M	$10^4 k'/s^{-1}$		
	$HClO_4$	HCl	H_2SO_4
3.00	2.46 (4.17)	2.49 (3.56)	3.44 (4.06)
4.00	2.67 (4.23)	2.65 (3.67)	3.52 (4.08)
5.00	2.74 (4.44)	2.75 (3.79)	3.55 (4.09)
6.00	2.79 (4.53)	2.79 (3.83)	3.60 (4.11)
7.00	2.73 (4.58)	2.82 (3.92)	3.68 (4.13)
8.00	2.83 (4.62)	2.90 (4.04)	3.75 (4.20)
9.00	2.84		3.82

Values in parentheses refer to HOCl oxidation of substrate.

Table 2. Effect of varying reactant concentrations on the rate of reaction at 30 °C. [HCl] = $[HClO_4]$ = 0.02M; $[H_2SO_4]$ = 0.01M; $[HOCl]_0$ = $[CAT]_0$ = 5.00 × 10^{-3}M; μ = 0.5M

$10^2[Mt]_0$/M	$10^4 k'/s^{-1}$		
	$HClO_4$	HCl	H_2SO_4
1.00	2.74 (4.44)	2.75 (3.79)	3.44 (4.06)
1.20	2.56 (4.31)	2.64 (3.66)	3.42 (4.02)
1.50	2.42 (4.27)	2.48 (3.52)	3.25 (4.01)
1.80	2.37 (4.13)	2.40 (3.47)	3.15 (4.01)
2.00	2.30 (4.07)	2.30 (3.35)	3.10 (3.98)
2.50	2.15 (3.96)	2.05 (3.26)	2.92 (3.93)
3.00	2.03 (3.89)	1.90 (3.15)	2.82 (3.80)

Values in parentheses refer to HOCl oxidation of substrate.

Effect of anions on the rate. Presence of Cl^-, ClO_4^-, and SO_4^{2-} ions had no effect on the rate. This was confirmed by further addition of NaCl, $NaClO_4$, and Na_2SO_4 to the reaction mixture.

Effect of varying ionic strength and addition of toluene-p-sulphonamide. Variation of the ionic strength of the medium (0.05—1.0M) or addition of the reaction product, $ArSO_2NH_2$ (up to 0.01M), had no significant effect on the rate.

Effect of changing the solvent compositions. The reaction was also studied in aqueous methanol of varying composition. An increase in methanol content (0—40%) retarded the rate of reaction. A plot of log k' against $1/D$, where D is the dielectric constant of the medium, gave a straight line with a negative

Table 3. Effect of $[H^+]$ on the rate of reaction. $[HOCl]_0 = [CAT]_0 = 5.00 \times 10^{-3}$M; $[Mt]_0 = 1.00 \times 10^{-2}$M; $\mu = 0.5$M; temp. 30 °C

	$10^4\ k'/s^{-1}$		
$10^2\ [H^+]$/M	$HClO_4$	HCl	H_2SO_4
1.00	1.36 (2.22)	1.38 (1.79)	(4.06)
2.00	2.74 (4.44)	2.75 (3.59)	3.85 (7.95)
3.00	4.01 (6.64)	4.12 (5.37)	5.24 (12.50)
4.00	5.27 (8.74)	5.53 (7.10)	6.88 (17.02)
5.00	6.72 (10.78)	6.92 (9.15)	
6.00	8.01	8.26	10.28
7.00	9.31	9.48	
8.00	10.78	10.09	12.98
10.00			16.31
12.00			20.32

Values in parentheses refer to HOCl oxidation of substrate.

(2) *Alkaline Medium.*—At constant NaOH and substrate concentrations, the reaction showed a first-order dependence on $[CAT]_0$ ($r > 0.9960$, Table 5). Values of k' increased with increase in $[Mt]_0$ and a plot of log k' against log $[Mt]_0$ was linear with a slope of unity (r 0.9992). The rate decreased with increase in [NaOH] (Table 5) and a plot of log k' *versus* log $[OH^-]_R$ was linear (r 0.992) with a slope of -0.52, indicating an inverse fractional order dependence of rate on hydroxide ion concentration. Here the reactive $[OH^-]_R$ was determined by the relation $[OH^-]_R = [OH^-]_T - [OH^-]_{Mt}$, where $[OH^-]_T$ was the total concentration and $[OH^-]_{Mt}$ was the hydroxide ion concentration neutralized by the amino acid.

Addition of chloride, or $ArSO_2NH_2$, to the reaction mixture had no effect on the rate. Also variation of ionic strength or the dielectric constant of the medium had no influence on the rate. The normal solvent isotope effect $(k')_{H_2O}/(k')_{D_2O}$ was 1.76 ± 0.005 since $(k')_{H_2O} = 6.30 \times 10^{-4}\ s^{-1}$ and $(k')_{D_2O} = 3.58 \times 10^{-4}\ s^{-1}$, showing a stronger retardation of rate in D_2O medium. Activation parameters in the temperature range of 30—45 °C were calculated (Table 4). Test for free radicals with aqueous acrylamide solution was found to be negative.

Table 4. Kinetic and thermodynamic parameters for the oxidation of methionine by CAT and HOCl in acids and alkaline media

	$HClO_4$	HCl	H_2SO_4	NaOH
log A	7.88 (7.39)	7.62 (8.43)	6.68 (7.49)	7.28
$\Delta H^‡$/kJ mol^{-1}	54.39 (49.92)	52.51 (56.40)	44.38 (49.08)	62.2
$\Delta S^‡$/J K mol^{-1}	−133.67 (−144.64)	−140.20 (−124.59)	−163.45 (−142.57)	−142.5
$\Delta G^‡$/kJ mol^{-1}	96.53 (94.81)	96.04 (95.10)	95.29 (93.35)	94.5

Values in parentheses refer to HOCl oxidation of substrate in acid media.

Table 5. Effect of varying concentrations of $[CAT]_0$, [Mt], and $[OH^-]$ on the rate of reaction at 35 °C. μ = 0.50M

$10^3\ [CAT]_0$/M	$10^2\ [Mt]_0$/M	$10^2\ [OH^-]_R$/M	$10^4\ k'/s^{-1}$
3.00	1.00	3.00	6.02
4.00	1.00	3.00	6.14
5.00	1.00	3.00	6.30
6.00	1.00	3.00	6.27
7.00	1.00	3.00	6.31
8.00	1.00	3.00	6.12[a]
5.00	1.00	3.00	6.30[b]
5.00	1.25	3.00	8.28
5.00	1.50	3.00	10.38
5.00	2.00	3.00	13.92
5.00	2.50	3.00	17.98
5.00	1.00	1.00	9.16
5.00	1.00	1.50	7.24
5.00	1.00	2.00	6.30
5.00	1.00	2.50	5.63
5.00	1.00	3.50	4.72

[a] Runs carried out at μ = 1.00M. [b] Runs carried out in the presence of 0.01M toluene-*p*-sulphonamide.

slope ($r > 0.9960$) supporting a rate-limiting step with charge dispersal.

Solvent isotope effect. Kinetic studies were carried out in D_2O containing $HClO_4$ and $(k')_{D_2O}$ was found to be $1.74 \times 10^{-4}\ s^{-1}$ while the corresponding $(k')_{H_2O}$ was $2.75 \times 10^{-4}\ s^{-1}$ leading to the normal solvent isotope effect, $k'_{H_2O}/k'_{D_2O} = 1.58 \pm 0.002$. For the oxidation of Mt by HOCl in acids, the ratio k'_{H_2O}/k'_{D_2O} was 1.540 ± 0.003.

Effect of temperature on the rate. The reaction was studied at different temperatures (30—45 °C) and from the plots of log k' against $1/T$ ($r > 0.9980$) activation parameters were computed (Table 4).

Test for free radicals. Addition of the reaction mixture to aqueous acrylamide solution did not initiate polymerization, showing the absence of free-radical species.

Discussion

Chloramine-T behaves like a strong electrolyte in aqueous solutions[11,12] and dissociates according to equation (1). The anion picks up a proton in acid solutions to give the free acid monochloramine-T [equation (2)]. Although the free acid has not been isolated there is experimental evidence for its formation in acid solutions. It undergoes disproportionation giving rise to toluene-*p*-sulphonamide and dichloramine-T [equation (3)]. Dichloramine-T and the free acid hydrolyse to give hypochlorous acid, HOCl [equations (4) and (5)]. Finally HOCl ionizes according to reaction (6). The possible oxidizing

$$ArSO_2NClNa \rightleftharpoons ArSO_2NCl^- + Na^+ \quad (1)$$

$$ArSO_2NCl^- + H^+ \overset{K_a}{\rightleftharpoons} ArSO_2NHCl \qquad K_a\ 2.82 \times 10^{-5}\ \text{at 25 °C} \quad (2)$$

$$2ArSO_2NHCl \overset{K_d}{\rightleftharpoons} ArSO_2NH_2 + ArSO_2NCl_2 \qquad K_d\ 5.8 \times 10^{-2}\ \text{at 25 °C} \quad (3)$$

$$ArSO_2NCl_2 + H_2O \overset{K_h}{\rightleftharpoons} ArSO_2NHCl + HOCl \qquad K_h\ 8.0 \times 10^{-7}\ \text{at 25 °C} \quad (4)$$

$$ArSO_2NHCl + H_2O \overset{K_h'}{\rightleftharpoons} ArSO_2NH_2 + HOCl \qquad K_h'\ 4.88 \times 10^{-8}\ \text{at 25 °C} \quad (5)$$

$$HOCl \overset{K_2}{\rightleftharpoons} H^+ + OCl^- \qquad K_2\ 3.3 \times 10^{-8}\ \text{at 25 °C} \quad (6)$$

species in acidified CAT solution are $ArSO_2NHCl$, $ArSO_2NCl_2$, HOCl, and probably H_2OCl^+ and in the alkaline solution $ArSO_2NHCl$, HOCl, $ArSO_2NCl^-$, and OCl^-. Bishop and Jennings[11] have calculated the order of concentrations of the

various species present at different pH in a 0.05M solution of chloramine-T.

It is seen from the data of Bishop and Jennings[11] that $ArSO_2HCl$ concentration in acid solutions with pH < 1.5 or $[H^+]$ > 0.03M is almost constant and hence insensitive to increases in $[H^+]$ beyond *ca.* 0.03M. However, a recent evidence[13] for the protonation of $ArSO_2NHCl$ [equation (7)] at pH < 2.8, has been obtained by a radiochemical method.

$$ArSO_2NHCl + H^+ \overset{K_p}{\rightleftharpoons} ArSO_2\overset{+}{N}H_2Cl \qquad K_p\ 1.03 \times 10^2 \text{ at } 25\,^\circ C \quad (7)$$

Protonated monochloramine-T produced in reaction (7) can undergo hydrolysis to form $ArSO_2NH_2$ and H_2OCl^+ [equation (8) where the forward step is rate determining].

$$ArSO_2\overset{+}{N}H_2Cl + H_2O \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} ArSO_2NH_2 + H_2OCl^+ \quad (8)$$

(1) *Mechanism in Acid Medium.*—As discussed earlier, in acid solutions of CAT, $ArSO_2NHCl$, $ArSO_2NCl_2$, HOCl, and H_2OCl^+ are the probable oxidizing species. The overall rate law for the oxidation of methionine by CAT appears to be (9),

$$-d[CAT]/dt = k[CAT][H^+] \quad (9)$$

indicating that the substrate is oxidized in a fast step. If $ArSO_2NCl_2$ were to be the reactive species, then the rate law predicts a second-order dependence of rate on CAT [equation (3)] but experimentally clean first-order plots were obtained for the disappearance of CAT. First-approximation calculations by Bishop and Jennings[11] on 0.05M solutions of CAT have shown that the concentrations of $ArSO_2NHCl$ and HOCl are *ca.* 10^{-2} and 10^{-7}M, respectively, at pH *ca.* 0—3. Experimental evidence can be quoted in support of a protonated HOCl.

(a) Swain and Crist[14] have pointed out in their studies on the chlorination of anisole by HOCl that hypochlorous acidium ion H_2OCl^+ formed in a prior equilibrium before the rate-limiting step is a better electrophile than HOCl.

(b) In the present studies, oxidation of methionine with HOCl in the presence of acids, show an H^+ ion dependence of the form, rate = $a + b[H^+]$ (r 0.9999). The magnitudes of a and b as computed from a linear plot of k' *versus* $[H^+]$ in the presence of H_2SO_4 are, $a = 0.58 \times 10^{-4}$ s^{-1}; $b = 4.3 \times 10^{-2}$ l mol^{-1} s^{-1}. This indicates (i) a rapid pre-equilibrium between protonated and deprotonated forms (*i.e.* $HOCl + H^+ \rightleftharpoons H_2OCl^+$), (ii) a small protonation equilibrium constant, (iii) that both the forms are reactive; and (iv) that the protonated form is more reactive. Blank experiments carried out with methionine and HOCl in the absence of acid indicated the value of a to be 0.8×10^{-4} s^{-1}.

(c) The rate of oxidation of methionine by CAT or HOCl decreases in D_2O medium which is contrary to expectations.[15] For a proton-catalysed reaction, the solvent isotope effect $k_{D_2O}/k_{H_2O} > 1$. But for a rate-limiting hydrolysis [equation (8)], the primary kinetic isotope effect $k_H/k_D > 1$. The observed solvent isotope effect $k_{D_2O}/k_{H_2O} < 1$ indicates a pre-equilibrium proton transfer followed by a rate-limiting hydrolysis, as it is a composite of solvent isotope effect of reaction (7) and primary isotope effect of reaction (8). This invariably points towards the formation of protonated HOCl in the hydrolysis step (8).

There is no retardation of rate by the added reaction product $ArSO_2NH_2$, indicating that k_8 is rate determining and subsequent steps of the reaction sequence are fast.

Thus assuming H_2OCl^+ as the reactive species, a reaction scheme can be formulated in which the substrate is attacked at the nucleophilic sulphur site by the oxidant to form a sulphurane-type intermediate and the latter is attacked by the dipolar solvent. Elimination of H^+ and HCl results in the formation of methionine sulphoxide, which is oxidized by the second molecule of the oxidant. The four-electron stoicheiometry shown for the reaction clearly rules out the formation of a sulphimide as a minor product which was observed during the oxidation of sulphides[9] by chloramine-B.

Mechanism in Alkaline Medium.—The oxidation potential of the chloramine-T–sulphonamide system is pH dependent and decreases with an increase in pH of the medium.[16] In alkaline solutions of CAT dichloramine does not exist, and the possible species are the anion $ArSO_2NCl^-$ and OCl^- which would be transformed into more reactive oxidizing species $ArSO_2NHCl$ and HOCl in the course of the reaction in alkaline medium. A retarding influence of OH^- ions on the reaction rate noticed in several chloraminometric reactions has been attributed to the formation of the conjugate acid $ArSO_2NHCl$ from $ArSO_2NCl^-$ in a base-retarding step.[17] An inverse fractional order dependence of rate on $[OH^-]$ observed in the oxidation of Mt by CAT indicates that a fraction of the overall reaction proceeds *via* an alternative path involving the anion $ArSO_2NCl^-$. Schemes 1 and 2 have been proposed to account for the observed kinetics for the oxidation of methionine by CAT in alkaline solutions. In Scheme 2 the substrate Mt may react in the form of zwitterion or the negative ion.

The rate laws from Schemes 3 and 4 are, respectively, (10) and (11). Scheme 1 and rate law (10) show a first-order dependence in [CAT] and [amino acid], and an inverse first-order dependence in [OH], while Scheme 2 and rate law (11), indicate the rate to be independent of $[OH^-]$. The net outcome of the two paths results in the combined rate law (12) from which the inverse fractional dependence of rate on $[OH^-]$ can be inferred.

$$-d[CAT]/dt = k_{10}k_{11}[CAT]_0[Mt]_0/k_{-10}[OH^-] \quad (10)$$

$$-d[CAT]/dt = k_{13}[CAT][Mt] \quad (11)$$

$$-d[CAT]/dt = k_{10}k_{11}[CAT][Mt]/k_{-10}[OH^-] + k_{13}[CAT][MT] \quad (12)$$

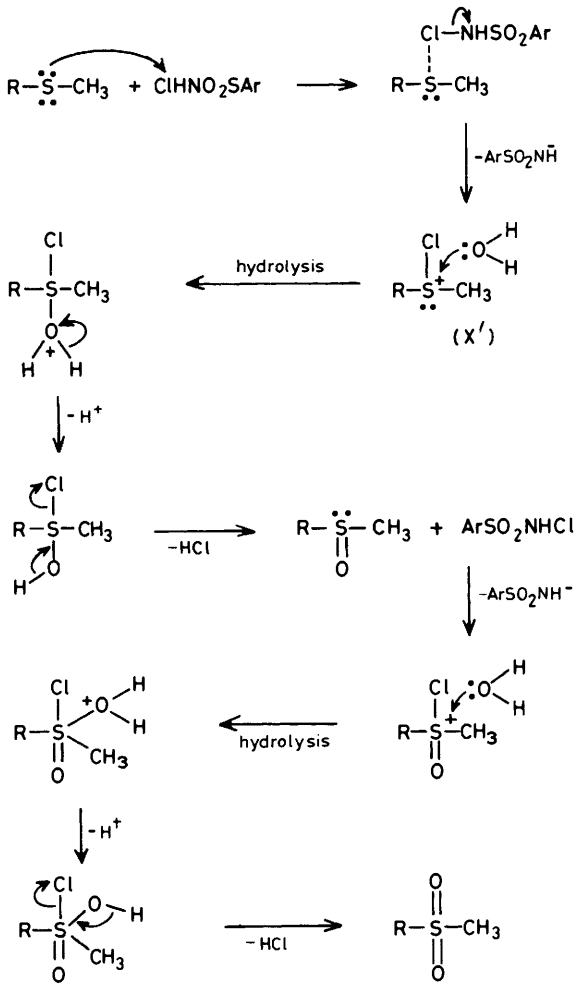
Detailed mechanisms involving the electron transfer during the oxidation of methionine by $ArSO_2NHCl$ (Scheme 3) or by $ArSO_2NCl^-$ (Scheme 4) are shown. There is an initial formation of halogenosulphonium ion (X′) which is attacked by the nucleophilic solvent. Elimination of H^+ and HCl leaves the

$ArSO_2NCl^- + H_2O$	$\overset{k_{10}}{\underset{k_{-10}}{\rightleftharpoons}}$	$ArSO_2NHCl + OH^-$	(i) slow
$ArSO_2NHCl + Mt$	$\xrightarrow{k_{11}}$	X′	(ii) r.d.s.
$X' + ArSO_2NHCl$	$\xrightarrow{k_{12}}$	Products	(iii) fast

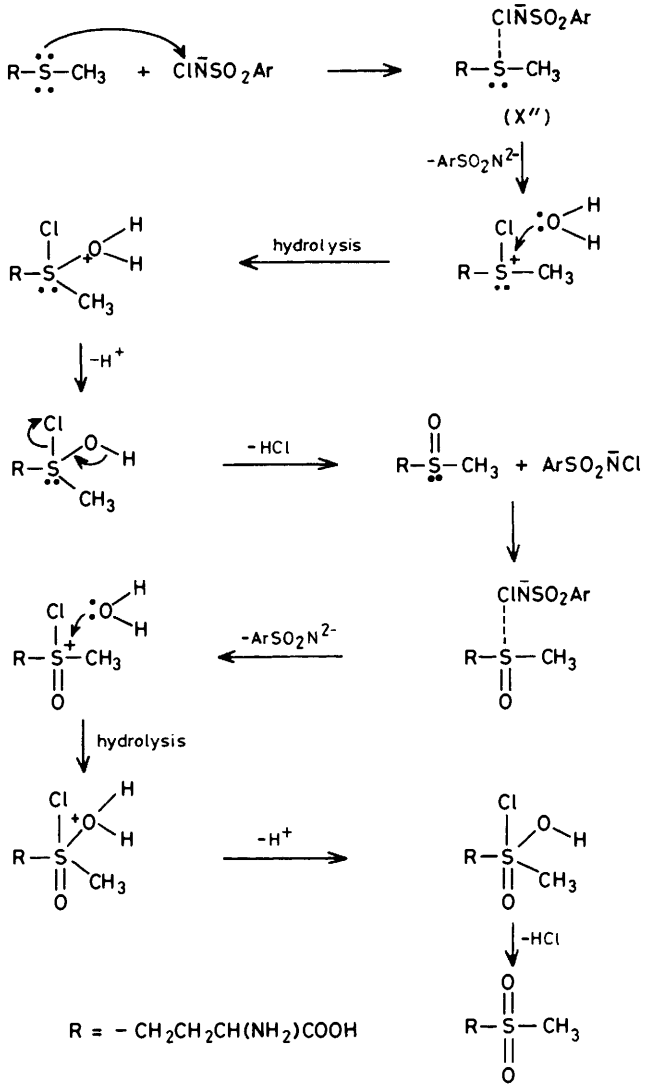
Scheme 1.

$ArSO_2NCl^- + Mt$	$\xrightarrow{k_{13}}$	$X'' + ArSO_2NH^-$	(i) slowest and r.d.s.
$X'' + ArSO_2NCl^-$	$\xrightarrow{k_{14}}$	Products	(ii)
$ArSO_2NH^- + H_2O$	$\xrightarrow{k_{15}}$	$ArSO_2NH_2 + OH^-$	(iii)

Scheme 2.



Scheme 3.



Scheme 4

sulphoxide which is rapidly oxidized by CAT to methionine sulphone.

The solvent isotope effect $(k')_{H_2O}/(k')_{D_2O} > 1$ is observed under alkaline conditions. This is generally correlated with the greater basicity of OD^- compared with OH^-. However, the magnitude of retardation in D_2O is small due to the hydrolysis step which tends to make the normal kinetic isotope effect $k_{1O}^{H}/k_{1O}^{D} > 1$ for the slow step (i) in Scheme 1.

Experimental

Chloramine-T (Merck) was purified by the method of Morris *et al.*[11] The purity of compound was checked by iodometric assay of the active halogen and by recording its Fourier transform ^1H and ^{13}C n.m.r. spectra. An aqueous solution of the compound was standardized by the iodometric method and preserved in brown bottles to prevent its photochemical deterioration.

Hypochlorous acid was prepared[18] by bubbling chlorine gas through a suspension of yellow HgO in water, until a test sample decolourized indigocarmine solution. The acid was obtained by distillation of the resulting mixture at low temperatures (< 30 °C). The distillate was collected in a receiver cooled in ice and was stored over yellow HgO in a brown bottle. Daily standardization through iodometry was required to assess the strength of the solution.

Chromatographically pure DL-methionine (Merck) was further assayed by the acetous perchloric acid method[19] and its aqueous solution was prepared. All other reagents were of AnalaR grade. Heavy water (D_2O, 99.2%) employed for solvent isotope studies was supplied by the Bhabha Atomic Research Center, Trombay, India. The ionic strength of the system was kept at a high value by using a concentrated solution of sodium perchlorate.

Stoicheiometry.—Varying ratios of CAT to amino acid in the presence of HCl, $HClO_4$, H_2SO_4, and NaOH were equilibrated at 30 °C for acids (and at 35 °C in alkali) for 24 h. The unchanged CAT in the reaction mixtures was determined by iodometric titrations. The analysis showed that one mole of methionine reacted with two moles of CAT. The observed reaction stoicheiometry is (13).

$$CH_3SCH_2CH_2CH(NH_2)COOH + 2ArSO_2NClNa + 2H_2O \longrightarrow CH_3\text{-}\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}\text{-}CH_2CH_2CH(NH_2)COOH + 2ArSO_2NH_2 + 2NaCl \quad (13)$$

Product Analysis.—Toluene-*p*-sulphonamide ($ArSO_2NH_2$) 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).] Identification of methionine sulphone was also made through paper chromatography.[20] [The solvent employed was n-butanol–glacial acetic acid–water (4:1:5 v/v), and a 0.2% solution of ninhydrin in butanol, water, and acetic acid (95:4:0.5 v/v) was used as the spray reagent (R_F 0.17).]

Kinetic Procedure.—In each kinetic run the reaction was carried out in a glass-stoppered Pyrex boiling tube whose outer surface was coated black to eliminate photochemical effects. Appropriate amounts of methionine, acid or NaOH, $NaClO_4$ solutions, and water (the total volume for all runs was kept constant) were placed in the tube and thermostatted at a known temperature for *ca.* 30 min for thermal equilibrium. The reaction was initiated by rapidly adding the required amount of CAT solution to the mixture in the tube. Portions (5 ml) of the reaction mixture were removed at varying time intervals and added to an ice-cold solution of KI (5%; 10 ml) containing H_2SO_4 (1M). The liberated iodine was titrated with sodium thiosulphate solution (0.001M) using starch indicator. The course of reaction was followed for, at least, two-half lives. The pseudo-first-order rate constants, k', were calculated graphically by plotting $\log_{10}$ titre against time. The values of k' were reproducible within $\pm$ 3% error.

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