Kinetics and Mechanism of the Oxidation of Amino Acids by Peroxomonosulphate. Part 2.† Effect of Formaldehyde

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The kinetics of oxidation of amino acids (S) by peroxomonosulphate (PMS) in the presence of formaldehyde (SH) were studied. Analysis of the results shows that the rate of oxidation can be represented by equation (i), at constant $[H^+]$. The effect of hydrogen ion on the rate and thermodynamic

$$-d[PMS]/dt = k'[S]\,[SH]\,[PMS] + k''[SH]\,[PMS] \quad (i)$$

parameters was also calculated. Perusal of the kinetic results shows that the first step in the reaction of amino acids with PMS in the presence of formaldehyde is the formation of a Schiff's base. This Schiff's base reacts more rapidly with PMS in the rate-determining step to give the products. The mechanism of the reaction is also discussed in terms of kinetic results.

The importance of the biologically active vitamin pyridoxal phosphate in the metabolism of amino acids is well established. It is the cofactor for enzymes catalysing a number of reactions of amino acids such as transfer of the amino group to an oxo acid.[1-3] The initial reaction of an amino acid with pyridoxal phosphate is the formation of an intermediate complex, a Schiff's base, by condensation between the α-amino group of the substrate and the 4'-formyl group of pyridoxal phosphate.[4-7] The electron-withdrawing effect of the heterocyclic nitrogen results in withdrawal of electrons from all three of the bonds about the α-carbon. The structural requirements for non-enzymic and coenzymic activity of pyridoxal phosphate analogues are the heterocyclic nitrogen, for electron withdrawal, the 4'-formyl group, and the phenolic hydroxy group.[8] Hence in the non-enzymic model reactions, we can replace the heterocyclic nitrogen (in the pyridine moiety) by an oxidant which will act as an electron withdrawer.

It has been observed that the thermal decarboxylation of α-amino acids in the presence of aldehydes and ketones is relatively fast.[9] In Part 1,[10] we noted that in the oxidation of α-amino acids, the product aldehyde enhances the rate of the reaction. Therefore a chemical model like pyridoxal-catalysed amino acid metabolism can also be constructed using formaldehyde and the oxidant peroxomonosulphate and the efficacy of the model can be measured from the oxidation of amino acids. In this paper, the results of the oxidation kinetics of α-amino-acids by peroxomonosulphate in the presence of formaldehyde are presented and discussed.

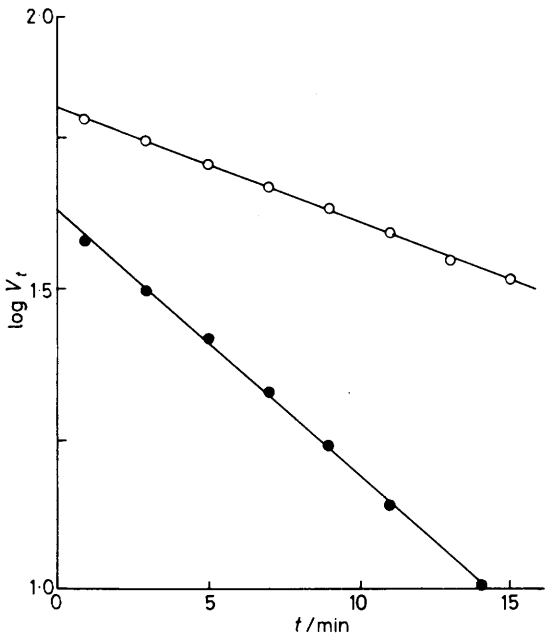


Figure 1. Plots of log V_t *versus* time: A, [alanine] 0.15M, [PMS] 2.12×10^{-3}M, pH 4.0, [SH] 2.82×10^{-3}M, μ 0.25, 31 °C; B, [butyrine] 0.10M, [PMS] 12.0×10^{-3}M, pH 4.0, [SH] 2.82×10^{-3}M, μ 0.25, 31 °C

Results and Discussion

All the experiments were carried out with amino acid concentrations always greater than the oxidant, at least 5—10 times, and at pH 4.0. Plots of the logarithm of the volume of thiosulphate consumed (log V_t) *versus* time were found to be linear even up to 80% conversion of PMS (Figure 1). The values of k_{obs} were evaluated from the slopes of the above plots. At constant $[H^+]$, [amino acid], and [HCHO] the values of k_{obs} were found to be independent of the initial concentration of PMS. This shows that the reaction is first order in PMS.

Values of k_{obs} were found to increase with an increase in the concentrations of amino acids at constant $[H^+]$, [PMS], and [HCHO]. Plots of k_{obs} *versus* [amino acid] were found to be linear with a definite positive intercept (Figure 2). This clearly proves that the reaction proceeds through two different steps; one is independent of amino acid and the other has first-order dependence on amino acid concentration. Therefore the rate law can be represented by equation (1).

$$-d[PMS]/dt = k'[\text{amino acid}]\,[PMS] + c\,[PMS] \quad (1)$$

Keeping $[H^+]$ and [amino acid] constant, increasing the concentration of formaldehyde increased the values of k_{obs}. Plots of k_{obs} *versus* [HCHO] were found to be straight lines passing through the origin (Figure 3). This shows that the disappearance of PMS can be expressed by equation (2).

$$-d[PMS]/dt = k''[HCHO]\,[PMS] \quad (2)$$

† Part 1, ref. 10.

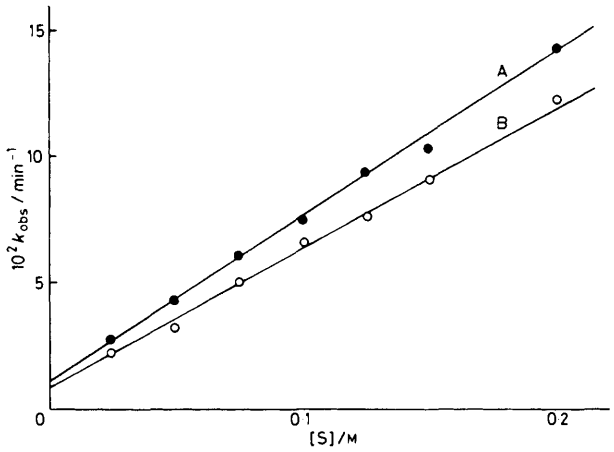


Figure 2. Plots of k_{obs} *versus* [amino acid]: A, alanine–PMS–HCHO: [PMS] 2.12 × 10^{-3}M, [SH] 2.82 × 10^{-3}M, pH 4.0, μ 0.25, 31 °C; B, butyrine–PMS–HCHO: [PMS] 8.48 × 10^{-3}M, [SH] 2.82 × 10^{-3}M, pH 4.0, μ 0.25, 31 °C

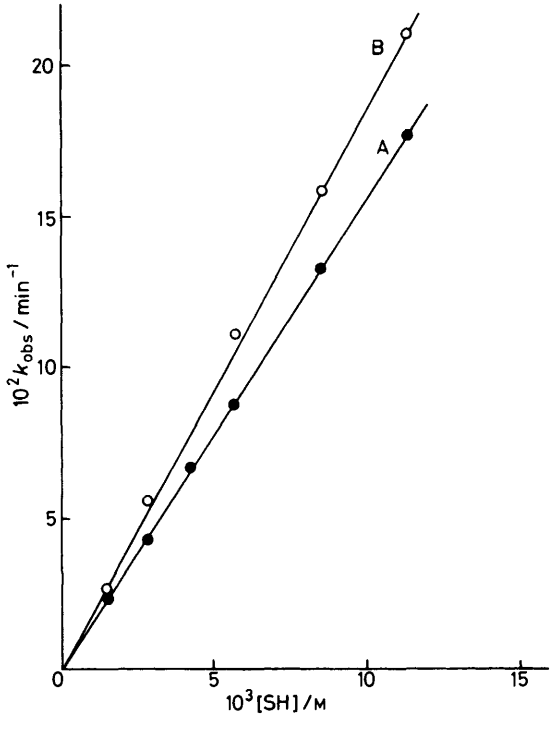


Figure 3. Plots of k_{obs} *versus* [HCHO]; A, alanine–PMS–HCHO: [alanine] 0.05M, [PMS] 2.12 × 10^{-3}M, pH 4.0, μ 0.25, 31 °C; B, butyrine–PMS–HCHO: [butyrine] 0.10M, [PMS] 8.48 × 10^{-3}M, pH 4.0, μ 0.25, 31 °C

Comparison of equations (1) and (2) shows that the disappearance of PMS is of the form (3).

$$-\mathrm{d[PMS]}/\mathrm{d}t = k_a\,[\text{amino acid}]\,[\mathrm{HCHO}]\,[\mathrm{PMS}] + k_b\,[\mathrm{HCHO}]\,[\mathrm{PMS}] \quad (3)$$

The values of k_{obs} were found to decrease with increasing H^+ concentration. Plots of k_{obs} *versus* $[H^+]^{-1}$ were found to be linear with a positive slope and a definite intercept showing an inverse first-order dependence on $[H^+]$ and consequently plots of k_{obs} $[H^+]$ *versus* $[H^+]$ were linear with positive intercepts (Figure 4).

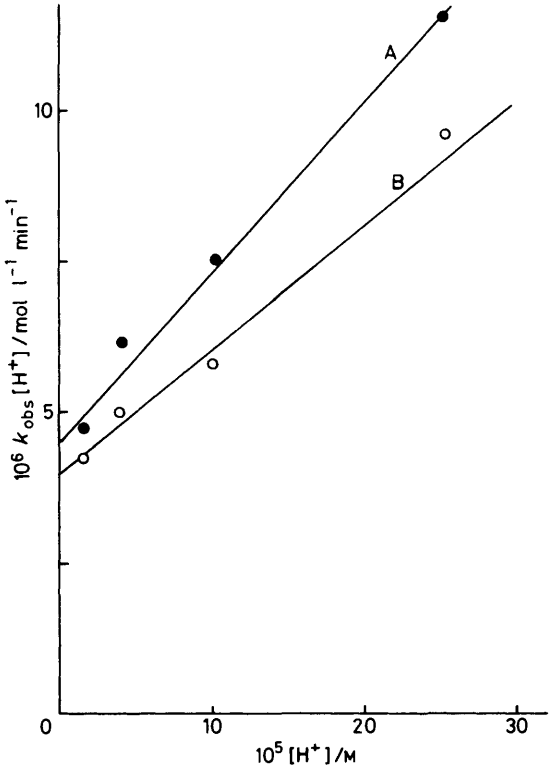
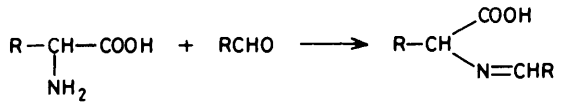


Figure 4. Plots of k_{obs} $[H^+]$ *versus* $[H^+]$: A, alanine–PMS–HCHO: [alanine] 0.10M, [PMS] 2.12 × 10^{-3}M, [SH] 2.82 × 10^{-3}M, μ 0.25, 31 °C; B, butyrine–PMS–HCHO: [butyrine] 0.10M, [PMS] 8.48 × 10^{-3}M, [SH] 2.82 × 10^{-3}M, μ 0.25, 31 °C



Scheme 1.

The change in ionic strength caused no pronounced effect on k_{obs} in all cases. The reactions were studied at three different temperatures (30—45 °C) and the activation parameters were calculated.

It is well known that amino acids react with aldehydes to give Schiff's compounds (Scheme 1). This reaction is used to estimate amino acids or to eliminate interference by aldehydes.[11,12] In the above reactions, a small amount of alkali is added to the aqueous solution of the amino acids because amino acids in aqueous solutions are present as zwitterions. This shows that aldehydes will react readily only with a free NH_2 group.

The pK_1 and pK_2 values, for all the amino acids used in this report, are *ca.* 2.3 and 9.9, respectively. Under our experimental conditions (at pH 4.0) all the amino acids are in the form of zwitterions. Also, from the experimental observation that the oxidation of formaldehyde occurs as a separate and independent reaction in addition to the formaldehyde-catalysed oxidation of amino acids, we can assume that the equilibrium in Scheme 2 exists. Based on the experimental observations, Scheme 3, at constant $[H^+]$, is more probable.

The rate law for the disappearance of PMS can be written as (4) where $[\mathrm{HCHO}]_{eq}$ represents the equilibrium concentration

$$-\mathrm{d[PMS]}/\mathrm{d}t = k_1\,[\text{aldimine}]\,[\mathrm{PMS}] + k_2\,[\mathrm{HCHO}]_{eq}\,[\mathrm{PMS}] \quad (4)$$

of formaldehyde. Substituting for the aldimine concentration, with the assumption that K_1 is very small, the rate equation (4) becomes (5) where [S] and $[HCHO]_T$ represent the amino acid

$$-d[PMS]/dt = k_1K_1[S][HCHO]_T[PMS] + k_2\{[HCHO]_T - K_1[S][HCHO]_T\}[PMS] \quad (5)$$

concentration and initial (total) concentration of formaldehyde, respectively. If $K_1[S] \ll 1$, then as an approximation $K_1[S][HCHO]_T$ can be neglected in comparison to $[HCHO]_T$. Therefore we have equation (6). Equation (7) explains the

$$-d[PMS]/dt = k_1K_1[S][HCHO]_T[PMS] + k_2[HCHO]_T[PMS]_T \quad (6)$$

$$k_{obs} = k_1K_1[S][HCHO]_T + k_2[HCHO]_T \quad (7)$$

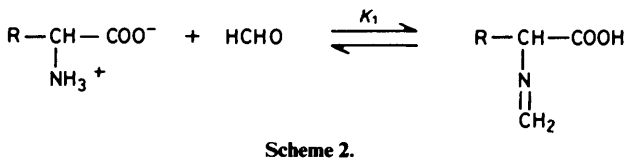
observed effects of amino acid and HCHO on k_{obs}. At constant [HCHO], the plot of k_{obs} *versus* [amino acid] should give the value of k_2 [HCHO] as intercept and k_1K_1 [HCHO] as slope. From the intercept we can calculate the value of k_2, the rate constant for the oxidation of formaldehyde by PMS. The values of k_2, calculated from various amino acids, are in Table 1 along with the value observed from the direct oxidation[13] of formaldehyde by PMS. Similarly from the slope of k_{obs} *versus* $[HCHO]_T$ we can get the value of $k_1K_1[S] + k_2$ and using the value of k_2 obtained from the plot of k_{obs} *versus* [amino acid], we can calculate the k_1K_1 values. The values of k_1K_1 obtained by the effects of HCHO and amino acid on k_{obs} are given in Table 1. Comparison of these values shows that the agreement is excellent for k_1K_1 whereas the values of k_2 obtained from different amino acids are of the same order of magnitude as that of k_2 obtained from the direct oxidation of formaldehyde. This clearly proves the validity of our assumption.

The effect of hydrogen ion on k_{obs} can be explained as follows. Since the rate was found to increase as the pH was increased and decrease as $[H^+]$ was decreased and also from the nature of the $k_{obs}[H^+]$ *versus* $[H^+]$ plots (Figure 4) leaving an intercept on the ordinate, Scheme 4 for the oxidation involving acid-independent and inverse acid-dependent paths may be proposed. The rate equation can be written as (8). This

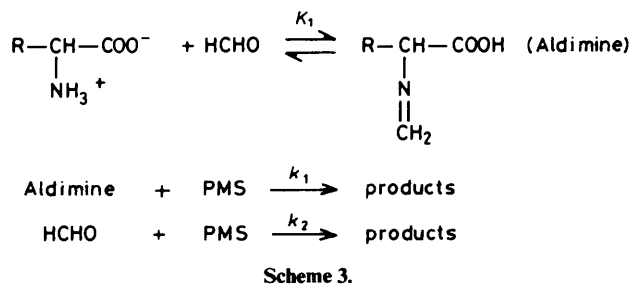
$$\text{Rate} = -d[PMS]/dt = (k_5K_1[S] + k_6)[HCHO]_T[SO_5^{2-}] + (k_3K_1[S] + k_4)[HCHO]_T[HSO_5^-] = (k_5K_1[S] + k_6)[HCHO]_T K[HSO_5^-]/[H^+] + (k_3K_1[S] + k_4)[HCHO]_T[HSO_5^-] \quad (8)$$

$$k_{obs} = \{k_5K_1[S] + k_6\}([HCHO]_T K/[H^+]) + \{k_3K_1[S] + k_4\}[HCHO]_T \quad (9)$$

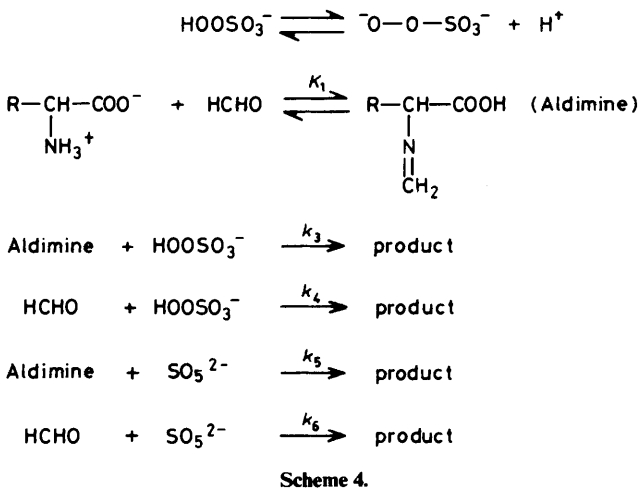
mechanistic scheme explains the acid dependence. The plots of $k_{obs}[H^+]$ *versus* $[H^+]$ were drawn and from the slopes of these plots and using the value of k_4 from the oxidation[13] of formaldehyde by PMS (k_4 4.0 × 10^2 l mol^{-1} s^{-1}) the values of k_3K_1 were calculated. From the intercepts the values of k_5K_1 were obtained using the literature value[14] of K 3.98 × 10^{10} and k_6 from the oxidation[13] of formaldehyde (k_6 3.7 × 10^4 l mol^{-1} s^{-1}). The values are in Table 1. Here we have made an



Scheme 2.



Scheme 3.



Scheme 4.

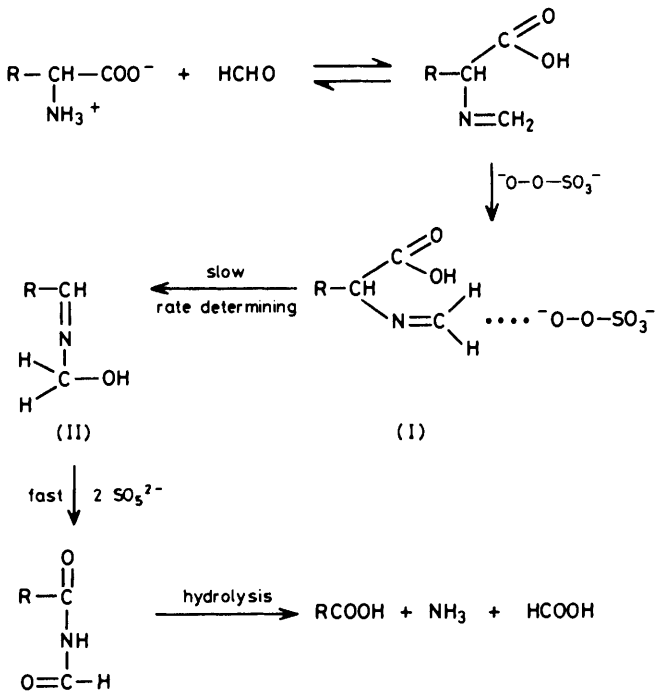
Table 1. Kinetic parameters for the oxidation of amino acids by PMS in the presence of HCHO

	Glycine[a]	Alanine	Butyrine	Valine	Leucine	Norleucine
k_1K_1[b]/l^2 mol^{-2} s^{-1}						
from amino acid variation	4.8	3.9	3.3	3.7	4.3	7.3
from HCHO variation	4.8	3.9	2.5	3.7	4.1	7.1
10^2k_2*[b]/l mol^{-1} s^{-1}	7.1	6.5	5.9	13.2	4.5	11.2
k_3K_1[c]/l^2 mol^{-2} s^{-1}	1.7	1.3	0.9		0.8	
$10^{-5}k_5K_1$[c]/l^2 mol^{-2} s^{-1}	5.5	3.0	2.2	3.7	2.8	3.5
$\Delta H^{\neq}$/kJ mol^{-1}[d]	64.9	57.7	59.8	59.4	65.7	56.5
$\Delta S^{\neq}$/J K^{-1} mol^{-1}[d]	−59.8	−82.9	−73.7	−69.9	−59.4	−83.7

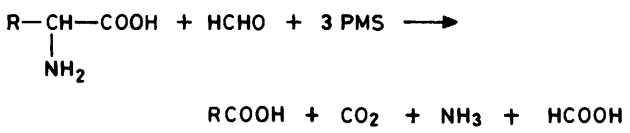
All the kinetics were carried out with [amino acid] 0.01—0.25M, [HCHO] 1.42—10.3 × 10^{-3}M, and [PMS] 1.7—8.56 × 10^{-4}M.

* k_2 For formaldehyde oxidation by PMS is 11.3 × 10^{-2} l mol^{-1} s^{-1} at pH 4.0; μ 0.25; 31.0 °C.

[a] In glycine–HCHO–PMS, all the kinetic runs were carried out at 35.0 °C and the derived values correspond to 35.0 °C. All other systems were at 31.0 °C. [b] k_1K_1 and k_2 values are at pH 4.0, μ 0.25. In the case of glycine the temperature was 35.0 °C and for other systems 31.0 °C. [c] k_3K_1 and k_5K_1 are at μ 0.25 and temperatures as above. [d] $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values are at pH 4.0 and μ 0.25.



Scheme 5.



Scheme 6.

important assumption that the values of K_1 do not depend upon pH in the range we have studied (3.6—4.8). Although this may not be correct, the fortuitous agreement of experimental observations with the rate equation shows that the change in K_1, if any, will be small in the pH range 3.6—4.8.

It can be observed from Table 1 that k_5K_1 is approximately five orders of magnitude higher than k_3K_1. This is similar to our observation on the oxidation of amino acids by PMS.[10] This higher reactivity of SO_5^{2-} than that of HSO_5^- may be considered in favour of nucleophilic attack by peroxide. Based on the foregoing kinetic results we can suggest a nucleophilic substitution mechanism on the CH_2 of the Schiff's base by PMS as shown in Scheme 5.

As pointed out earlier, aldehydes react with amino acids to give Schiff's bases. By comparison with enzyme-catalysed decarboxylation–deaminations of amino acids,[4–7] we can assume that the oxidant reacts with the CH_2 group of $-N{=}CH_2$ to give the activated complex (I), which in the rate-determining step rearranges to give (II) and CO_2. If the oxidant reacts with any other carbon atom in the imine, *e.g.* the α-carbon atom, then this will be reflected in $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values as observed in the oxidation of amino acids by PMS. But approximately equal values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for all the amino acid–HCHO–PMS systems irrespective of the structure of the amino acid (Table 1) support our assumption that PMS reacts with the CH_2 group of $-N{=}CH_2$. Similarly the production of aldehyde in any intermediate step can be eliminated as it would complicate the kinetics by catalysis and the observed kinetics would not be as simple as observed.

Because of the difficulty in obtaining the values of rate constants (k_1, k_3, and k_5) and K_1 separately, we can't compare the rate of oxidation of various amino acids. However, this study brings out the importance of Schiff's bases (aldimines) in the oxidative decarboxylation–deamination of amino acids. A comparison of the results of oxidation of amino acids by PMS in the presence of aldehyde with that of amino acids alone shows that the aldehyde-catalysed decarboxylative deaminative oxidation is very fast (*ca.* 10^5 times). Had it not been for the formation of Schiff's base, the observed rate constant should be of the order of the oxidation of formaldehyde since the second-order rate constant for formaldehyde oxidation[13] is 10^3 times greater than that of amino acids. However, the values of k_1K_1 (Table 1) are 10^2 times greater than that for formaldehyde oxidation. This is in accord with Snell[15] who showed that the reaction of amino acids normally catalysed by pyridoxal phosphate-dependent enzymes occurred in aqueous solution at 100 °C in the presence of non-phosphorylated cofactor and metal ions, the relative extents of decarboxylation, racemization, transamination *etc.* depending on pH.

Table 2. Stoicheiometric studies in the oxidation of amino acids by PMS in the presence of formaldehyde

Amino acid	[S]/M	[HCHO]/M	$[PMS]_{consumed}$/M
Glycine	0.01	0.0113	0.0426
	0.005	0.0113	0.0353
Alanine	0.01	0.0113	0.0328
	0.005	0.005 65	0.156
Butyrine	0.01	0.0013	0.034
	0.005	0.005 65	0.0164
Valine	0.01	0.0113	0.0378
	0.005	0.0113	0.0322
	0.005	0.005 65	0.180
Leucine	0.011	0.0113	0.035
	0.005	0.0056 65	0.0164

All the stoicheiometric reactions were carried out without maintaining pH. Time allowed for the completion of the reaction is 24 h.

Experimental

Potassium peroxomonosulphate was from the Dupont Chemical Co., and was found to be 96% pure. The absence of free hydrogen peroxide was confirmed. Amino acids of biochemical grade were from Loba-Chemie Indo Austranal Co. Formaldehyde (S. Merck, India; 30% solution) was used as such without further purification. The strength of formaldehyde was estimated by the hypoiodite method.[11] Other chemicals used were of analytical grade.

Experiments were carried out in buffered media (acetic acid–sodium acetate) and a high concentration of the buffer (0.1M) was maintained in the reaction mixture since the product HSO_4^- is a stronger acid than the oxidant HSO_5^-. Under our experimental conditions no self-decomposition of PMS was observed. Formaldehyde and oxidant solutions were prepared daily and estimated. Amino acid solutions were also prepared daily by gravimetry. The kinetics of the reaction was followed by iodometry at different time intervals.

The stoicheiometry of the reactions were obtained by taking a known excess concentration of PMS over amino acids and formaldehyde and allowing the reaction to go to completion at room temperature. The concentration of formaldehyde was always slightly greater than that of the amino acids. Different ratios of amino acids, formaldehyde, and peroxomonosulphate were taken and after the reaction was over, the remaining PMS was estimated by iodometry. The results are shown in Table 2. Analysis of the results shows that the stoicheiometry can be written as in Scheme 6. If more than three equiv. PMS are

consumed this is due to the oxidation of formic acid by PMS which was also confirmed.[13] The evolution of CO_2 and NH_3 was detected by tests with lime water and Nessler's reagent, respectively. In the formaldehyde-catalysed oxidation of amino acids, the formation of formic acid was detected by spot tests.[16] In the case of alanine oxidation, the presence of acetic acid was also detected.[17]

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