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Oxidation of Some α-Amino Acids by Pyridinium Bromochromate in an Aquo-Acetic Acid Medium—A Kinetic and Mechanistic Study[1]

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Abstract—Oxidation of α-amino acids by pyridinium bromochromate (PBC) was studied in acetic acid–water mixture containing perchloric acid. The reaction rate is first order in [PBC] and inverse first order in [H^+] and has aldehyde as a product. The results are contrary to that of Karim and Mahanti, who observed first order with [H^+] and cyanide as the product in the oxidation of amino acids by quinolinium dichromate. Michaelis–Menten type kinetics has been observed with respect to α-amino acids. The rate of reaction increases with a decrease in the polarity of solvent indicating an ion–dipole interaction in the slow step. The reactions exhibit no primary kinetic isotope effect. The activation parameters have been evaluated. The reaction mechanism involving the formation of chromate-ester between unprotonated PBC and unprotonated amino acid followed by C–C bond fission in the slow step has been suggested. The value of the Michaelis constant (substrate–oxidant complex formation constant) increases as the number of carbon atoms increases in the amino acid.

INTRODUCTION

Mahanti and Banerji [1] have reviewed the synthetic and mechanistic aspect of the use of chromium(VI) halochromates as mild and selective reagents in synthetic organic chemistry. Our preliminary studies on the oxidation of α-amino acids by pyridinium bromochromate (PBC) indicated an inhibition of the rate of oxidation with increasing H^+ ion concentration. These observations, together with the products of oxidation, were inconsistent with the observation of Karim and Mahanti [2–4] in the oxidation of α-amino acids by PBC. The present study is a continuation of the earlier published work [5] on the oxidation of α-amino acid (glycine) by PBC. We have tried to correlate the structure and reactivity in these oxidations.

EXPERIMENTAL

Materials and methods. All the amino acids were commercial products of the highest purity, and the concentration of all the amino acids was determined by Sorensen formal titration. PBC was prepared by the reported method [6], and its purity checked by m.p. (481 K), IR spectrum (KBr, cm^{-1}: 1045, 1025, 950, 770, 670, 383, 260, and 230), and elemental analysis (found, %: C, 23.6; H, 2.3; calcd., %: C, 23.1; H, 2.3). Acetic acid was purified by distillation over CrO_3, and fractional distillation in the presence of acetic anhydride and fraction was collected over 491 K. All other reagents used were of "AnalaR" grade.

Kinetic measurements. The reactions were carried out under pseudo-first-order conditions by keeping a large excess of α-amino acid over PBC. Stock solutions of PBC in acetic acid and reaction mixtures containing known volumes of substrate, perchloric acid, and acetic acid were brought to the thermostat temperature (±0.02 K) separately. Perchloric acid was used as a source of hydrogen ions. The reaction was started by rapidly adding a predetermined volume of thermostated PBC solution to the reaction mixtures. The rate of oxidation was followed iodometrically, taking all precautions to avoid air oxidation of the iodide. The rate constants were computed from the linear plots of log [hypo] against time using the linear least-squares method. The results were reproducible within ±2%. The order with respect to each reactant was determined by the Ostwald isolation method.

Detection of free radicals. Induced polymerization with acrylonitrile was used to detect free radicals in the oxidation of α-amino acids by PBC in an inert atmosphere. No milky appearance was observed, indicating the absence of free radicals formation.

Stability of PBC in solution. Solution of oxidant (PBC) in acetic acid–water–perchloric acid mixture obeys Beer–Lambert's law at λ_{max} = 370 nm. It was found that there is no change in λ_{max} and optical density on long standing and heating up to 60°C. This indicated PBC is stable in solution in an acetic acid–water solution.

[1] This article was submitted by the authors in English.

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Table 1. Variation of rate with concentration of pyridinium bromochromate. $[HClO_4] = 1.0 \times 10^{-1}$ mol/dm^3, [α-amino acid] = 2.0×10^{-2} mol/dm^3, acetic acid = 30 vol %, T = 303 K

PBC × 10^3, mol/dm^3	$k_1 \times 10^5$, s^{-1}					
	glycine	alanine	valine	*iso*-leucine	*nor*-leucine	phenylalanine
0.80	6.20	5.30	3.33	5.21	5.20	6.03
1.20	6.29	5.27	3.35	5.24	5.20	6.03
1.60	6.18	5.32	3.37	5.20	5.26	6.08
2.00	6.20	5.29	3.35	5.22	5.22	6.18
2.80	6.20	5.30	3.35	5.22	5.26	6.16
3.20	6.18	5.30	3.32	5.20	5.22	6.19
4.00	6.12	5.28	3.35	5.22	5.22	6.18

Product study. The oxidation of α-amino acids by PBC gives the corresponding aldehydes. This was detected and estimated as its 2,4-dinitrophenylhydrazone. The product study was carried under kinetic conditions. After the completion of the reaction, the reaction mixture was neutralized by $NaHCO_3$ and treated with a 2,4-dinitrophenylhydrazene solution. The precipitate was filtered, washed with cold water, and dried in vacuum. Blank and parallel runs were performed and the correction was applied to the results of the estimation. The yield of hydrazone obtained using an authentic sample of the corresponding aldehyde was 75–80%. Ammonium ion and carbon dioxide were detected as products of oxidation by Nesslar's reagent and lime water, respectively.

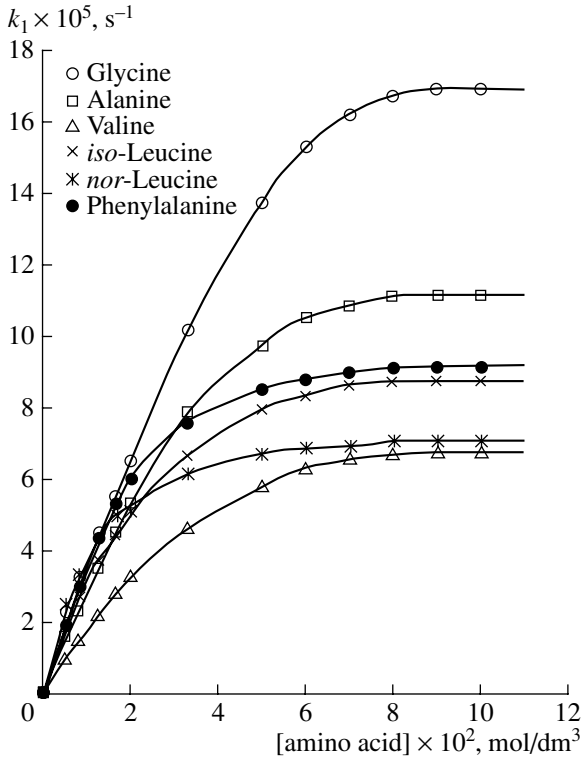


Fig. 1. Variation of rate with amino acid concentration (plot of k_1 vs. [amino acid]) at $[HClO_4] = 1.0 \times 10^{-1}$ mol/dm^3, [PBC] = 2.0×10^{-3} mol/dm^3, acetic acid = 30 vol %, T = 303 K.

Stoichiometry. Reaction mixtures containing known slight excess of PBC over α-amino acids containing 1.0 mol/dm^3 $[HClO_4]$ in 30 vol % acetic acid–70 vol % water mixtures (v/v) were allowed to stand at room temperature in the dark. When the reaction was completed, the unreacted PBC in the reaction mixture was determined iodometrically. Three moles of α-amino acids were found to require 2 mol of PBC for complete oxidation. Therefore, the reaction may be represented stoichiometrically as

$$3RCH(NH_2)COOH + 2Cr(VI) + 3H_2O \quad \text{(PBC)}$$
$$\longrightarrow 3RCHO + 2Cr(III) + 3NH_4^+ + 3CO_2 + 3H^+. \quad \text{(I)}$$

RESULTS AND DISCUSSION

Effect of oxidant. When α-amino acids were in excess, the rate at which PBC disappears followed the first-order rate law. The first-order rate constants are independent of the initial concentration of the PBC when varied in the range $(0.8–4.0) \times 10^{-3}$ mol/dm^3 at 303 K (Table 1).

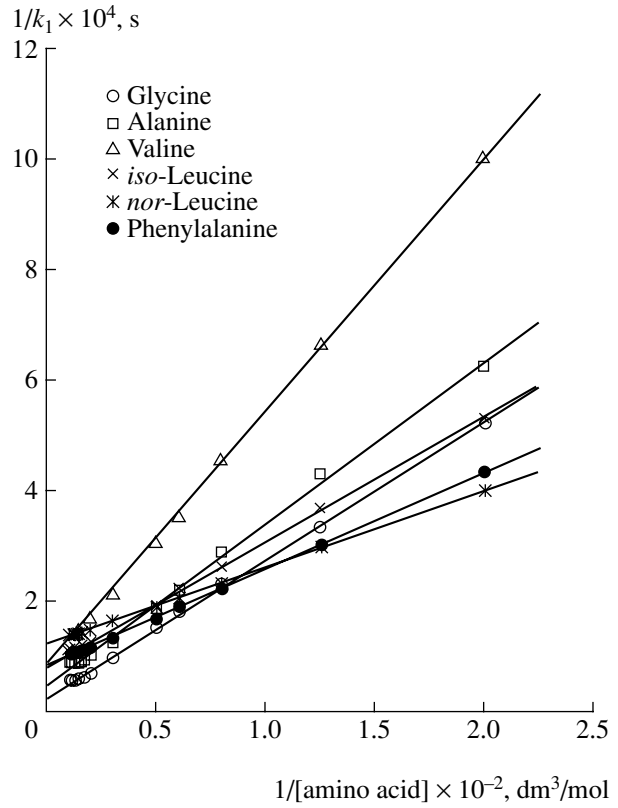


Fig. 2. Variation of rate with amino acid concentration (plot of 1/[amino acid] vs. $1/k_1$) at $[HClO_4] = 1.0 \times 10^{-1}$ mol/dm^3, [PBC] = 2.0×10^{-3} mol/dm^3, acetic acid = 30 vol %, T = 303 K.

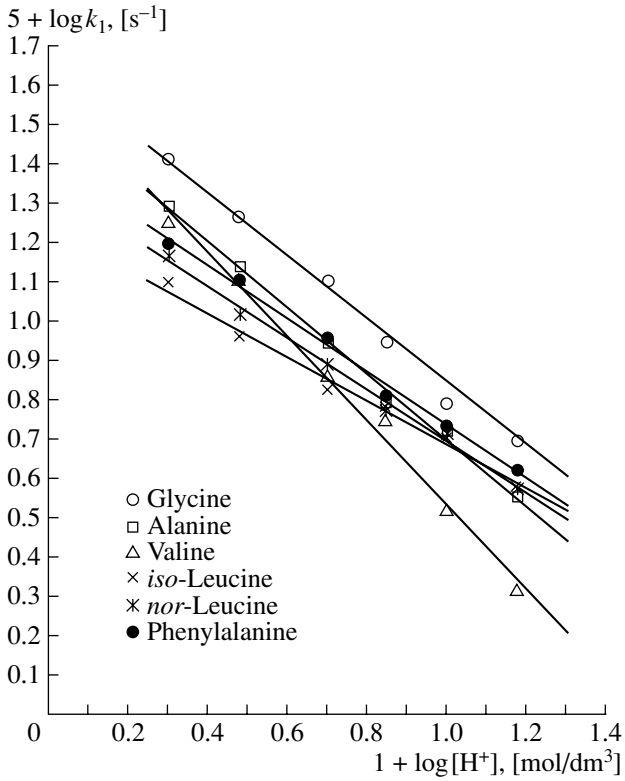


Fig. 3. Variation of rate with perchloric acid concentration at [amino acid] = 2.0×10^{-2} mol/dm^3, [PBC] = 2.0×10^{-3} mol/dm^3, acetic acid = 30 vol %, T = 303 K.

Effect of substrate. At constant PBC concentration, the reaction rate increased with an increase in the concentration of amino acids from 0.005–0.100 mol/dm^3. At lower concentrations of amino acids, determining the initial reaction rate and dividing it by the concentration of the amino acid was used to calculate the first-order rate constants. Plots of k_1 against [amino acid] (Fig. 1) are nonlinear in all the cases and approach a maximum value. A plot of $1/k_1$against 1/[amino acid] (Fig. 2) gives a straight line and intercept on the rate ordinate, indicating the oxidation of amino acids follows Michaelis–Menten type kinetics and proceeds through the formation of a complex between the oxidant and the α-amino acid. A similar phenomenon has been observed in the oxidation of α-amino acids by pyridinium hydrobromide perbromide [7]. The variation of the rate of oxidation of α-amino acids with PBC can be expressed as

$$\frac{d[PBC]}{dt} = \frac{k[\text{amino acid}][PBC]}{K_M + [\text{amino acid}]}.$$

In spite of the variation of substituents in the amino acids, the first-order rate constant variation is not significantly different (cf. k^1 at 2.0×10^{-2} mol/dm^3 amino acid). This may be due to different structural effects on the equilibrium constant $K = \frac{k'_1}{k'_{-1}}$ and the specific rate constant k.

Effect of H^+ ion. The rate decreased with an increase in the concentration of H^+ from 0.2 to 1.5 mol/dm^3 at constant ionic strength. A plot of $\log k_1$ against $\log[H^+]$ (Fig. 3) is a straight line with a negative slope ≈1. This suggests that the reactive species in the oxidation of an amino acid is a simple molecular amino acid. By increasing the H^+ ion concentration, the protonation of the amino acid will increase, which does not take part in the oxidation process. Since protonated amino acid cannot form a coordinate bond with the oxidant, i.e., no complex formation, the rate decreases. This is contradictory to the results obtained by Karim and Mahanti [2–4], who observed first order with H^+ in the oxidation of amino acids by quinolinium dichromate, and cyanide as a product. We have not used this oxidant and, unless the system is repeated and observations confirmed, we cannot comment on it. There is no report on the oxidation of amino acids by halochromates except the present one. In most of the cases, oxidation by PBC is catalyzed by H^+, and, in the present study, retardation has been observed. Pending confir-

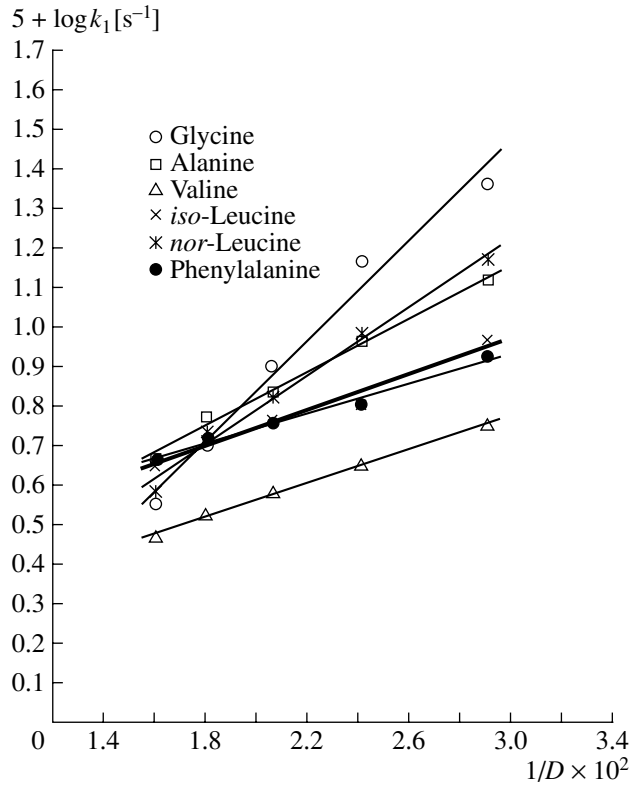


Fig. 4. Variation of rate with solvent composition at [$HClO_4$] = 1.0×10^{-1} mol/dm^3, [amino acid] = 2.0×10^{-2} mol/dm^3, [PBC] = 2.0×10^{-3} mol/dm^3, T = 303 K.

mation of the work of Karim and Mahanti, we would like to offer our explanation on the [H^+] dependence as follows.

The reaction of a PBC substrate other than amino acids follows the rate expression $w = a + b[H^+]$, in the presence of H^+ ion. The expression indicates a direct reaction of PBC with the substrate (alcohol, diols, and methionine). There is no direct reaction of PBC with amino acids. The reaction starts when protons are added. This indicates that the reaction of PBC with amino acid is a reaction not of a zwitterion or protonated amino acid as the reaction is retarded by the further addition of protons; thus, a chelate formation is a necessary condition in the oxidation of amino acids as protonated nitrogen (donor of electron pair). Hence, the mode of mechanism with amino acid follows a different path than that adopted by other substrates, hence, the difference in the rate dependence on H^+ ion concentration.

Effect of ionic strength. The reaction rate remained almost unaffected by changing the concentration of Na_2SO_4, $NaNO_3$, and $NaClO_4$, indicating that the interaction in the rate-determining step is not an ion–ion type.

Effect of solvent composition. The reaction rate increased with an increase in the percentage of acetic acid, suggesting that a low dielectric medium favors the oxidation. A plot of $\log k_1$ against $1/D$ (dielectric constant) is linear (Fig. 4) with a positive slope, which ranges from 20.0–66.7 for the amino acids under study. This indicates an ion–dipole type of interaction in the rate-determining step [8].

Effect of pyridine. The addition of pyridine has no effect on the reaction rate. This also proves the stability of PBC in a medium of acetic acid–water containing perchloric acid in the concentration range studied.

Kinetic isotope effect. The rate of oxidation of α-deuterio phenyl alanine $C_6H_5CD(NH_2)COOH$ is nearly the same as the rate of oxidation of the protonated analogue. The absence of primary kinetic isotope effect ruled out the cleavage of α-C–H fission in the rate-determining step.

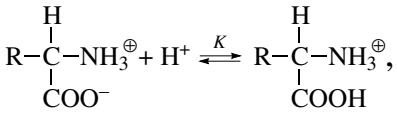
Effect of temperature. The rate increased with an increase in temperature (Table 2). A plot of $\log k_1$ against $1/T$ was found to be linear. The extra thermodynamic parameters were calculated. These are collected in Table 3.

Table 2. Variation of rate with temperature. [$HClO_4$] = 1.0×10^{-1} mol/dm^3, [α-amino acid] = 2.0×10^{-2} mol/dm^3, [PBC] = 2.0×10^{-3} mol/dm^3, acetic acid = 30 vol %

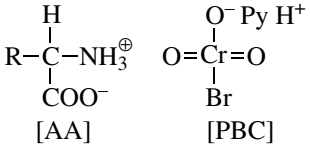
Temperature, K	$k_1 \times 10^5$, s^{-1}					
	glycine	alanine	valine	*iso*-leucine	*nor*-leucine	phenylalanine
303	6.26	5.50	3.35	5.37	5.18	5.47
308	8.66	10.05	7.00	8.47	8.30	8.88
313	12.15	16.00	12.00	13.50	12.50	14.60
318	20.15	20.51	23.70	20.30	20.50	20.30
323	32.62	38.30	36.30	31.00	32.30	28.70

The value of the activation energy is approximately 63 to 98 kJ/mol. The entropy values are all negative and high (except valine) suggesting that the transition state is more rigid and extensively solvated than the reactants. The negative entropy also suggests the formation of cyclic intermediate from acyclic species. The plot of $\Delta S^{\#}$ against $\Delta E_a^{\#}$ is nearly linear suggesting that all the amino acids studied follow a similar mechanism.

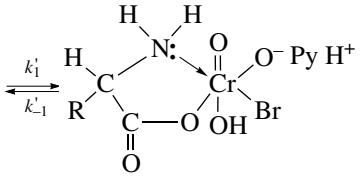
The following reaction scheme explains all the observed experimental results:



(II)

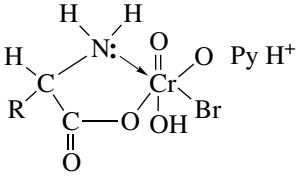


[AA] [PBC]

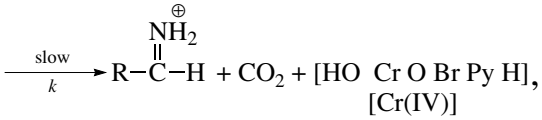


(III)

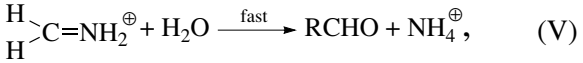
Complex **C**



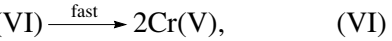
Complex **C**



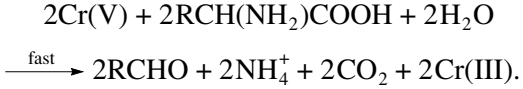
[Cr(IV)] (IV)



(V)



(VI)



(VII)

The overall reaction may be represented as (I).

The rate law, derived based on the above mechanism, is as follows:

Rate of reaction $w = k[\mathbf{C}]$.

Concentration of complex [**C**] can be calculated by applying steady-state concept. This gives

Table 3. Thermodynamic parameters

α-Amino acid	Energy of activation $\Delta E_a^{\#}$, kJ/mol	Entropy of activation $\Delta S^{\#}$, J/(mol K)	Free energy $\Delta F^{\#}$, kJ/mol
Glycine	63.2 ± 2.8	–106.1 ± 4.2	95.3 ± 2.9
Alanine	76.6 ± 3.1	–65.1 ± 3.8	96.3 ± 2.9
Valine	97.9 ± 3.8	–4.4 ± 2.9	99.2 ± 2.8
iso-Leucine	81.5 ± 3.9	–55.7 ± 4.3	98.9 ± 2.6
nor-Leucine	66.4 ± 2.9	–115.3 ± 4.0	101.3 ± 3.0
Phenylalanine	68.9 ± 2.9	–97.5 ± 2.6	98.5 ± 3.2

$$[\mathbf{C}] = \frac{k_1'[\mathrm{AA}][\mathrm{PBC}]}{k_{-1}' + k + k_1'[\mathrm{AA}]} = \frac{[\mathrm{AA}][\mathrm{PBC}]_t}{K_m + [\mathrm{AA}]},$$

where $K_M = \dfrac{k_{-1}' + k}{k_1'}$.

Hence,

$$w = k[\mathbf{C}] = \frac{k[\mathrm{AA}][\mathrm{PBC}]_t}{K_m + [\mathrm{AA}]} = k_1[\mathrm{PBC}]_t.$$

The observed pseudo-first-order rate constants, k_1, will then be

$$k_1 = \frac{k[\mathrm{AA}]}{K_m + [\mathrm{AA}]}$$

or

$$\frac{1}{k_1} = \frac{K_m}{k}\frac{1}{[\mathrm{AA}]} + \frac{1}{k}.$$

Therefore, K_m can be calculated by the slope/intercept of plot $1/k_1$ vs. 1/[AA].

K_m values by calculation are 0.125, 0.0722, 0.0480, 0.0262, 0.0114, and 0.02212 for glycine, alanine, valine, *iso*-leucine, *nor*-leucine, and phenylalanine, respectively. This infers that the alkyl group in an amino acid increases the value of K_m and decreases the stability of the substrate–PBC complex, since $1/K_m$ measures the stability of the complex.

This rate law is consistent with the results. A decrease in the reaction rate can be explained based on equation (II), i.e., the amino acid concentration will increase as H^+ increases and, hence, a decrease in the rate of reaction.

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REFERENCES

1. Mahanti, M.K. and Banerji, K.K., *J. Ind. Chem. Soc.*, 2002, vol. 79, p. 31.
2. Karim, E. and Mahanti, M.K., *Oxid. Commun.*, 1998, vol. 21, p. 559.
3. Karim, E. and Mahanti, M.K., *Oxid. Commun.*, 1996, vol. 19, p. 582.
4. Karim, E. and Mahanti, M.K., *Oxid. Commun.*, 1992, vol. 15, p. 211.
5. Nalwaya, N., Jain, A., and Hiran, B.L., *J. Ind. Chem. Soc.*, 2002, vol. 79, p. 587.
6. Narayanan, N. and Balasubramanian, T.R., *J. Chem. Res.*, 1986, p. 336.
7. Devi, J., Kothari, S., and Banerji, K.K., *Ind. J. Chem. A*, 1995, vol. 34, p. 116.
8. Amiss, E.S., *Solvent Effect on Reaction Rates and Mechanism*, New York: Academic, 1967.