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Research Article

Kinetics and Mechanism of Oxidation of L-isoleucine by Tributylammonium Chlorochromate in acetic acid medium.

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ABSTRACT

Kinetics and Mechanism of Oxidation of L-isoleucine (Ile) by tributyl ammonium chlorochromate in the acetic acid medium were studied spectrophotometrically at 445nm. The reaction was first-order concerning both oxidant and substrate and positive fractional order for H^+. The reaction between isoleucine and oxidant exhibits 1:1 stoichiometry. By changing the salts had no significant effect on the rate of reaction. The reaction was studied at different temperatures and the activation parameters were calculated. The main product was identified as the corresponding carbonyl compound by spot test. A possible mechanism was proposed to explain the results of kinetic studies, reaction stoichiometric, and product analysis.

KEYWORD

Tributyl ammonium chlorochromate, Isoleucine, Oxidation.

1. INTRODUCTION

Isoleucine is an essential amino acid that is best known for its ability to increase endurance and help in healing and repairing of muscle tissue [1] and encourage clotting at the site of injury. It also keeps energy levels stable by helping to regulate blood sugar, a deficiency of ISL produces symptoms similar to those of hypoglycemia, and may include headaches, dizziness, fatigue, depression, confusion, and irritability. Hence oxidation of isoleucine may help in understanding some aspects of enzyme kinetics. Amino acid residues are the main constituents of proteins and the study of its sensitivity towards Oxidation opens up a new area to understand the mechanism involved in the protein and amino acid modification [2]. Chromium (VI) reagents are widely used in organic chemistry for the oxidation of various organic compounds. In past years, some new chromium (VI) based reagents like tetramethyl ammonium fluorochromate[3], N-methyl benzyl ammonium fluorochromate and benzyl trimethyl ammonium fluorochromate[4], tripropyl ammonium fluorochromate[5], benzyl triethyl ammonium chlorochromate[6], tetrapropyl ammonium bromochromate and tetrabutyl ammonium bromochromate[7] were proposed. Tributyl ammonium chlorochromate (TriBACC) is also one of such compounds used for the oxidation of organic substrates. It is a more efficient and mild oxidizing agent [8-9] and has certain advantages over similar oxidizing agents in terms of the amount of oxidant and solvent required, short reaction times and high yields. The present investigation is a part of our broad program of studying mechanistic aspects of the oxidation of isoleucine by TriBACC. There are many reports on the kinetics of oxidation of amino acids by various oxidants, such as *N*-bromoacetate, benzyl dimethyl ammonium tribromide [10], *N*-bromosuccinimide[11], chloramine-T [12-14], Chromium (III) [15], etc. The literature survey reveals that there are no reports on mechanistic studies of L-isoleucine oxidation by TriBACC. Hence we have considered it to study the kinetics and mechanism of oxidation of isoleucine by TriBACC in acid medium.

2. MATERIALS AND METHODS

Reagent grade chemicals and double distilled water were used throughout the work. Acetic acid was purified by distillation over CrO_3 (S.D. Fine chemicals) followed by fractionation in the presence of acetic anhydride. The pure acetic acid fraction was collected over 118ºC. A solution of L-isoleucine (S.D. Fine chemicals) was prepared by dissolving an appropriate amount of recrystallized sample in double-distilled water. The TriBACC was synthesized as previously reported [5] and a solution of the TriBACC was prepared in acetic acid and stored in a brown bottle to prevent its photochemical degradation. All other reagents were of analytical grade and their solutions were prepared by dissolving the requisite amount of the samples in doubly distilled water. The reaction was carried out by a UV-VIS spectrophotometer with a 1 cm quartz cell at 445 nm and Kinetics of the reaction was followed in the temperature range 303-323 K.

2.1. Kinetic measurements

The reactions were followed under pseudo-first-order conditions by keeping large excess (x 10 or greater) of the L-Isoleucine over TriBACC. The reactions were followed at constant temperatures (303K), by monitoring the decrease in absorbance of [TriBACC]

spectrophotometrically at 445 nm. The pseudo-first-order rate constant kobs were evaluated from the linear (r = 0.990-0.999) plots of log [TriBACC] against time for up to 80% completion of the reaction. Duplicate kinetic runs showed that the rate constants were reproducible to within ±3%.

3. RESULTS AND DISCUSSION

3.1. Stoichiometry and product analysis

Different sets of reaction mixtures that contain varying ratios of TriBACC to L-isoleucine in the presence of a constant amount of H_2SO_4 were kept for 2 hr. at 298 K in a nitrogen atmosphere in a closed vessel. The remaining concentration of TriBACC has estimated spectrophotometrically at 445 nm the results indicate that the one moles of TriBACC consumed one mole of L-isoleucine as shown in Scheme 1.Product analysis was carried out under kinetic conditions. In this experiment, the mixture of L-isoleucine (0.1 mol dm^{-3}) and TriBACC (0.01 mol dm^{-3}) was made up of 50 ml with acetic acid in the presence of HCl (0.8 mol dm^{-3}). The mixture was kept in the dark for twelve hours until completion of oxidation. It was then treated overnight with an excess (125 ml) of a freshly prepared saturated solution of 2, 4-dinitrophenylhydrazine in 2M HCl. The precipitated 2, 4-dinitrophenylhydrazone (DNP) was collected by filtration, dried and recrystallized from ethanol and weighed. The product was found identical m.p and mixed m.p with an authentic sample of DNP of 2-Methyl butanal. Ammonia was identified by Nessler's reagent. The presence of corresponding aldehyde and ammonium ions were also confirmed by the spot tests [16], with chromotropic acid and p-nitrobenzene diazonium chloride respectively.

3.2. Effect of oxidant, amino acid and sulphuric acid concentration on the rate of reaction

The effect of oxidant was studied by keeping all other concentration constant at 298 K. The concentration of oxidant was varied from 2.0 x 10^{-3} to 6.0 x 10^{-3}mol $dm^{-3}$and given in Table-1. The pseudo-first-order rate constant, kobs, are found to increase with an increase in the concentration of oxidant. The plot of log kobs versus log [TriBACC] is linear with a positive slope indicating the first-order dependence of the reaction on the [TriBACC] as shown in Fig.1. The pseudo-first-order rate constants are found to increase with an increase in the concentration of L-isoleucine varied from 1x10^{-2} to 5x10^{-2}mol dm^{-3} at a constant concentration of TriBACC and sulphuric acid, the plot of kobs against [L-isoleucine] are also linear indicating the first-order dependence of the reaction rate on the [L-isoleucine] Fig. 2. The rate of reaction has also been studied by varying sulphuric acid concentration. It is observed that the rate of reaction increases with increasing the concentration of sulphuric acid and a plot of log (H_2SO_4) vs. Log (Kobs) is also linear with a less than unit slope indicates the fractional-order dependence of rate on [H^+] Fig. 3.

Table 1. Effect of concentration of L-isoleucine, TriBACC and H^+ on the kobs values at 303 K. [KCl] = 3.0 × 10^{-3}mol dm^{-3}.

10^{-3}TriBACC (mol dm^{-3})	**10^{-2} L-isoleucine (mol dm^{-3})**	**H^+ (mol dm^{-3})**	**10^{-4}Kobs S^{-1}**
1.0	1.0	1	12.5

1.0	2.0	1	13.4
1.0	3.0	1	14.1
1.0	4.0	1	15.1
1.0	5.0	1	16.2
2.0	1.0	1	12.7
3.0	1.0	1	13.1
4.0	1.0	1	13.3
5.0	1.0	1	14.1
6.0	1.0	1	14.9
1.0	1.0	2	13.3
1.0	1.0	3	14.3
1.0	1.0	4	15.8
1.0	1.0	5	16.9
1.0	1.0	6	17.1

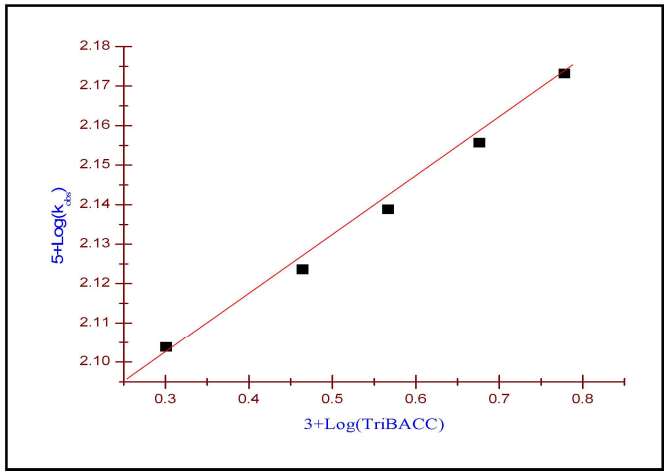


Fig. 1. 3+log (TriBACC) verses 5+log (kobs).

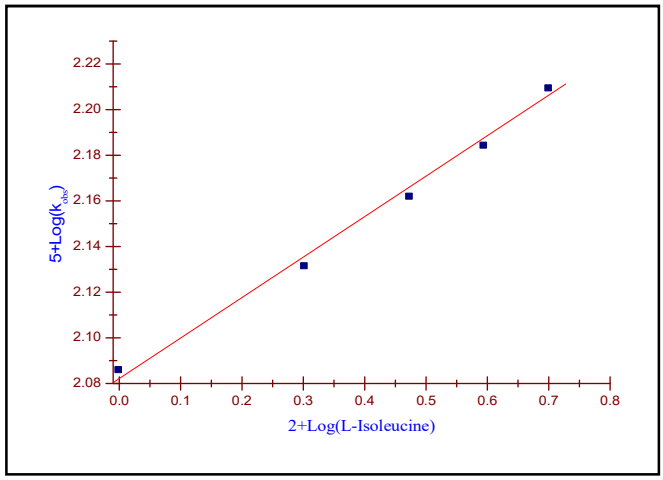


Fig. 2. 2 +log (L-isoleucine) verses 5+log (kobs).

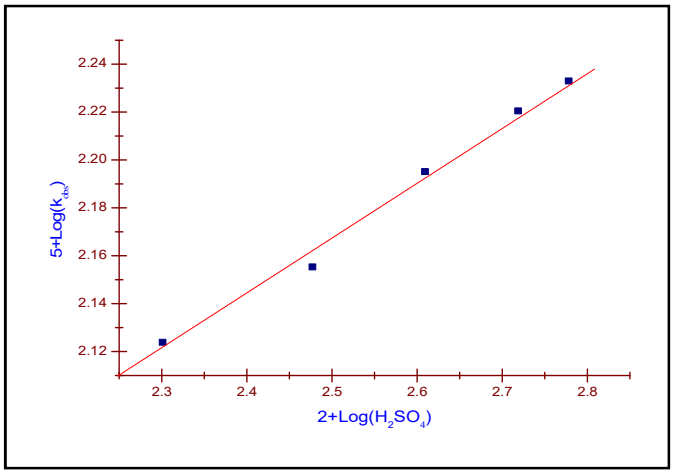


Fig. 3. 2 +log (H_2SO_4) verses 5+log (kobs).

3.3. Effect of salts

The effect of added salts on the reaction rate was studied by adding 0.001 mol dm^{-3} of the salts, by keeping the concentrations of isoleucine, H_2SO_4 and TriBACC constant. It was observed that the rate of oxidation does not alter by the addition of salts Table-2.

3.4. Effect of Temperature

To study the effect of temperature on the rate of oxidation of isoleucine by TriBACC has been investigated at 303-323K by keeping the concentration of isoleucine, TriBACC& H_2SO_4 constant. Rate constants are given in Table-3. The Arrhenius plots of log k vs. 1/T were found to be linear Figure 4.The activation energy (Ea) was calculated from the slope of the plots. From this value, the thermodynamic parameters $\Delta H^{\#}$, $\Delta S^{\#}$, $\Delta G^{\#}$ was evaluated Tabel-4.

Table 2. Effect of variation of [salts] on reaction rate.
L-isoleucine $1x10^{-2}$ mol dm^{-3}; [TriBACC] = 1 $x10^{-3}$ mol dm^{-3}; [H_2SO_4] = 1 mol dm^{-3}, Temperature. = 303K

Salts 10^{-3}mol dm^{-3}	**k_{obs} S^{-1}**
KCl	0.00133
NaCl	0.00139
$CaCl_2$	0.00142
$MgSO_4$	0.00134
$Ag(NO_3)_2$	0.00137

Table 3. Effect of variation of Temperatures on reaction rate.
Isoleucine 1x10^{-2} mol dm^{-3}; [TriBACC] = 1 x10^{-3} mol dm^{-3}; [H_2SO_4] = 1mol dm^{-3}.

Temperature	**k_{obs} 10^{-4} S^{-1}**
303 K	15.9
308 K	18.7
313 K	22.3
318 K	24.7
323 K	26.1

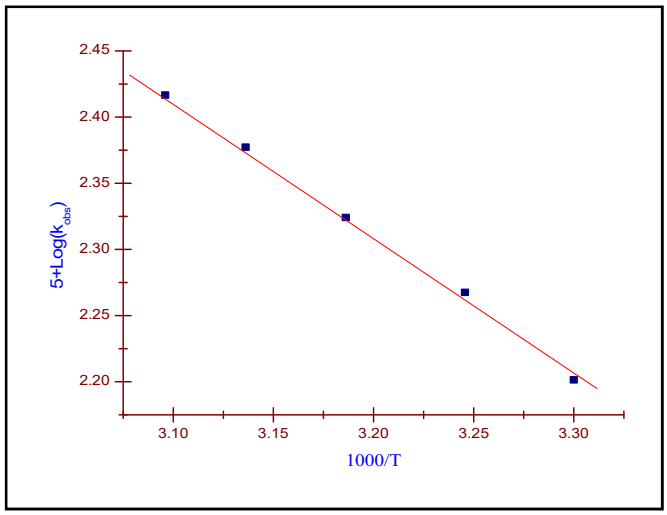


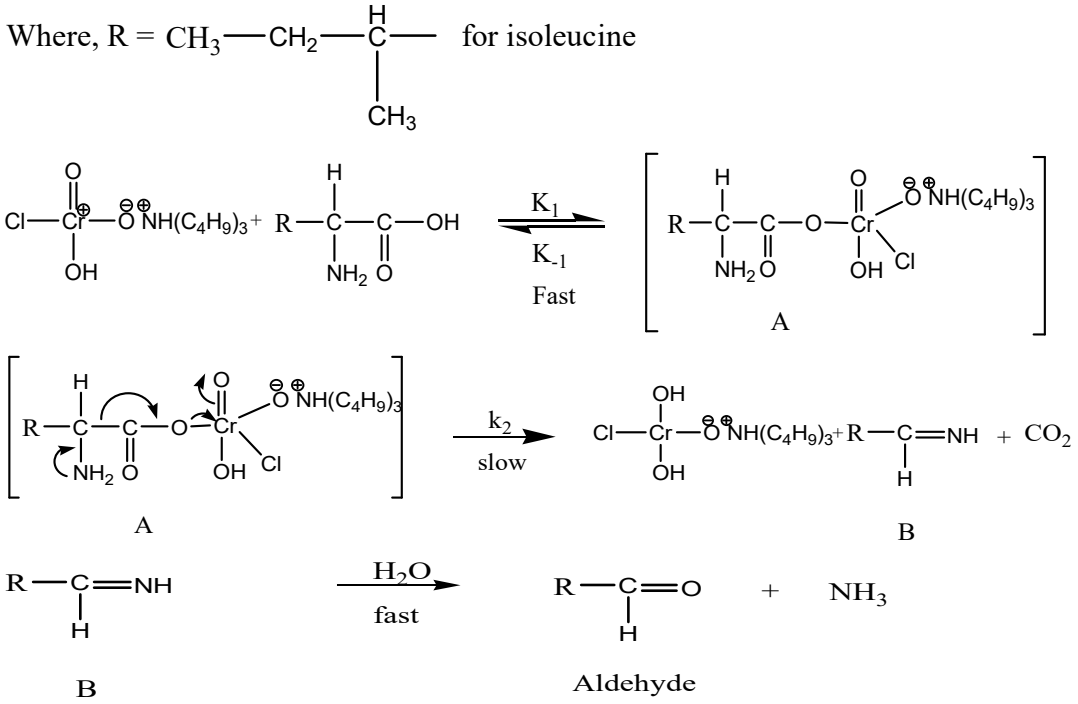
Fig. 4. 5+log k_{obs}verses1000/T.

Table 4. Activation Parameters.
Isoleucine 1x10^{-2} mol dm^{-3}; [TriBACC] = 1 x10^{-3} mol dm^{-3}; [H_2SO_4] = 1 mol dm^{-3}, Temperature. = 303K

Activation Parameters	
Ea KJ mol^{-1}	13.23
$\Delta H^{\#}$ KJ mol^{-1}	10.54
$\Delta S^{\#}JK^{-1}$ mol^{-1}	-263.7
$\Delta G^{\#}$ KJ mol^{-1}	95.74

3.5. Effect of acrylonitrile as the free radical detector

The addition of acrylonitrile to the reaction mixture at 35 and 45^{0}C did not alter the reaction rate and there was absolutely no polymer or change in the viscosity was detected, showing the absence of free radicals in the reaction mechanism.



Scheme 1. Mechanism of oxidation of isoleucine by TriBACC.

4. CONCLUSION

Sulphuric acid-catalyzed the oxidation of isoleucine by Tributyl ammonium chlorochromate was studied in an acid medium at 303 K. It shows first-order dependence of rate to TriBACC, isoleucine and fractional order on sulphuric acid. Isoleucine was oxidized into the corresponding carbonyl compound. An added salt does not alter the rate of reaction and also the addition of acrylonitrile did not polymerize the reaction. The thermodynamic parameters have been evaluated. 2-Methyl butanal is the main product of the reaction and a suitable kinetic mechanism has been proposed (Scheme 1).

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