KINETICS OF OXIDATION OF SOME ORGANIC SUBSTRATES

ABSTRACT

DIDCY LALOO, M. Sc.

DEPARTMENT OF CHEMISTRY
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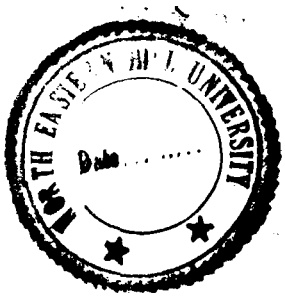
A THESIS SUBMITTED
IN
FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY



To

THE NORTH-EASTERN HILL UNIVERSITY
SHILLONG
INDIA

NOVEMBER, 1987

SUMMARY

1. KINETICS OF OXIDATION OF GLYCINE, ALANINE, VALINE, LEUCINE AND PHENYLALANINE BY ALKALINE HEXACYANOFERRATE(III)

The kinetics of oxidation of some amino acids (glycine, alanine, valine, leucine and phenylalanine) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate (II) ions, and the addition of salts, did not have any effect on the rates of these reactions.

The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical

intermediates in the rate determining step. The radical underwent further reaction, by way of the imino acid, to yield the products. The products formed from the oxidation of these amino acids were the respective α-keto acids, which were characterized by chemical methods.

2. KINETICS OF OXIDATION OF SERINE AND THREONINE BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (serine and threonine) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

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The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical intermediates in the rate determining step. The radical underwent further reaction, by way of the ionic acid, to yield the products. The products formed from the oxidation of these amino acids were the respective α-keto acids, which were characterized by chemical methods.

3. KINETICS OF OXIDATION OF CYSTEINE AND METHIONINE BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (cysteine and methionine) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

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The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical intermediates in the rate determining step. The radical underwent further reaction, by way of dimerization, to yield the products. The products formed from the oxidation of these amino acids were the disulfide (from cysteine) and the sulfoxide (from methionine), which were characterized by chemical methods.

4. KINETICS OF OXIDATION OF TYROSINE AND TRYPTOPHAN BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (tyrosine and tryptophan) by potassium hexacyanoferrate(III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

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5. KINETICS OF OXIDATION OF GLUTAMIC ACID AND ASPARTIC ACID BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (glutamic acid and aspartic acid) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

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6. KINETICS OF OXIDATION OF LYSINE, ARGININE AND HISTIDINE BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (lysine, arginine and histidine) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength,

under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

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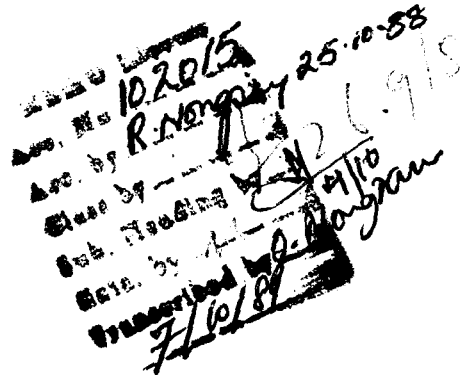
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Dr. Mahendra K. Mahanti

I certify that the thesis entitled "KINETICS OF OXIDATION OF SOME ORGANIC SUBSTRATES" submitted by MISS DIDCY LALOO for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by her under my supervision. She has been duly registered, and the thesis presented is worthy of being considered for the Award of the Ph.D. Degree. This work has not been submitted for any Degree of any other University.

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November 4, 1987.

DEDICATED

TO

THE MEMORY OF MY PARENTS

WHO HAD A GREAT INFLUENCE

ON MY LIFE AND CAREER

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Didcy Laloo.
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CONTENTS

INTRODUCTION

INTRODUCTION

Metal ion oxidants can function either as one-equivalent or two-equivalent reagents. One-equivalent oxidants are those which accept a single electron by direct transfer or by interaction with a hydrogen atom. Two-equivalent oxidants can accept two electrons from the substrate.

In one-equivalent redox processes, the change of valency can be brought about by either an inner-sphere or an outer-sphere mechanism (1).

1. Inner-sphere mechanism:

The inner-sphere or bonded mechanism envisages a direct contact between the oxidant and the reductant, and the transition state is characterised by a ligand which is bonded to both metal ions. It can therefore act as a bridge between them for the transfer of an electron. The typical reaction is (2):

$$Cr^{2+}(aq) + (NH_3)_5\ Co^{III}\ Cl^{2+} \longrightarrow$$

$$CrCl^{2+}(aq) + Co^{2+}(aq) + 5\ NH_3 \quad .$$

The formation of an inner-sphere transition state would lead to considerable distortion of the ions, which may thus asist electron transfer by reducing the energy terms involved. In order that an inner-sphere mechanism can operate,

the ligand present should behave in a bidentate manner. The ligand must possess available pi-orbitals, and one of the reagents involved must have a ligand which can be easily displaced.

2. Outer-sphere mechanism:

Outer-sphere electron exchange reactions constitute the simplest class of electron transfer reactions. In an outer-sphere mechanism, the inner co-ordination shell of both reagents is preserved intact in the transition state. Since the metal-ligand distances will be affected by valency change, some distortion of the inner shells would occur, but no metal-ligand bond would be broken or formed.

The criteria for outer-sphere or non-bonded reactions are as follows:

(a) there must be no observed transfer of ligands between the reagents;

(b) the rate of the reactions may have any magnitude, but if it is faster than any reasonable rate of substitution of the ligands of either oxidant or reductant, then the reaction can be classified as an outer sphere reaction;

(c) the activation energy of this type of reaction should be much less than that anticipated for a mechanism involving ligand-metal bond fission;

(d) the kinetic law of the reaction must show that the transition state has the same composition as the sum of the reagent molecules.

It would therefore be expected that most outer sphere processes would be fast reaction between complexes which are substitution-inert, in solvents which are not themselves providing ligands.

Most organic reaction processes are explicable in terms of electron shifts accompanying bond formation and bond breaking. The rates and activation energies of such reactions are therefore of considerable interest.

The essential steps in these reactions would be:

(a) the approach of two reagent ions, which may be aided or hindered by electrostatic forces;

(b) before electron transfer can occur, two conditions must be obeyed (3), in accordance with the Franck-Condon principle:

(i) the electron transfer must not alter the energy of the system. This electron transfer occurs in a very short time, and the only possible loss or gain of energy would be by radiation. Electron transfer can occur only if the oxidant and reductant have been vibrationally excited to the same total energy. Thus, when the two valency states of a reagent are closely similar in bond energy and geometry, the outer-sphere process would be expected to be most facile.

(ii) the overall spin angular momentum should not be altered.

(c) The process of electron transfer itself. If the reagent complexes are sufficiently close and of suitable symmetry, such that there is some interaction between the orbitals involved in electron transfer, then the probability of electron transfer would be essentially unity. The requirement of orbital overlap can be related to the orbitals of the reagents. This would suggest that in octahedral complexes, t_{2g} electrons might be more easily transferred than e_g electrons which are in orbitals directed along the metal-ligand axis.

In order to establish that an outer-sphere or non-bonded electron transfer can occur from an organic compound to a metal oxidant, it is necessary to choose a suitable model system in which the probability of non-bonded mechanism would be a maximum, and that of the competing bonded process a minimum. The oxidant should be so chosen such that it possesses ligands which are slow or difficult to replace. The organic substrate should be so chosen such that it is not likely to displace ligands from a metal ion complex. Examples of such exchange-inert oxidants are the iron (III) tris-o-phenanthroline complex (4), Ir Cl_6^{2-} ion (5), Mn (III) tris-acetylacetone

complex (6), ceric ions (7), and the hexacyanoferrate (III) ion (8,9).

In the reactions of one-equivalent oxidants with organic substrates, the most frequently encountered oxidation process would seem to correspond to an electron transfer between substrate and oxidant, accompanied by the breaking of a C-H bond and loss of a proton to give a substrate radical as for example (10):

$$Co\ (III) + C_6H_5CH_3 \longrightarrow Co\ (II) + C_6H_5\dot{C}H_2 + H^+$$

It would therefore be expected that the loss of a proton would be slower than electron transfer, and hence would correspond to the rate determining step.

The presence of radicals may be inferred by their oxidation or reduction of added inorganic ions, or by their ability to cause polymerisation to occur with added monomer, as for example, acrylamide or acrylonitrile. If the radical is present in sufficiently high concentrations, its presence can be detected by electron spin resonance spectroscopy.

Although the radical may undergo many other processes, it is most probable that in the presence of an excess of oxidant, the radical will be oxidised further. Examples are known where the main mechanisms of this step may be: (a) a non-bonded electron transfer (conversion of a neutral radical to a cation), as for example the oxidation of 2,6-dimethylphenol by hexachloroiridate ion (5), and the

oxidation of hydroquinones by ferric ions (11); (b) bonded electron transfer or the transfer of a ligand from the oxidant to the radical, as for example, the reduction of $IrCl_6^{2-}$ by Cr^{2+} ions (12); (c) redox substitution, in which the radical remains attached to the complex, as for example, the phenylation of the ferricenium complex (13,14); (d) redox addition, where the radical remains attached to the complex, as for example, the reaction of hexacyanoferrate (III) with isobutyraldehyde (15).

Oxidation of organic substrates with potassium hexacyanoferrate (III).

Potassium hexacyanoferrate (III), $K_3Fe(CN)_6$, is essentially a substitution-inert transition metal complex (16). It does not exchange its ligands at a rate fast enough to compete with rapid electron transfer. Therefore, oxidations by hexacyanoferrate (III) ion occur by means of a non-bonded electron transfer or outer sphere process, whereby an electron is transferred from the substrate to the metal ion through the cyano ligand.

In acidic medium, potassium hexacyanoferrate (III) has been used for the oxidation of sulfur containing compounds (17-27), toluene and substituted toluenes (28), diphenylmethane and triphenylmethane (29) fluorene (30), unsaturated

systems (31) and polynuclear systems (32).

In alkaline medium, Potassium hexacyanoferrate(III) has been extensively used for the oxidation of various kinds of organic substrates such as aldehydes (33-38), ketones(33,34,39-45), alcohols and diols (46-59), sulphur compounds (60-68), hydroxyl ion (69), acids (70-81), sugars (82-85), hydrazines (86-89), acyloins (90-92) As(III) (93-95), hypophosphite (96,97), hydrocarbons (98), Phenols (99-116), amino acids (117,118), amines (119-130), and the 10-metnyl-acridinium cation (131).

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SCOPE OF THE PRESENT INVESTIGATION

SCOPE OF THE PRESENT INVESTIGATION

The present investigation is a detailed kinetic probe into the oxidation of amino acids by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere.

The purpose of this kinetic investigation has been to attempt to extend the scope of this extremely efficient and versatile one-electron oxidizing agent, potassium hexacyanoferrate (III), in alkaline medium, and to explore and establish mechanistic pathways of reactions involving the oxidation of amino acids.

The purpose of the present study was:

(1) To study the kinetic features of the oxidation of amino acids; and

(2) To demonstrate the usefulness of potassium hexacyanoferrate (III) as a reagent which can bring about the oxidation of amino acids, in a manner similar to that observed in the enzymatic oxidation of amino acids.

In the present investigation, the amino acids chosen for the purposes of oxidation by potassium hexacyanoferrate (III), in alkaline medium, have included:

(1) Glycine, alanine, valine, leucine and phenylalanine.

(2) Serine and threonine.

(3) Cysteine and methionine.

(4) Tryptophan and tyrosine.

(5) Glutamic acid and aspartic acid.

(6) Lysine, arginine and histidine.

For each oxidation reaction, the stoichiometry of the reaction has been determined. The concentrations of the substrate, oxidant and alkali have been varied, and the effects of these variations on the reaction rates have been studied. Changes in the temperature of the reaction medium have been made, and the activation parameters were evaluated. Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate (II) ions, the addition of salts, and the effect of added product on the rates of the reactions have been studied.

The presence of radical intermediates has been detected and characterized by ESR spectroscopy. For each oxidation reaction, the products were isolated and characterized by chemical methods. The mechanistic pathways for the oxidation of these amino acids by potassium hexacyanoferrate (III), in alkaline medium, have been suggested.

EXPERIMENTAL

PURIFICATION OF MATERIALS AND PREPARATION OF COMPOUNDS

Conductivity Water:

Conductivity water was prepared by the following method: tap water was distilled first with alkaline potassium permanganate and then redistilled with Merck "Pro Analysi" sulphuric acid from an all-glass vessel. This sample of double distilled water was further distilled from an all-quartz vessel (Sunvic, U.K.). The conductivity water thus prepared was utilised for the preparation of all the solutions used in the kinetic determinations.

Sodium hydroxide:

E. Merck sample was used.

Potassium hexacyanoferrate (III):

E. Merck sample was used.

Potassium hexacyanoferrate (II):

E. Merck sample was used.

Sodium perchlorate:

Sodium perchlorate was prepared by neutralising 70% perchloric acid (E. Merck) with sodium hydroxide (E.Merck). The solution was concentrated, when crystals of sodium perchlorate were obtained. The crystals were filtered,

and recrystallised from water. The recrystallised product was dried over silica gel under vacuum. This sample of sodium perchlorate was used for the preparation of stock solutions which were employed for maintaining the ionic strength of the medium.

Substrates

All amino acids used were the L-amino acids. L-Glycine was obtained from Loba Chemical Co.. L-Cysteine and L-histidine were E.Merck samples. L-Lysine, L-phenylalanine, L-leucine, L-arginine, L-valine, L-serine, L-threonine, L-tyrosine and L-tryptophan were BDH samples. L-Methionine, L-alanine, L-glutamic acid and L-aspartic acid were obtained from SISCO Research Laboratories.

The melting points and the spectral data obtained for each of the substrates used, are summarised in Table 1.

TABLE 1

Substrate	Melting Point(°C)	uv(nm)*
(1)	(2)	(3)
Glycine	262	220(W)
Alanine	295	215(W)
Valine	298	210(W)
Leucine	293	190(W)
Phenylalanine	284	258(M)
Serine	228	225(M)
Threonine	251	240(M)
Cysteine	240	236(M)
Methionine	283	208(M)
Tyrosine	310	295(M)
Tryptophan	290	290(M)
Glutamic acid	224	206(M)
Aspartic acid	324	
Lysine	224	220(M)
Arginine	207	205(M)
Histidine	287	210(M)

*W = Water; M = Methanol.

All uv/visible spectra were recorded on an UV-26 (Beckman) spectrophotometer, and esr spectra on an E-4 (Varian) EPR spectrometer.

Kinetic method:

All the standard flasks and reaction vessels were of pyrex glass with well-ground stoppers. The reaction vessels used were stoppered conical flasks which were painted black on the outside to prevent any photochemical change. All the glass apparatus used were tested for loss of solvent, and the loss was found to be negligible. The standard flasks, reaction vessels and the pipettes used were standardised, using conductivity water, and the correction was found out and applied.

An electrically operated thermostatic water-bath was used. It was provided with sufficient thermal lagging, suitable heaters and stirrers with proper cooling arrangements for continuous work. A xylene-filled regulator, working in conjunction with an electronic relay, was used to maintain the required temperatures accurately, with fluctuations of not more than $\pm$ 0.1^{o}C. The temperatures were recorded by means of an accurate sensitive thermometer, reading to tenths of a degree. The bath-liquid was water, covered with a layer of liquid paraffin to minimise evaporation of water and loss of heat due to radiation.

Spectrophotometers:

For absorption measurements, the spectrophotometers used were (a) Digital spectrophotometer type 106, MK II model (Systronics), and (b) uv-26 (Beckman) UV-Visible spectrophotometer.

(a) The MK II model (systronics) spectrophotometer was a single beam spectrophotometer having a grating of 600 lines/mm and a wavelength range from 340 nm to 960 nm. The nominal spectral slit width was 20 nm, constant over the entire range. The full scale deflection could be obtained over the wavelength range of 340 nm to 600 nm. By the addition of a red filter and interchanging of the phototube, the range could be extended to 960 nm. In order to ensure maximum sensitivity of the instrument, and to minimise the errors in measurements of optical density due to fluctuations in voltage, the spectrophotometer was connected to the mains through an external voltage-stabiliser. This was in addition to the in-built voltage-stabiliser within the instrument itself. The light source was a 15 watt tungsten lamp operated by a regulated power supply. The instrument was calibrated, as specified in the instruction manual, over the range of concentrations of K_2CrO_4 in KOH solutions, so as to verify Beer's law at 370 nm.

(b) The UV-26 (Beckman) UV-Visible spectrophotometer was a single beam monochromator, having a filter-grating of 1200 lines/mm, and a wavelength range from 190nm to 900nm. This instrument employs a tungsten light source in the visible region of the spectrum (340nm to 900nm). This spectrophotometer had a thermostatic control arrangement to maintain the temperature, and the absorbance value was directly displayed on the digital display and on the recorder. As specified in the instruction manual, photometric linearity was checked over the range of concentrations of potassium chromate in KOH solutions, so as to verify Beer's law at 370nm.

Absorption cells:

The absorption cells were of 'Corning' glass and of 8 ml capacity for the spectrophotometer 106 MK II model (Systronics). Quartz cells of 5 ml capacity were used for spectral determinations with the UV-26 spectrophotometer (Beckman). All the cells were thoroughly cleaned by aqueous ethanol and acetone, and dried before they were used for the spectral measurements. After the transfer of the solution to the cell, care was taken to see that no solution adhered to the outer surface of the cell. During the measurements, the cells were covered.

Solutions of hexacyanoferrate (III) in methanol-water mixtures (60-40 to 75-25%, v/v) were prepared. The absorbance of each of these solutions was scanned over the range of wavelengths from 350 nm to 700 nm. The maximum absorption in each case was located at 420 nm (Fig.1). At this wavelength of 420 nm, the absorption due to $Fe(CN)_6^{3-}$ has been observed to be a maximum (1), the absorption due to $Fe(CN)_6^{4-}$ being negligible (2).

At 420 nm, Beer's law was obeyed over the range of concentrations of solutions used. A typical graph of the optical density against the concentration of $Fe(CN)_6^{3-}$ is shown (Fig.2).

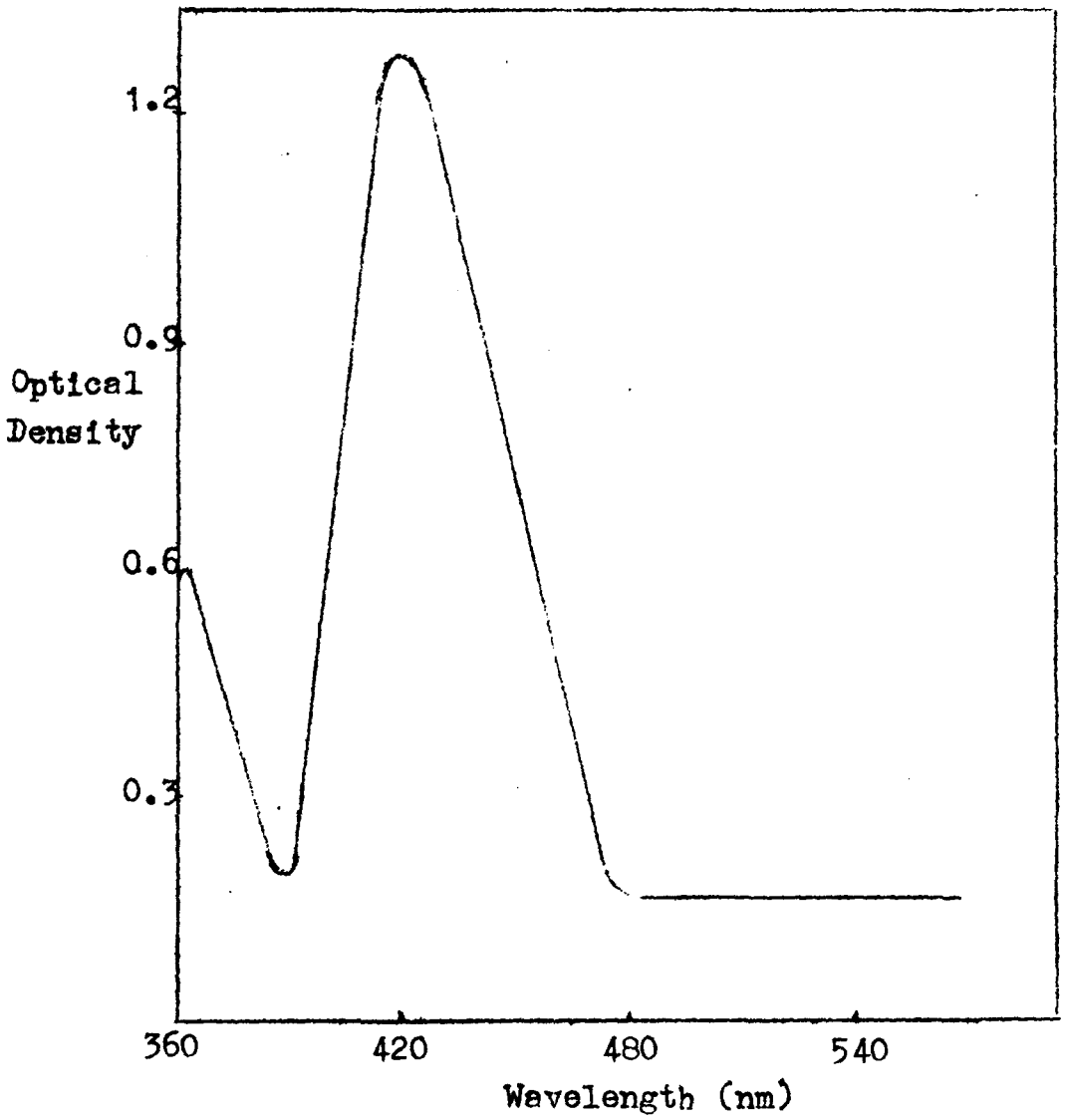

Fig. 1 Plot of absorbance against wavelength (nm) of Potassium Hexacyanoferrate(III).

(Determination of maximum absorption)

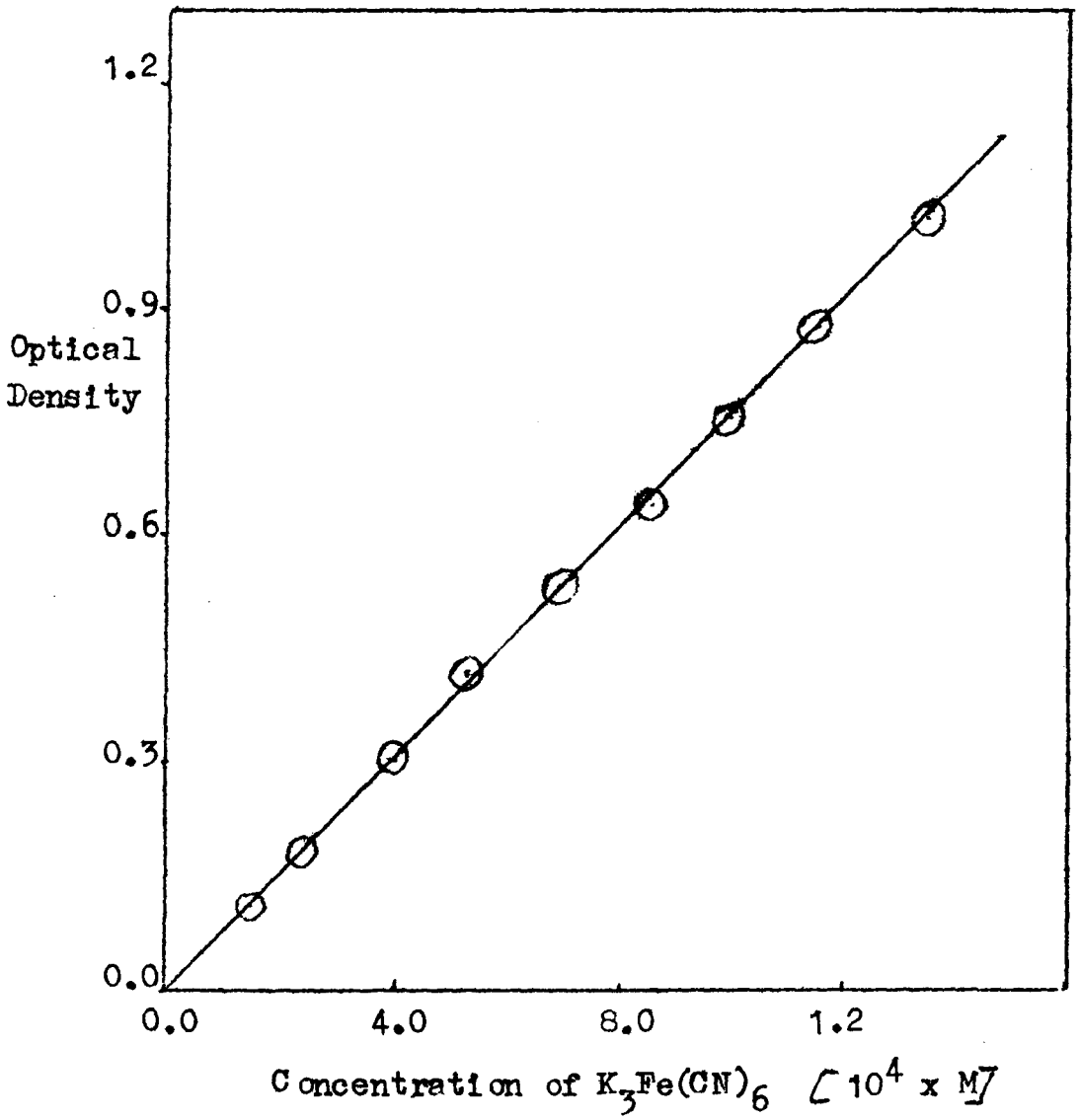

Fig. 2 Verification of Beer-Lambert's Law. Plot of concentration against optical density at 420nm .

In this investigation, all the optical density measurements were carried out at a wavelength of 420 nm.

Rate measurements

A known amount of the substrate was weighed accurately into a 10 ml standard flask and made up in water. Potassium hexacyanoferrate(III) was accurately weighed out into a 10 ml standard flask, and dissolved in a small volume of water. The requisite amount of sodium hydroxide solution, whose strength had been determined, was added to maintain the required alkalinity. Sodium perchlorate was added so as to maintain a constant ionic strength of the medium. The solution was then made up in water. Sufficient time was allowed to compensate for any change of heat during dilution. The two reactant solutions were separately thermostated at the required temperature for 1 hour, under a nitrogen atmosphere. The solutions were then mixed in equal volumes, by syringing into the spectrophotometric cell. The reaction mixture was homogeneous throughout the duration of the reaction.

The progress of the reaction was followed by observing the disappearance of hexacyanoferrate (III). Readings were taken at regular intervals of time, by noting the decrease in optical density at 420 nm, spectrophotometrically.

All the kinetic experiments were carried out in triplicate, and the rate constants which were determined were found to be reproducible to within ± 3%.

Calculations:

(a) Rate constants:

For all the kinetic determinations, pseudo-first order reaction conditions have been used, wherein the concentration of the substrate has been taken in a very large excess over that of the concentration of the oxidant.

The pseudo-first order rate constant, k_{obs}, expressed as sec^{-1}, were calculated from the equation (3):

$$k_{obs} = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad \ldots \quad (1)$$

where D_o was the initial optical density of the reaction mixture, and D_t was the optical density at time, t.

The logarithmic plots of optical densities against time were linear, and extrapolation to zero time gave the values of D_o.

The values of the second order rate constant, k_2, expressed in $M^{-1}\ s^{-1}$, were computed by dividing the pseudo-first order rate constant (k_{obs}, s^{-1}) by the concentration of the substrate (M).

All values of rate constants were the average of three experiments, with agreement being within ± 3%.

(b) Thermodynamic activation parameters:

These parameters were determined from a study of the effect of temperature on the rate of the reaction.

The various parameters have been calculated as follows:

(i) Activation energy (E)

From the linear plot of log k_{obs} against the reciprocal of temperature (T),

$$\text{slope} = - \frac{E}{2.303\ R}$$

$$E = - \text{slope} \times 2.303\ R \ (\text{kJ mol}^{-1})$$

(ii) Frequency factor (A)

$$k_{obs} = A\ e^{-E/RT}$$

$$\log A = \log k_{obs} + \frac{E}{2.303\ RT}$$

(iii) Enthalpy of Activation ($\triangle H^{\neq}$)

$$\triangle H^{\neq} = E - RT$$

(iv) Entropy of Activation ($\triangle S^{\neq}$)

$$k_{obs} = \frac{kT}{h}\ e^{\triangle S^{\neq}/R} \cdot e^{-\triangle H^{\neq}/RT},$$

$$\triangle S^{\neq} = 2.303\ R \left[\log k_{obs} + \frac{\triangle H^{\neq}}{2.303\ RT} - \log \frac{kT}{h} \right],$$

where k is the Boltzmann constant, and h is the Planck's constant.

Stoichiometry:

Reaction mixtures containing the substrate and an excess of hexacyanoferrate (III), taken in water, containing the requisite amounts of sodium hydroxide and sodium perchlorate, were allowed to react to completion at a particular temperature. The hexacyanoferrate (III) which was left, was analysed spectrophotometrically at 420 nm. The individual stoichiometric regulations are shown along with the reactions of each of the substrates with the oxidant.

Product Analysis

(1) Products obtained from the oxidation of glycine, alanine, Valine, leucine, phenylalanine, serine, threonine, lysine, arginine, histidine, tyrosine, tryptophan, glutamic acid and aspartic acid.

Using the same experimental conditions that were used for the kinetic determinations, solutions of substrate and oxidant, taken in NaOH (ionic strength adjusted by the requisite amounts of $NaClO_4$), were mixed and kept at the required temperature for 24 hours under nitrogen.

(i) The evolution of ammonia was shown by partial distillation of the reaction mixture. The ammonia formed was absorbed in an excess of standard acid (0.1N HCl). The excess of

acid was then back-titrated (against base), in the presence of methyl red indicator (4).

(ii) The reaction mixture was extracted with ether, washed with water, dried over anhydrous $MgSO_4$, and then concentrated. The product obtained was the corresponding keto acid. The keto acid as the reaction product, in each case, was detected by spot tests (5), in agreement with earlier work (6).

(iii) The reaction mixture was treated with an acidic solution of sodium bisulfite, and cooled in ice. 25 ml. of 2,4-dinitrophenylhydrazine solution (0.05 M) was added, and the mixture allowed to stand overnight at $0^{\circ}C$. The solid compound formed was filtered, dried, recrystallized from a mixture of ethyl acetate and petroleum ether, and weighed as the 2,4-dinitrophenylhydrazone derivative of the corresponding keto acid (yields, 70%-80%, in all cases).

For the oxidation of the amino acids by alkaline hexacyanoferrate(III), the products obtained are shown in Table 2.

TABLE 2

Product Analysis

Amino Acid	Keto acid (Oxidation Product)	Melting point 2,4 DNP derivative (°C)
(1)	(2)	(3)
Glycine	Glyoxylic acid	203
Alanine	Pyruvic acid	216
Valine	α-ketoisovaleric acid	196
Leucine	α-ketoisocaproic acid	162
Phenylalanine	β-phenylpyruvic acid	162
Serine	β-hydroxypyruvic acid	162
Threonine	α-keto-β-hydroxy butyric acid	157
Lysine	α-keto-ε-aminocaproic acid	212
Arginine	α-keto-δ-guanidinovaleric acid	216
Histidine	β-imidazolylpyruvic acid	190
Tyrosine	β-(p-hydroxyphenyl)pyruvic acid	178
Tryptophan	β-indolylpyruvic acid	155
Glutamic acid	α-ketoglutaric acid	220
Aspartic acid	Oxaloacetic acid	218

(2) Product from the oxidation of cysteine

Using the same experimental conditions that were used for the kinetic determinations, cysteine and hexacyanoferrate (III) solutions, in NaOH(ionic strength adjusted by the addition of $NaClO_4$), were allowed to react at 30°C for 24 hours, under nitrogen. The reaction mixture was taken in ether, washed with water, the ether evaporated, and the residue refluxed with toluene for 1 hour. The solution was concentrated and allowed to cool overnight. Crystals of the disulfide were precipitated, which were recrystallized from ether (mp. 260°C).

(3) Product from the oxidation of methionine

Using the same experimental conditions that were used for the kinetic determinations, methionine and hexacyanoferrate (III) solutions in NaOH (ionic strength adjusted by the addition of $NaClO_4$) were allowed to react at 30°C for 24 hours, under nitrogen. To the reaction mixture was added 5 ml of benzoyl chloride and 10 ml of sodium bicarbonate solution. A precipitate of N-benzoyl methionine sulfoxide was obtained, which was filtered, washed with water, and recrystallized from ether (mp 183°C).

Tests for Radical formation:

Most of the oxidation reactions investigated were observed to proceed via radical intermediates formed in the rate determining step of these reactions. The presence of these radical intermediates was confirmed by the following tests:

(a) Reduction of inorganic ions, $R^{\bullet} + M^{(n+1)+} \longrightarrow R^{+} + M^{n+}$. Mercuric chloride was easily reduced by these radicals to isoluble mercurous chloride, which was relatively inert towards reoxidation by the oxidant M^{n+}.

(b) Polymerisation of an added olefinic monomer, such as acrylonitrile or acrylamide.

Acrylamide and the substrate were placed in the lower part of a Thunberg tube (7), with the oxidant solution placed in the upper portion of the tube. The system was evacuated, filled with dry nitrogen, and then sealed. The two solutions were mixed and allowed to stand at the reaction temperature. After 30 minutes, there was the formation of a white opalescence, indicating the formation of a polymer.

ESR measurements

The presence of radical intermediates formed in the rate determining steps of these reactions was detected and confirmed by esr measurements.

Using the requisite reaction conditions, the radicals were generated, in a flow system, by mixing the substrate and oxidant, by volume, in an esr sample tube just outside the cavity of the spectrometer. The mixture was placed under high vacuum, in order to expel dissolved oxygen, and the sample tube was placed in the cavity of the spectrometer. The conditions for obtaining the spectrum at room temperature were as follows:
Scan range 4000 G, field set 3300G, modulation amplitude 6.3G, microwave frequency 9.45 GHz , time constant 0.3 sec, scan time 4 minutes.

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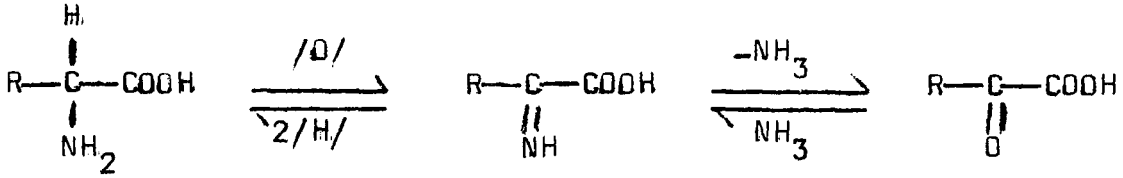
DISCUSSION

CHAPTER 1

KINETICS OF OXIDATION OF GLYCINE, ALANINE, VALINE, LEUCINE AND PHENYLALANINE

Amino acids are the building blocks in protein synthesis. The fact that the important naturally occurring amino acids have α-hydrogen atoms suggests that the biosynthesis and degradation of amino acids occur by way of α-imino acids and α-keto acids, thus:



In metabolism, amino acids are subjected to many reactions, and can supply precursors for various endogenous substances, as for example, hemoglobin in blood. Amino acids undergo various kinds of reactions, depending on whether the particular amino acids contain non-polar groups, polar substituents, acidic or basic substituents.

Various earlier proposals for the derivation of alkaloid structures from common amino acids were considered by Robinson (1) and later expanded (2). Biochemical experiments confirmed the predicted pathways based on Robinson's scheme of biosynthesis. The fundamental skeleton of alkaloids was

derived from common amino acids and other small biological molecules. Simple amines which have been found to occur in plants have been shown to be derived by the decarboxylation of amino acids. Examples are methyl amine from glycine, ethanol amine from serine, isobutyl amine from valine, isopentylamine from leucine, and cadavarine from lysine.

Many naturally occuring nitrogen compounds have basic prpperties in common with the alkaloids, but their structures are relatively simple. Their nitrogen atoms are not incorporated into heterocyclic skeletons. This seems to be derived from amino acids by simple reactions. Decarboxylation of amino acids produces amines. Such amines can be modified by the introduction of methyl groups or hydroxyl groups. Some proto alkaloids are possible precursors of typical alkaloids. Proto alkaloids related to aromatic amino acids, such as tyrosine and dihydroxy phenylalanine, are important precursors of alkaloids. The utilization of nicotine in the sybthesis of amino acids and proteins has been reported (3). Correlation of alkaloid content with the concentration of free amino acids in different kinds of lupine alkaloids have shown the presence of arginine, threonine, glutamic acid, histidine, tyrosine and lysine (4). It has been suggested that arginine could be a normal precursor of the lupine alkaloids (5). Tracer experiments have confirmed that the carbon skeletons of all

the major lupine alkaloids have been derived from lysine (6,7). Many of the indole alkaloids derived their indole nucleus from tryptophan (8). Threonine and isoleucine have been reported to be good precursors of the pyrrolizidine alkaloids (9). There is evidence that alkaloids having the imidazole ring are probably made from histidine (10-12).

The kinetics of oxidations of amino acids have become important, both from a purely chemical point of view and from the point of view of its bearing on the mechanism of amino acid metabolism.

In general, the dissociation of amino acids depends on the pH. of the medium. In strongly acidic or alkaline media, the following equilibria exist:

$$RCH(NH_2)COO^- \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} RCH(\overset{+}{N}H_3)COO^- \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} RCH(\overset{+}{N}H_3)COOH$$

(S^-)	$(S^\pm)$	$(S\overset{+}{H})$
Anion	Zwitterion	Cation.

Amino acids have been oxidized by a variety of oxidizing agents such as persulfate (13), peracids (14), peroxydisulfate (15), chloramine - B (16,17), manganese (III) ion (18), acidic permanganate ion (19), bromate ion (20), peroxomonosulfate (21), N-bromoacetamide (22,23), chloramine-T (24), ceric ions (25), N-bromosuccinimide (26), bromamine-T (27), and by Frémy's salt (28).

EARLIER WORK

Glycine is the precursor for methylamine which has been found to occur in plants. Glycine plays a significant role in the biosynthesis of diterpenoid alkaloids (29), and in the pathways of choline metabolism (30). Glycine is one of the major constituents of silk fibroin and collagen. The formation of δ-aminolevulinic acid from succinyl CoA and glycine is the first step leading specifically to the biosynthesis of porphyrins, which are present in chlorophyll, haemoglobin and cytochromes.

In the biosynthesis of the piperidine alkaloid, mimosine, the alanine substituent has been observed to play an important role (31). Alanine has been found to be among the possible pyridine ring precursors in the biosynthesis of nicotinic acid (32).

On decarboxylation, valine gives isobutylamine, which has been found to occur in many plant species. Some enzyme preparations have been used to carry out such decarboxylation reactions (33).

The decarboxylation of leucine gives isopentylamine which is found to be present in many plant species. Certain enzymes have also been successful in carrying out such decarboxylation reactions (33). Leucine has been shown

to be a precursor for some purine alkaloids (34).

Phenylalanine has been shown to be a precursor in the biosyntheses of tropane alkaloids (35), norbelladine (36,37), colchicine (38,39), and ergotamine (40). Tracer feeding experiments have shown that in the taxine group of alkaloids, the phenylpropane moiety originates from phenylalanine, with an α,β-migration of the amino group (41). The biosynthesis of alkaloids such as galanthamine(42), known to possess analgesic activity comparable to morphine (43), was achieved starting from phenylalanine. The pathway had involved a crucial phenolic coupling step, as predicted by Barton (44). The biosynthesis of rotenone (45), starting from phenylalanine, illustrates the sequential formation of different types of oxygen heterocycles. The construction of tryptophan uses the initial conversion of glucose to phenylalanine (46). Indeed, the literature is replete with instances of phenylalanine being effectively used for the biosynthesis of a wide variety of alkaloids (47-51).

Glycine, alanine, valine, leucine and phenylalanine have been oxidized by a variety of oxidizing agents such as ceric ions (52,53), Fenton's reagent (54), hexacyanoferrate(III) catalyzed by osmium (VIII) ions (55), manganese (III) sulfate (56), Fe^{2+} ions (57), peroxydisulfate catalyzed by Cu^{2+} ions (58), Co^{3+} ions catalyzed by Ag^{+} ions (59), acidic permanganate (60), aquopentacyanoferrate (II)

ion (61), periodate (62), permanganate catalyzed by bromide ion (63), aquomanganese (III) ion in acid medium (64), peroxydisulfate catalyzed by Ag^{+} and Cu^{2+} ions (65-67), N-bromosuccinimide (68-70), chloramine - T (71,72), bromamine - B (73,74), N-bromoacetamide (75), lead tetra-acetate (76) peroxomonosulfate (77), acid bromate (78), phenyliodosoacetate (79,80), potassium bromate (81), bromine catalyzed by osmium (VIII) ion (82), and by Ag^{2+} ions (83).

Alanine has been oxidized by a variety of oxidizing agents such as N-bromosaccharin (84), chloramine - B (85), N-chlorobenzamide (86), peracids (87,88), chloramine - T(89), N-bromosuccinimide (90), peroxydisulfate catalyzed by Cu^{2+} ion (91), and by Fenton's reagent (92).

Valine has been oxidized by chloramine-T (93), phenyliodosoacetate and lead tetraacetate (94), manganese (III) sulfate (95), and by N-bromosuccinimide (96).

Leucine has been oxidized by metaperiodic acid (97), trinitrobenzene sulforic acid (98) manganese (III) sulfate (99), and by hexacyanoferrate (III) catalyzed by ruthenium(VI) ion (100).

Phenylalanine has been oxidized by acidic permanganate (101-102), and by aqueous hydrogen peroxide (103).

PRESENT WORK

The present work is a detailed kinetic investigation of the oxidation of amino acids by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere. The amino acids chosen for purposes of oxidation were glycine, alanine, valine, leucine and phenylalanine.

Stoichiometry (Vide 'Experimental').

The stoichiometry of each of the reactions was determined to be:

$$R.CH(NH_2)\,COOH + 2Fe(CN)_6^{3-} + 2\,OH^- \longrightarrow R.CO.\,COOH + 2Fe(CN)_6^{4-} + NH_3 + H_2O$$

Here R = - H (glycine), - CH_3 (alanine),

- $CH\begin{matrix}\diagup CH_3\\ \diagdown CH_3\end{matrix}$ (valine),

- $CH_2\text{-}CH(CH_3)_2$ (leucine),

- $CH_2\text{-}C_6H_5$ (phenylalanine).

Effect of substrate and oxidant

The rates of the reactions were found to be dependent on the first powers of the concentrations of both, substrate and oxidant (Table 1-4).

Table 1 : Effect of substrate and oxidant

/ Glycine / (10^2 x M)	/ $K_3Fe(CN)_6$ / (10^3 x M)	10^5 x k_{obs} (s^{-1})
1.0	1.0	1.9
2.5	1.0	4.7
5.0	1.0	9.6
10.0	1.0	19.5
25.0	1.0	47.0
50.0	1.0	96.0
1.0	0.75	1.9
1.0	0.50	1.8
1.0	0.25	1.8
1.0	0.10	1.9

/ NaOH / = 0.1 M ; μ = 0.05 M; temp. = 55°C.

Table 2: Effect of substrate and oxidant

/ Alanine / (M)	/ $K_3Fe(CN)_6$ / (10^2 x M)	10^5 x k_{obs} (s^{-1})
0.1	1.0	1.6
0.5	1.0	8.2
0.75	1.0	12.0
1.0	1.0	16.0
0.5	0.75	8.0
0.5	0.50	8.5
0.5	0.25	8.0
0.5	0.10	8.0

/ NaOH / = 1.0 M; μ = 0.5 M; temp. = 75°C.

Table 3: Effect of substrate and oxidant

/ Valine / (10^2 x M)	/ $K_3Fe(CN)_6$ / (10^3 x M)	10^5 x k_{obs} (s^{-1})
1.0	1.0	1.8
5.0	1.0	3.3
10.0	1.0	6.8
25.0	1.0	16.5
50.0	1.0	34.0
5.0	0.75	3.2
5.0	0.50	3.3
5.0	0.25	3.5
5.0	0.10	3.3

/ NaOH / = 0.5 M; μ = 0.05M ; temp. = 55°C.

Table 4: Effect of substrate and Oxidant

/ Substrate / (10^3 x M)	/ $K_3Fe(CN)_6$ / (10^4 x M)	Phenylalanine (10^5 x k_{obs} , s^{-1})	Leucine
2.5	1.0	3.5	1.2
5.0	1.0	5.7	2.3
10.0	1.0	9.8	4.7
25.0	1.0	24.5	12.0
50.0	1.0	49.0	24.0
10.0	0.75	9.7	4.5
10.0	0.50	9.9	4.8
10.0	0.25	9.5	4.5
10.0	0.10	9.5	4.6

/ NaOH / = 0.1M ; μ = 0.05 M; temp. = 55°C.

Plots of k_{obs}, the pseudo first order rate constant, against a 20-fold range of concentration of substrates, gave straight lines passing through the origin, indicating that the rate of oxidation was dependent on first power of the concentrations of the substrates. This was further seen by the constant values of k_2, the second rate order constant.

When a constant concentration of substrate (large excess) was used, k_{obs} did not show any appreciable variation with changing concentrations of oxidant (ten fold range), indicating a first order dependence of the reaction on the concentration of the oxidant (Tables 1-4).

Effect of NaOH

The rate of the reaction showed a first order dependence on the concentration of alkali, in the range studied (Tables 5-7).

Table 5: Effect of NaOH

/ NaOH / (M)	$10^5 \times k_{obs}$ (s^{-1}) Glycine	Valine
0.1	1.9	0.6
0.25	4.5	1.6
0.50	9.0	3.3
0.75	13.0	5.0
1.0	18.0	6.5

/ Glycine / = 1×10^{-2}M; / Valine / = 5×10^{-2} M;
/ $K_3Fe(CN)_6$ / = 1×10^{-3}M; μ = 0.05 M; temp. = 55°C.

Table 6 : Effect of NaOH

/ NaOH / (M)	$10^5 \times k_{obs}$ (s^{-1})
0.1	1.6
0.25	4.0
0.50	8.0
1.0	16.0

/ Alanine / = 0.5 M; / $K_3Fe(CN)_6$ / = 1 x 10^{-2} M;

μ = 0.5 M; temp. = 75°C.

Table 7 : Effect of NaOH

/ NaOH / (M)	Phenylalanine ($10^5 \times k_{obs}$, s^{-1})	Leucine
0.1	9.8	4.7
0.25	24.0	12.0
0.50	48.0	23.0
0.75	72.0	35.0
1.0	97.0	47.0

/ Substrates / = 1x10^{-2} M; / $K_3Fe(CN)_6$ / = 1 x 10^{-4} M;

μ = 0.05 M; temp. = 55°C.

Rate law

Under the present experimental conditions, the rate law could be expressed as:

$$\text{Rate} = -\frac{d/Fe(CN)_6^{3-}/}{dt} = k_{obs} / \text{Amino Acid} / /Fe(CN)_6^{3-} / / OH^- /$$

--------(1)

The pseudo first order rate constant, k_{obs}, was calculated from the equation (104):

$$k_{obs} = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad \ldots\ldots \quad (2)$$

(Vide 'Experimental' : Calculations).

Effect of temperature

The rate of the reaction was influenced by changes in temperature, and an increase in temperature resulted in an increase in the rate of the reaction (Tables 8-10).

Table 8 : Effect of temperature

Temp. ($\pm 0.1^{o}C$)	$10^5 \times K_{obs}$ (s^{-1}) Glycine	Valine
35.0	0.5	-
40.0	0.8	0.3
45.0	1.1	0.9
50.0	1.4	2.1
55.0	1.9	3.3
60.0	2.6	-

/Glycine / = 1×10^{-2} M; / $K_3Fe(CN)_6$ / = 1×10^{-3} M;

/ NaOH / = 0.1 M ; μ = 0.05 M.

/VALINE / = 5×10^{-2} M; / $K_3Fe(CN)_6$ / = 1×10^{-3} M;

/ NaOH / = 0.5 M; μ = 0.05 M.

Table 9 : Effect of temperature

Temp. ($\pm$ 0.1^{o}C)	$10^5 \times K_{obs}$ (s^{-1}) Alanine
65.0	3.2
70.0	4.7
75.0	8.2
80.0	13.7

/ Alanine / = 0.5 M; / $K_3Fe(CN)_6$ / = 1×10^{-2} M;

/ NaOH / = 1.0 M ; μ = 0.5 M.

Table 10 : Effect of temperature

Temp. ($\pm$ 0.1°C)	$10^5 \times k_{obs}$ (s^{-1}) Phenylalanine	Leucine
40.0	1.5	0.6
45.0	2.8	1.2
50.0	5.5	2.1
55.0	9.8	4.7
60.0	20.0	10.0

/ Substrates / = 1×10^{-2} M; / $K_3Fe(CN)_6$ / = 1×10^{-4} M;

/ NaOH / = 0.1 M ; μ = 0.05 M.

Plots of log k_{obs} against the reciprocal of temperature were linear (Figs. 1-2), suggesting the validity of the Arrhenius equation. The slopes of the plots were used to calculate the activation energies of the reactions . The other activation parameters were calculated (vide 'Experimental' : Calculations) and have been shown in Table 11.

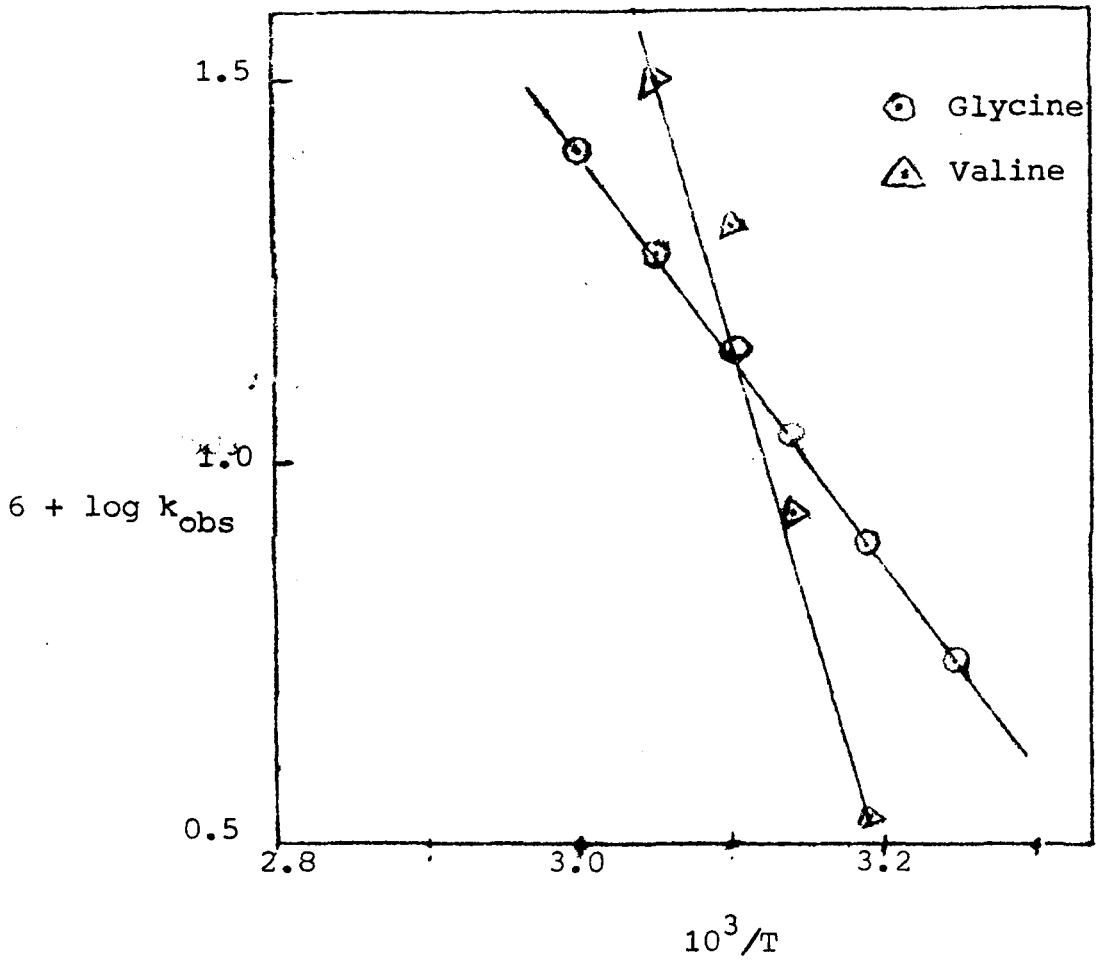


Fig. 1 : Plot of log k_{obs} against the reciprocal of temperature.

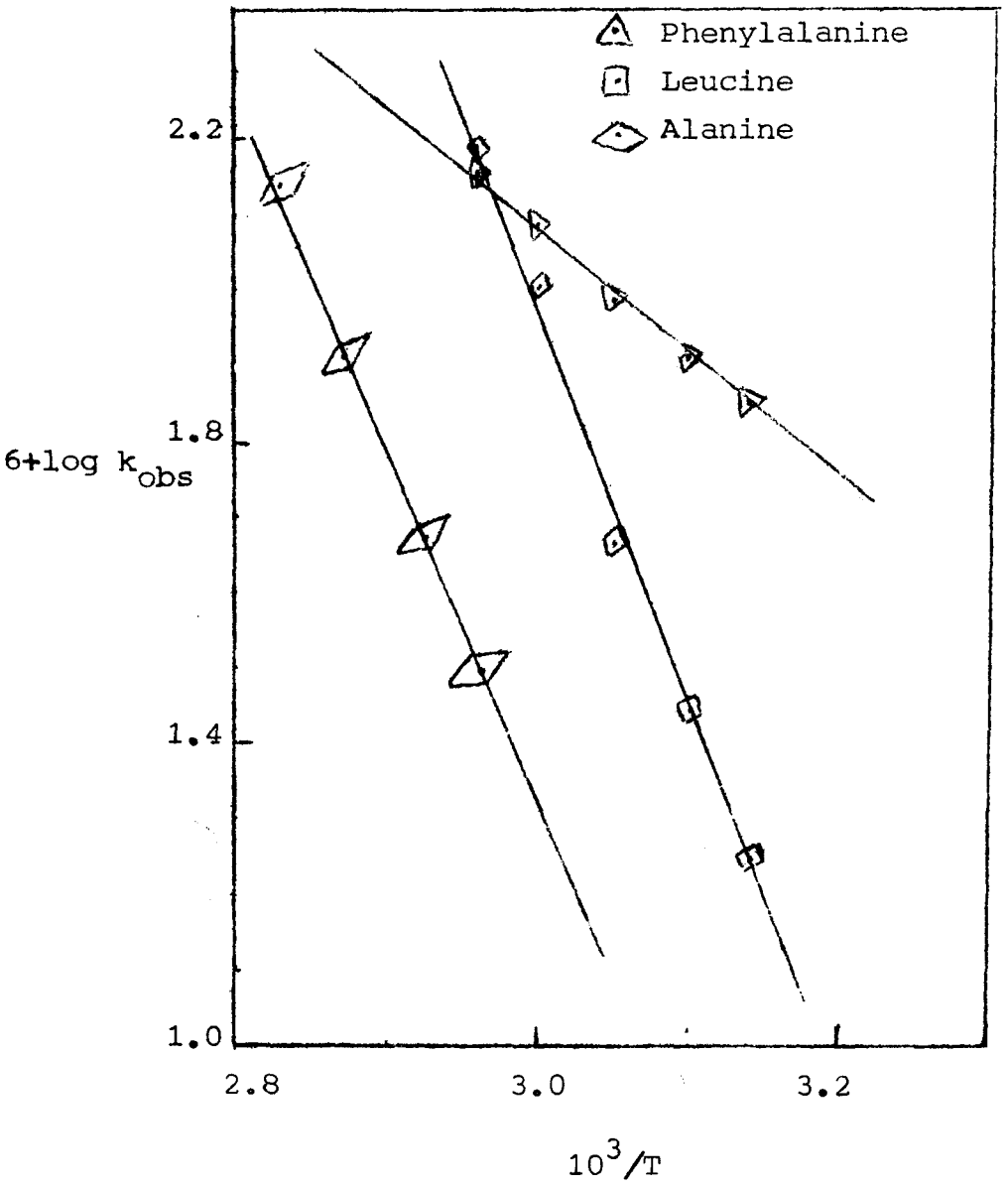


Fig. 2 : Plot of log k_{obs} against the reciprocal of temperature.

Table 11 : Activation Parameters

Substrate	E ($kJmol^{-1}$)	A (s^{-1})	$\Delta H^{\neq}$ ($kJmol^{-1}$)	$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)
Glycine	10±2	$1x10^3$	7±2	-150±4
Alanine	12±2	$6x10^3$	9±2	-155±4
Valine	15±2	$3x10^3$	12±2	-150±4
Leucine	13±2	$1x10^4$	10±2	-160±4
Phenylalanine	12±2	$3x10^4$	9±2	-160±4

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were favourable for electron abstraction processes. The favourable enthalpy for electron abstraction may be in part due to the release of energy on solvation of charges created in the transition state. Values of $\Delta S^{\neq}$ in this range for radical reactions have been ascribed (105) to the forbidden nature of electron - pairing and electron unpairing processes, and to the loss of degrees of freedom, formerly available to the reactants, on the formation of a rigid transition state.

Effect of ionic strength

Variations in the ionic strength of the medium using $NaClO_4$ (μ = 0.01 M to 0.50 M) did not have any effect on the rates of these reactions.

Effect of added $K_4Fe(CN)_6$

The addition of $K_4Fe(CN)_6$ in the concentration range, 1.0×10^{-4} M to 1.0×10^{-3} M, did not have any influence on the rates of these reactions.

Effect of added salts

The addition of slats such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4, $MgSO_4$ (concentration range of 1.0×10^{-4} M to 5.0×10^{-3} M), did not have any effect on the rates of these oxidation reactions.

Radical intermediates

The esr spectra of the corresponding radicals, generated from the oxidation of each of the substrates, were obtained (Vide 'Experimental' : ESR measurements).

The esr spectrum, obtained from the oxidation of glycine, gave a seven-line spectrum. The number of lines accounted for by a radical species consisting of four equivalent protons and a nitrogen atom, all having nearly equal coupling constants. This seven-line spectrum had relative intensities (within 10%) of 1:5:11:14:11:5:1 .

Mechanism

Oxidation of Glycine, Alanine, Valine, Leucine and Phenylalanine

The rate of the reaction between the substrate and hexacyanoferrate (III), in alkaline medium, was dependent on the first powers of the concentrations of each of the reacting species (Tables 1-4).

The dissociation of amino acids depends upon the pH of the medium. It is well known that amino acids exist as zwitterions in aqueous solution. In strongly acidic or alkaline media, the following equilibria exist:

$$RCH(NH_3^+)COOH \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(NH_3^+)COO^- \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(NH_2)COO^-$$

(SH^+) (S^o) (S^-)

Cation Zwitterion anion

In alkaline solutions, the zwitterion is converted to the anion, $RCH(NH_2)COO^-$, which is the reactive species under the present experimental conditions. The pK values for the system

$$RCH(NH_3^+)COO^- \rightleftharpoons RCH(NH_2)COO^- + H^+ ,$$

have been reported (106).

The ionization constants and the pH values at the isoelectric points of glycine, alanine, valine, leucine and phenylalanine at 25°C are given in Table 12.

Table 12: Ionization constants(106)and pH values at isoelectric points

Amino Acid	pK_1	pK_2	pH_i
Glycine	2.34	9.60	5.97
Alanine	2.34	9.69	6.01
Valine	2.32	9.62	5.96
Leucine	2.36	9.60	5.98
Phenylalanine	1.83	9.13	5.48

For these amino acids, $pH_i = \frac{pK_1 + pK_2}{2}$, where pH_i is the isoelectric point.

Since all the kinetic studies were carried out at high concentrations of NaOH (Tables 5-7), it may be assumed that the amino acids would be completely dissociated into their anions.

The addition of hexacyanoferrate (II) ions did not have any effect on the rates of the reactions. This showed that the step involving the reaction between the substrate

and oxidant (the electron abstraction step) was an irreversible step.

The addition of salts did not have any effect on the rates of the reactions, indicating that the reaction was between an ion and a dipolar species.

The reaction pathway was via the formation of radical intermediates, as detected by ESR spectroscopy.

Since potassium hexacyanoferrate (III) is a one-electron oxidant, it would be justified to postulate that the reaction betwedn the substrate and oxidant would give rise to a radical intermediate, analogous to enzymatic oxidation reactions which also proceed via radical intermediates (107). This would imply that potassium hexacyanoferrate (III), as a chemical oxidant, was capable of mimicking enzymatic behaviour.

In the present investigation, the formation of radical intermediates was supported by the following experimental observations:

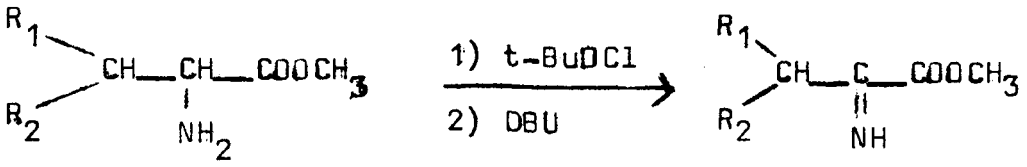
(a) the rate of the reaction was dependent on the first powers of the concentrations of substrate and oxidant;
(b) the lack of any effect on the rate of the reaction by the addition of hexacyanoferrate (II) ions;

(c) favourable enthalpy and entropy factors which were characteristic of radical processes;

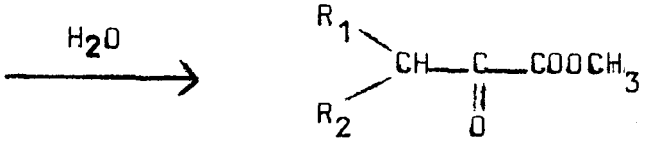
(d) detection of radical intermediates by ESR spectroscopy.

The subsequent steps involved the rapid reaction of the radical with the oxidant, yielding the imino compound, which, on hydrolysis, gave the keto acid and ammonia. No other intermediate(s) could be isolated from the reaction mixture.

This mechanistic pathway for the oxidation of amino acids to the keto acids, via the intermediate formation of the imino acid, has been well established in the synthesis of α-keto acid esters, wherein the following pathway has been suggested (108) :



(1) (2)



(3)

The metabolic relationship between α-amino acids and α-ketoacids has been recognised. Specific amino acid oxidases have been found to oxidise amino acids according to the following equations:

$$\underset{(4)}{R{-}CH(NH_2){-}COOH} + O_2 \xrightarrow{\text{enzymatic}} \underset{(5)}{R{-}\overset{\overset{NH}{\|}}{C}{-}COOH} + H_2O_2$$

$$\underset{(5)}{R{-}\overset{\overset{NH}{\|}}{C}{-}COOH} + H_2O \xrightarrow{\text{non-enzymatic}} \underset{(6)}{R{-}\overset{\overset{O}{\|}}{C}{-}COOH} + NH_3$$

$$\underset{(6)}{R{-}\overset{\overset{O}{\|}}{C}{-}COOH} + H_2O_2 \longrightarrow \underset{(7)}{R.COOH} + CO_2 + H_2O \quad .$$

The amino acid, 4 , is oxidised by the action of amino acid oxidase to the corresponding α-imino acid, 5 , with the generation of hydrogen peroxide. The α-imino acid, 5 , is spontaneously (non enzymatically) hydrolysed, usually within a few seconds, to the corresponding α-keto acid, 6 , and

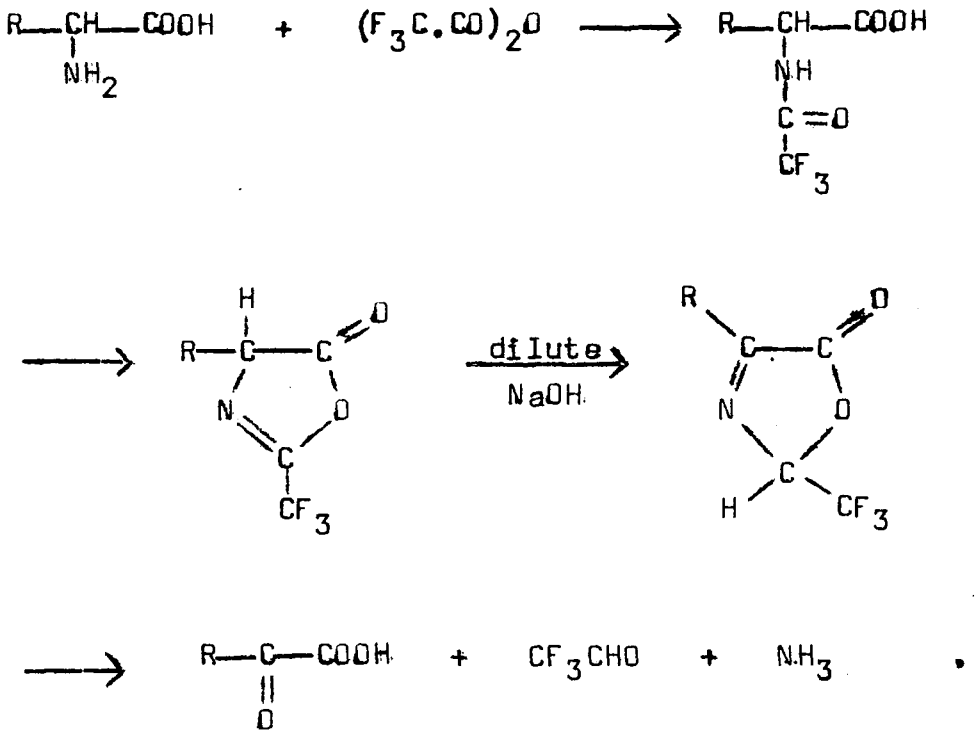
ammonia. The α-keto acid, 6 , is susceptible to oxidative decarboxylation by hydrogen peroxide, so that unless hydrogen peroxide is rapidly removed (e.g., by the action of catalase), a considerable amount of carboxylic acid, 7 , containing one less carbon than the parent amino acid, is generated. The relative concentration of α-keto acid, 6 , to carboxylic acid, 7 , in the final product, depends on the conditions employed for the oxidation of the amino acid. In the presence of catalase, oxidation results in the stoichiometric formation of α-keto acid, 6 , and ammonia.

Enzymatic methods have been adapted for large scale synthesis of α-keto acids. It has been shown that immobilised yeast cells containing D-amino acid oxidase could be used to prepare α-keto acids from the corresponding D-amino acids (109).

The α-keto acid analogues of the naturally occurring amino acids are of major importance in intermediary metabolism. Pyruvic acid is a metabolite involved in many enzyme-catalyzed intracellular phenomena. The α-keto acid analogues of the protein amino acids are often the penultimate products formed in the biosynthesis of amino acids, and are commonly the first product formed in the degradative metabolism of amino acids. α-keto acids have been used in the therapy of certain conditions such as

uremia and nitrogen accumulation disorders. Thus, α-keto acids are of continuing interest as intermediates in chemical syntheses, in the development of enzyme inhibitors and drugs, as model substrates of enzymes, and in various other ways. In recent years, interest in the biochemical importance of α-keto acids has increased substantially.

The azlactone method has been used for the synthesis of α-ketoisovaleric acid, α-ketoisocaproic acid and α-phenylpyruvic acid, starting from the respective amino acids — valine, leucine and phenylalanine (110).



The acid catalyzed cleavage of α-acetylamino α-methyl esters has been used for the synthesis of α-keto-isovaleric acid and α-phenylpyruvic acid (111).

The reaction of acylimidazolides with the appropriate Grignard reagent, followed by the hydrolysis of the resulting α-keto acid ester gave good yields of α-ketoisocaproic acid (112).

The oxidation of α-keto aldehydes, in the presence of catalytic amounts of cyanide and an oxidizing agent, has been used to convert methyl-glyoxal to pyruvate (113),

$$CH_3\ COCHO \xrightarrow[O_2]{CN^-} CH_3\ CO\ COOH$$

This method has been used to synthesize steroid α-keto acids (114).

The reaction of the appropriate ketone with 2,2-difluoro-1-(tosyloxy) vinyl lithium, gave good yields of α-ketoisovaleric acid and α-phenyl-pyruvic acid (115),

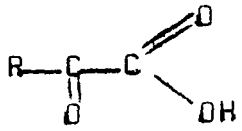
$$CF_3\ CH_2\text{-}OTs \xrightarrow[\text{diisopropylamide (LDA) in THF}]{\text{Lithium}} CF_2 = CLi - OTs$$

$$\xrightarrow{RR'\ C = O} RR'\ C\ (OH)\ C\ (\ OTs) = CF_2$$

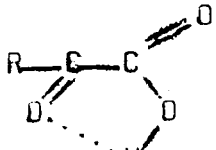
$$\xrightarrow{95\%\ H_2SO_4} RR'\ C = C\ (OTs\ )\ COOH \xrightarrow[2.\ H^+]{1.\ OH^-}$$

RR' CH CO COOH

α-keto acids have been shown to form proton chelates in solution (116-119). The ratio of proton chelate, 1A , to open form 1 , was found to depend on concentration, temperature, solvent and the nature of R.



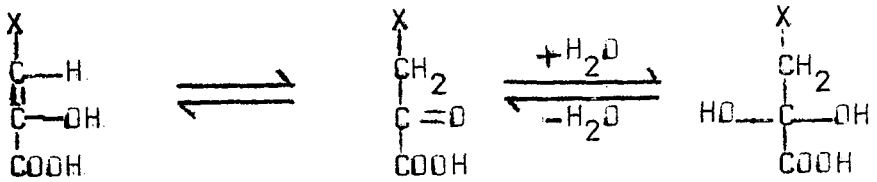
(1)



(1A)

For example, for pyruvic acid and α-ketoisovaleric acid, "proton chelates" are important contributions in nonpolar solvents (CCl_4) and probably in acid aqueous solutions, but are unimportant in polar solvents such as dioxane. The chelates cause a major bathochromic shift of the $n \longrightarrow \pi^*$ bands of the carbonyl group, compared to the nonchelated carbonyl. The intensity of the $n \longrightarrow \pi^*$ transition of various α-keto acids was in the order pyruvic acid $>$ α-ketoisovaleric acid. Glyoxylic acids were shown to form proton-chelates in both nonpolar (CCl_4) and polar solvents such as water (119). The infrared frequencies of the α-carboxyl and hydroxyl bands of the substituted glyoxylic acids were strongly dependent on the nature of the ring substituents (118). It was observed that the infrared frequency correlates well with the σ^+ values of ring substituents of the "proton chelate", whereas the frequency correlates with the Hammett σ-function in the open form (117-119).

It is well known that α-keto acids, 2 , can form enols, 2A , or add water to form hydrates or gem-diols.



(2A) (2)

Although β-arylpyruvic acids (Ar CH_2CO COOH) readily enolize in strong base, the concentration of the enol form is generally low at physiological pH values. However, in the presence of borate buffer ($\sim$ pH = 3.0), enolization is promoted via formation of an enol-borate complex (120). The enzyme, arylpyruvate keto-enol tautomerase, catalyzes the rapid interconversion between keto and enol forms of β-phenylpyruvic acid (120).

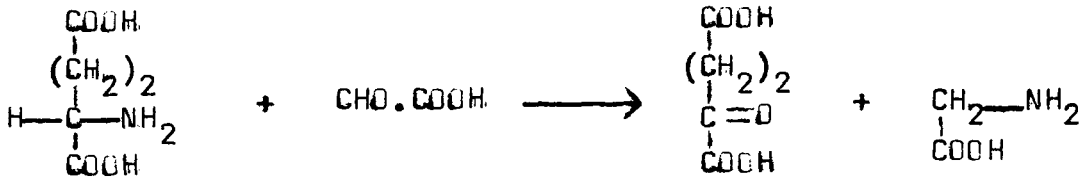
Generally, for those α-keto acids in which an enol contribution is unimportant, the percentage of the gem-diol is low in solution (5-10%) at neutral pH(121). However, when the α-carboxyl is protonated, the percentages of the gem-diol increases ($\geq$ 60%). Glyoxylic acid is $\geq$ 99% hydrated at pH = 0.5 (121).

$$HCO\ COOH \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} HC(OH)_2\ COOH$$

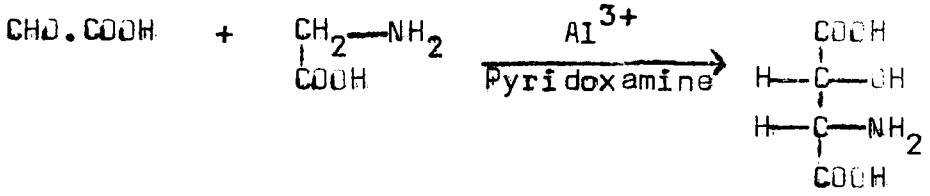
The degree of hydration of an α-keto acid correlates well with the inductive effect of the substituents adjacent to the carbonyl (12). Although pure pyruvic acid exists in the keto form, the lithium monohydrate salt is in the gem-diol form ($CH_3\ C(OH)_2\ COO\ Li$), and is not a true hydrate (122).

α-keto acids (glyoxylic acid, pyruvic acid and α-keto isocaproic acid) have been observed to undergo photofragmentation (123).

Oxidative deamination reactions of amino acids, in the presence of compounds such as isatin, alloxan, quinones and ninhydrin, have been well documented (124). Glyoxylic acid undergoes transamination with glutamic acid at physiological temperature and pH (125). The products are α-ketoglutaric acid and glycine; decarboxylation did not occur.



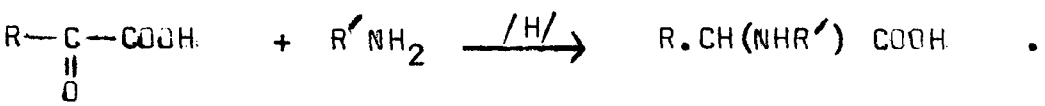
When glyoxylic acid was heated with an appropriate amino donor (Al^{3+} and pyridoxamine), considerable amount of β-hydroxyaspartic acid was generated (126).



Enzymes have been used to catalyze the reductive amination of α-keto acids. Thus, L-alanine dehydrogenase (127), and L-leucine dehydrogenase (128) catalyze the reductive amination of pyruvic acid and α-ketoisocaproic acid, respectively, to the corresponding L-amino acids (alanine and leucine).

It has been shown that alanine could be produced by the reduction of pyruvic acid phenyl hydrazone with aluminium amalgam (129).

It was later shown that α-keto acids could be converted to amino acids or N-methylamino acids by catalytic reduction in the presence of ammonia or methylamine (130),



More recently, amino acids have been generated from α-keto acids by electrolytic reduction in aqueous ammonia solutions with Hg, Pt/C , or Pd/C electrodes (131).

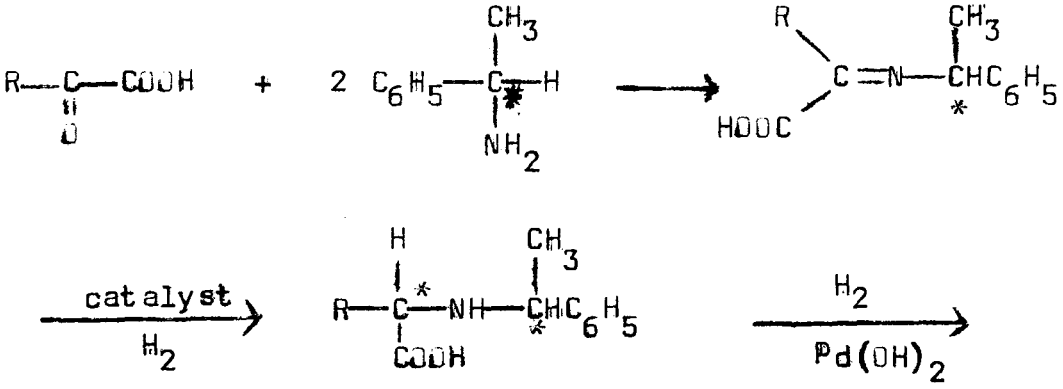
Pyruvate was easily reduced to alanine in the presence of sodium cyanoborohydride and ammonia (132). Sodium cyanoborohydride was shown to be particularly effective in reducing α-keto acid oximes to the N-hydroxy compounds (133).

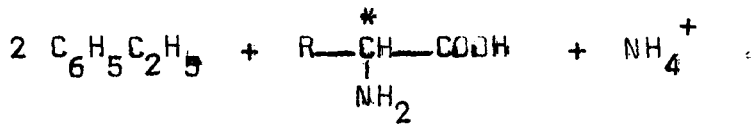
It has been suggested that the reduction of α-keto acid derivatives could be used for the purpose of identifying α-keto acids in biological materials; the most suitable derivative for this purpose was shown to be the 2,4-dinitrophenyl-hydrazones (134). Several investigations have established the reductive amination of α-keto acid 2,4-dinitrophenylhydrazones to α-amino acids as a possible aid in the detection and estimation of α-keto acids(135-140). The catalytic hydrogenation of β-phenylpyruvic 2,4-dinitrophenylhydrazone gave good yields of phenylalanine(141).

Several investigations have been carried out to establish the possibility of using the reductive amination of α-keto acids to produce optically active α-amino acids. Thus, reduction of α-keto acids in the presence of L- or D-

methylbenzylamine results in amino acids with varying

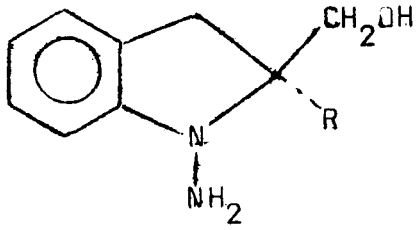
degrees of optical purity (142).





A novel modification of the reductive amination technique, described as "hydrogenolytic transamination", has been introduced (143). This technique involves the use of an optically active α-amino acid, as a nitrogen donor, in place of an amine (143).

Using the chiral reagents, N-amino-2-hydroxymethylindolines, (S) - 9 and (S) - 16 , optical purities of a high degree have been obtained following the reductive amination of α-keto acids (144,145),



(S) - 9, R = H

(S) - 16, R = CH_3

α-keto acids can undergo aldol condensation with another molecule of α-keto acid, with aldehydes or with ketones. Phenylpyruvic acid forms a crystalline aldol product with benzyl methyl ketone (146), yields a lactone with benzaldehyde (147,148), and condenses with acetone in alkaline solution to give benzyl-maleic acid (149). Pyruvic acid condenses with benzaldehyde to yield benzalpyruvic acid, a β,γ-unsaturated α-keto acid (150-152). Pyruvic acid reacts with aliphatic aldehydes to yield an α-keto-γ-hydroxy acid that cyclizes to a lactone in acid medium, but which undergoes dehydration in alkaline medium to give an α-keto-β,γ-unsaturated acid (153). Glyoxylic acid reacts with acetophenones to yield 2,2-disubstituted acetic acids (154).

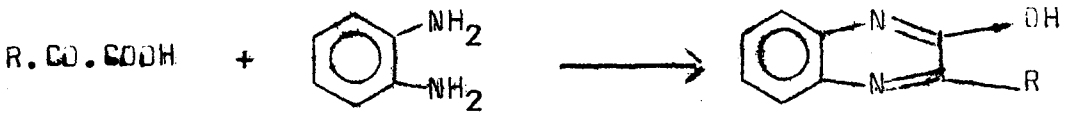
Model reactions of ferredoxin-dependent bacterial CO_2 fixation have been described which apparently involve an α-keto acid intermediate (155,156), thus,

$$CH_3CO \cdot S \cdot C_8H_{17} \xrightarrow[Na_2S_2O_4\ ,\ NaHCO_3]{CO_2} CH_3CO\ COOH$$

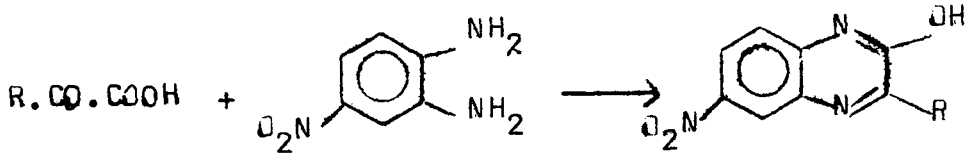
in THF - MeOH - H_2O

$$\xrightarrow[NH_4^+ \text{ or } Na_2S_2O_4]{\text{Pyridoxamine, } Zn^{2+}} CH_3CH(NH_2)COOH \quad .$$

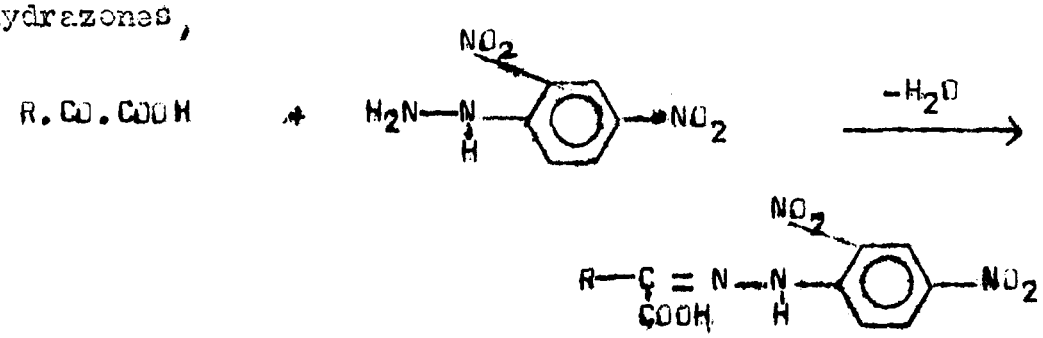
It has been known that α-keto acids condense with o-phenylene-diamine to yield stable quinoxalinols (157),



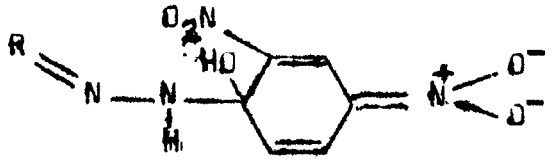
This reaction has beenme very important for the estimation of α-keto acids, which can be detected as fluorescent spots on paper chromatograms by spraying with o-phenylenediamine (158), or by preparing the derivative of the α-keto acid prior to chromatography (159-161). The 4-nitro derivative of o-phenylenediamine has been used, yielding highly coloured products which can be separated by peper chromatography on alumina columns (162,163),



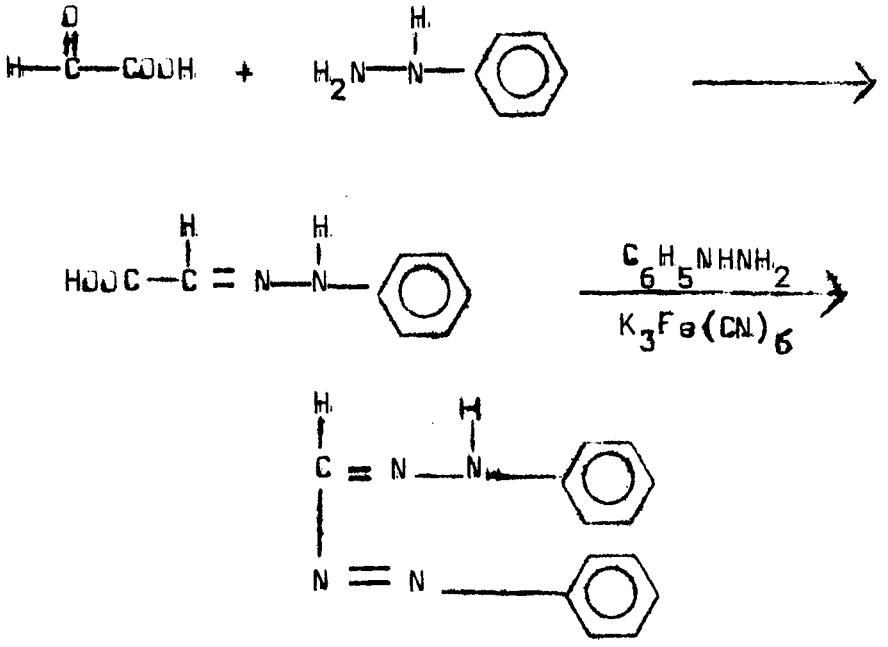
α-Keto acids form a large number of crystalline derivatives. The most useful derivatives have been the 2,4-dinitrophenylhydrazones,



Paper chromatography of 2,4-dinitrophenylhydrazone derivatives has been used in identifying α-keto acids in biological samples (164). Subsequent investigations have highlighted the identification and semiquantitative analysis of α-keto acids by paper chromatography and by thin layer chromatography (136-140; 165-171). The 2,4-dinitrophenylhydrazone derivatives are generally stable in acid medium, and yield intensely coloured products in basic medium. This colour reaction is very useful for the estimation of α-keto acids (172,173). The species responsible for the colour, in basic medium, is probably a quinoid type structure (174),



α-keto acids form other crystalline derivatives, such as the p-nitrophenylhydrazones (173) and phenylhydrazones (129). It has been shown that glyoxylic acid phenylhydrazone is oxidized by potassium hexacyanoferrate (III) to give a highly coloured product (175). This reaction is specific for the estimation of glyoxylic acid, and the coloured product formed is now known to be 1,5-diphenylformazan (175),



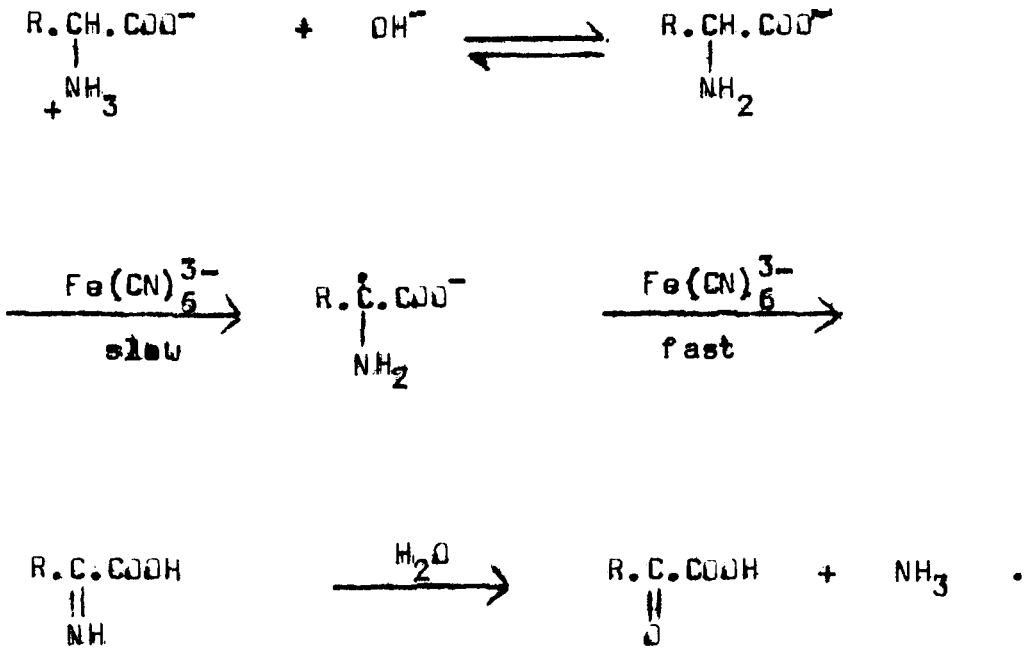
A GC method has been introduced for the determination of α-keto acids based on the reaction with pentafluorophenylhydrazine followed by reaction with diazomethane to produce the methyl ester (176).

Enzymatic methods have been used for the determination of pyruvic acid (177). The enzyme, B. subtilis leucine dehydrogenase, has been used to estimate total branched-chain α-keto acids in rat tissues (178). Analytical methods, based on the formation of fluorescence products, have been used for the estimation of total α-keto acid or simple mixtures of α-keto acids. These methods have utilized reagents such as o-phenylenediamine (179), pyridoximine and zinc (180), and α-hydrazino-2-stilbazole (181). A GC method, using the technique of postcolumn mass spectrometry (182), has been used for the measurement of branch - chain α-keto acids in biological materials (183,184).

HPLC techniques, using post-column derivatization with N-methylnicotinamide chloride, have been used for the fluorometric determination of pyruvic acid (185). It has been shown that glyoxylic acid and pyruvic acid can be separated on a C_{18} column by reversed-phase HPLC as the 4-(bromomethyl)-7-coumarin derivatives (186). It has been reported that a seven-component homologous series of α-keto acid 2,4-dinitrophenylhydrazones of increasing chain length, could be separated by HPLC techniques (187). In some cases, resolution of cis and trans forms was achieved (187). This method has been used to detect pyruvic acid in biological samples (187).

In the present investigation, the reaction sequence for the oxidation of the amino acids (glycine, alanine, valine, leucine and phenylalanine) by potassium hexacyanoferrate (III), in alkaline medium, is shown in the Scheme.

SCHEME



The products obtained from the oxidation of the amino acids were the corresponding keto acids (glyoxylic acid from glycine; pyruvic acid from alanine; α-ketoisovaleric acid from valine; α-ketoisocaproic acid from leucine; and β-phenylpyruvic acid from phenylalanine), which were characterized by their corresponding 2,4-dinitrophenyl hydrazone derivatives (vide 'Experimental': Product Analysis).

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CHAPTER 2

KINETICS OF OXIDATION OF SERINE AND THREONINE

Serine has been used in the pathway of choline metabolism (1), as also in the biosynthetic pathways for various alkaloids such as ricinine (2) and mimosine (3). The reaction involving the decarboxylation of serine (4) provides the nitrogen atom and its attached C_2 unit in the biosynthesis of atisine, a diterpenoid (5). Serine is present at the active centre in elastase (6). The interatomic distance (3.0 Å) measured from a model of chymotrypsin gave clear evidence for hydrogen bonding (7) between histidine 57 and serine 195, indicating that histidine 57 and serine 195 were involved in the charge relay mechanism of chymotrypsin (8). The hydroxyl group of serine thus plays a direct role in the catalytic action of chymotrypsin (9). The enzymic activity of trypsinogen has been attributed partly to the presence of serine residue at position 183 (10-12).

In the pathway for vitamin B_{12} synthesis (13), the conversion of cobyric acid to cobinamide involved amide formation with 1-amino-2-propanol. It has been demonstrated that threonine was the precursor of 1-amino-2 propanol (14).

Threonine has been observed to be present in many lupine alkaloids (15). Threonine has also been established as a good precursor in the biosynthesis of seneciphyllic acid, which is an important pyrrolizidine alkaloid (16).

The kinetics of oxidation of serine and threonine have become important because of their biological significance. Serine has been oxidised by a variety of oxidising agents such as chloramine - T(17-19), N-bromosuccinimide (20-21), peroxy-disulfate (22), Fenton's reagent (23), ceric ions (24,25), chloramine-B (26), Chromic acid (27), N-Bromoacetamide (28) and by periodate in acid medium (29).

Threonine has been oxidised by a variety of oxidising agents such as N-bromosuccinimide (20), Ceric ions (30), Co(III) ion catalysed by Ag^{+} ion (31), peroxy disulfate catalysed by Cu (II) ion (32), acidic $KMnO_4$ solution (33) chloramine - T (34-37), peroxomono sulfate (38) and by Mn (III) sulfate (39),

PRESENT WORK

The present work is a detailed kinetic investigation of the oxidation of serine and threonine by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere.

Stoichiometry (vide 'Experimental')

The stoichiometry of each of the reactions was determined to be:

$$R.CH(NH_2)\,COOH + 2Fe(CN)_6^{3-} + 2\,OH^- \longrightarrow R.CO.COOH + 2Fe(CN)_6^{4-} + NH_3 + H_2O$$

Here, R = $-CH_2OH$ (serine),

= $-CHOH.CH_3$ (threonine).

Effect of substrate and oxidant.

The rates of the reactions were observed to be dependent on the first powers of the concentrations of both, substrate and oxidant (Table 1).

Table 1: Effect of substrate and oxidant

/Substrate / (10^2 x M)	/ $K_3Fe(CN)_6$ / (10^3 x M)	$10^5 x k_{obs}$ (s^{-1}) Serine	Threonine
5.0	1.0	2.1	3.2
10.0	1.0	4.1	6.7
25.0	1.0	10.8	16.0
50.0	1.0	21.5	32.0
100.0	1.0	44.0	65.0
10.0	0.5	4.0	6.5
10.0	0.25	4.2	6.8
10.0	0.1	4.4	6.5
10.0	0.05	4.0	6.3

/ NaOH / = 1.0 M; μ = 0.50M; temp. = 55°C.

Plots of k_{obs}, the pseudo first order rate constant, against a 20-fold range of concentrations of substrates, gave straight lines passing through the origin, indicating that the rate of oxidation was dependent on the first power of the concentrations of the substrates. This was further seen by the constant values of k_2, the second order rate constant.

When a constant concentration of substrate (large excess) was used, k_{obs} did not show any appreciable

variation with changing concentrations of oxidant (20-fold range), indicating a first order dependence of the reaction on the concentration of the oxidant (Table 1).

Effect of alkali

The rate of the reaction was dependent on the first power of the concentration of alkali in the range studied (Table 2).

Table 2 : Effect of NaOH

NaOH (M)	$10^6 \times k_{obs}$ (s^{-1}) Serine	Threonine
0.10	4.1	6.7
0.25	10.0	17.0
0.50	20.0	33.0
1.0	41.0	67.0

/ Substrates / = 0.1 M; / $K_3Fe(CN)_6$ / = 1×10^{-3} M;

μ = 0.50 M ; temp. = 55°C.

Rate law

Under the present experimental conditions, the rate law could be expressed as:

$$\text{Rate} = -\frac{d/Fe(CN)_6^{3-}/}{dt} = k_{obs} / \text{Amino Acid} / /Fe(CN)_6^{3-}/ / OH^- / \quad \ldots\ldots\ldots (1)$$

The pseudo first order rate constant, k_{obs}, was calculated from the equation (40):

$$k_{obs} = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad \ldots\ldots\ldots \quad (2)$$

(vide 'Experimental' : Calculations)

Effect of temperature

The rate of the reaction was influenced by changes in temperature, the rate showing an increase with an increase in the temperature (Table 3).

Table 3 : Effect of temperature

Temp. ($\pm$ 0.1°C)	$10^5 \times k_{obs}$ (s^{-1}) Serine	Threonine
45.0	2.0	4.8
50.0	2.8	5.8
55.0	4.1	6.7
60.0	6.2	7.8
65.0	8.5	9.2

/ Substrates / = 0.1 M; / $K_3Fe(CN)_6$ / = 1×10^{-3} M;

/ NaOH / = 1.0 M ; μ = 0.5 M.

Plots of log k_{obs} against the reciprocal of temperature were linear (Fig.1), suggesting the validity of Arrhenius equation. The slopes of these plots were used to calculate the activation energies of the reactions. The other activation parameters were calculated (vide 'Experimental' : Calculations), and have been shown in Table 4.

Table 4 : Activation Parameters

Parameter	Serine	Threonine
E($kJmol^{-1}$)	50 ± 3	45±3
A (s^{-1})	4 x 10^5	2x10^5
$\Delta H^{\neq}$ ($kJmol^{-1}$)	47 ± 3	42 ± 3
$\Delta S^{\neq}$ ($Jk^{-1}\ mol^{-1}$)	- 140 ± 5	-145 ± 5

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were both favourable for electron abstraction processes, suggesting the formation of radical intermediates in the rate determining step of the reaction (41).

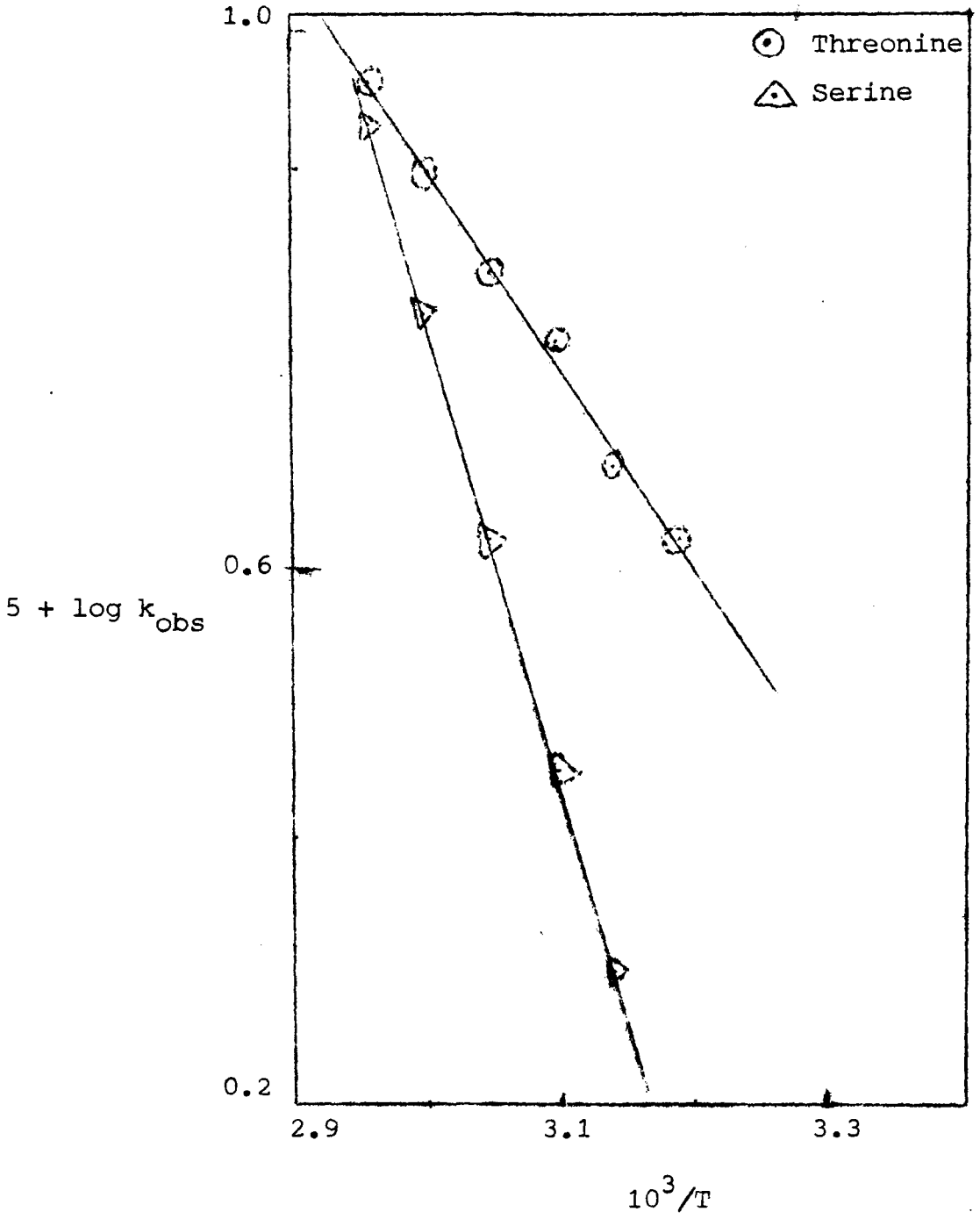


Fig. 1 : Plot of log k_{obs} against the reciprocal of temperature.

Effect of added $K_4Fe(CN)_6$

The addition of $K_4Fe(CN)_6$ in the concentration range, 1.0×10^{-4}M to 1.0×10^{-3}M, did not have any effect on the rates of the reactions.

Effect of ionic strength

Variations in the ionic strength of the medium using $NaClO_4$ (μ= 0.01M to 0.50 M), did not have any influence on the rates of the reactions.

Effect of added salts

The addition of salts such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4, $MgSO_4$ (concentration range of 1.0×10^{-4} M to 5.0×10^{-3} M), did not have any effect on the rates of these oxidation reactions.

Radical intermediates

The esr spectra of the corresponding radicals, generated from the oxidation of each of the substrates, were obtained (vide 'Experimental : ESR measurements).

The esr spectra of the radicals, obtained from the oxidation of serine and threonine, each gave a six-line spectrum, accounted for by a radical species consisting of three equivalent protons and a nitrogen atom, all having nearly equal coupling constants. No further splitting of the signals was observed.

Mechanism

The rate of the reaction between the substrate and hexacyanoferrate (III), in alkaline medium, was dependent on the first powers of the concentrations of each of the reacting species — substrate, oxidant and alkali (Tables 1-2). Since all the kinetic studies were carried out at high concentrations of NaOH (Tables 1-2), it may be assumed that the amino acids would be completely dissociated into their anions.

The addition of hexacyanoferrate (II) ions did not have any effect on the rates of these reactions. This showed that the step involving the reaction between the substrate and the oxidant (the electron abstraction step) was an irreversible step.

The addition of salts did not have any effect on the rate of the reaction, indicating that the slow step involved the reaction between an ion and a dipolar species.

The reaction pathway was via the formation of radical intermediates, as detected by ESR spectroscopy.

The subsequent steps involving the reaction of the radical with the oxidant were rapid, giving the imino compound, which on hydrolysis yielded the corresponding keto acid and ammonia. No other intermediate(s) could be isolated from the reaction mixture.

Based on the inductive effect of the substituents, the order of reactivity should be as threonine > serine. This has been observed (Table 1). Comparable values for $\Delta H^{\neq}$ and $\Delta S^{\neq}$, respectively, for the amino acid - hexacyanoferrate (III) systems, irrespective of the structures of the amino acids, supports the observation that the reactions between the amino acids and hexacyanoferrate (III) proceed via the same pathway for the systems under consideration (serine and threonine).

Since potassium hexacyanoferrate (III) is a one - electron oxidant, the reaction between the substrate and the oxidant would involve the formation of radical intermediates, analogous to enzymatic oxidation reactions which are known to proceed via the formation of radical intermediates (42). This would help to establish that hexacyanoferrate (III), as a chemical oxidant, was similar in its behavior to that of enzyme systems.

In the present investigation, the formation of radical intermediates was supported by the following experimental observations:

(a) The rate of the reaction was dependent on the first powers of the concentrations of the substrate and oxidant;

(b) The lack of any effect on the rate of the reaction by the addition of hexacyanoferrate (II) ions;

(c) favourable enthalpy and entropy factors; and

(d) signals observed by ESR spectroscopy.

The ionization constants and pH values at the isoelectric points of serine and threonine at 25°C are given in Table 5.

Table 5: Ionization constants[43] and pH values at isoelectric points

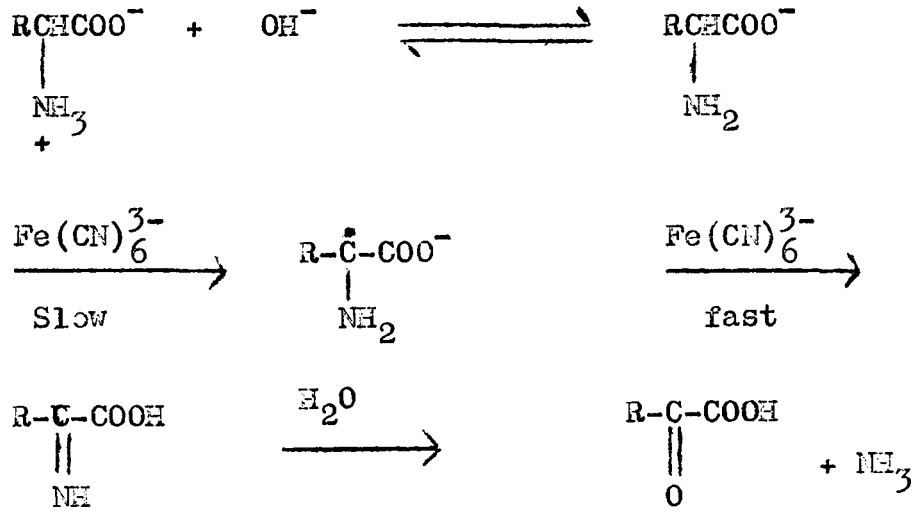
Amino Acid	pK_1	pK_2	pH_i
Serine	2.21	9.15	5.68
Threonine	2.71	9.62	6.16

pH_i (isoelectric point) is given by $pH_i = \frac{pK_1 + pK_2}{2}$

Amino acids exist as zwitterions in aqueous solution. In alkaline solutions, the zwitterion is converted to $RCH(NH_2)COO^-$, which is the reactive species, under the present experimental conditions.

The reaction sequence for the oxidation of these amino acids (serine and threonine) by potassium hexacyanoferrate (III), in alkaline medium, is shown in the Scheme.

SCHEME



The products obtained from the oxidation of each of these amino acids were the corresponding keto acids (β -hydroxypyruvic acid from serine, and α-keto-β-hydroxy-butyric acid from threonine), which were isolated and characterized as their respective 2,4-dinitro-phenyl hydrazone derivatives (vide 'Experimental' : Product Analysis).

It has been reported that α-keto acids can exist in equilibrium with their enol forms. Generally, for those α-keto acids in which an enol contribution is unimportant, the percentage of the gem-diol is also low in solution(5-10%) at neutral pH (44). However, when the α-carboxyl is protonated, the percentage of the gem-diol increases ($\geq$ 60%). The degree of hydration of an α-keto acid

correlates well with the inductive effect of the substituents adjacent to the carbonyl group (44). It has been shown that β-hydroxypyruvic acid (the oxidation product of serine) exists in the pure form as the keto acid, but the lithium hydrate, $RC(OH)_2COOLi$, has the gem-diol form (45).

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CHAPTER 3

KINETICS OF OXIDATION OF CYSTEINE AND METHIONINE

Pulse radiolysis studies have shown the presence of transient species when thiols are irradiated at a pH where some ionisation of the thiol group has occured. These species have an absorption band from approximately 350 to 500 nm with a maximum at 400 to 450 and an extinction coefficient of the order of 10^4 l mol^{-1} cm^{-1}. It was shown that the transient species formed was R-S-$\bar{S}$-R which resulted from the reactions of thiyl radical with the thiolate ion (1).

$$R\dot{S} + RS^- \rightleftharpoons RS\bar{S}R \qquad (1)$$

The rate of disappearance of the thiol was controlled by the dimerisation of free thiyl radicals,

$R\dot{S} + R\dot{S} \rightleftharpoons$ RSSR, as observed for cysteine (2)

In the case of cysteine the extinction coefficient at 420 nm was of the order 9 x 10^3 l mol^{-1} cm^{-1} (3).

The radical formed by dissociative electron capture has been detected directly for the mercapto acetate ion (3). The radical, $^-SCH_2\dot{C}(NH_2)COO^-$, was detected in alkaline solutions of cysteine, abstraction from the β-carbon atom, with respect to Sulphur, being attributed to the extra

stability of a tertiary radical (4). Homocysteine readily lactonises readily in acidic solutions. The reaction of the OH radical with the lactone form of homocysteine leads to oxidative deamination and keto acid formation in a manner similar to that for amino acids (5). This shows that hydrogen atom abstraction occurs from the tertiary carbon atom, rather than from that to sulphur, as found in ESR studies with cysteine (4).

When neutral and slightly alkaline solutions of cysteine saturated with N_2O were irradiated (6), it was found that oxygen markedly decreased the amount of RSSR formed, immediately after the pulses, as well as greatly increasing its rate of decay by the reaction:

$$RSSR + O_2 \longrightarrow RSSR + O_2 \quad .$$

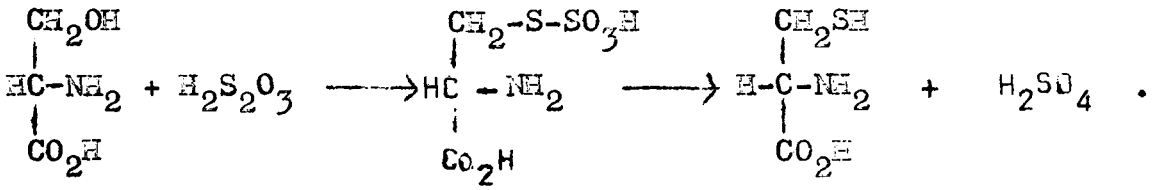
A weak transient, λ_{max} at 530 nm was detected when acidic solutions of cysteine were irradiated in the presence of oxygen, the amount formed increasing marginally with cysteine concentration and markedly with oxygen concentration (7).

ESR studies on single crystals of cysteine hydrochloride monohydrate gave an isotropic doublet as the main radical species with a high anisotropic , but axial, symmetric g factor, attributed to the $SCH_2CH(COOH)NH_3^+ Cl^-$ radical (8). The radical recombination in irradiated

poly-crystaline cysteine hydrochloride monohydrate at 340-390 K has been reported (9). The decay mechanism involved dimerisation of thiol radicals, and hydrogen atom transfer between the thiol groups and thiyl radicals occured above 378 K (9).

Sulfide entry into organic linkage is through a reaction with serine to form cysteine, the central compound of intermediary thiol metabolism (10).

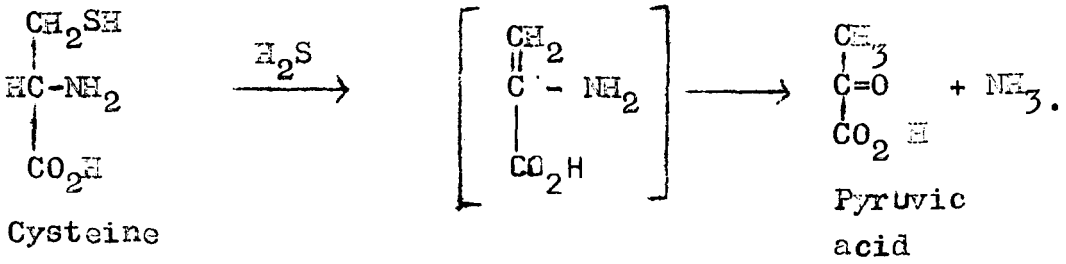
The direct H_2S-H_2O interchange enzyme, serine sulfhydrase is responsible for the sulfuration or desulfuration of cysteine (10). Cysteine formation through the addition of thiosulphate to serine or o-acetyl serine may be important in the sulfur metabolism of some organisms, with S-sulpho-cysteine serving as an intermediate,



Serine 5-Sulphocysteine Cysteine.

The only thiol oxidation reaction to oxy-derivatives of general biochemical significance is that of cysteine to cysteine sulphinic acid (10,11). This is thought to be the initial reaction in the main path way for the utilization of cysteine sulphur for sulphate production (10,11).

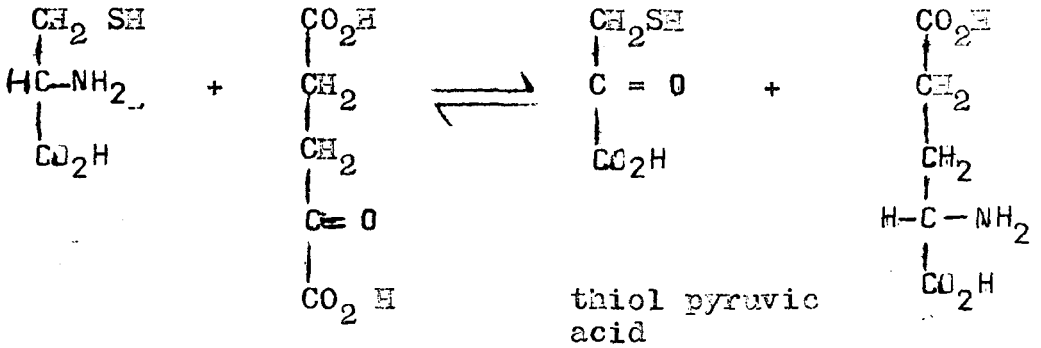
Desulphuration of cysteine may play a role in thiol catabolism.



Cysteine

Pyruvic acid

It has been suggested that the removal of thiol group from cysteine was through the intermediate formation of thiol pyruvic acid, which is the α-keto acid derived from cysteine by trans amination.



thiol pyruvic acid

α-ketoglutaric acid

Glutamic acid

The product can react with an enzyme which transfers sulfur to a variety of acceptors to generate thiosulphate, thiocyanate and organic per sulphides (10-12).

Cysteine can donate its sulphur to form homocysteine which can be methylated to give methionine. Thiol groups enter some biologically important thiol compounds by the direct incorporation of cysteine, and most frequently this involves the peptide bond formation. An example in which a portion of the cysteine carbon chain is directly incorporated into the protein has been shown in the synthesis of Biotin by microorganisms (13).

Cysteine is the pivotal compound in the thiol metabolism. Sulphate and other oxidise forms of sulphur are reduced to the level of sulphide which enters organic linkage as cysteine. All biological thiols and their derivatives such as disulphides, thioesters, thioethers and sulphonium salts derive sulphur through cysteine. This is accomplished either by trans sulphuration or by incorporation of the cysteine structure directly.

Electrochemical methods have been used for the quantitative analysis of thiol and disulphide groups in organic compounds as well as in proteins (14-19). The polorography of thiols is characterised by a well defined anodic wave (20-23). In the case of cysteine, the shape of the polarogram depended strongly on pH and buffer (24). It was shown that the anodic wave of cysteine was due to the formation of mercurous mercaptide (24). It was observed that cysteine was oxidised by a 1- electron process to cystine, and further oxidation gave cysteic acid (25-26).

Methionine is one of the twenty amino acids utilized for protein synthesis. N-formyl methionine is an important chain initiator in protein synthesis (27). Methionine reacts with ATP to produce S-adenosyl methionine, with the release of both, an orthophosphate and a pyrophosphate residue. S-adenosyl methionine is an "active methyl" compound, and serves as a methyl donor for biological synthesis. It has been shown that the methyl groups have been derived from methionine in the biosyntheses of various alkaloids such as choline (28,29), gramine (30), sedamine (31), ricinine (32), nicotine (33), isoquinoline alkaloids (34), indole alkaloids(35), quinoline alkaloids (36,37), purine alkaloids (38), and diterpenoids (39).

Thiols have been oxidised to disulphides by peroxidic compounds (40), DMSO (41), halogens (42), diethyl azodicarboxylate (43), nitro and nitroso compounds (44), iodosobenzene (45), transition metal ions (46-51), LTA (52), metal oxides (53,54), flavine derivatives (55), and by photo oxidation (56).

The oxidation of cysteine has been carried out by various oxidising agents such as DMSo(57), auto oxidation in the presence of Cu^{2+} ion (58,59), chromic acid (60,61), permanganate (62), radiolytic oxidation (63), bromide and iodide radical anions (64), super oxide ion (65), and by the 12-tungsto cobaltate (III) ion (66).

Methionine has been oxidised by oxidising agents such as chromate ion (67), Au^{3+} ion (68), chromic acid (60), halogen radical anions (69), auto oxidation in the presence of ascorbic acid (70), periodate (71), hydrogen peroxide (72), Chloramine - B (73,74), Chloramine-T (75), and by Bromamine-T in aqueous solution (76).

PRESENT WORK

The present work is a kinetic investigation of the oxidation of cysteine and methionine by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen utmosphere.

Stoichiometry (Vide 'Experimental').

The stoichiometry of each of the reactions was determined to be:

(a) Cysteine

$$2\ RSH + 2Fe(CN)_6^{3-} + 2OH^- \longrightarrow RSSR + 2Fe(CN)_6^{4-} + 2H_2O$$

(b) Methionine

$$R.CH(NH_2)COOH + 2Fe(CN)_6^{3-} + 2OH^- \longrightarrow R'.CH(NH_2)COOH + 2Fe(CN)_6^{4-} + H_2O$$

(Sulfoxide)

Effect of substrate and oxidant

The rate of the reaction was dependent on the first powers of the concentrations of substrate and oxidant (Tables 1-2).

Table 1 : Effect of substrate and oxidant

Cysteine (10^3 x M)	$K_3Fe(CN)_6$ (10^4 x M)	10^5 x k_{obs} (s^{-1})
5.0	5.0	21.0
10.0	5.0	40.2
25.0	5.0	105.0
50.0	5.0	202.0
5.0	2.5	20.5
5.0	1.0	21.0
5.0	0.5	20.8
5.0	0.1	22.0

/NaOH / = 0.25M; μ = 0.25 M; temp. = 30°C.

Table 2 : Effect of substrate and oxidant

/ Methionine / (10^3 x M)	/ $K_3Fe(CN)_6$ / (10^4 x M)	10^5 x k_{obs} (s^{-1})
1.0	1.0	4.6
5.0	1.0	23.2
10.0	1.0	46.2
25.0	1.0	115.0
1.0	0.5	4.5
1.0	0.25	4.8
1.0	0.1	4.6

/ NaOH / = 0.5M; μ = 0.5M; temp. = 55°C.

Plots of k_{obs} against the concentration of substrate were linear passing through the origin, indicating that the rate of oxidation was dependent on the first power of the concentrations of the substrates. This was further seen by the constant value of k_2, the second order rate constant.

When a constant concentration of the substrates (large excess) was used, k_{obs} did not show any appreciable variation with changing concentration of oxidant, indicating a first order dependence of a reaction on the concentration of oxidant (Table 1).

Effect of alkali

The rate of the reaction was dependent on the first power of the concentration of the alkali in the range studied (Table 3).

Table 3: Effect of NaOH

NaOH (M)	Cysteine ($10^5 \times k_{obs}$, s^{-1})	Methionine
0.05	4.0	0.4
0.10	8.2	0.9
0.25	21.0	2.2
0.50	42.5	4.6
0.75	64.0	6.8

/ Cysteine / = 5×10^{-3}M; / $K_3Fe(CN)_6$ / = 5×10^{-4} M; μ = 0.25 M; temp. = 30°C.

/ Methionine / = 1×10^{-3}M; / $K_3Fe(CN)_6$ / = 1×10^{-3}M; μ = 0.5 M; temp. = 55°C.

Rate Law

Under the present experimental conditions, the rate law could be expressed as:

$$\text{Rate} = -\frac{d/Fe(CN)_6^{3-}/}{dt} = k_{obs}/\text{ Amino Acid }/\,/Fe(CN)_6^{3-}/\,/\,OH^-/$$

..... (1)

The psuedo first order rate constant k_{obs}, was calculated from the equation (77):

$$k_{obs} = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad .... \quad ..... \quad (2)$$

(vide 'Experimental': Calculations).

Effect of Temperature

The rate of the reaction was influenced by changes in temperature, the rate showing an increase with an increase in the temperature (Tables 4-5).

Table 4 : Effect of temperature

Temp.^{o}C ($\pm$0.1^{o}C)	10^5 x k_{obs} (s^{-1})
30.0	21.0
35.0	23.0
40.0	29.0
45.0	38.0
50.0	50.0

/ Cysteine / = 5×10^{-3} M; / $K_3Fe(CN)_6$ / = 5×10^{-4}M;
/ NaOH / = 0.25M; μ = 0.25 M.

Table 5: Effect of temperature

Temp. ($\pm$0.1^{o}C)	10^5 x k_{obs} (s^{-1})
45.0	2.3
50.0	3.2
55.0	4.6
60.0	5.9

/ Methionine / = 1×10^{-3} M; / $K_3Fe(CN)_6$ / = 1×10^{-4} M;
/ NaOH / = 0.5 M; μ = 0.5 M.

From the linear plots of log k_{obs} against the reciprocal of temperature (Fig.1), the activation energies were calculated . The other activation parameters were calculated and have been shown (Table 6) .

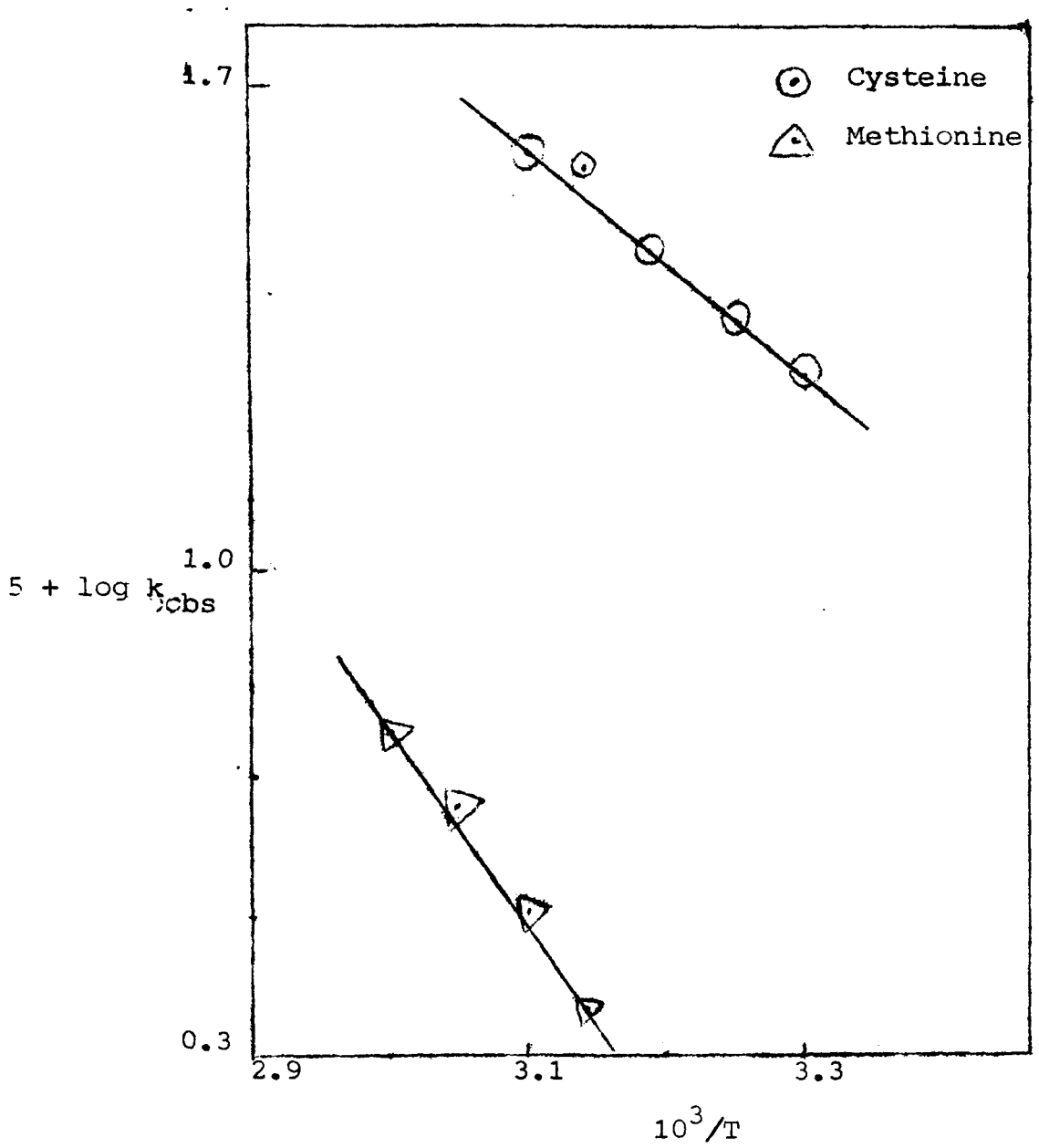


Fig. 1 : Plot of log k_{obs} against the reciprocal of temperature.

Table : Activation Parameters

Parameter	Cysteine	Methionine
E(kJ mol^{-1})	25± 2	55±3
A (s^{-1})	$5x10^3$	$2x10^4$
$\Delta H^{\neq}$ (kJ mol^{-1})	22±2	52±3
$\Delta S^{\neq}$ (JK^{-1} mol^{-1})	-190±6	-170±4

Values of $\Delta S^{\neq}$ in this range for radical reactions have been ascribed to the nature of electron - pairing and electron - unpairing processes (both of which are inherently forbidden processes), and to the loss of degrees of freedom, formerly available to the reactants on the formation of rigid transition state (78).

Effect of added $K_4Fe(CN)_6$

The addition of $K_4Fe(CN)_6$ in the concentration range, 1.0×10^{-4} M to 1.0×10^{-3}M, did not have any effect on the rates of these reactions.

Effect of ionic strength

Variations in the ionic strength of the medium using $NaClO_4$ (μ = 0.01M to 0.50M), did not have any effect on the rates of these reactions.

Effect of added salts

The addition of salts such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4, $MgSO_4$ (concentration range of $1.0x10^{-4}$ M to $5.0x\ 10^{-3}$ M), did not affect the rates of these reactions.

Effects of addition of product

The addition of the product ($1.0x10^{-4}$M to $1.0x10^{-3}$M) did not have any effect on the rates of these reactions.

Radical intermediates

The esr spectrum of the radical generated from the oxidation of cysteine was obtained (Vide 'Experimental': ESR measurements). The radical intermediate, detected in the slow step of the reaction, was a 10.0 G triplet, which was further split by one nitrogen (5.4 G), and two protons which were unequivalent (0.5G and 1.2G), having the value of $g = 2.0037 \pm 0.0002$. It could be assigned to the radical species, $[^{-}SCH_2\ \dot{C}(NH_2)COO^{-}]$, abstraction from the β-carbon atom (with respect to the sulfur) being attributed to the extra stability of the tertiary radical. The two hydrogens of the amino group are not equivalent, as has been earlier reported for radicals obtained from other amino acids (79). In the present investigation, the value of $g = 2.0037 \pm 0.0002$ indicated that the thiyl radical intermediate was absent. Thiyl radicals typically have a

large g-factor anisotropy (principal values such as 2.003, 2.025 and 2.053 have been observed for L-cystine dihydrochloride (80), with anisotropic value of 2.027). No thiyl radical was detected, probably because such radicals could react with thiolate anions (81),

$$RS^{\cdot} + RS^{-} \rightleftharpoons RSSR^{\cdot -} ,$$

and the g-factor of RSSR might cause such line broadening to make it undetectable by ESR spectroscopy.

Mechanism

The rates of the oxidation reactions of cysteine and methionine by hexacyanoferrate (III) were dependent on the first powers of the concentrations of the substrate and oxidant (Tables 1-2). This indicated that the reaction was directly between the substrate and oxidant.

The addition of hexacyanoferrate (II) ions did not have any effect on the rates of these reactions, indicating that the reaction between the substrate and oxidant (the electron abstraction step), was an irreversible one.

The addition of salts did not have any effect on the rate of the reaction, indicating that the reaction was between an ion and a dipolar species.

The reaction pathway was via the formation of a radical intermediates as detected by ESR spectroscopy.

Since potassium hexacyanoferrate (III) is a one-electron oxidant, it would be justified to postulate that the reaction between the substrate and oxidant would give rise to a radical intermediate, analogous to many enzymatic oxidation reactions which also proceed via radical intermediates (82). This would imply that hexacyanoferrate(III), as a chemical oxidant, was capable of mimicking enzymic behaviour.

In the present investigation, the formation of radical intermediates was supported by the following experimental observations:

(a) the rate of the reaction was dependent on the first powers of the concentrations of the substrates and oxidant;

(b) the lack of any effect on the rate by the addition of hexacyanoferrate (II) ions;

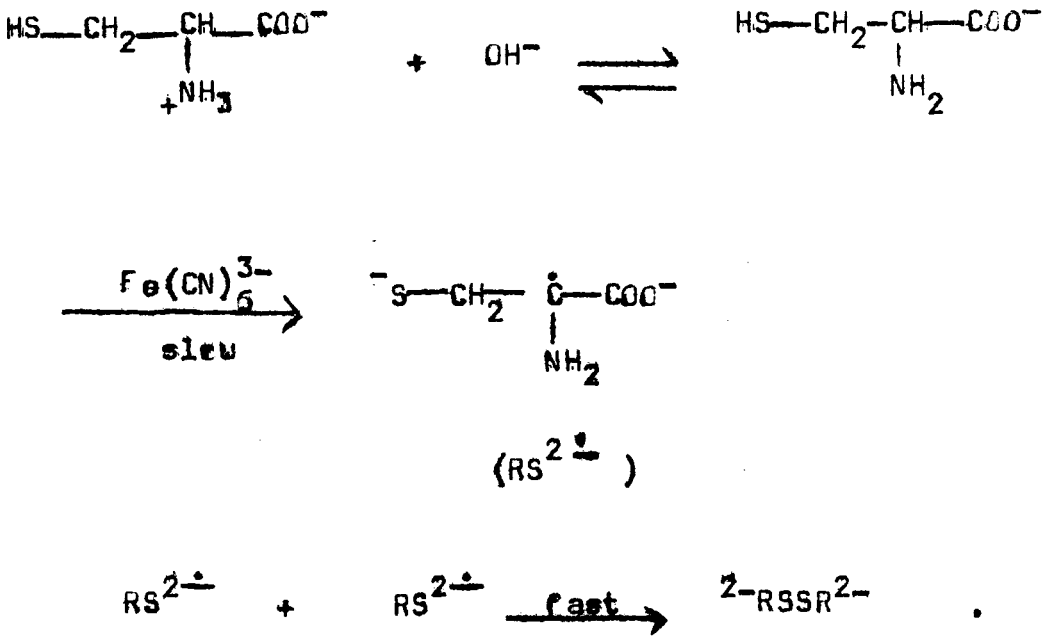
(c) favourable enthalpy and entropy factors which were characteristic of radical processes; and

(d) detection of radical intermediates by ESR spectroscopy.

The pK_a value for cysteine has been reported (83) to be 10.28. It can be assumed that cysteine existed mostly in the ionised form in aqueous medium. Since disulphide was the final product of the oxidation reaction, the sulfhydryl group ($-SH$) provides the site of attack. The reaction

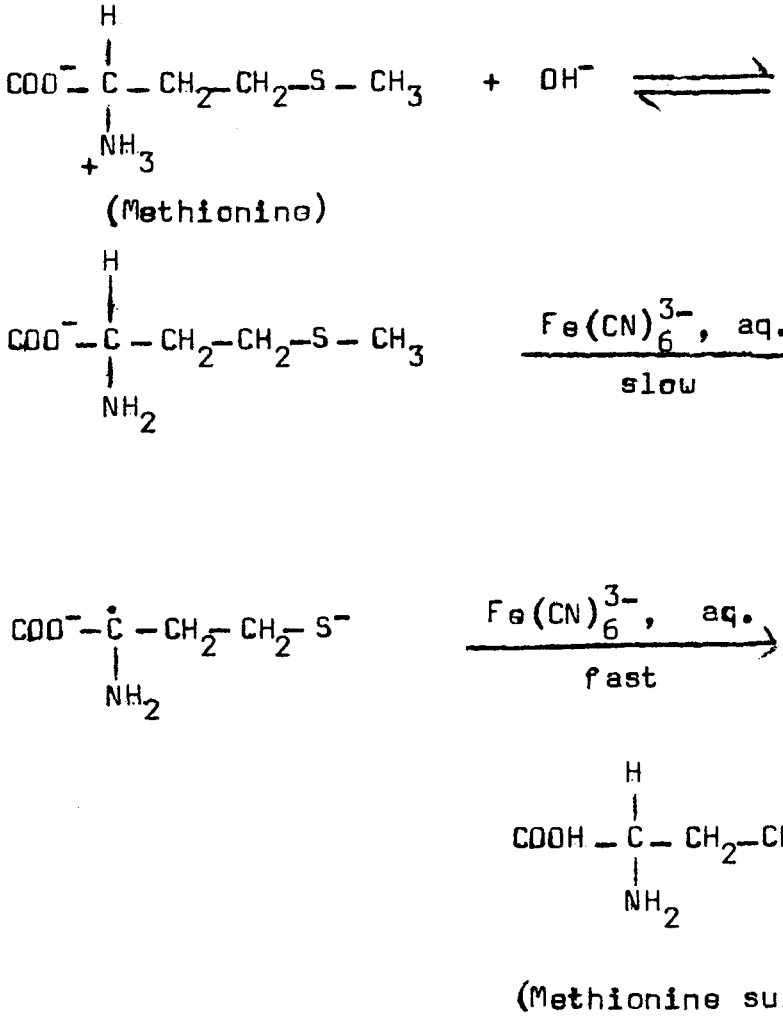
sequence for the oxidation of cysteine by hexacyanoferrate(III), in alkaline medium, is shown in Scheme 1.

SCHEME 1



The reaction sequence for the oxidation of methionine by hexacyanoferrate (III), in alkaline medium, is shown in Scheme 2.

Scheme 2



Earlier investigations had shown that methionine was oxidised to the sulfoxide by oxidising agents such as $KBrO_3$ and ICl (84), bromine (85) and iodine (86).

The products obtained (the disulfide from cysteine, and the sulfoxide from methionine), were isolated and characterised (vide 'Experimental' : Product analysis).

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CHAPTER 4

KINETICS OF OXIDATION OF TRYPTOPHAN AND TYROSINE

Tryptophan is an important synthon in the creation of a very large number of natural products. It is a precursor of the important plant harmones related to indole-3-acetic acid as well as of the indole alkaloids (1). Substituted tryptamines have been found to be widely distributed in several plants (2,3). Tracer experiments have shown that the degradation of gramine by barley plants apparently leads to an indole derivative that can be reconverted to tryptophan(4). The pathway to nicotinic acid from tryptophan, anthranilic acid, is well elucidated in animals, and seems to be used by fungi (5), and by yeast growing aerobically (6). Tracer experiments have shown that the C-3 of tryptophan was specifically converted to C-3 of tropic acid (7). Acid hydrolysis of tryptophan - containing proteins gave rise to harmene and its derivatives, which are important indole alkaloids (8). Many plant alkaloids containing the indole and quinoline nuclei have been shown to be derived from tryptophan (9,10). It has been confirmed that tryptophan combines with anthranilic acid in one of the initial steps in the biosynthesis of the alkaloid, rutaecarpine (11).

The construction of the anthramycin framework by Nature highlights the gainful utilization of the metabolic products of tryptophan (12,13). Melatonin, found in high concentrations in the pineal gland and which is responsible for the construction of melanophores, is an indole derivative arising from tryptophan (14,15). Serotonin occurs widely in animals and plants, and it arises from tryptophan by hydroxylation in the 5-position and subsequent decarboxylation (16,17). Tryptophan has been observed to be at the active sites of many enzymes such as streptococcal proteinase, papain, and trypsinogen (18,19), and in egg white lysozyme (20-24).

Protoalkaloids related to tyrosine are important precursors of various groups of alkaloids (25,26). Tracer experiments with barley have demonstrated the origin of tyramine and hordenine from tyrosine (27-29). High contents of tyrosine were observed in many lupine alkaloids (30). Isoquinoline alkaloids have been derived by the condensation of a carbonyl compound with a derivative of tyrosine (31-33). Tracer feeding experiments showed that tyrosine-2-^{14}C was a precursor of aporphine and morphinan alkaloids in poppy seeds, with the label appearing at C-9 and C-16 of morphine (34-37). Tyrosine has been shown to be a precursor of betanidin, an alkaloid of the imidazole group (38).

The kinetics of oxidation of tryptophan and tyrosine have become important because of their biological significance.

Tryptophan has been oxidized by periodate (39), photooxidation (40), autooxidation (41), inorganic radical ions (42,43), enzymes (44), sodium hypochlorite (45), Osmium tetroxide - pyridine reagent (46), and by chloramine-T(47).

Tyrosine has been oxidized by Cu^{2+}/ H_2O_2(48), peroxidase (49-51), and by chymotrypsin (52).

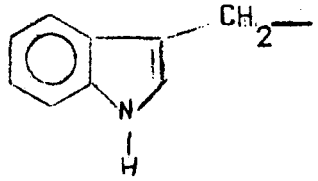
PRESENT WORK

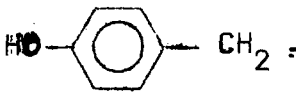
The present work is a detailed kinetic investigation of the oxidation of tryptophan and tyrosine by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere.

Stoichiometry (vide 'Experimental').

The stoichiometry of each of the reactions was determined to be:

$$R.CH(NH_2).COOH + 2Fe(CN)_6^{3-} + 2\ OH^- \longrightarrow R.CO.COOH + 2Fe(CN)_6^{4-} + NH_3 + H_2O \quad .$$

R =  (Tryptophan)

=  (Tyrosine)

Effect of substrate and oxidant

The rate of the reaction was observed to depend on the first powers of the concentrations of both, substrate and oxidant (Table 1).

Table 1: Effect of substrate and oxidant

/ Substrate / (10^3 x M)	/ $K_3Fe(CN)_6$ / (10^4 x M)	10^3 x k_{obs} (s^{-1}) Tyrosine	Tryptophan
1.0	1.0	7.8	5.2
5.0	1.0	38.0	25.0
10.0	1.0	77.0	52.0
25.0	1.0	190.0	130.0
1.0	0.75	7.9	5.5
1.0	0.50	8.0	5.7
1.0	0.25	7.5	5.0
1.0	0.10	7.5	5.3

/ NaOH / = 0.5 M; μ = 0.5 M; temp. = 55°C

Plots of k_{obs} against the concentrations of substrates were linear passing through the origin, suggesting a first order dependence of the rate of oxidation on the concentrations of the substrates. This was further substantiated by the constant value of k_2 (the second rate order constant).

When a constant concentration of substrate (large excess) was used, k_{obs} did not show any appreciable variation with changing concentrations of oxidant, indicating a first order dependence of the reaction on the

concentration of the oxidant (Table 1).

Effect of NaOH

The rate of the reaction showed a first order dependence on the concentration of NaOH in the range studied (Table 2).

Table 2 : Effect of NaOH

/ NaOH / (M)	$10^3 \times k_{obs}$ (s^{-1}) Tyrosine	Tryptophan
0.05	0.7	0.5
0.10	1.5	1.0
0.25	3.8	2.5
0.50	7.8	5.2
1.0	16.0	10.0

/ Substrates / = 1 x 10^{-3} M; / $K_3Fe(CN)_6$ / = 1 x 10^{-4} M;

μ = 0.5 M; temp. = 55°C.

Rate Law:

Under the present experimental conditions, the rate law could be expressed as :

$$\text{Rate} = -\frac{d/Fe(CN)_6^{3-}/}{dt} = k_{obs} / \text{Amino Acid} / / Fe(CN)_6^{3-} / / OH^- / \quad \ldots\ldots\ldots\ldots \quad (1)$$

The psuedo first order rate constant, k_{obs}, was calculated from the equation (53).

$$k_{obs} = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad \ldots\ldots \qquad (2)$$

(vide 'Experimental' : Calculations)

Effect of temperature

The rate of the reaction was influenced by changes in temperature and an increase in temperature resulted in an increase in the rate of the reaction (Table 3).

Table 3 : Effect of temperature

Temp. ($\pm 0.1^{\circ}C$)	$10^3 \times k_{obs}$ (c^{-1}) Tyrosine	Tryptophan
45.0	3.5	3.2
50.0	5.0	4.3
55.0	7.8	5.2
60.0	12.7	6.0

/ Substrates / = 1 x 10^{-3} M; / $K_3Fe(CN)_6$ / = 1 x 10^{-4} M;

/ NaOH / = 0.5 M; μ = 0.5 M.

Plots of log k_{obs} against the reciprocal of temperature were linear (Fig.1), suggesting the validity of the Arrhenius equation. The slopes of the plots were used to calculate the activation energy of the reactions. The other activation parameters were calculated (vide 'Experimental': Calculations) and have been shown in Table 4.

Table 4 : Activation Parameters

Parameter	Tyrosine	Tryptophan
E (kJ mol^{-1})	20±2	25±2
A (s^{-1})	$1x10^4$	5×10^3
$\Delta H^{\neq}$ (kJ mol^{-1})	17±2	22±2
$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	-190±6	-190±6

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were both favourable for electron abstraction processes. The favourable enthalpy was due to the release of energy on solvation of charges created in the transition state. Values of $\Delta S^{\neq}$ in this range for radical processes have been ascribed (54) to the nature of electron pairing and electron unpairing processes and to the loss of degrees of freedom formerly available to the reactants, on the formation of a rigid transition state.

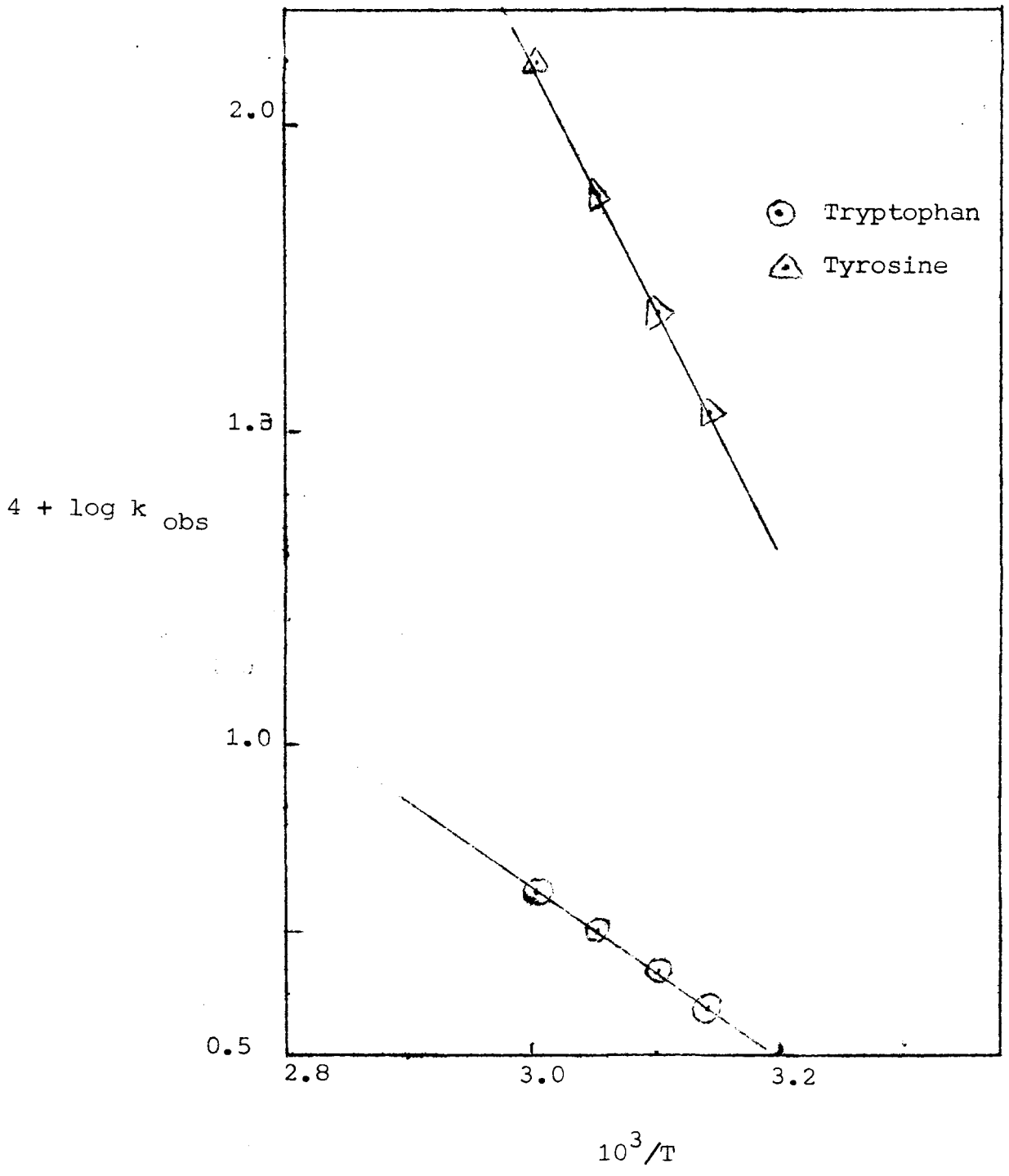


Fig. 1 : Plot of log k_{obs} against the reciprocal of temperature.

Effect of added $K_4Fe(CN)_6$

The addition of $K_4Fe(CN)_6$ in the concentration range, 1.0×10^{-4} M to 1.0×10^{-3} M, did not have any effect on the rates of these reactions.

Effect of ionic strength

Variations in the ionic strength of the medium using $NaClO_4$ (μ = 0.01M to 0.50 M), did not have any influence on the rates of these oxidation reactions.

Effect of added salts

The addition of salts such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4, $MgSO_4$ (Concentration range of 1.0×10^{-4} M to 1.0×10^{-3} M), did not affect the rates of these reactions.

Radical intermediates

The esr spectra of the radicals generated from the oxidation of each, tyrosine and tryptophan, were obtained (Vide 'Experimental' : ESR measurements).

The esr spectra showed a six-line spectra for the radical intermediates obtained from the oxidation of each of the substrates. This was accounted for by a radical intermediate consisting of three equivalent protons and a nitrogen atom, all having nearly equal coupling constants. There was no further splitting of the signals.

Mechanism

The rate of the reaction between the substrate and hexacyanoferrate (III), in alkaline medium, was dependent on the first powers of the concentrations of the reacting species — substrate, oxidant and alkali (Tables 1-2). Since all the kinetic studies were carried out at high concentrations of NaOH (Table 2), it may be assumed that the amino acids would be completely dissociated into their anions.

The addition of hexacyanoferrate (II) ions did not have any effect on the rates of the reactions, indicating that the reaction between the substrate and oxidant (the electron abstraction step) was an irreversible step.

The addition of salts did not have any influence on the rates of reactions, indicating that the reaction was between an ion and a dipolar species.

The reaction pathway was via the formation of radical intermediates as detected by ESR spectroscopy.

The subsequent steps involved the rapid reaction of the radical with the oxidant yielding the imino compound, which underwent hydrolysis to give the corresponding keto acid and ammonia. No other intermediate could be isolated from the reaction mixture.

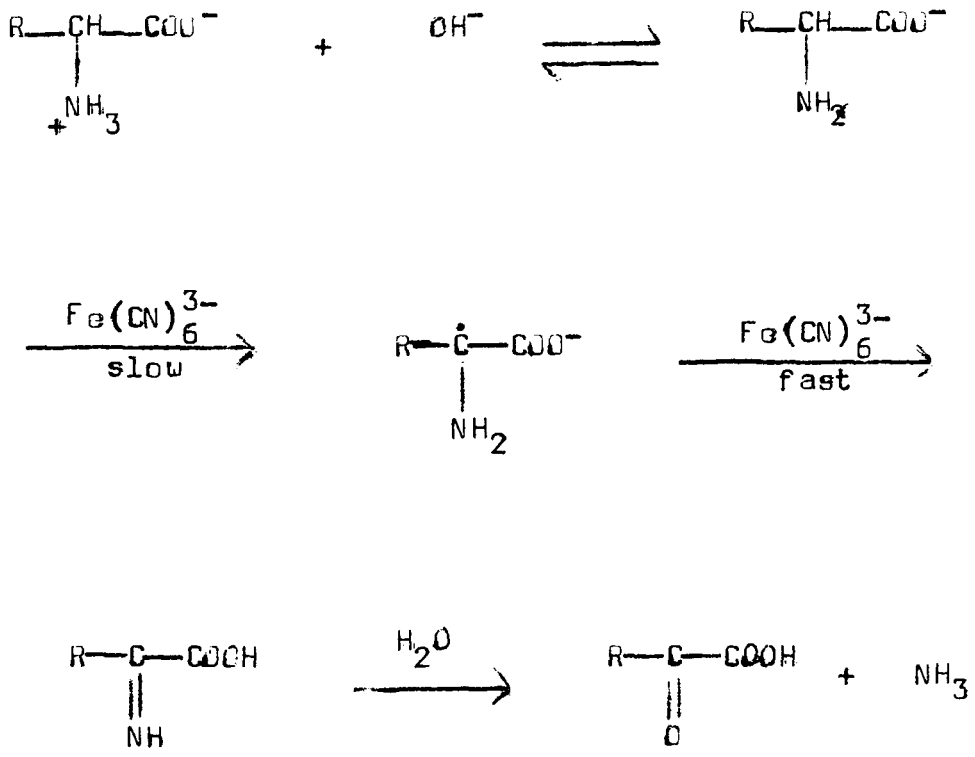
Since potassium hexacyanoferrate (III) is a one electron oxidant, it would be justified to postulate that the reaction between the substrate and oxidant would give rise to a radical intermediate analogous to enzymatic oxidation reaction which are also known to proceed via radical intermediates (55). This would suggest that hexacyanoferrate (III) as a chemical oxidant, was capable of simulating enzymic behavior.

In the present investigation, the formation of radical intermediates was supported by the following experimental observations:

(a) the rate of the reaction was dependent on the first powers of the concentrations of substrate and oxidant;
(b) the lack of any effect on the rate of the reaction by addition of hexacyanoferrate (II) ions,
(c) favourable enthalpy and entropy factors which were characteristic of radical processes;
(d) detection of radical intermediates by ESR spectroscopy.

The reaction sequence for the oxidation of these amino acids (tryptophan and tyrosine) by potassium hexacyanoferrate (III), in alkaline medium, is shown in Scheme I.

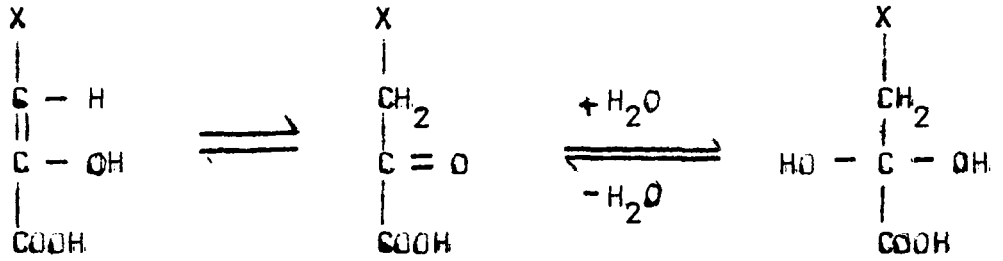
SCHEME



The products obtained (the corresponding keto acids in each case) were isolated and characterised (vide 'Experimental': Product analysis).

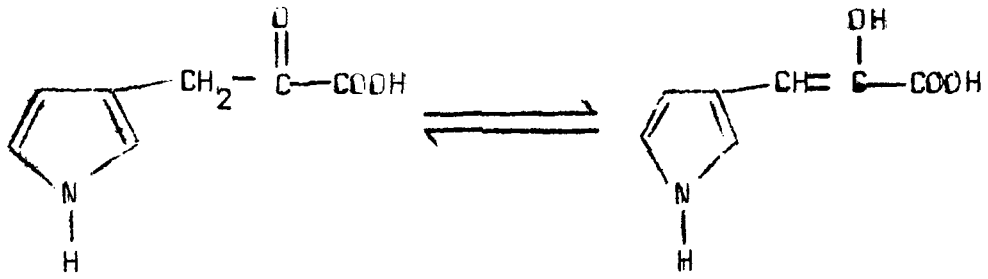
It is known that α-keto acids, I , can form enols, II , or add water to form hydrates or gem-diols,

III, thus (56)



(II) (I) (III)

If X (in structure I) is electron-withdrawing (such as indolyl, IV), the enol is an important contribution, particularly in alkaline solutions (57-59). Some evidence has been presented that β-indolylpyruvic acid, IV (obtained from the oxidation of tryptophan), and β-(p-hydroxyphenyl) pyruvic acid, V (obtained from the oxidation of tyrosine), crystallize as the enol, IVa and Va, respectively (60,61),



(IV) (IVa)

(V) (Va)

Under anaerobic conditions, two forms of β-indolylpyruvic acid could be detected by paper chromatography, presumably the keto (IV) and enol (IVa) forms (62).

The enzyme, arylpyruvate tautomerase, has been observed to catalyze the rapid interconversion between the

keto and enol forms of β-(p-hydroxyphenyl) pyruvic acid, V, and to a lesser extent, β-indolylpyruvic acid, IV (63). The presence of tautomerase has been found to be useful for the spectroscopic determination of enzymatically generated aromatic α-keto acids in borate buffer (63).

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CHAPTER 5

KINETICS OF OXIDATION OF GLUTAMIC ACID AND ASPARTIC ACID

Glutamic acid has been used as a precursor in the biosynthesis of the pyrrolidine and piperidine rings of nicotine and anabasine (1-3). Glutamic acid has been found to be a constituent of various lupine alkalinds (4). The active site in trypsin has been found to contain glutamic acid in position 173(5). In the enzyme, carboxypeptidase A, the glutamic acid residue at position 72 is linked to a zinc ligand, while the glutamic acid in position 270 is present as a nucleophile (6). Glutamic acid at position 35, and aspartic acid at position 52, have been observed to be directly involved in the catalytic function of the enzyme, lysozyme (7).

In the biosynthesis of nicotinic acid from anaerobic yeast (8), bacteria (9), and higher plants (10), the pyridine ring is built from a C_3 unit closely related to glycerol and a second unit closely related to aspartin acid. It was suggested that in the biosynthesis of nicotinic acid , a condensation of glyceraldehyde-3-phosphate with aspartic acid was an important step (11,12). In

chymotrypsin, the aspartic acid residue at position 102 was involved in a charge relay mechanism, while the aspartic acid residue at position 194 functioned as an ion pair which was involved in the activation process (13,14). The aspartic acid residue at position 177 was responsible for the catalytic function of the enzyme, trypsin (15).

Glutamic acid and aspartic acid have been oxidised by a variety of oxidising agents such as ceric ions (16,17), chloramine - T(18), bromamine - B(19), chloramine - B(20), Co (III) catalysed by Ag^{+} ion (21), peroxydisulfate catalysed by Cu^{2+} ion (22), $KMnO_4$ in acid medium in the presence and absence of Ag^{+} ion (23), hexacyanoferrate(III) catalysed by Ru(VI) ion (24), peroxydisulfate ion (25,26), N-bromoacetamide (27), periodate (28), acidic permanganate ion (29), Fenton's reagent (30), and by Fe^{2+} ions (31).

PRESENT WORK

The present work is a detailed kinetic investigation of the oxidation of glutamic acid and aspartic acid by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere.

Stoichiometry (Vide 'Experimental').

The stoichiometry of the reactions was determined to be:

$$R.CH(NH_2)\,COOH + 2Fe(CN)_6^{3-} + 2\,OH^- \longrightarrow R.CO.\,COOH + 2Fe(CN)_6^{4-} + NH_3 + H_2O$$

Here, R = $-CH_2-COOH$ (aspartic acid)

= $-CH_2-CH_2\,COOH$ (glutamic acid)

Effect of substrate and oxidant

The rate of the reaction was observed to be dependent on the first powers of the concentrations of both, substrate and oxidant (Table 1).

Table 1 : Effect of substrate and oxidant

/Substrate /	/$K_3Fe(CN)_6$ /	$10^5 \times k_{obs}$ (s^{-1})	
		Aspartic acid	Glutamic acid
0.05	1.0	4.7	2.1
0.10	1.0	9.3	4.5
0.25	1.0	24.0	10.5
0.50	1.0	47.0	21.0
0.25	0.75	23.0	10.0
0.25	0.50	25.0	10.5
0.25	0.25	24.0	10.0
0.25	0.10	24.0	10.0

/ NaOH / = 1.0 M; μ = 0.5 M; temp. = 65°C.

Plots of k_{obs}, the psuedo first order rate constant, against the concentrations of the substrates, were linear passing through the origin, indicating that the rate of oxidation was dependent on the first power of the concentrations of the substrates. This was further seen by the constant values of k_2, the second order rate constant.

When a constant concentration of the substrate (large excess) was employed, k_{obs} did not show any appreciable variation with changing concentrations of oxidant, indicating

The pseudo first order rate constant, k_{obs}, was calculated from the equation (32):

$$k_{obs} = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad \ldots\ldots \qquad (2)$$

(vide 'Experimental' : Calculations).

Effect of temperature:

The rate of the reaction was influenced by changes in temperature, the rate showing an increase with an increase in the temperature (Table 3).

Table 3: Effect of temperature

Temp. ($\pm 0.1^{\circ}C$)	$10^5 \times k_{obs}$ (s^{-1})	
	Aspartic Acid	Glutamic Acid
50.0	2.6	3.9
55.0	3.7	5.7
60.0	6.5	6.9
65.0	9.3	10.5
70.0	12.0	13.8

/ Aspartic Acid / = 0.1 M; / Glutamic Acid / = 0.25 M;

/ $K_3Fe(CN)_6$ / = 1 x 10^{-3} M; NaOH = 1.0M; μ = 0.5 M.

Plots of log k_{obs} against the reciprocal of temperature were linear (Fig.1). The slopes of these plots were used to calculate the activation energies of the reactions. The other activation parameters were calculated (vide 'Experimental' : Calculations), and have been shown in Table 4.

Table 4: Activation Parameters

Parameter	Aspartic Acid	Glutamic Acid
E($kJmol^{-1}$)	55±3	57±3
A (s^{-1})	$4x10^4$	$3x10^4$
$\Delta H^{\neq}$ ($kJmol^{-1}$)	52±3	54±3
$\Delta S^{\neq}$ (JK^{-1} mol^{-1})	-160±5	-155±5

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were both favourable for electron abstraction processes, suggesting the formation of radical intermediates in the rate determining step of the reaction (33).

Effect of added $K_4Fe(CN)_6$

The addition of $K_4Fe(CN)_6$ in the concentration range, 1.0×10^{-4} M to 1.0×10^{-3} M, did not have any effect on the rates of the reactions.

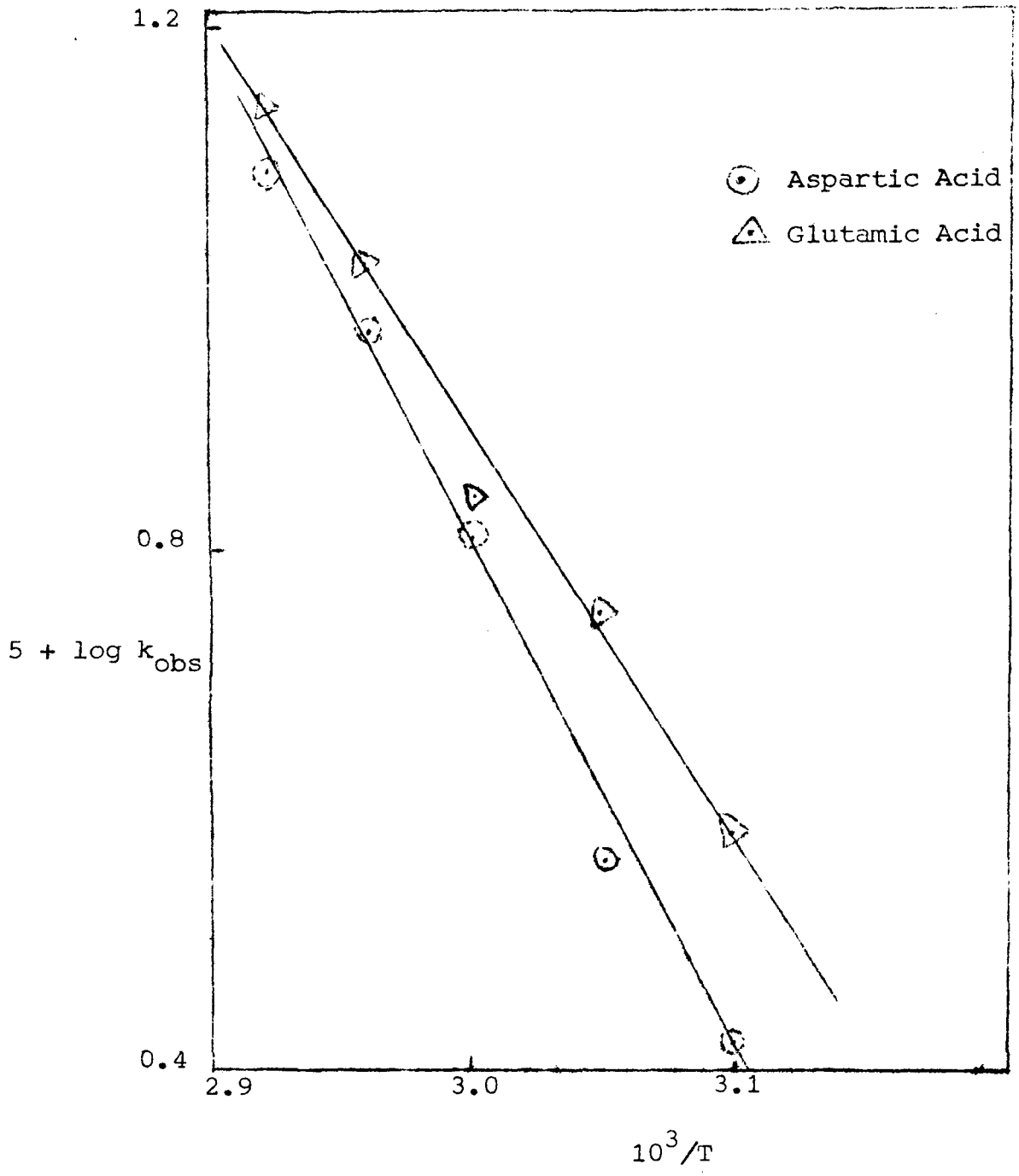


Fig. 1 : Plot of log k_{obs} against the reciprocal of temperature.

Effect of ionic strength

Variations in the ionic strength of the medium using $NaClO_4$ (μ= 0.01M to 0.50 M), did not influence the rates of the reactions.

Effect of added salts

The addition of salts such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4 and $MgSO_4$ (concentration range of 1.0×10^{-4} M to 5.0×10^{-3} M), did not affect the rates of these reactions.

Radical intermediates

The esr spectra of the corresponding radicals, generated from the oxidation of each of the substrates, were generated (vide 'Experimental' : ESR measurements).

The esr spectra of the radicals, obtained from the oxidation of aspartic acid and glutamic acid, each gave a six-line spectrum. This was accounted for by a radical species consisting of two equivalent protons and a nitrogen atom, all having nearly equal coupling constants. No further splitting of the signals was observed.

Mechanism

The rate of the reaction was dependent on the first powers of the concentrations of each of the reacting

species — substrate, oxidant and alkali (Tables 1-2).

Amino acids exist as zwitterions in aqueous solution. Since the kinetic runs were carried out in alkaline solutions, the zwitterion would be quantitatively converted to $RCH(NH_2)COO^-$, which is the reactive species under the present experimental conditions.

The addition of hexacyanoferrate (II) ions did not have any effect on the rates of these reactions, indicating that the electron abstraction step was an irreversible step.

The addition of salts did not have any influence on the rate of the reaction, suggesting that the slow step of the reaction was between an ion and a dipolar species.

The reaction pathway was via the formation of radical intermediates, as detected by ESR spectroscopy.

The subsequent fast steps involved the reaction of the radical with the oxidant, giving the imino compound, which on hydrolysis afforded the corresponding keto acid and ammonia. No other intermediate(s) could be isolated from the reaction mixture.

Since potassium hexacyanoferrate (III) is a one electron oxidant, the reaction between the substrate and the oxidant would involve the formation of radical intermediates analogous to enzymatic oxidation reactions which are known

to proceed via the formation of radical intermediates (34). This would help to establish that hexacyanoferrate (III), as a chemical oxidant, was capable of mimicking enzymatic behaviour.

In the present investigation, the formation of radical intermediates was supported by the following observations:

(a) The rate of the reaction showed a first order dependence on the concentrations of each, substrate and oxidant;

(b) the rate of the reaction was unaffected by the addition of hexacyanoferrate (II) ions;

(c) favourable entropy and enthalpy factors; and

(d) signals observed by ESR spectroscopy.

The reaction sequence for the oxidation of glutamic acid and aspartic acid by potassium hexacyanoferrate (III), in alkaline medium, is shown in the Scheme.

SCHEME

$$R\text{-}\underset{\underset{\overset{+}{N}H_3}{|}}{CH}\text{-}COO + OH^- \rightleftharpoons R\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COO^-$$

$$\xrightarrow[\text{slow}]{Fe(CN)_6^{3-}} R\text{-}\underset{\underset{NH_2}{|}}{C}\text{-}COO^- \xrightarrow[\text{fast}]{Fe(CN)_6^{3-}} R\text{-}\underset{\underset{NH}{||}}{C}\text{-}COOH$$

$$\xrightarrow{H_2O} R\text{-}\underset{\underset{O}{||}}{C}\text{-}COOH + NH_3$$

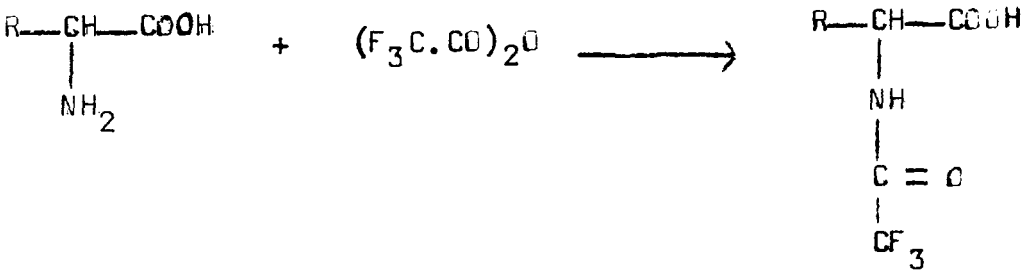
R = $-CH_2COOH$ (Aspartic Acid)

= $-CH_2\text{-}CH_2COOH$ (Glutamic Acid).

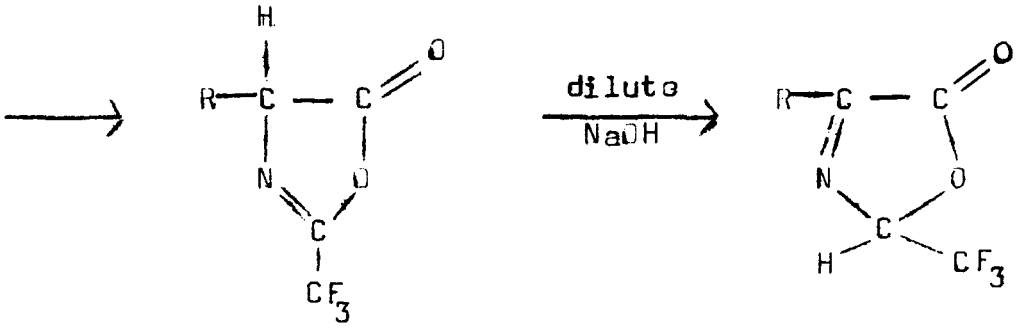
The product obtained in each case (the corresponding keto acid) was isolated and characterised (vide 'Experimental'. Product analysis).

The α-keto acid analogues of the naturally occurring amino acids are of major importance in intermediary metabolism. Oxaloacetic acid and α-ketoglutaric acid are intermediates in the tricarboxylic acid cycle. Interest in the biochemical importance of α-keto acids has increased substantially.

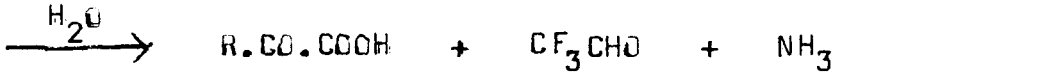
The azlactone method has been employed for the synthesis of oxaloacetic acid and α-ketoglutaric acid, starting from the respective amino acids, aspartic acid and glutamic acid (35). The appropriate amino acid was gently heated with trifluoro acetic anhydride to yield the corresponding N-(trifluoroacetyl) amino acid, 1 , which rapidly cyclized to a 4-substituted-2-(trifluoromethyl)-5-oxazolone, 2 . This compound was readily converted to the corresponding α-keto acid via a novel intramolecular oxidation - reduction, followed by hydrolysis,



(1)



(2)

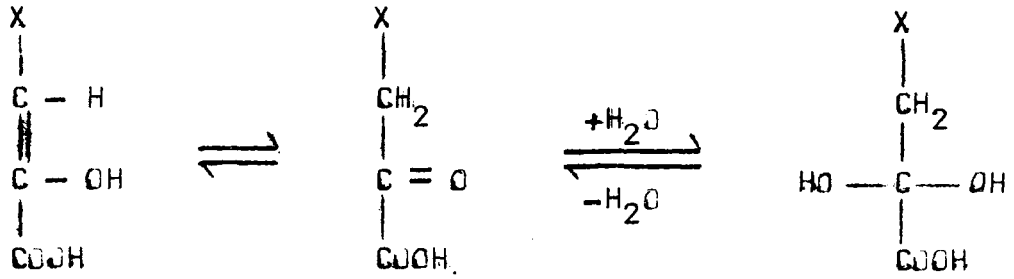


(I)

I : R = $-COCH.CH_2$ (oxaloacetic acid)

R = $-COCH.CH_2.CH_2$ (α-ketoglutaric acid)

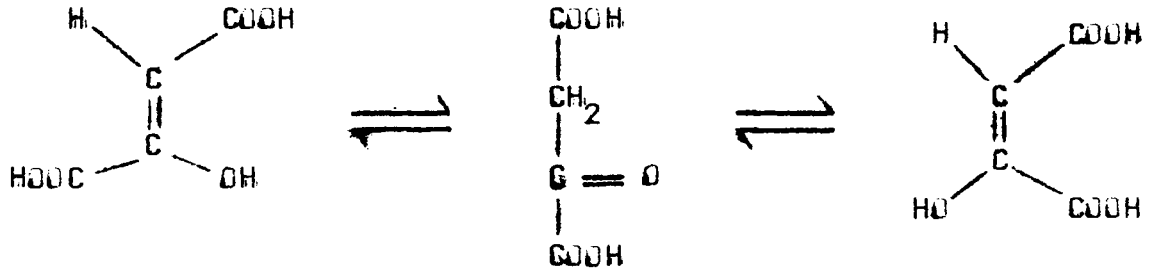
It is known that α-keto acids, I , can form enols, II , or add water to form hydrates or gem-diols, III,



(II) (I) (III)

When X is a simple aliphatic group or is linked through one or more methylenes (e.g., as in α-ketoglutaric acid), the enol is quantitatively unimportant in neutral or acidic solutions (36). When X is electron withdrawing (X = COOH, as in oxaloacetic acid), the enol is an important contribution particularly in alkaline solutions (37-39). These α-keto acids absorb strongly in the uv region (300 nm) in base, due to conjugation of the enol with the carboxyl carbonyl.

Pure oxaloacetic acid, 3 , is probably in the enol form (40,41), while in solution it contains both cis and trans enolic forms (38,40,41), that is, hydroxyfumaric acid, 3a , and hydroxymaleic acid, 3b .



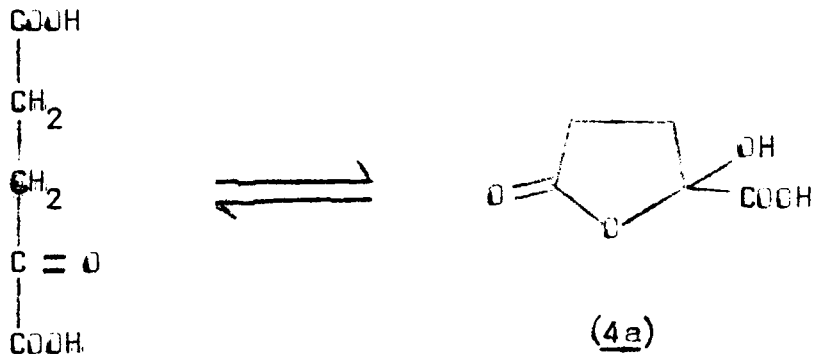
(3a) (3) (3b)

The mechanism of the interconversion between keto and enol forms of oxaloacetic acid, 3 , has been extensively studied (42).

The degree of hydration of an α-keto acid correlates well with the inductive effect of the substituents adjacent to the carbonyl group (43). The kinetics of the hydration reaction of oxaloacetic acid, 3 , have been studied (44).

α-ketoglutaric acid, 4 , exhibits an anomalous ^1H NMR spectrum in D_2O at pH = 0.5 (43). This was attributed to an equilibrium between α-keto acid and

lactol, 4a (43),

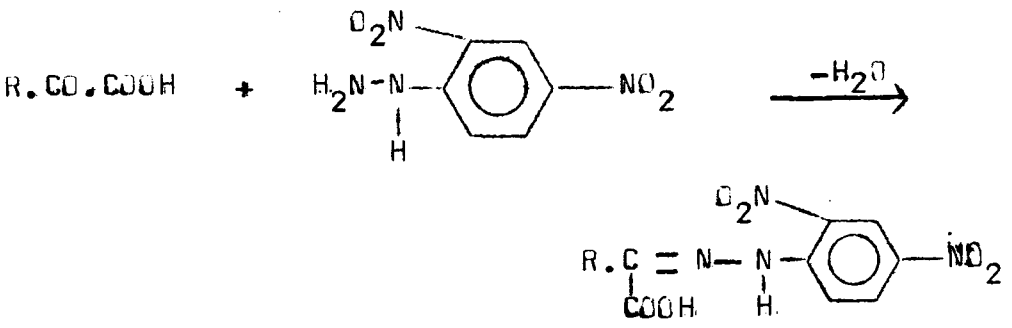


(4) (4a)

Later evidence showed that at neutral pH, α-ketoglutaric acid was in equilibrium with a lactol, although the lactol was present in small amounts (45,46).

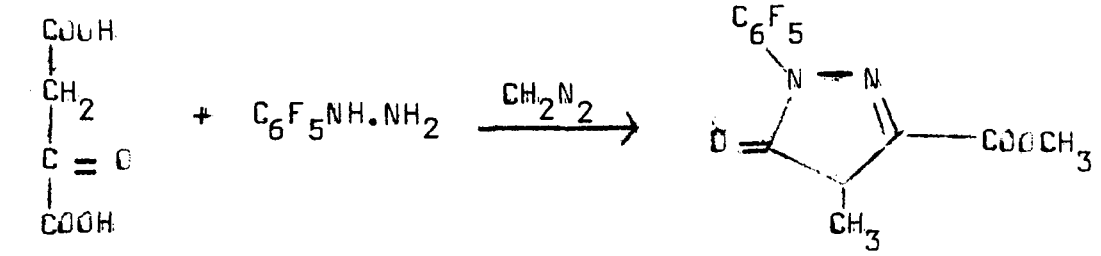
α-Ketoglutaric acid underwent photofragmentation when subjected to EPR analysis (47).

α-Keto acids form a large number of crystalline derivatives, the most useful being the 2,4-dinitrophenylhydrazones.

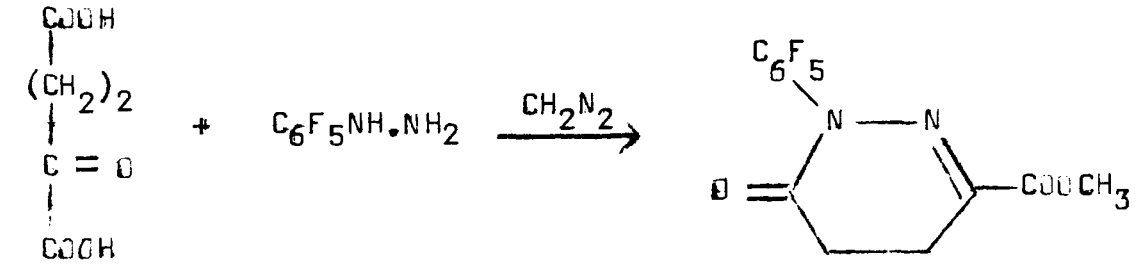


Both the cis and trans forms of α-ketoglutaric acid 2,4-dinitrophenyl-hydrazones have been prepared (48). The kinetics of the isomerization reaction of cis-α-keto acid 2,4-dinitrophenyl-hydrazones have been investigated(49).

A GC method has been developed for the determination of α-keto acids based on the reaction with pentafluorophenylhydrazzine, followed by the reaction with diazomethane to produce the methyl ester (50). Oxaloacetic acid and α-ketoglutaric acid gave cyclic products (50),



(Oxaloacetic) acid



(α-ketoglutaric) acid

Enzymatic methods have been used for the determination of oxaloacetic acid and α-ketoglutaric acid (51).

Analytical methods, based on the formation of fluorescence products, have been used for the estimation of total α-keto acid or simple mixtures of α-keto acids. These methods have used reagents such as o-phenylenediamine (52), pyridoxamine and zinc (53), and α-hydrazino-2-stilbazole (54).

HPLC techniques, using post-column derivatization with N-methyl-nicotinamide chloride, have been used for the fluorometric determination of α-ketoglutaric acid (55).

It has been shown that oxaloacetic acid and α-ketoglutaric acid can be separated on a C_{18} column by reversed-phase HPLC as the 4-(bromomethyl)-7-coumarin derivatives (56).

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CHAPTER 6

KINETICS OF OXIDATION OF LYSINE, ARGININE AND HISTIDINE.

The biosynthesis of stachydrine and homostachydrine was studied using lysine as a precursor (1). The piperidine alkaloids, such as isopelletierine and coniine, have been synthesized starting from lysine (2). The pyridone ring of mimosine, a piperidine alkaloid, has been derived from lysine (3-5). Sedamine has derived from lysine (6). Lysine has been used in the synthesis of anabasine, a tobacco alkaloid (7). Lysine has been used as a possible pyridine ring precursor in the synthesis of nicotinic acid (8). High lysine contents have been found in some lupine alkaloids (9). Tracer feeding experiments have led to the overall conclusion that the carbon skeletons of all the major lupine alkaloids were derived from lysine (10-14). Alkaloids with the quinazoline nucleus are present in several plant families, and the combination of anthranilic acid with a lysine derivative gives the mackinlaya alkaloids (15). The biosynthesis of alkaloids such as lycopodine (16) and spartine (17), have been achieved starting from lysine. These pathways stress the importance of creating highly condensed nitrogen heterocydes from simple precursors. The first

evidence implicating lysine in the activity of the enzyme, ribonuclease, was obtained by Hirs and coworkers (18-20), who suggested that lysine at position 41 was part of an anion binding site at or near the active site of ribonuclease.

Arginine has been used in syntheses of some protoalkaloids (21), tropane alkaloids (22) and lupine alkaloids (23).

In some plant species, histidine has been converted to urocaric acid (24,25). High histidine contents have been observed in different lupine alkaloids (26). Various alkaloids having an imidazole ring are presumably made from histidine, as for example pilocarpine (27). Some imidazole alkaloids(28) seem to be derived from the N-acylation of histamine (the decarboxylation product of histidine), whereas some unusual sulfur containing alkaloids have been formed by methylation, thiomethylation, and ring closure of histamine (29). The importance of the histidine residue has been shown in biological reactions. In hemoglobin (as in many other hemoproteins), one histidine residue is near the iron atom. In many enzyme protins, histidine acts as a proton donor or acceptor. In nature, apparently complicated transformations are common, and their genesis could be related to the maximum optimization in the construction of molecular frameworks, particularly those which play an

important role in life processes. This fact is best illustrated with the "ATP-imidazole" cycle, which is related to the biosynthesis of histidine.

Lysine has been oxidised by chloramine - T(30), and by hexacyanoferrate (III) catalysed by OS(VIII) ion (31,32).

Arginine has been oxidised by chloramine - T (30, 33-37), Potassium permanganate in acidic medium (38), N-Bromo succinimide in aqueous perchloric acid medium (39), and by N-bromo acetamide in acid medium (40).

Histidine has been oxidized by chloramine - T (30,33,35,37,41), photo oxidation (42), aquapentacyanoferrate (II) ion (43), Diethyl pyrocarbonate in aqueous solution (44), and by N-bromoacetamide in acid medium (40).

PRESENT WORK

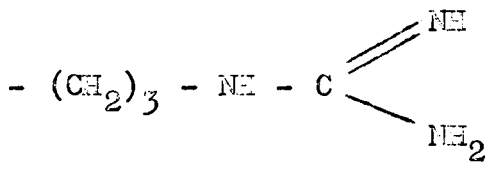
The present work is a detailed kinetic investigation of the oxidation of lysine, arginine and histidine by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere.

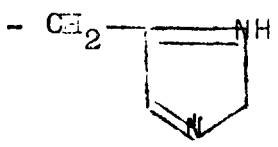
Stoichiometry (vide 'Experimental').

The stoichiometry of each of the reactions was determined to be

$$R.CH(NH_2).COOH + 2Fe(CN)_6^{3-} + 2\,OH^- \longrightarrow R.CO.COOH + 2Fe(CN)_6^{4-} + NH_3 + H_2O \quad .$$

R = $-(CH_2)_4-NH_2$ (lysine),


(arginine),


(histidine).

Effect of substrate and oxidant

The rate of the reaction was dependent on the first powers of the concentrations of both, substrate and oxidant (Tables 1-3).

Table 1 : Effect of substrate and oxidant

/ Lysine / (10^2 x M)	/ $K_3Fe(CN)_6$ / (10^3 x M)	10^5 x K_{obs} (s^{-1})
1.0	1.0	15.2
2.5	1.0	38.5
5.0	1.0	76.0
10.0	1.0	150.0
25.0	1.0	380.0
1.0	0.75	16.0
1.0	0.50	15.5
1.0	0.25	15.0
1.0	0.10	16.0

/ NaOH / = 0.5 M; μ = 0.5 M; temp. = 55°C

Table 2: Effect of substrate and oxidant

/ Arginine / (10^2 x M)	/ $K_3Fe(CN)_6$ / (10^3 x M)	10^4 x k_{obs} (s^{-1})
1.0	1.0	0.3
2.5	1.0	0.8
5.0	1.0	1.6
10.0	1.0	3.3
25.0	1.0	8.5
10.0	5.0	3.2
10.0	2.5	3.3
10.0	0.5	3.3
10.0	0.25	3.4

/ NaOH / = 0.1 M; μ = 0.5 M; temp. = 35°C.

Table 3: Effect of substrate and oxidant

/Histidine / (10^3 x M)	/ $K_3Fe(CN)_6$ / (10^4 x M)	10^4 x k_{obs} (s^{-1})
5.0	5.0	8.1
10.0	5.0	16.5
25.0	5.0	41.0
50.0	5.0	83.0
100.0	5.0	165.0
10.0	1.0	16.7
10.0	2.5	16.5
10.0	10.0	16.2

/ NaOH / = 0.5M; μ = 0.5; temp. = 55°C.

Plots of k_{obs} against the concentrations of substrates were linear passing through the origin, suggesting a first order dependence of the rate of oxidation on the concentrations of the substrates. This was further substantiated by the constant values of k_2, the second rate order constant.

When a constant concentration of substrate (large excess) was used, k_{obs} did not show any appreciable variation with changing concentrations of oxidant, indicating a first order dependence of the reaction on the concentration of the oxidant (Tables 1-3).

Effect of NaOH

The rate of the reaction showed a first order dependence on the concentration of alkali in the range studied (Table 4).

Table 4: Effect of NaOH

/NaOH / (M)	10^4 x k_{obs} (s^{-1}) Lysine	arginine	Histidine
0.05	1.5	1.5	1.7
0.10	3.0	3.3	3.3
0.25	7.6	8.2	8.5
0.50	15.2	16.0	16.5
0.75	24.0	25.0	26.0
1.0	30.0	33.0	34.0

/ Lysine / = 1 x 10^{-2} M; / $K_3Fe(CN)_6$ / = 1x10^{-3} M ;
μ = 0.5 M; temp. = 55°C.
/ Arginine / = 0.1 M; / $K_3Fe(CN)_6$ / = 1x10^{-3} M;
μ = 0.5 M; temp. = 35°C.
/ Histidine / = 1x10^{-2}M; / $K_3Fe(CN)_6$ / = 5x10^{-4} M;
μ = 0.5 M; temp. = 55°C.

Rate Law

Under the present experimental conditions, the rate law could be expressed as:

$$\text{Rate} = -\frac{d/Fe(CN)_6^{3-}/}{dt} = k_{obs}\ /\text{Amino Acid}/\ /Fe(CN)_6^{3-}/\ /OH^-/ \quad \ldots\ldots\ldots \quad (1)$$

The pseudo first order rate constant, k_{obs}, was calculated from the equation (45).

$$k_{obs} = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad \ldots\ldots \qquad (2)$$

(vide 'Experimental' : Calculations).

Effect of temperature

The rate of the reaction was influenced by changes in temperature, and an increase in temperature resulted in an increase in the rate of the reaction (Tables 5-7).

Table 5 : Effect of temperature

Temp. ($\pm$ 0.1°C)	$10^5 \times k_{obs}$ (s^{-1}) Lysine
45.0	7.1
50.0	10.0
55.0	15.2
60.0	24.1
65.0	34.0

/ Lysine / = 1×10^{-2} M; / $K_3Fe(CN)_6$ / = 1×10^{-3} M;

/ NaOH / = 0.5 M; μ = 0.5 M.

Table 6 : Effect of temperature

Temp. (± 0.1°C)	$10^4 \times k_{obs}$ (s^{-1}) Arginine
30.0	2.4
35.0	3.3
40.0	4.5
45.0	6.2
50.0	8.5

/ Arginine / = 0.1 M; / $K_3Fe(CN)_6$ / = 1 x 10^{-3} M;

/ NaOH / = 0.1 M; μ = 0.5 M.

Table 7: Effect of temperature

Temp. (± 0.1°C)	$10^4 \times k_{obs}$ (s^{-1}) Histidine
40.0	7.7
45.0	9.3
50.0	12.7
55.0	16.5
60.0	20.5

/ Histidine / = 1 x 10^{-2} M; / $K_3Fe(CN)_6$ / = 5x10^{-4} M;

/ NaOH / = 0.5 M; μ = 0.5 M.

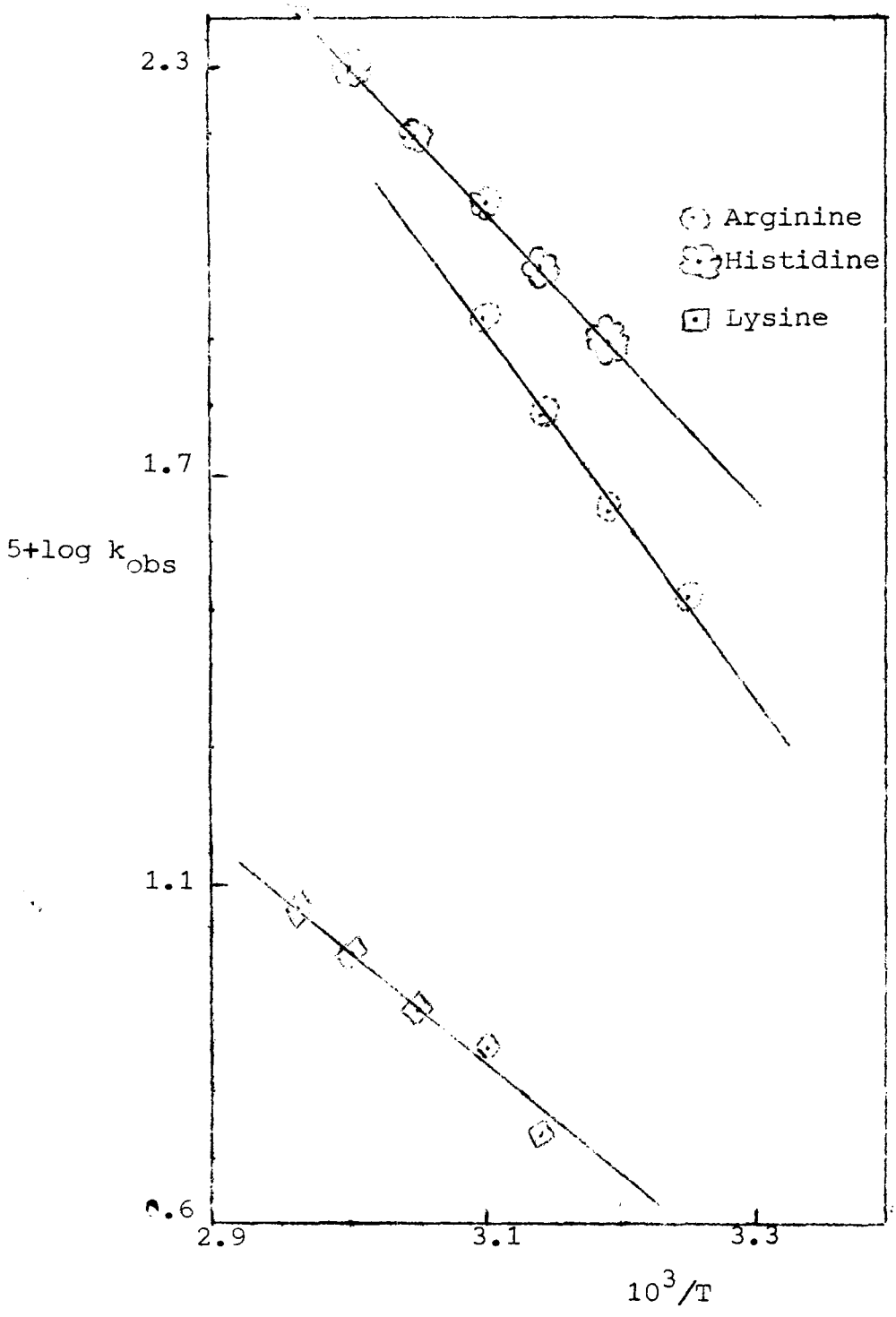


Fig. 1 : Plot of log k_{obs} against the reciprocal of temperature.

Plots of log k_{obs} against the reciprocal of temperature were linear (fig.1), suggesting the validity of the Arrhenius equation. The slopes of the plots were used to calculate the activation energies of the reactions. The other activation parameters were calculated (vide **'Experimental'** : Calculations) and have been shown in Table 8.

Table 8 ; Activation Parameters

Parameter	Lysine	Arginine	Histidine
E($kJmol^{-1}$)	44±2	49±2	43±2
A(s^{-1})	$4x10^4$	$3x10^4$	$1x10^4$
$\Delta H^{\neq}$ ($kJmol^{-1}$)	41±2	46±2	40±2
$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	-170±5	-160±5	-175±6

The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were both favourable for electron abstraction processes. The favourable enthalpy was due to the release of energy on solvation of charges created in the transition state. Values of $\Delta S^{\neq}$ in this range for radical processes have been ascribed (46) to the nature of electron pairing and electron unpairing processes, and to the loss of degrees of freedom, formerly available to the reactants, on the formation of a rigid transition state.

Effect of added $K_4Fe(CN)_6$

The addition of $K_4Fe(CN)_6$ in the concentration range of 1.0×10^{-4} M to 1.0×10^{-3} M, did not have any effect on the rates of these reactions.

Effect of ionic strength

Variations in the ionic strength of the medium using $NaClO_4$ (μ= 0.01 M to 0.5 M), did not affect the rates of these reactions.

Effect of added salts

The addition of salts such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4 and $MgSO_4$ (concentration range of 1.0×10^{-4}M to 5×10^{-3}M), did not have any influence on the rates of these reactions.

Radical intermediates

The esr spectra of the corresponding radicals, generated from the oxidation of each of the substrates, were obtained (vide 'Experimental' : ESR measurements).

The radical, generated from the oxidation of each of the substrates, gave a six-line spectrum, having intensity ratios of 1:3:5:5:3:1. This radical intermediate was formed by the loss of a hydrogen atom from the carbon atom of the methylene group. The number of lines was accounted for by

the radical species,

$R - \overset{\bullet}{\underset{\underset{NH_2}{|}}{C}} - COO^-$, which corresponds to a radical with two equivalent protons and a nitrogen atom, all having nearly equal coupling constants.

Mechanism

The rate of the reaction between the substrate and hexacyanoferrate (III), in alkaline medium, was dependent on the first powers of the concentrations of the reacting species - substrate, oxidant and alkali (Tables 1-2). Since all the kinetic studies were carried out at high concentrations of NaOH (Table 2), it may be assumed that the amino acids would be completely dissociated into their anions.

The addition of hexacyanoferrate (II) ions did not have any effect on the rates of the reactions, indicating that the reaction between the substrate and oxidant (the electron abstraction step) was an irreversible step.

The addition of salts did not have any influence on the rates of the reactions, indicating that the reaction was between an ion and a dipolar species.

The reaction pathway was via the formation of radical intermediates, as detected by ESR spectroscopy.

The subsequent steps involved the rapid reaction of the radical with the oxidant yielding the imino compound, which underwent hydrolysis to give the corresponding keto acid and ammonia. No other intermediate(s) could be isolated from the reaction mixture.

Since potassium hexacyanoferrate (III) is a one electron oxidant, it would be justified to postulate that the reaction between the substrate and oxidant would give rise to a radical intermediate, analogous to enzymatic oxidation reactions, which are also known to proceed via radical intermediates (47). This would suggest that hexacyanoferrate (III), as a chemical oxidant, was capable of simulating enzymic behaviour.

In the present investigation, the formation of radical intermediates was supported by the following experimental observations:-

(a) the rate of the reaction was dependent on the first powers of the concentrations of the substrate and oxidant;

(b) the lack of any effect on the rate of the reaction by the addition of hexacyanoferrate (II) ions;

(c) favourable enthalpy and entropy factors, which were characteristic of radical processes

(d) detection of radical intermediates by ESR spectroscopy.

The ionization constants and the pH values at the isoelectric points of lysine, arginine and histidine at 25°C are given in Table 9.

Table 9: Ionization constants[48] and pH values at isoelectric points

Amino Acid	pK_1	pK_2	pK_3	pH_i
Lysine	2.18	9.12	10.53	9.82
Arginine	2.17	9.04	12.48	10.76
Histidine	1.82	6.00	9.17	7.59

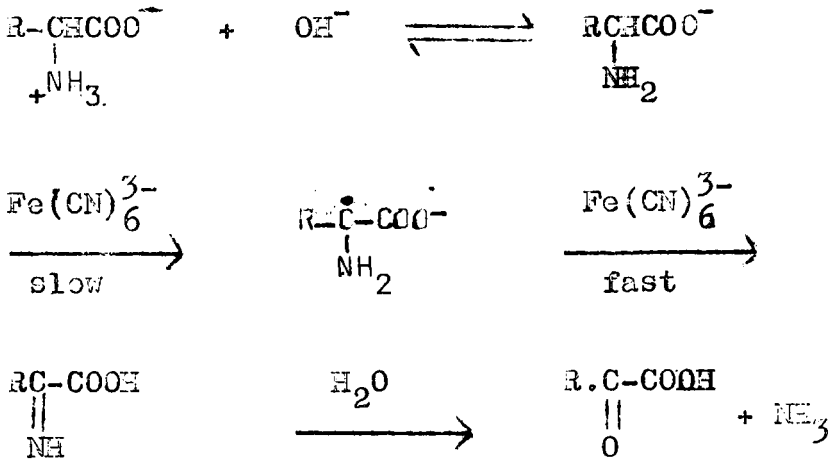
For these basic amino acids,

$pH_i = \frac{pK_2 + pK_3}{2}$, where pH_i is the isoelectric point.

Amino acids exist as zwitterions in aqueous solution. In alkaline solutions, the zwitterion is converted to $RCH(NH_2)\,COO^-$, which is the reactive species, under the present experimental conditions.

The reaction sequence for the oxidation of these basic amino acids (lysine, arginine and histidine) by potassium hexacyanoferrate (III), in alkaline medium, is shown in the Scheme.

SCHEME

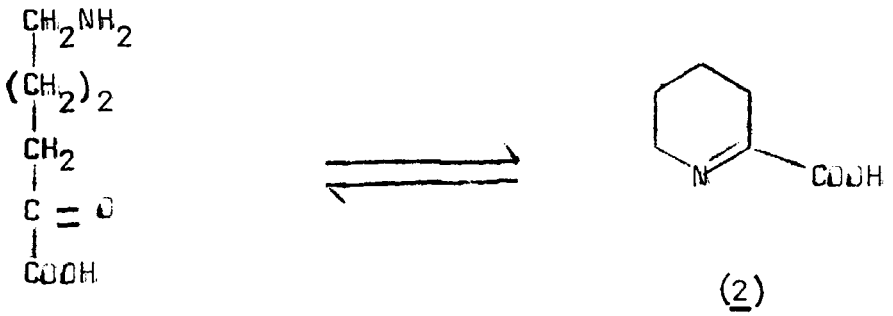


The ϵ-amino group of lysine was unaffected during the course of the oxidation reaction, in agreement with an earlier observation (49).

The products obtained from the oxidation of each of these amino acids were the corresponding α-keto acids (α-keto-ϵ-aminocaproic acid from lysine; α-keto-δ-guanidinovaleric acid from arginine; and β-imidazolyl-pyruvic acid from histidine), which were isolated and characterized as their respective 2,4-dinitrophenyl-hydrazone derivatives (vide 'Experimental': Product Analysis).

There have been reports of α-keto acids with unusual properties, in that the α-keto acids can exist in equilibrium with their respective cyclic forms.

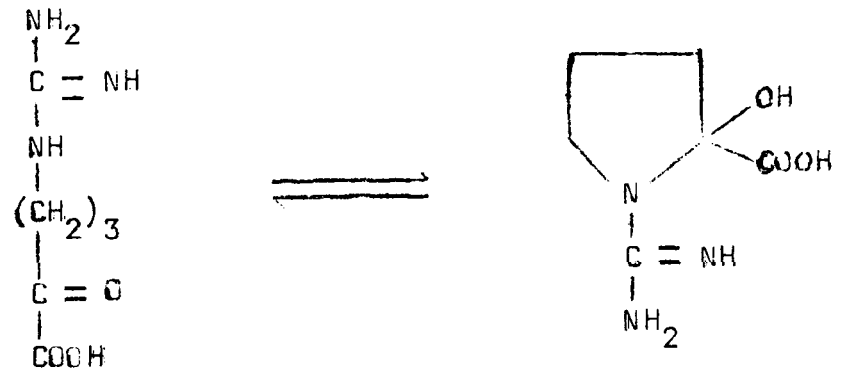
It has been shown that the α-keto acid analogues of lysine and arginine can exist in equilibrium with their respective cyclic forms. The α-keto acid analogue of lysine (α-keto-ϵ-aminocaproic acid, 1) can exist in equilibrium with Δ^1- piperidine-2-carboxylic acid, 2), as observed in an earlier investigation (49), thus:



(1) (2)

Similarly, the α-keto acid analogue of arginine (α-keto-δ-guanidinovaleric acid, 3), has been shown to exist in equilibrium with its cyclic form, 1-amidino-2-hydroxy-pyrrolidine-2-carboxylic acid, 4 , as shown in an earlier

work (50), thus:



(3) (4)

These cyclization reactions explain the earlier observations that the α-keto acid analogue of arginine, 3 , reacts more slowly with 3-quinolylhydrazine (51) and with γ-glutamylhydrazide (52), than acyclic α-keto acids at comparable concentrations.

These kinds of cyclization reactions of α-keto acids indicate their unusual properties which have been highlighted from time to time.

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RECORD OF

EXPERIMENTAL VALUES

Glycine

$[K_3Fe(CN)_6] = 1.0 \times 10^{-3}$ M, [NaOH] $= 1.0 \times 10^{-1}$ M,
T = (55±0.1)°C, OD(420 nm) for [Glycine] at

t_m	0.01M	0.025M	0.05M	0.1 M	0.25 M	0.5 M
0	0.344	0.344	0.344	0.344	0.344	0.344
5	0.342	0.339	0.334	0.325	0.298	0.258
10	0.340	-	0.325	0.306	0.259	0.193
15	0.338	0.330	0.316	0.289	0.224	0.145
20	0.337	0.325	0.306	0.272	0.195	0.108
25	0.334	0.321	0.298	0.257	0.169	0.082
30	0.332	0.316	0.290	0.242	0.146	0.061
35	0.331	0.312	-	0.228	0.127	0.046

/ $K_3Fe(CN)_6$ / $= 1.0 \times 10^{-3}$ M, / Glycine / $= 1.0 \times 10^{-2}$ M,
OD(420 nm) T = (55±0.1)°C, for /NaOH / at

t_m	0.1 M	0.25M	0.5 M	0.75M	1.0M	1.5 M
0	0.344	0.344	0.344	0.344	0.344	0.344
5	0.342	0.342	0.342	0.342	0.342	0.342
10	0.340	0.340	-	0.340	0.340	0.340
15	0.338	0.338	0.339	0.338	-	0.338
20	0.337	0.336	0.336	0.336	0.337	0.336
25	0.334	0.335	0.334	0.334	0.334	0.334
30	0.332	0.332	0.332	0.332	-	-
35	0.331	0.330	0.330	0.329	-	-

/ Glycine / = 1.0×10^{-2}M, / NaOH / = 1.0×10^{-1} M, T = $(55 \pm 0.1)^{\circ}$C, OD (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.001 M	0.00075 M	0.0005M	1.00025M	0.0001 M
0	0.344	0.305	0.291	0.158	0.087
5	0.342	0.303	0.289	0.157	-
10	0.340	0.302	0.288	0.156	0.086
15	0.338	0.300	0.286	0.156	-
20	0.337	0.298	0.284	-	-
25	0.334	-	0.283	0.154	0.085
30	0.332	0.295	0.282	0.152	-
35	0.331	0.293	0.281	0.151	0.083

/ Glycine / = 1.0×10^{-2} M, / $K_3Fe(CN)_6$ = 1.0×10^{-3}M, / NaOH / = 1.0×10^{-1}M, OD (420nm) for temperature at

t_m	35°C	40°C	45°C	50°C	55°C	60°C
0	0.344	0.344	0.344	0.344	0.344	0.344
5	-	-	-	-	0.342	-
10	0.343	0.342	0.342	0.341	0.340	0.339
15	0.342	0.342	0.341	0.340	0.338	0.336
20	0.342	0.341	0.339	0.338	0.337	0.333
25	0.341	0.340	0.338	0.337	0.334	0.331
30	-	0.339	0.337	-	0.332	0.328
35	0.340	0.338	0.336	0.334	0.331	0.326

Alanine

/ NaOH / = 1.0M, / $K_3Fe(CN)_6$ / = 5.0 x 10^{-4} M,
T = (75±0.1)°C, OD (420 nm) for / Alanine / at

t_m	0.1M	0.5M	0.75M	1.0 M
0	0.158	0.158	0.158	0.158
5	0.155	0.154	0.152	0.151
10	0.152	0.150	0.148	0.145
15	0.149	0.147	0.142	0.139
20	0.146	0.143	0.137	0.136
25	0.144	0.141	0.132	0.126
30	0.141	0.137	0.128	0.120

/ $K_3Fe(CN)_6$ / = 5.0 x 10^{-4} M, / Alanine / = 5.0 x 10^{-2} M,
T = (75±0.1)°C, OD(420 nm) for / NaOH / at

t_m	0.25M	0.5 M	1.0 M
0	0.158	0.158	0.158
5	0.157	0.156	0.154
10	0.155	0.153	0.150
15	0.153	0.151	0.147
20	0.152	0.149	0.143
25	0.151	0.147	0.141
30	0.150	0.145	0.137

/ NaOH / = 1.0 M, / Alanine / = 5 x 10^{-1} M,
T = (75±0.1)°C, OD (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.0001	0.0005	0.001 M
0	0.036	0.158	0.279
5	-	0.154	0.275
10	0.032	0.150	0.272
15	0.03	0.147	0.270
20	0.028	0.143	0.267
25	0.026	0.141	0.264
30	0.024	0.137	0.260

/ $K_3Fe(CN)_6$ / = 5.0 x 10^{-4} M, / Alanine / = 5.0 x 10^{-1} M,
/ NaOH / = 1.0 M, OD (420 nm) for Temperature at

t_m	75°C	80°C
0	0.158	0.158
5	0.154	0.151
10	0.150	0.146
15	0.147	0.140
20	0.143	0.134
25	0.141	0.129
30	0.137	0.124

Valine

/ NaOH / = 5.0 x 10^{-1} M, / $K_3Fe(CN)_6$ / = 1.0x10^{-3} M,
T = (55 ± 0.1)°C, OD(420 nm) for /Valine / at

t_m	0.01M	0.05 M	0.1 M
0	0.41	0.41	0.41
5	-	0.406	0.399
10	0.408	0.402	0.394
15	-	0.398	0.392
20	0.407	0.395	0.387
25	-	0.390	0.375
30	0.405	0.386	0.364
35	0.404	0.383	0.360

/ $K_3Fe(CN)_6$ / = 1.0x10^{-3} M, / valine / = 5.0 x 10^{-2} M,
T = (55±0.1)°C, OD (420 nm) for / NaOH / at

t_m	1.0M	0.75M	0.5 M	0.25 M
0	0.41	0.41	0.41	0.41
5	0.402	0.406	0.406	0.408
10	0.394	0.402	0.402	0.407
15	0.386	0.398	0.398	0.405
20	0.379	0.393	0.395	0.404
25	0.371	0.389	0.390	0.402
30	0.363	0.385	0.386	0.401
35	0.357	0.380	0.383	0.399

/ NaOH / = 5.0×10^{-1} M, / Valine / = 5.0×10^{-2} M,
T = $(55 \pm 0.1)^{\circ}$C, OD(420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.001 M	0.0005 M	0.0001 M
0	0.41	0.268	0.069
5	0.406	0.265	-
10	0.402	0.262	0.068
15	0.398	0.260	0.067
20	0.395	0.257	0.066
25	0.390	0.255	0.065
30	0.386	0.251	0.064
35	0.383	0.249	0.064

/ $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, / Valine / = 5.0×10^{-2} M,
/ NaOH / = 5×10^{-1} M, OD (420 nm) for temperature at

t_m	40°C	45°C	50°C	55°C
0	0.41	0.41	0.41	0.41
5	-	0.399	0.407	0.406
10	0.409	0.389	0.405	0.402
15	0.409	0.379	0.403	0.398
20	-	0.369	0.400	0.395
25	0.408	0.360	0.397	0.390
30	0.407	0.350	0.395	0.386
35	0.407	-	0.393	0.383

Leucine

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / NaOH / = 1 M,

T = $(55 \pm 0.1)^{\circ}C$, O D(420 nm) for / Leucine / at

t_m	0.01 M	0.025 M	0.05 M	0.1 M
0	0.048	0.048	0.048	0.048
10	0.047	0.045	0.043	0.038
15	0.046	0.044	0.040	0.032
20	0.045	0.043	0.038	0.029
25	-	0.042	0.035	0.025
35	0.043	0.039	0.031	0.021

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / Leucine / = 1.0×10^{-2} M,

T = $(55 \pm 0.1)^{\circ}C$, OD (420nm) for / NaOH / at

t_m	1.0M	0.5 M	0.25 M	0.1 M
0	0.048	0.048	0.048	0.048
10	0.047	-	0.047	-
15	0.046	0.046	-	0.046
20	0.045	0.045	-	-
25	-	-	0.045	0.045
35	0.043	0.044	0.044	-

/ Leucine / = 1.0×10^{-2} M, / NaOH / = 1 M, T = (55±C.1)°C,
O D (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.0001 M	0.00025 M	0.0005 M
0	0.048	0.092	0.165
10	0.047	0.090	0.161
15	0.046	0.088	0.158
20	0.045	0.086	0.156
35	0.043	0.084	0.150

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / NaOH / = 1.0 M,
/ Leucine / = 1.0×10^{-2} M, O D (420 nm) for Temperature at

t_m	50°C	55°C	60°C	65°C
0	0.048	0.048	0.048	0.048
10	-	0.047	0.045	0.044
15	0.047	0.046	0.044	0.042
20	0.046	0.045	0.043	0.040
25	0.046	-	0.041	0.038
35	0.045	0.043	0.039	0.035

Phenylalanine

/ $K_3Fe(CN)_6$ / = 1.0 x 10^{-4} M, / NaOH / = 1.0 x 10^{-1} M,

T = (55±0.1)°C, O D (420 nm) for / Phenylalanine / at

t_m	0.0025 M	0.005 M	0.01 M	0.025 M
0	0.043	0.043	0.043	0.043
10	0.042	-	0.041	0.037
15	0.042	0.041	0.039	0.036
20	0.041	0.040	0.038	0.035
25	0.041	-	0.037	0.034
30	0.040	0.039	0.036	0.033
35	0.040	0.038	0.035	0.032

/ $K_3Fe(CN)_6$ / = 1.0 x 10^{-4} M,/ Phenylalanine / = 1.0x10^{-2} M,

T = (55 ± 0.1)°C, O D (420 nm) for / NaOH / at

t_m	0.05 M	0.1 M	0.25 M	0.5 M
0	0.043	0.043	0.043	0.043
10	-	0.041	0.041	0.040
15	0.039	0.039	0.039	0.040
20	0.038	0.038	0.039	0.039
25	0.036	0.037	0.038	0.038
30	0.036	0.036	0.037	-
35	0.035	0.035	0.037	0.034

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / Phenylalanine / = 1.0×10^{-2} M, / NaOH / = 1.0×10^{-1} M, O D (420 nm) for temperature at

t_m	45°C	50°C	55°C	60°C	65°C
0	0.043	0.043	0.043	0.043	0.043
10	0.041	0.041	0.041	0.040	0.039
15	-	0.040	0.039	0.038	0.038
20	0.040	0.039	0.038	0.037	0.036
25	0.039	0.038	0.037	0.036	0.035
30	0.038	0.037	0.036	0.035	0.034
35	0.037	0.036	0.035	-	-

Serine

/ $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, /NaOH / = 1.0 M,
O D (420 nm) T = (55 ± 0.1)°C, for / Serine / at

t_m	0.05M	0.1M	0.25 M	0.5 M
0	0.135	0.135	0.135	0.135
5	0.134	-	0.131	0.127
10	0.133	0.132	0.126	0.119
15	0.132	0.130	0.122	0.112
20	0.131	0.128	0.119	0.105
25	0.130	0.127	0.115	0.100
30	0.130	0.125	0.111	0.093

/ $K_3 Fe(CN)_6$ / = 1.0 x 10^{-3}M, / Serine / = 1.0x10^{-1}M,
O D (420 nm) T = (55±0.1)°C, for / NaOH / at

t_m	0.25 M	0.5 M	1.0 M
0	0.135	0.135	0.135
5	0.133	0.133	-
10	0.133	0.131	0.132
15	0.131	0.130	0.130
20	-	0.130	0.128
25	0.126	0.128	0.127
30	0.120	0.125	0.125

/ NaOH / = 1.0 x 10^{-3}M, / Serine / = 1.0 x 10^{-1} M,
T = (55±0.1)°C, O D (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.0001 M	0.001 M	0.0005 M
0	0.02	0.135	0.093
5	0.01	-	0.088
10	0.005	0.132	0.083
15	0.002	0.130	0.079
20	-	0.128	0.076
25	-	0.127	0.072
30	-	0.125	0.069

/ $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, / Serine / = 1.0×10^{-1} M,

/ NaOH / = 1.0 M, O D(420 nm) for temperature at

t_m	45°C	50°C	55°C	60°C	65°C
0	0.135	0.135	0.135	0.135	0.135
5	-	0.134	-	0.132	0.132
10	0.133	0.133	0.132	0.130	0.128
15	-	0.132	0.130	0.128	0.125
20	0.132	0.130	0.128	0.126	0.122
25	-	0.129	0.127	0.124	0.119
30	0.131	0.128	0.125	0.121	0.116

Threonine

/ NaOH / = 1.0 M, / $K_3Fe(CN)_6$ / = 1.0×10^{-3}M,

/ Threonine / = 1.0×10^{-1} M, T = (60±0.1)°C

O D (420 nm) for Temperature at

t_m	40°C	45°C	50°C	55°C	60°C
0	0.098	0.098	0.098	0.098	0.098
10	0.096	0.095	0.095	0.094	0.094
15	0.094	0.094	0.093	0.093	0.091
20	0.093	0.092	0.091	0.091	0.089
25	0.092	0.091	0.090	0.089	0.087
30	0.091	0.090	0.088	0.087	0.085
35	0.090	0.089	0.087	0.084	0.083

Cysteine

/ $K_3Fe(CN)_6$ / = 5.0x10^{-4} M, / NaOH / = 2.5 x 10^{-1} M,

T = (30±0.1)°C, O D (420 nm) for / cysteine / at

t_m	0.0035M	0.005 M	0.007 M
0	0.032	0.032	0.032
5	0.031	-	0.028
10	0.029	0.028	0.026
15	0.028	0.027	0.023
20	0.027	0.026	0.021
25	0.026	0.024	0.018
30	0.025	0.023	0.015
35	-	0.022	0.013

/ $K_3Fe(CN)_6$ / = 5.0 x 10^{-4}M, / cysteine / = 5.0 x 10^{-3} M,

T = (30±0.1)°C, O D (420nm) for / NaOH / at

t_m	0.5M	0.25M	0.1M
0	0.032	0.032	0.032
5	0.027	-	0.031
10	0.024	0.028	0.029
15	0.021	0.027	0.028
20	0.018	0.026	0.028
25	0.016	0.024	0.027
30	0.013	0.023	0.026
35	0.012	0.022	0.024

/ NaOH / = 2.5 x 10^{-1} M, / Cysteine / = 5 x 10^{-3} M,
T = (30±0.1)°C, O D (420 nm) for ($K_3Fe(CN)_6$ / at

t_m	0.0005 M	0.0007 M
0	0.032	0.051
5	-	0.048
10	0.028	0.045
15	0.027	0.042
20	0.026	0.040
25	0.024	0.038
30	0.023	0.036
35	0.022	0.034

/ $K_3Fe(CN)_6$ / = 5.0 x 10^{-4} M, / NaOH / 2.5 x 10^{-1}M,
/ Cysteine / = 5.0 x 10^{-3} M, O D (420 nm) for Temp. at

t_m	30°C	35°C	40°C	45°C	50°C
0	0.032	0.032	0.032	0.032	0.032
5	-	0.030	0.029	0.028	0.028
10	0.028	0.028	0.027	0.026	0.025
15	0.027	0.026	0.025	0.023	0.022
20	0.026	0.024	0.022	0.020	0.020
25	0.024	0.023	0.021	0.018	0.018
30	0.023	0.021	0.019	0.016	0.016
35	0.022	0.019	0.018	0.015	0.014

Methionine

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / NaOH / = 0.5 M,

T = (55±0.1)°C, O D(420 nm) for / Methionine / at

t_m	0.001 M	0.005 M	0.01 M
0	0.05	0.05	0.05
5	-	0.046	0.045
10	-	0.044	0.040
15	0.048	0.041	0.035
20	0.047	0.038	0.032
25	0.047	0.035	0.029
30	0.046	0.033	0.025

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / Methionine / = 1.0×10^{-3} M,

O D (420 nm) T = (55±0.1)°C, for / NaOH / at

t_m	0.05 M	0.1 M	0.25 M	0.5 M
0	0.05	0.05	0.05	0.05
10	0.049	0.049	0.049	-
15	0.048	0.048	0.048	0.048
20	0.047	-	-	0.047
25	0.046	0.047	0.046	0.047
30	-	-	-	0.046
35	0.045	0.044	0.045	-

/ Methionine / = 1.0×10^{-3} M, / NaOH / = 5.0×10^{-1} M, T = $(55 \pm 0.1)^{\circ}C$, O D (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.0001 M	0.0005 M
0	0.05	0.018
15	0.048	-
20	0.047	0.017
25	0.047	-
30	0.046	-
40	-	0.016

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / NaOH / 0.5 M, / Methionine / = 1.0×10^{-3} M, T = $(55 \pm 0.1)^{\circ}C$ O D (420 nm) for / kcl / at

t_m	0.01 M	0.05 M	0.1 M
0	0.05	0.05	0.05
5	0.049	-	0.049
10	0.049	-	-
15	0.048	0.048	0.048
20	0.047	-	-
25	0.046	0.046	0.047
30	-	-	0.046
35	0.046	0.045	-

/ $K_3Fe(CN)_6$ / = 1.0 x 10^{-4} M, / NaOH / = 0.5 M,

/ Methionine / = 1.0 x 10^{-3} M, O D (420 nm) for Temp. at

t_m	45^o	50^o	55^o	60^o
0	0.05	0.05	0.05	0.05
10	-	0.049	-	0.048
15	0.049	-	0.048	-
20	-	0.048	0.047	0.047
25	-	0.048	0.047	0.046
30	0.048	0.047	0.046	0.045
35	-	-	-	0.044

Tyrosine

/ $K_3Fe(CN)_6$ / = 1.0x10^{-3} M, / NaOH / = 5.0 x 10^{-1} M,

Temp = (55±0.1)^{o}C, for / Tyrosine / at

t_m	0.001 M	0.0005 M	0.00025 M
0	0.250	0.250	0.250
5	0.088	0.180	0.197
10	-	0.154	0.191
15	0.034	0.144	0.190
20	0.022	0.136	0.188
25	0.018	0.129	-

/ Tyrosine / = 1.0×10^{-3} M, / $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, O D (420 nm) T = $(55 \pm 0.1)^{\circ}C$, for / NaOH / at

t_m	0.05 M	0.10 M	0.5 M
0	0.250	0.250	0.250
5	0.191	0.105	0.088
10	0.152	0.080	-
15	0.130	0.055	0.034
20	0.110	0.035	0.022
25	0.097	0.028	0.018

/ Tyrosine / = 1.0×10^{-3} M, / NaOH / = 5.0×10^{-1} M, Temp. = $(55 \pm 0.1)^{\circ}C$, O D (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.0005 M	0.001 M
0	0.128	0.250
5	0.021	0.088
10	0.008	-
15	0.004	0.034
20	0.001	0.022
25	-	0.018

/ Tyrosine / = 1.0×10^{-3} M, / $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, / NaOH / = 5.0×10^{-1} M, O D (420 nm) for temperature at

t_m	45°C	50°C	55°C	60°C
0	0.250	0.250	0.250	0.250
5	0.126	0.096	0.088	-
10	0.072	0.068	-	0.028
15	0.058	0.045	0.034	0.020
20	0.052	0.034	0.022	0.016
25	0.039	0.029	0.018	0.013

Tryptophan

/ $K_3Fe(CN)_6$ / = 1.0×10^{-4} M, / NaOH / = 1.0×10^{-2} M,

T = (55±0.1)°C, O D (420 nm) for / Tryptophan / at

t_m	0.001 M	0.0025 M
0	0.057	0.057
5	0.049	0.039
10	0.042	0.027
15	0.036	0.018
20	0.030	0.012
25	0.026	0.008
30	0.022	0.006
35	0.019	0.004

/ $K_3Fe(CN)_6$ / = 1.0x10^{-4} M, / Tryptophan / = 1.0x10^{-3} M,
T = (55±0.1)°C, O D = (420 nm) for / NaOH / at

t_m	0.005 M	0.01 M	0.05 M
0	0.057	0.057	0.057
5	0.049	0.049	0.05
10	0.042	0.042	-
15	0.036	0.036	0.039
20	0.031	0.030	0.028
25	0.027	0.026	0.028
30	0.023	0.022	0.027
35	0.020	0.019	0.026

/ NaOH / = 1.0x10^{-2} M, / Tryptophan / = 1.0x10^{-3} M,
T = (55±0.1)°C , O D (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.00005 M	0.00001 M	0.001 M
0	0.024	0.005	0.057
5	0.021	-	0.049
10	0.017	-	0.042
15	0.015	0.003	0.036
20	0.012	-	0.030
25	0.010	--	0.026
30	0.009	0.002	0.022
35	0.007	-	0.019
45	-	0.001	-

/ $K_3Fe(CN)_6$ / = 1.0x10^{-4} M, / NaOH / = 1.0 x 10^{-2} M,
/ Tryptophan / = 1.0 x 10^{-3} M, O D (420 nm) for Temp. at

t_m	45°C	50°C	55°C	60°C
0	0.057	0.057	0.057	0.057
5	0.051	0.050	0.049	0.048
10	0.045	0.048	0.042	0.040
15	0.040	0.036	0.036	0.034
20	0.036	0.033	0.030	0.028
25	0.032	0.030	0.026	0.024
30	0.029	0.026	0.022	0.020
35	0.026	0.023	0.019	0.017

Histidine

/ $K_3Fe(CN)_6$ / = 5.0x10^{-4} M, / NaOH / = 5.0x10^{-1} M,
O D(420 nm) T = (55±0.1)°C, for / Histidine / at

t_m	0.01 M	0.025 M	0.05 M
0	0.134	0.134	0.134
2	0.109	0.080	0.049
4	0.090	0.047	0.018
6	0.074	0.028	0.006
8	0.060	0.017	0.003
10	0.049	0.010	0.001
12	0.040	0.006	-

/ Histidine / = 1.0×10^{-2} M, / $K_3Fe(CN)_6$ / = 5×10^{-4} M,

O D (420 nm) for / NaOH / at.

t_m	0.05 M	0.10 M	0.25 M	0.50 M
0	0.134	0.134	0.134	0.134
2	-	0.129	0.121	0.109
4	0.128	0.123	0.110	0.090
6	0.126	0.118	0.099	0.074
8	0.123	0.113	0.09	0.060
10	0.121	0.109	0.081	0.049
12	0.118	0.104	0.072	0.040

/ Histidine / = 1.0×10^{-2} M, / NaOH / 5.0×10^{-1} M,

O D (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.0001 M	0.00025 M	0.0005 M	0.001 M
0	0.017	0.032	0.134	0.198
2	0.013	0.023	0.109	0.160
4	0.009	0.017	0.090	0.129
6	0.008	0.012	0.074	0.104
8	0.006	0.009	0.060	0.083
10	0.004	0.006	0.049	0.067
12	-	0.005	0.040	0.054

/ Histidine / = 1.0×10^{-2} M, / NaOH / = 5.0×10^{-1} M,

/ $K_3Fe(CN)_6$ / = 5.0×10^{-4} M, O D(420 nm) for

Temperature at

t_m	45°C	50°C	55°C	60°C
0	0.134	0.134	0.134	0.134
2	0.120	0.115	0.109	0.105
4	0.107	0.099	0.09	0.078
6	0.096	0.085	0.074	0.068
8	0.086	0.073	0.060	-
10	0.077	0.062	0.049	0.039
12	0.068	0.054	0.040	0.031

Arginine

/ $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, / NaOH / = 2.0 M,

O D (420 nm) T = (35±0.1)°C, for / arginine / at

t_m	0.05 M	0.10 M	0.25 M
0	0.051	0.051	0.051
5	0.048	0.046	0.037
10	0.047	0.042	0.028
15	0.044	0.038	0.021
20	0.042	0.034	0.014
25	0.040	0.031	0.010
30	0.039	0.028	0.008
35	0.037	0.026	-

/ $K_3Fe(CN)_6$ / = $1.0x10^{-3}$ M, / arginine / = $1x10^{-1}$ M,

O D (420 nm) T = $(35\pm0.1)^{o}C$, for / NaOH / at

t_m	1.0 M	2.0 M	3.0 M
0	0.051	0.051	0.051
5	0.049	0.046	0.042
10	0.047	0.042	0.036
15	0.046	0.038	0.030
20	0.045	0.034	0.027
25	0.043	0.031	0.023
30	0.041	0.028	0.020
35	0.040	0.026	0.017

/ Arginine / = $1.0x10^{-1}$ M, / NaOH / = 2.0 M,

O D (420 nm) T = $(35\pm0.1)^{o}C$, for / $K_3Fe(CN)_6$ / at

t_m	0.0025 M	0.0005 M	0.00075M	0.001 M
0	0.059	0.028	0.036	0.051
5	0.057	0.022	0.030	0.046
10	0.055	0.016	0.025	0.042
15	0.053	0.013	0.021	0.038
20	0.052	0.009	0.018	0.034
25	0.050	0.008	0.015	0.031
30	0.048	0.006	0.012	0.028
35	0.047	-	-	0.026

/ $K_3Fe(CN)_6$ / = 1.0 x 10^{-3} M, / NaOH / = 2.0 M,
/ Arginine / = 1.0 x 10^{-1} M, O D (420 nm) for temperature at

t_m	35°C	40°C	45°C	50°C
0	0.051	0.051	0.051	0.051
5	0.046	0.044	0.042	0.039
10	0.042	0.040	0.036	0.032
15	0.038	0.035	0.029	0.025
20	0.034	0.029	0.024	0.018
25	0.031	0.028	0.020	0.014
30	0.028	0.022	0.017	0.011
35	0.026	0.020	0.014	-

Glutamie acid

/ $K_3Fe(CN)_6$ / = 1.0x10^{-3} M, / NaOH / = 1.0 M, T=(65±0.1)°C,
O D (420 nm) for / glutamie acid / at

t_m	0.05 M	0.1 M	0.25 M
0	0.3	0.3	0.3
5	0.298	0.295	0.286
10	0.297	0.289	0.284
15	0.292	0.286	0.274
20	0.290	0.282	0.266
25	0.287	0.276	0.263
35	0.279	0.267	0.247

/ $K_3Fe(CN)_6$ / = 1.0 x 10^{-3} M, / Glutamic acid / = 2.5x10^{-1} M,

T = (65±0.1)°C, O D (420 nm) for / NaOH / at

t_m	1 M	2 M
0	0.3	0.3
5	0.286	0.29
10	0.284	0.281
15	0.274	0.271
20	0.266	0.262
25	0.263	0.254
25	0.247	0.237

/ Glutamic acid / = 2.5 x 10^{-1} M, / NaOH / = 1.0 M,

T = (65±0.1)°C, O D (420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.001 M	0.0005 M	0.0001 M
0	0.3	0.194	0.049
5	0.286	0.185	-
10	0.284	0.179	0.041
15	0.274	0.170	0.038
20	0.266	0.160	0.032
25	0.263	0.157	0.028
35	0.247	0.128	0.026

/ Glutamic acid / = 2.5×10^{-1} M, / NaOH / = 1.0 M,

/ $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, O D (420 nm) for Temp. at

t_m	50°C	55°C	60°C	65°C	70°C
0	0.3	0.3	0.3	0.3	0.3
5	0.295	0.295	-	0.286	0.287
10	0.295	0.290	0.288	0.284	0.270
15	0.292	0.284	0.282	0.274	0.265
20	0.289	0.278	0.277	0.266	0.253
25	0.283	0.276	0.269	0.263	0.250
30	-	0.273	0.265	-	0.240
35	0.276	0.268	0.259	0.247	0.232

Aspartic acid

/ $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, / NaOH / = 1.0 M,

T = $(65 \pm 0.1)^{\circ}C$, O D (420 nm) for / Aspartic acid / at

t_m	0.05 M	0.1 M	0.2 M
0	0.266	0.266	0.266
5	0.262	0.259	0.251
10	0.257	0.251	0.236
15	0.254	0.245	0.224
20	0.250	0.238	0.209
25	0.246	0.231	0.200
30	0.243	0.224	0.187

/ $K_3Fe(CN)_6$ / = 1.0×10^{-3} M, / Aspartic acid / = 1.0×10^{-1} M,
T = $(65 \pm 0.1)^{\circ}C$, O D (420 nm) for / NaOH / at

t_m	0.75 M	1.0 M	2.0 M
0	0.266	0.266	0.266
5	0.258	0.259	0.259
10	0.251	0.251	0.253
15	0.243	0.245	0.247
20	0.236	0.238	0.240
25	0.229	0.231	0.234
30	0.222	0.224	0.228

/ NaOH / = 1.0 M, / Aspartic acid / = 1.0×10^{-1} M,
T = $(65 \pm 0.1)^{\circ}C$, O D(420 nm) for / $K_3Fe(CN)_6$ / at

t_m	0.001 M	0.0005 M	0.0001 M
0	0.266	0.224	0.062
5	0.259	0.207	0.055
10	0.251	0.192	0.049
15	0.245	0.180	0.043
20	0.238	0.165	0.038
25	0.231	0.155	0.034
30	0.224	0.141	0.030

/ NaOH / = 1.0 M, / Aspartic acid / = 1.0×10^{-1} M,

/ $K_3Fe(CN)_6$ / = 1×10^{-3} M, O D (420 nm) for temperature at

t_m	50°C	55°C	60°C	65°C	70°C
0	0.266	0.266	0.266	0.266	
5	0.264	-	0.261	0.259	0.257
10	0.262	0.260	0.256	0.251	0.247
15	0.260	0.257	0.251	0.245	0.238
20	0.258	0.255	0.246	0.238	0.229
25	0.255	0.252	0.241	0.231	0.221
30	0.253	0.250	0.237	0.224	0.213

SUMMARY

SUMMARY

1. KINETICS OF OXIDATION OF GLYCINE, ALANINE, VALINE, LEUCINE AND PHENYLALANINE BY ALKALINE HEXACYANOFERRATE(III)

The kinetics of oxidation of some amino acids (glycine, alanine, valine, leucine and phenylalanine) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate (II) ions, and the addition of salts, did not have any effect on the rates of these reactions.

The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical

intermediates in the rate determining step. The radical underwent further reaction, by way of the imino acid, to yield the products. The products formed from the oxidation of these amino acids were the respective α-keto acids, which were characterized by chemical methods.

2. KINETICS OF OXIDATION OF SERINE AND THREONINE BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (serine and threonine) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate(II) ions, and the addition of salts, did not have any effect on the rates of these reactions.

The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical intermediates in the rate determining step. The radical underwent further reaction, by way of the ionic acid, to yield the products. The products formed from the oxidation of these amino acids were the respective α-keto acids, which were characterized by chemical methods.

3. KINETICS OF OXIDATION OF CYSTEINE AND METHIONINE BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (cysteine and methionine) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate(II) ions, and the addition of salts, did not have any effect on the rates of these reactions.

The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical intermediates in the rate determining step. The radical underwent further reaction, by way of dimerization, to yield the products. The products formed from the oxidation of these amino acids were the disulfide (from cysteine) and the sulfoxide (from methionine), which were characterized by chemical methods.

4. KINETICS OF OXIDATION OF TYROSINE AND TRYPTOPHAN BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (tyrosine and tryptophan) by potassium hexacyanoferrate(III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate(II) ions, and the addition of salts, did not have any effect on the rates of these reactions.

The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical intermediates in the rate determining step. The radical underwent further reaction, by way of the imino acid, to yield the products. The products formed from the oxidation of these amino acids were the respective α-keto acids, which were characterized by chemical methods.

5. KINETICS OF OXIDATION OF GLUTAMIC ACID AND ASPARTIC ACID BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (glutamic acid and aspartic acid) by potassium hexacyanoferrate (III), in alkaline medium, at constant ionic strength, under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate (II) ions, and the addition of salts, did not have any effect on the rates of these reactions.

The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical intermediates in the rate determining step. The radical underwent further reaction, by way of the imino acid, to yield the products. The products formed from the oxidation of these amino acids were the respective α-keto acids, which were characterized by chemical methods.

6. KINETICS OF OXIDATION OF LYSINE, ARGININE AND HISTIDINE BY ALKALINE HEXACYANOFERRATE (III).

The kinetics of oxidation of some amino acids (lysine, arginine and histidine) by potassium hexacyano-ferrate (III), in alkaline medium, at constant ionic strength,

under a nitrogen atmosphere, has been studied.

The rates of the reactions were found to be dependent on the first powers of the concentrations of each reactant (substrate, oxidant and alkali).

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated.

Variations in the ionic strength of the medium, changes in the concentrations of added hexacyanoferrate (II) ions, and the addition of salts, did not have any effect on the rates of these reactions.

The presence of radical intermediates, formed in the rate determining step of the reaction, has been detected and characterized by ESR spectroscopy.

The reaction pathway has been mechanistically visualized as proceeding via the formation of radical intermediates in the rate determining step. The radical underwent further reaction, by way of the imino acid, to yield the products. The products formed from the oxidation of these amino acids were the respective α-keto acids, which were characterized by chemical methods.

LIST OF PUBLICATIONS

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1. Kinetics of Oxidation of methionine by alkaline hexacyanoferrate (III), D. Laloo and M.K. Mahanti, Afinidad, 42 , 593 (1985).
2. Kinetics of Oxidation of valine by alkaline hexacyanoferrate (III), D. Laloo and M.K. Mahanti, Polish J. Chem., 59, 931 (1985).
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7. Kinetics of oxidation of amino acids by alkaline hexacyanoferrate (III), Oxidation of serine and threonine, D. Laloo and M.K. Mahanti, Afinidad, in press.

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