KINETICS OF OXIDATION OF AMINO ACIDS

SUMMARY

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

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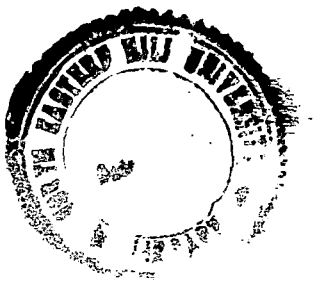

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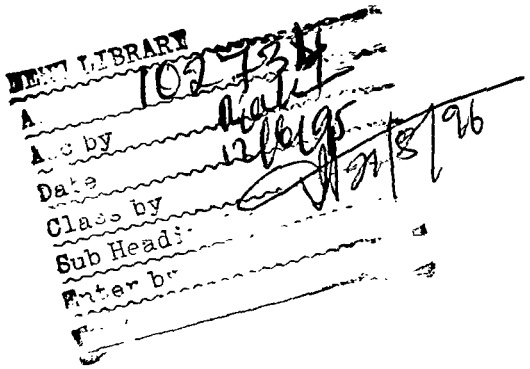

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SUMMARY

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Hexavalent chromium compounds have been widely used as oxidizing agents, reacting with diverse kinds of organic substrates. The mechanism of oxidation varies with the nature of the chromium(VI) species and the solvent used. The development of newer chromium(VI) reagents for the oxidation of organic substrates continues to be a subject of interest. A number of novel chromium(VI) oxidizing agents have been introduced, especially for complex or highly sensitive substances where great selectivity and effectiveness, coupled with mildness of conditions, are prerequisites for success.

Some of the chromium(VI) reagents which have been used as efficient oxidizing agents are:
chromium trioxide; chromyl chloride; Jones reagent —— a solution of Cr(VI) oxide in concentrated sulfuric acid(1); Collins' reagent —— dipyridinium Cr(VI) oxide in dichloromethane(2); Corey's reagent —— pyridinium chlorochromate(3); pyridine oxodiperoxy chromium(VI) reagent(4); pyridinium dichromate(5); bis tetrabutylammonium dichromate(6); Chaudhuri's reagent —— pyridinium fluorochromate(7); 4-(dimethylamino)-pyridinium chlorochromate(8); Cr(VI) oxide diperoxide(9); chlorotrimethylsilane-Chromium trioxide (10); chromium peroxide complexes(11); imidazolium dichro-

mate(12); pyridinium bromochromate(13); biphosphonium dichromate(14); and 3-carboxy pyridinium dichromate(15).

New procedures have been emerging involving non-aqueous chromium(VI) reagents with the general idea that anhydrous conditions are more conducive to mild oxidation.

The reagent employed in the present investigation, quinolinium dichromate(QDC) . $(C_9H_7N^+H)_2Cr_2O_7^{2-}$, has emerged as a very useful and versatile oxidant(16), which is clearly deserving of widespread application.

The oxidation of amino acids has become important, both from a chemical point of view and in trying to explore the various transformations involved in the metabolism of amino acids. Owing to the differing nature of the hydrocarbon portion, amino acids can undergo various kinds of reactions depending on whether the particular amino acids contain non-polar groups, polar substituents, acidic or basic substituents.

In the present investigation, the kinetics of oxidation of amino acids by quinolinium dichromate (QDC), in acid medium, at constant ionic strength have been studied. The amino acids which have been used for the purposes of oxidation have included :

1. Glycine, alanine, valine, leucine and phenylalanine: Chapter 1.
2. Serine, threonine and tyrosine: Chapter 2.
3. Methionine and cysteine: Chapter 3.
4. Aspartic acid and glutamic acid: Chapter 4.
5. Lysine, arginine and histidine: Chapter 5.

All the oxidation reactions were performed under a nitrogen atmosphere. The stoichiometries of the individual kinetic reactions were determined. For all the kinetic runs, the progress of the oxidation reaction was followed by monitoring the disappearance of Cr(VI) at 440nm, spectrophotometrically. The rates of all the reactions were found to be dependent on the first power of the concentrations of each — substrate, oxidant and acid. The first order dependence of the rate on acid concentration indicated that a protonated Cr(VI) species was involved in the rate determining step of the reaction.

The rate of the reaction showed an increase, with increasing proportions of acetic acid. Plots of log k_1 (the pseudo-first-order rate constant) against the reciprocal of the dielectric constant were linear, with positive slopes, indicating an ion-dipole type of reaction. This was in consonance with the observation that the use of more polar solvents required larger reaction times. This

also indicated that, in the presence of an acid, the rate determining step involved a protonated Cr(VI) species.

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated. The reactions were characterized by negative entropies of activation ($\Delta S^{\neq}$). This suggested a highly ordered transition state, relative to the reactants. Although current views do not attach much physical significance to isokinetic temperatures, a linear correlation between $\Delta H^{\neq}$ and $\Delta S^{\neq}$ has been considered a necessary condition for the validity of linear free energy relationships. Plots of $\Delta H^{\neq}$ vs $\Delta S^{\neq}$ were linear, indicating that the oxidation reactions of amino acids were controlled by both these parameters. The isokinetic temperatures(β) obtained were 338K (for glycine, alanine, valine, leucine and phenylalanine); 320K (for serine, threonine and tyrosine); 438K (for lysine, arginine and histidine). Further, the free energies of activation ($\Delta G^{\neq}$) were nearly constant, indicating that the same mechanism operated for the oxidation of all these amino acids.

The kinetic rates of oxidation were in accordance with the theory of electronic substituent effects. Structure-reactivity correlations were carried out for some amino acids (glycine, alanine, valine, leucine and phenylalanine). It was observed that the Taft equation, which

could be applied to these amino acids, was of the form:

$$\log k/k_0 = -1.48 \quad \sigma^* + 0.84 \; E_s \qquad (1)$$

The validity of this relationship indicated that both, the polar effect ($\rho^* = -1.48$) and the steric effect ($\delta = +0.84$), influenced the rate of the reaction. Since the reaction centre was near the site of substitution, the magnitudes of the reaction constants were expected to be fairly high. This has been observed in these oxidation reactions.

The solvent isotope effects, k_{D_2O}/k_{H_2O}, in the oxidation of all these amino acids, have been observed to be greater than unity. This indicated that the reactions were catalyzed by acid. This would support the protonation of the oxidant (QDC), as seen from the acid dependence on the rate of the reaction.

The oxidation of the deuterated amino acids (deuterated at the α-carbon atom) yielded values of the kinetic isotope effect, k_H/k_D, which were close to unity. The absence of a primary kinetic isotope effect indicated that, in the rate determining step of the reaction, there was no cleavage of the carbon-hydrogen bond. The oxidation of deuterated methionine (by deuterating the methyl group of methionine) did not show a primary kinetic isotope effect. This indicated that the carbon-hydrogen bond (of the methyl

group in methionine) was not cleaved in the rate-determining step of the reaction. During the course of the reaction, there was no induced polymerization of acrylonitile, no reduction of mercuric chloride, and no ESR signals could be detected. This showed the absence of radical species.

Variations in the ionic strength of the medium, and the addition of salts had no effect on the rates of these reactions. This indicated a direct reaction between the substrate and oxidant, in acid medium, to give an intermediate which on further reaction gave the product.

The dissociation of amino acids depends upon the pH of the medium. In aqueous solution, amino acids exist as dipolar ions (zwitterions). In strongly acidic or alkaline media, the following equilibria exist:

$$RCH(N^+H_3)COOH \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(N^+H_3)COO^- \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(NH_2)COO^- \qquad (2)$$

(cation) (zwitterion) (anion)

In acid solution, amino acids exist as a mixture of the zwitterionic [$RCH(N^+H_3)COO^-$] and cationic [$RCH(N^+H_3)COOH$] forms. In the present investigation, the reactions were carried out in acid media. The zwitterion would be converted to the cation [$RCH(N^+H_3)COOH$], which was the reactive species, under the present experimental conditions.

Based on the observed kinetic data, the mechanistic pathway for the oxidation of these amino acids by quinolinium dichromate (QDC), in acid medium, would proceed via a direct interaction between the cationic form of the amino acid and the oxidant to yield an intermediate, which would then undergo further reaction to yield the product.

For the oxidation of the sulfur containing amino acids (cysteine and methionine) by quinolinium dichromate, in acid medium, since disulfide was the final product of oxidation (from cysteine), and sulfoxide was the final product of oxidation (from methionine), the sulfhydryl group (-SH) of cysteine and the S-methyl group ($S-CH_3$) of methionine would provide the site of attack. Considering the mechanism of oxidation of cysteine and methionine, the attack could occur either at nitrogen or at sulfur. The products obtained from the oxidation of these amino acids (the disulfide from cysteine, and methionine sulfoxide from methionine), would suggest that the oxidant (QDC) attacks the sulfur group. Further, sulfur is more nucleophilic than nitrogen, and the sulfhydryl group (-SH) could donate electrons more readily than the amino group ($-NH_2$). Therefore, the mechanism was via an electron-pair donation by sulfur present in methionine (and cysteine) to the Cr=0 bond of the oxidant.

The major products obtained in these oxidation reactions in good yield (∿75-80%) were:

a) the corresponding nitriles, which were characterized by chemical and spectral methods;

b) trace amounts of the corresponding aldehydes, which were characterized by their respective 2,4-dinitrophenyl-hydrazone derivatives;

c) methionine sulfoxide (from methionine) which was characterized as N-benzoyl methionine sulfoxide;

d) cystine (from cysteine), which was characterized by chemical methods.

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1. Kinetics of oxidation of sulfur containing amino acids by quinolinium dichromate

   E. Karim and M.K. Mahanti, Oxidation Commun., 14, 157(1991).

2. Kinetics of oxidation of amino acids by quinolinium dichromate,

   E. Karim and M.K. Mahanti, Polish J. Chem., 66, 000(1992).

3. Kinetics of oxidation of α-amino acids by quinolinium dichromate,

   E. Karim and M.K. Mahanti, Oxidation Commun., 15, 000(1992).

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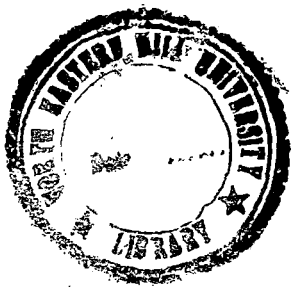

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Dr. Mahendra K. Mahanti
PROFESSOR

I certify that the thesis entitled "KINETICS OF OXIDATION OF AMINO ACIDS" submitted by Mr. Enamul Karim for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. He 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.

Mahendra K. Mahanti

SHILLONG
the 22nd July 1992

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Dedicated
to
My Parents

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Enamul Karim

ENAMUL KARIM

CONTENTS

INTRODUCTION

Oxidation is an essential operation in organic syntheses and several reagents have been developed for a wide variety of transformations(1,2). Hexavalent chromium compounds have been widely used as oxidizing agents reacting with diverse kinds of organic substrates. In the course of these reactions, the Cr(VI) compounds are reduced to the Cr(III) species.

The earliest known Cr(VI) oxidants are chromium trioxide and chromyl chloride. Chromium trioxide has been used in various kinds of reaction media such as water, sulphuric acid, acetic anhydride, t-butyl alcohol and pyridine. Chromyl chloride has been generally used in carbon tetrachloride and carbon disulphide.

In the recent past, a large number of novel Cr(VI) oxidizing agents have been introduced, mostly in response to the needs of mildness and selectivity. The usefulness of "Jones reagent"(3) for the oxidation of organic compounds has been well established.

One of the earliest and most widely employed Cr(VI) oxidants was "Collins reagent" - dipyridinium-Cr(VI)

oxide in dichloromethane(4). The use of dichloromethane as the reaction medium constituted a major breakthrough in oxidation with dipyridinium-Cr(VI) oxide.

For oxidation purposes, the most widely used Cr(VI) complex with pyridine has been pyridinium chlorochromate(PCC) popularly known as "Corey's reagent"(5). Its principal advantage is that this reagent is not air or moisture sensitive, and oxidation with it does not involve handling a large volume of solvent. Studies on the kinetics of oxidation of primary alcohols by PCC have provided important information on the mechanism of the process(6). Involvement of protonated chromium species in the rate determining step was indicated by the catalysis of the reaction by acid, the acid catalysed reaction being first order. PCC does not polymerise acrylonitrile, and a hydrogen transfer hypothesis was thus not tenable. A substantial kinetic isotope effect, $k_H/k_D=5.71$, at 303K suggested a hydride transfer in the rate determining step. The transfer could occur directly between the alcohol and the protonated species or intramolecularly after the initial formation of a chromate ester. A few representative examples of oxidation of primary and secondary alcohols by PCC are given in Table 1.

Table 1. Oxidation of Primary and Secondary Alcohols by PCC (ref.5).

Alcohol	Product	% yield
1-Heptanol	Heptanal	78
1-Decanol	Decanal	92
1,6-Hexane diol	Hexandial	68
Oct-2-yn-1-ol	Oct-2-ynal	84
Citronellol	Citronellal	82
Benzhydrol	Benzophenone	100

The difficulties in handling Collins reagent and the problem arising out of the acidic nature of Corey's reagent were overcome by the use of pyridinium dichromate, $PyCr_2O_7$ (PDC), which was recognised as a mild and selective oxidizing agent(7). This reagent is soluble in a number of solvents, though an aprotic medium is necessary for getting satisfactory results. PDC is generally used either in solution in DMF or as a suspension in dichloromethane. Anhydrous conditions were used during oxidation with PDC, and when the oxidation was performed in DMF, the carbonyl compounds were isolated by ether extraction after pouring the reaction mixture in water. PDC shows remarkable selectivity as an oxidizing agent. When dissolved in DMF, it clearly oxidizes allylic alcohols to the corresponding α,β-unsaturated aldehydes in excellent yields.

PDC in dichloromethane oxidized primary and secondary alcohols efficiently. The aldehydes obtained as products from primary alcohols do not undergo further oxidation.

Cr(VI) oxide-3,5-dimethyl pyrazole(8) is a Cr(VI) complex which has been used as an oxidant with mixed success. The reagent is presumed to form a cyclic chromate ester that generates the carbonyl compound through intramolecular elimination. Despite the high yields of some simple aldehydes and ketones from the corresponding alcohols and near quantitative oxidation of geraniol, this reagent proved to be unsatisfactory in a number of cases(9,10).

Pyridine oxodiperoxy chromium, $C_5H_5N:CrO_5$, a complex of chromium pentoxide with pyridine, has also been used for the oxidation of primary and secondary alcohols(11).

In order to protect acid sensitive functional groups during oxidation of alcohols with Cr(VI)oxide, various polar aprotic solvents have been used. At least three such solvents, namely DMF(12), hexamethyl phosphoramide or HMPT(13-15) and dimethyl sulphoxide(16), have been used with some success. A solution of Cr(VI) oxide in DMF containing a trace of concentrated sulfuric acid

was able to oxidize alcohols containing acid sensitive protecting groups. The presence of catalytic amounts of sulfuric acid was essential, accompanied by the presence of an ice bath and an inert nitrogen atmosphere. Oxidation with Cr(VI) oxide in HMPT showed excellent selectivity. When a solution of Cr(VI) oxide was added to an equal volume of the substrate dissolved in HMPT, simple axial and equatorial hydroxyl functions were oxidized, the latter at a much faster rate(13). Under the same experimental conditions, Cr(VI)oxide in HMPT was found to oxidize allylic hydroxyl functions in preference to other alcoholic groups(13). A series of primary and secondary alcohols were oxidized in 80-90% yields by a solution of sodium dichromate dihydrate in concentrated sulfuric acid in DMSO at 70°C. DMSO acts as a solvent in these oxidations and not as an oxidant, as shown by the negligible oxidation of the substrate in the absence of dichromate.

The technique of using reagents intercalated in, or adsorbed on, a solid support(17) has also been exploited in oxidations with Cr(VI) oxidizing agents. The solid supports used have included graphite, silica, alumina, silica gel, celite and various reagents. As in the case of other Cr(VI) reagents, attempts were made to achieve

mild reaction conditions, better selectivity and convenient isolation of the oxidation products. On heating with graphite under reduced pressure, Cr(VI)oxide was claimed to be uniformly intercalated and the resulting substance was found to oxidize primary alcohols to aldehydes in high yields(18). Later work showed that the oxidizing agent was a surface deposit of Cr(VI) oxide on graphite (19-20).

Chromyl chloride adsorbed on silica-alumina was found to be an effective oxidizing agent for primary and secondary alcohols under neutral non-aqueous conditions (21). It has been reported that chromic acid adsorbed on silica gel was able to bring about the instantaneous oxidation of primary and secondary alcohols(22). Collins reagent adsorbed on celite has been used to oxidize allylic alcohols to the corresponding aldehydes(23-24). Chromic acid supported on an ion-exchange resin has been used to oxidize primary and secondary alcohols(25). This polymer supported reagent is prepared by adding the chloride form of the resin to an aqueous solution of Cr(VI)oxide under stirring. PCC, adsorbed on alumina, has been claimed (26) to be a better oxidizing agent than in dichloromethane suspension. Better efficiency has also been claimed(27)

for the oxidation of primary and secondary alcohols using PCC supported on polymer. The reagent, poly[Vinyl(pyridinium chlorochromate)], (PVPCC), is prepared by adding Cr(VI) oxide and concentrated hydrochloric acid to polyvinyl pyridine suspended in water.

Several facile oxidations of secondary alcohols with chromic acid in a two-phase system of ether and water have been reported (28-30). This method has proved particularly suitable for the synthesis of ketones, which are susceptible to epimerization under oxidizing conditions(28).

Cr(VI)oxide in a mixture of ether and dichloromethane oxidizes several secondary alcohols in the presence of celite(31).

There have been several reports on the oxidation of primary and secondary alcohols by Cr(VI) oxidants under phase transfer catalysis(32-36).

Allylic and benzylic alcohols were efficiently oxidized to the corresponding aldehydes with bis-tetrabutyl ammonium dichromate (TBADC) in refluxing dichloromethane(37).

The 2,2'-bipyridine complex of chlorochromic acid is a useful oxidizing agent and the use of this reagent had resulted in simplified procedures for the purification of the resulting carbonyl compounds(38). The 2,2'-bipyridinium chloro chromate and the 2,2'-bipyridine-chromium trioxide complex have both proved to be specially useful in oxidations of compounds with acid-sensitive protecting groups, due to the internal buffering of the 2,2'-bipyridyl system. These results indicated that synthetically useful changes in the properties and reactivity of Cr(VI) reagents could be brought about by varying the amine ligand associated with chromium trioxides. Another Cr(VI) reagent which has proved useful as a mild selective reagent for the oxidation of complex allylic and benzylic alcohols to the corresponding carbonyl compounds was 4-(dimethylamino)pyridinium chlorochromate(39). Secondary alcohols proved to be more reactive towards this reagent than primary alcohols. The ready preparation of this oxidizing agent, its selectivity, and the ease of using this reagent indicated that it may prove to be a useful alternative to other reagents in the oxidation of complex allylic and benzylic alcohols.

Since the process of oxidation in organic chemistry is of great value as a fundamental process in a wide

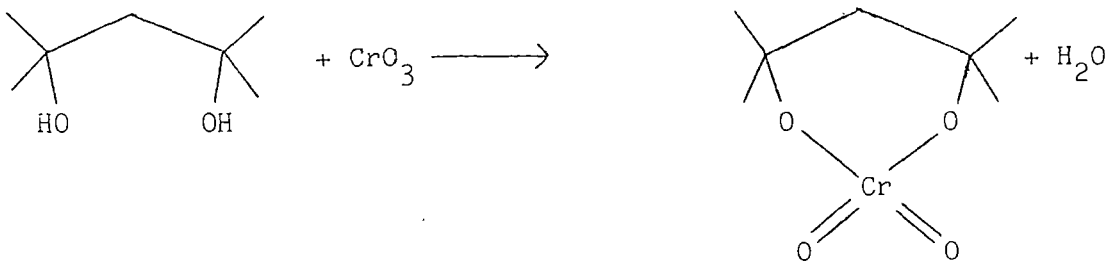
scope of chemical conversions, there has been considerable interest in the development of newer Cr(VI) reagents for the oxidation reactions. Since the appearance of pyridinium chlorochromate(5,40), other similar oxidizing agents have been developed varying the amine ligand associated with the chlorochromate anion.

A Cr(VI) reagent which was found to have certain advantages over similar oxidizing agents in terms of amounts of oxidant and solvent required, short reaction times, and high yield was pyridinium fluorochromate, PFC(41). In dichloromethane as solvent, PFC was found to oxidize primary and secondary alcohols to the corresponding aldehydes or ketones, and was also found to oxidize benzoin to benzil, as also anthracene and phenanthrene to their corresponding quinones(41).

A new class of Cr(VI) reagents derived from chromium trioxide and halosilanes has been developed(42). These reagents are highly efficient for the oxidation of alcohols to carbonyl compounds, for the oxidative coupling of mercaptans into disulphides and for a mild cleavage of oximes to carbonyl compounds. Chlorotrimethylsilane-chromium trioxide has been shown to be an efficient oxidizing

agent for the conversion of arylmethanes to benzaldehyde, and for the oxidative cleavage of some benzyl esters(42).

The oxidation of primary and secondary alcohols to the corresponding carbonyl compounds is most frequently accomplished in synthetic practice by the use of Cr(VI) reagents in amounts ranging from stoichiometric to large excess over stoichiometric(43). A new and highly effective reagent combination for the catalytic oxidation of secondary alcohols to ketones has been used(44). The reagent consisted of peroxy acetic acid as the stoichiometric oxidant and the Cr(VI) ester(1) as the catalyst with carbon tetrachloride-methylene chloride mixture as solvent. A solution of Cr(VI) ester(1) was prepared from 2-4 dimethylpentan-2,4-diol and CrO_3 in dry CCl_4.



(1)

The efficient oxidation of alcohols to carbonyl compounds under mild conditions has been a necessary theme in organic syntheses. An improved procedure was described for the extremely rapid and efficient oxidation of alcohols, by the addition of a small quantity of anhydrous acetic acid to pyridinium dichromate(PDC) and freshly activated molecular sieve powder in dichloromethane at room temperature(45).

Chromium peroxide complexes have been used as general oxidants in organic syntheses. 2,2'-bipyridyl chromium peroxide has been used to convert different classes of alcohols to the carbonyl compounds, for C-C bond cleavages in 1,2-diols, for the quantitative decarboxylation of α-hydroxy acids, for the conversion of oximes to their carbonyl compounds, thiols to disulphides, dihydroxy phenolic compounds to quinones, benzylamine to benzaldehyde, aromatic amines to their azo compounds, and for the conversion of anthracene and phenanthrene to their quinones(46). Pyridine chromium peroxide has been used to convert different classes of alcohols to the carbonyl compounds, thiols to disulphides, anthracene to anthraquinone, and for the decarboxylation of mandelic and benzylic acids(46). Chromium peroxide etherate has also been used as an effective reagent for the oxidation

of different classes of alcohols to their carbonyl compounds(46).

Imidazolinium dichromate(IDC) has been shown to be very useful and reliable for the oxidation of allylic and benzylic alcohols to the corresponding carbonyl compounds in high yields under mild conditions(47).

The phase transfer catalysed oxidation of benzylic alcohols using benzyltriethylammonium chlorochromate has been reported(48).

Pyridinium bromochromate has been reported as an efficient oxidant for the oxidation of benzyl alcohols, fluorenols and benzoin, all these reactions being performed in chloroform(49).

The biphosphonium dichromate reagent, $(C_6H_5)_3\ P^+CH_2P^+(C_6H_5)_3Cr_2O_7^{2-}$, was a particularly mild and selective reagent for the oxidation of primary and secondary alcohols(50). The oxidation of primary alcohols to aldehydes occurs without further oxidation to acid, and without double bond isomerisation or migration for such alcohols as geraniol(50).

The oxidation kinetics of alcohols by pyridinium fluorochromate (PFC) indicated that PFC was an efficient two-electron oxidant which was capable of participating in oxygen-transfer oxidations(51).

3-Carboxy pyridinium dichromate(NDC), readily prepared from nicotinic acid and chromium trioxide, is an efficient reagent for the oxidation of alcohols to carbonyl compounds in the presence of pyridine(52). The optimum molar ratio of substrate:oxidant:pyridine to ensure complete oxidation of starting material in a short reaction time was found to be 1:2.5 : 20 respectively.

The Cr(VI)oxide diperoxide oxidation of organic sulphides(53) and of tertiary amines(54) have been reported. The rate law observed suggested a mechanism involving a preliminary coordination of the amine to the metal. The oxidation rate of the amines and organic sulphides indicated a mechanism having some single-electron-transfer (SET) character.

Quinolinium dichromate(QDC) having the structure, $(C_9H_7NH^+)_2Cr_2O_7^{2-}$, has been used for the oxidation of alcohols and aldehydes(55). QDC is a stable orange solid,

which has been prepared by dissolving CrO_3 in water, adding quinoline and collecting the product. It has been shown that quinolinium dichromate(QDC) works as efficiently as Collins' reagent(4) and activated manganese dioxide(56). The data in Table 2 shows the details of the oxidation of some alcohols and aldehydes by QDC in dichloromethane and dimethyl formamide solvents.

Table 2. Oxidation of alcohols and aldehydes by QDC (ref.55)

Compound	Product	Yield (%) In CH_2Cl_2	Yield (%) In DMF
n-Butanol	n-Butanal	69	74
Benzyl alcohol	Benzaldehyde	45	45
Benzhydrol	Benzophenone	55	48
Benzaldehyde	Benzoic acid		55
Cinnamaldehyde	Cinnamic acid		52
Crotonaldehyde	Crotonic acid		85

Quinolinium dichromate (QDC) has emerged as a very useful and versatile oxidant, which is clearly deserving of widespread application. QDC in dimethyl formamide-water mixtures, in the presence of acid, has been used for the oxidation of a variety of organic substrates. Some of the organic substrates which have been oxidized by QDC,

in acid medium, have included benzyl alcohols(57), aryl-alkanes(58), toluene and substituted toluenes(59,60), fluorene(61), polynuclear aromatic hydrocarbons(59,62), and diphenylamines(63).

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SCOPE OF THE PRESENT INVESTIGATION

The development of newer chromium(VI) reagents for the oxidation of organic substrates continues to be a subject of interest. There exists a need for new methods, especially for complex or highly sensitive substances where selectivity and effectiveness, coupled with mildness of conditions, are prerequisites for success. New procedures are emerging, involving non-aqueous chromium(VI) reagents, with the general idea that anhydrous conditions are more conducive to mild oxidation. The reagent employed in this investigation, quinolinium dichromate, QDC, $(C_9H_7N^+H)_2Cr_2O_7^{2-}$, has emerged as a useful and versatile oxidant, which is clearly deserving of widespread application.

Amino acids play a pivotal role in the synthesis of proteins, and can undergo various kinds of reactions, depending on the nature of their intact hydrocarbon portions. The oxidation of amino acids is of importance, both from a chemical point of view and with regard to the mechanism of amino acid metabolism.

The present investigation is a detailed kinetic probe into the oxidation of amino acids by quinolinium

dichromate(QDC), in acid medium, at constant ionic strength, under a nitrogen atmosphere. The purpose of this investigation has been to attempt to extend the scope of this oxidising agent, quinolinium dichromate(QDC), in acid medium, and to explore and establish mechanistic pathways of reactions involving amino acids.

The purpose of the present study was:

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

(b) To demonstrate the usefulness of quinolinium dichromate(QDC) as a reagent which could bring about the oxidation of amino acids.

In the present investigation, the amino acids chosen for the purposes of oxidation by quinolinium dichromate, in acid medium, have included:

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

(ii) Serine, threonine and tyrosine.

(iii) Cysteine and methionine.

(iv) Glutamic acid and aspartic acid.

(v) Lysine, arginine and histidine.

For each oxidation reaction, the stoichiometry of the reaction has been determined. The concentrations of substrate, oxidant and acid have been varied and the effects of these variations on the reaction rates have

been studied. The solvent composition has been varied in order to study the effect of a change in the dielectric constant of the medium on the rate of the reaction. Changes in the temperature of the reaction medium have been made, and the activation parameters have been evaluated. For each oxidation reaction, the products have been isolated and characterized by chemical and spectral methods. Based on the observed experimental data, mechanistic pathways for the oxidation of amino acids by quinolinium dichromate (QDC), in acid medium, have been proposed.

EXPERIMENTAL

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" sulfuric 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.

Sulfuric Acid

E. Merck sample was used.

Acetic acid

Acetic acid (E. Merck) was refluxed for 3 hours with chromic oxide, with the addition of a quantity of acetic anhydride corresponding to the water content of the acetic acid. The solids that separated out were filtered off, and the acid was distilled from an all-glass apparatus. Large head and tail fractions were rejected and the fraction distilling at 116°C was collected.

Methanol

Methanol (E. Merck) was distilled before use (b.p. 65°C).

Sodium perchlorate

Sodium perchlorate was prepared by neutralizing 70% perchloric acid (E. Merck) with sodium hydroxide (BDH reagent grade). The solution was concentrated, when crystals of sodium perchlorate were obtained. The crystals were filtered, and recrystallized from water. The recrystallized 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.

Quinolinium dichromate $(C_9H_7N^+H)_2Cr_2O_7^{2-}$

To a stirred solution of CrO_3 (100g) in water (100 ml), cooled in ice, quinoline (86 ml) was added in small portions. The solution was diluted with acetone (400 ml), cooled to -20°C, and the orange solid which separated out was filtered, washed with acetone, dried in vacuo and recrystallized from water (m.p. 160°C). The purity of the compound was further checked by spectral analysis. Infrared spectrum (KBr) exhibited bands at 930, 875, 765 and 730 cm^{-1}, characteristic of the dichromate ion.

Acrylonitrile

The monomer (BDH) was washed with 5% sodium hydroxide olution to remove the inhibitor (hydroquinone), and then with 3% orthophosphoric acid to remove any basic impurities. It was then washed with water, dried over anhydrous calcium chloride, and distilled under reduced pressure in an atmosphere of nitrogen. The middle fraction was collected (b.p. 77°C) and used.

Other reagents

All other reagents used were of AnalaR grade, and were purified before use, and their boiling points/ melting points were checked, and found to agree with those given in the literature.

Substrates

All amino acids used were the L-amino acids. L-Glycine was obtained from Loba Chemical Company. L-Cysteine and L-histidine were E. Merck samples. L-Lysine, L-phenylalanine, L-leucine, L-arginine, L-valine, L-serine, L-threonine and L-tyrosine were BDH samples. L-Methionine, L-alanine, L-glutamic acid and L-aspartic acid were obtained from SISCO Research Laboratories. The amino acids were assayed by the acetic acid-perchloric acid method(1) and their aqueous solutions were used. The data obtained for each

of the substrates used, have been summarized in Table 1.

Table-1

Substrate	Melting Point (°C)	(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	25(M)
Threonine	251	240(M)
Tyrosine	310	5(M)
Cysteine	240	36(M)
Methionine	283	208(M)
Glutamic acid	224	206(M)
Aspartic acid	324	-
Lysine	224	20(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. All IR spectra were recorded on an IR-983 (Perkin Elmer) spectrophotometer, and all NMR spectra were recorded on an EM 390 (90 MHz, Varian) NMR 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 the loss of solvent, and the loss was found to be negligible. The standard flasks, reaction vessels and the pipettes used were standardized, 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 temperature accurately, with fluctuations of not more than ±0.1°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 minimize evaporation of water and loss of heat due to radiation.

Spectrophotometers

For absorption measurements, the spectrophotometers used were: (a) Digital spectrophotometer Type 106 (Systronics)

and (b) UV 26 (Beckman) UV-visible spectrophotometer.

(a) The Type 106 Digital spectrophotometer was a single beam spectrophotometer having a grating of 600 lines/mm and a wave length 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 minimize the errors in measurements of optical density due to fluctuations in voltage, the spectrophotometer was connected to the mains through an external voltage stabilizer. This was in addition to the in-built voltage stabilizer 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 monochromator, having a filter grating of 1200 lines/mm, and a wavelength range from 190 nm to 900 nm. This spectrophotometer had a thermostatic control arrangement

and the absorbance value was displayed directly on the digital display and on the recorder. Photometric linearity was checked over the range of concentrations of K_2CrO_4 in KOH solutions, as specified in the instruction manual, so as to verify Beer's law at 370 nm.

Absorption cells

The absorption cells were of 'Corning' glass and of 8 ml capacity for the spectrophotometer Type 106 (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.

Rate measurements

A known amount of the substrate was weighed accurately into a 10 ml standard flask, dissolved and made up with sulfuric acid of known strength, so as to make the solutions of the required molarity. Quinolinium dichromate was accurately weighed out into a 10 ml standard flask and dissolved in a small volume of water. Sodium perchlorate

was added so as to maintain a constant ionic strength of the medium. Sufficient time was allowed to compensate for any change of heat during dilution. The oxidant solution was made up with water. The two solutions were separately thermostated at the required temperature for 1 hour under a nitrogen atmosphere. Equal volumes of the two solutions of oxidant and substrate were mixed. The reaction mixture was homogeneous throughout the duration of the reaction.

The progress of the reaction was followed by observing the disappearance of Cr(VI). Readings were taken at regular intervals of time, by noting the decrese in optical density at 440 nm, spectrophotometrically.

All the kinetic experiments were carried out in duplicate or in triplicate, and the rate constants which were determined were found to be reproducible to within ±3%. All reactions were performed under a nitrogen atmosphere.

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 , expressed in sec^{-1}, was calculated from the equation (2):

$$k_1 = \frac{2.303}{t} \log \frac{D_o}{D_t} \quad \ldots\ldots (1),$$

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 density 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_1, s^{-1}) by the concentration of the substrate (M).

All values of rate constants were the average of two or more 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_1 against the reciprocal of temperature(T),

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

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

(ii) Enthalpy of activation ($\Delta H^{\neq}$)

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

(kJ mol^{-1})

(iii) Entropy of activation ($\Delta S^{\neq}$)

$$k_1 = \frac{kT}{h} e^{\Delta S^{\neq}/R} e^{-\Delta H^{\neq}/RT}$$

$$\Delta S^{\neq} = 2.303R \left[\log k_1 + \frac{\Delta H^{\neq}}{2.303RT} - \log \frac{kT}{h}\right]$$

$(\text{JK}^{-1} \text{mol}^{-1})$

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

(iv) Free energy of activation ($\Delta G^{\neq}$)

$$\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$$

(kJ mol^{-1})

Stoichiometry

The stoichiometric experiments were carried out under nitrogen at the particular temperature, under the conditions of $[QDC]_o > [Substrate]_o$ at varying acid concentrations. The disappearance of Cr(VI) was followed, until the absorbance values became constant. The $[QDC]_\infty$ was estimated. The stoichiometric ratio, $\Delta[QDC]/\Delta[Substrate]$, was obtained for each oxidation reaction studied. The individual stoichiometric equations have been shown along with the reactions of each of the substrates with the oxidant.

Product analysis

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

Using the same experimental conditions that were employed for the kinetic determinations, solutions of substrate and oxidant (QDC), taken in sulfuric acid (ionic strength adjusted by the addition of the requisite amount of $NaClO_4$), were allowed to react at the particular temperature for 24 hours, under nitrogen.

(1) The evolution of CO_2 was detected by the lime water test.

(2) The reaction mixture was treated with $NaHCO_3$ to neutralize the acid, the product formed was extracted with ether, washed with water and dried over anhydrous Na_2SO_4. The solvent was removed by distillation under reduced pressure.

(a) The major product, obtained in each case, was the corresponding nitrile (yields ∿75-80%), which was detected and characterized by:

(i) its colour reaction with hydroxylamine and ferric chloride (3);

(ii) IR analysis; and

(iii) NMR analysis.

(b) Trace amounts of the corresponding aldehyde were obtained (yields ∿5-10%), which were detected and characterized by their 2,4-dinitrophenylhydrazone derivatives.

The characterization of the major product (the corresponding nitrile) has been shown in Table 2.

Table 2

Amino acid	Oxidation product	Characterization
1	2	3
1. Glycine	Hydrogen cyanide	--
2. Alanine	Acetonitrile	B.P. : 81°C IR : 2270 cm^{-1} (-C ≡ N str.) H^1 NMR : δ (ppm) 1.9 (singlet)
3. Phenylalanine	Phenyl acetonitrile	B.P. : 232°C IR : 2254 cm^{-1} (-C ≡ N str.) H^1 NMR : δ (ppm) 3.7(Singlet 2H) 7.3(singlet 5H)
4. Leucine	3-methyl butyronitrile	B.P. : 130°C IR : 2265 cm^{-1} (-C ≡ N str.) H^1 NMR : δ (ppm)1.1(doublet 6H) 2.0(Multiplet H) 2.3(doublet 2H)
5. Valine	3-methyl propanonitrile	B.P. : 103°C IR : 2223 cm^{-1} (-C ≡ N str.) H^1 NMR : δ (ppm)1.3 (doublet 6H) 2.7 (Multiplet H)
6. Serine	Hydroxy acetonitrile	B.P. : 118°C IR : 3452 cm^{1} (-OH str.) 2220cm^{-1} (-C≡N str. H^1 NMR : δ (ppm) 4.2 (singlet 2H) 4.7 (singlet H)

Table 2 contd.

1	2	3
7. Threonine	2-hydroxy propanonitrile	B.P.: 180°C IR : 3422 cm^{-1}(-OH str.) 2250cm^{-1}(-C≡N str.) H^1NMR : δ (ppm) 1.6 (doublet 3H) 4.1 (quartet H) 4.6 (singlet H)
8. Tyrosine	4-hydroxy phenyl acetonitrile	M.P. : 69°C IR : 3550 cm^{-1}(-OH str.) 2264 cm^{-1} (-C≡N str.)
9. Aspartic acid	Malano mononitrile	M.P. : 68°C IR : 2265cm^{-1}(-C≡N str.) 1720cm^{-1}(>C=O str.) H^1NMR : (in DMSO-d_6) δ (ppm)3.8(singlet 2H) 11.8(singlet H)
10. Glutamic acid	3-cyano propanoic acid	M.P. : 48°C IR : 1710 cm^{-1} (>C=O str.) 2230cm^{-1} (-C≡N str.)
11. Arginine	N-(3-cyanopropyl) Guanidine	IR : 2200cm^{-1}(-C≡N str.) 3400cm^{-1}(-N-H str.)
12. Lysine	5-Amino valeronitrile	IR : 2246cm^{-1} (-C≡N str.) 3450cm^{-1}(-N-H str.)
13. Histidine	5-(cyano methyl) imidazole	IR : 2220cm^{-1} (-C≡N str.)

Table 2 contd..

1	2	3
14. Methionine	Methionine sulfoxide	N-benzoyl methionine sulfoxide M.P. : 183°C
15. Cysteine	Cystine	M.P. : 260°C

(B) Product obtained from the oxidation of methionine

Using the same experimental conditions that were employed for the kinetic determinations, solutions of substrate and oxidant (QDC), taken in sulfuric acid (ionic strength adjusted by the addition of the requisite amount of $NaClO_4$), were allowed to react at 313 K for 24 hours, under nitrogen. To the reaction mixture was added 5 ml of benzoyl chloride and 10 ml of $NaHCO_3$ solution (0.1N). A precipitate of N-benzoyl methionine sulfoxide was obtained, which was filtered, washed with water and recrystallized from ether (m.p. 183°C; yield ~75%).

(C) Product obtained from the oxidation of cysteine

Using the same experimental conditions that were employed for the kinetic determinations, solutions of substrate and oxidant (QDC), taken in sulfuric acid (ionic strength adjusted by addition of the requisite amount

of $NaClO_4$), were allowed to react at 313 K 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 (cystine) were precipitated, which were recrystallized from ether (m.p. 260°C; yield∿ 70-80%).

Tests for radical formation

Various tests were performed to determine whether radical intermediates were formed during the course of the oxidation reactions of amino acids by quinolinium dichromate (QDC) in acid medium, under nitrogen. The following tests were carried out :

(a) Reduction of mercuric chloride(4); it was observed that there was no reduction of mercuric chloride, thus indicating the absence of radical intermediates during the process of oxidation by QDC.

(b) Polymerization of an added olefinic monomer, such as acrylonitrile(4).

1 ml of acrylonitrile (0.02M) and 2 ml of substrate solution (0.2M) in H_2SO_4 (4.0M) were taken in a 10 ml conical flask. 2 ml of QDC solution (0.02M) was taken

in a test-tube. The two reactant solutions were placed under nitrogen, and then mixed and allowed to stand at the particular temperature for 30 minutes. There was no formation of a white opalescence, indicating the absence of any polymer formation. Each experiment was accompanied by a blank control.

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

Amino acids are the building blocks in protein synthesis. 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 proposals for the derivation of alkaloid structures from common amino acids were considered by Robinson(1). 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 from the decarboxylation of amino acids. Example are methylamine from glycine, ethanolamine from serine, isobutylamine from valine, isopentylamine from leucine, and cadavarine from lysine.

Many naturally occurring nitrogen compounds have basic properties in common with the alkaloids, but their structures are relatively simple. Their nitrogen atoms

are not incorporated into heterocyclic skeletons. Instead, they seem 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 protoalkaloids are possible precursors of typical alkaloids. Protoalkaloids related to aromatic amino acids, such as tyrosine and dihydroxy phenylalanine, are important precursors of alkaloids. The utilization of nicotine in the synthesis of amino acids and proteins has been reported(2). The 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(3). It has been suggested that arginine could be a normal precursor of the lupine alkaloids(4). Tracer experiments have confirmed that the carbon skeletons of all the major lupine alkaloids have been derived from lysine(5). Threonine and isoleucine have been reported to be good precursors of the pyrrolizidine alkaloids(6). There is evidence that alkaloids having the imidazole ring are probably made from histidine(7-9).

The kinetics of oxidation of amino acids have become important, both from a 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:

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

(S^-)	(S°)	(SH^+)
Anion	Zwitterion	Cation

Amino acids have been oxidized by a variety of oxidizing agents such as persulfate(10), peracids(11), peroxydisulfate(12), chloramine-B(13), manganese(III) and cerium(IV)ions(14), acidic permanganate ion(15), bromate ion(16), peroxomonosulfate(17), N-bromoacetamide(18,19), chloramine-T(20), N-bromosuccinimide(21), bromamine-T(22), and by Fremy's salt(23).

Glycine plays a significant role in the pathways of choline metabolism(24), and in the biosynthesis of diterpenoid alkaloids(25). It 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, hemoglobin and cytochromes. Alanine has been found to be among the possible pyridine ring precursors in the biosynthesis of nicotinic acid(26). Leucine has been shown to be a

precursor for some purine alkaloids(27). Phenylalanine has been recognized as a precursor in the biosynthesis of tropane alkaloids(28). 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(29). The biosynthesis of alkaloids such as galanthamine(30), known to possess analgesic activity comparable to morphine(31), was achieved starting from phenylalanine. The pathway had involved a crucial phenolic coupling step, as predicted by Barton(32). The biosynthesis of rotenone(33), 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(34). Indeed, the literature is replete with instances of phenylalanine being effectively used for the biosynthesis of a wide variety of alkaloids(35-39).

Glycine, alanine, valine, leucine and phenylalanine have been oxidized by a variety of oxidizing agents such as ceric ions(40,41), Fenton's reagent(42), hexacyanoferrate(II catalyzed by osmium(VIII) ions(43), manganese(III) sulfate(44), Fe^{2+}ions(45), peroxydisulfate catalyzed by Cu^{2+}ions(46), Co^{3+} ions catalyzed by Ag^{+} ions(47), acidic permanganate(48), aquopentacyanoferrate(II) ion(49), periodate(50), aquomanganese(III) ion in acid medium(51), peroxydisulfate

catalyzed by Ag^{+} and Cu^{2+} ions(52-54), N-bromosuccinimide(55,56), chloramine-T (57-60), bromamine-B(61,62), N-bromoacetamide (63,64), lead tetraacetate(65), peroxomonosulfate(66), acid bromate(67), phenyl iodosoacetate(68,69), bromine catalysed by osmium(VIII) ion(70), Ag^{2+} ions(71), potassium hexacyanoferrate(III) in alkaline media(72), N-chloro sulphonamide(73), bromamine-T(74-76), dichloramine-T(77,78), N-bromobenzamide(79), peroxo diphosphate catalysed by ruthenium(III) ions(80), N-bromosulphonamide(81), chloramine-T catalysed by ruthenium(III) ions(82), N-chlorobenzamide(83), N-chlorosuccinimide(84) and by alkaline hexachloroiridate(IV) (85).

Alamine has been oxidized by N-bromosaccharin(86), chloramine-B(87), N-chlorobenzamide(88), peracids(89), chloramine-T(90), N-bromosuccinimide(91), Fenton's reagent(92), cerium(IV) catalysed by Ag(I) ions(93), and by permanganate ions(94).

Valine has been oxidized by chloramine-T(95), phenyliodoso acetate(68,69), lead tetraacetate(65), manganese(III) sulfate(96), and by potassium hexacyanoferrate(III) in alkaline media(72a).

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

Phenylalanine has been oxidized by acidic permanganate(101,102,103), aqueous hydrogen peroxide(104), potassium hexacyanoferrate(III) in alkaline media(105), and by cerium(IV) in presence of Mn(II) ions(106).

PRESENT WORK

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

Stoichiometry (vide "Experimental")

The stoichiometries of all the oxidation reactions were determined. A stoichiometric ratio, $\Delta[QDC]/\Delta[Substrate]$ of 1.07 was obtained (Table 1).

Table 1. Stoichiometry of the oxidation of amino acids; [substrate]=0.005M, T=348K.

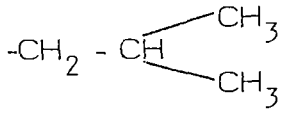
$[H_2SO_4]$(M)	1.0	3.0	4.0	5.0
10^2[QDC](M)	2.50	2.60	2.70	2.80
Δ [QDC]/Δ[Glycine]	1.06	1.01	1.05	1.08
Δ [QDC]/Δ[Alanine]	1.08	1.05	1.02	1.10
Δ [QDC]/Δ[Leucine]	1.15	1.03	1.07	1.12
Δ [QDC]/Δ[Valine]	1.05	1.01	1.08	1.03
Δ [QDC]/Δ[Phenylalanine]	1.11	1.02	1.06	1.09

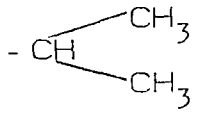
The stoichiometry conformed to the overall equation:

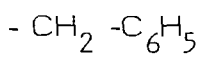
$$RCH(NH_2)COOH + 2Cr^{VI} \longrightarrow RCN + CO_2 + 2Cr^{IV} + 4H^+ \qquad (1),$$

which envisaged a two-electron transfer, in agreement with Brown's observations(107).

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


(leucine)


(valine)


(Phenylalanine)

Effect of substrate and oxidant

The rate of the reaction was found to be dependent on the concentrations of both, substrate and oxidant. The order of the reaction with respect to substrate concentration was obtained by changing the substrate concentration and observing the effect on the rate, at constant [QDC] and $[H^+]$. The results have been recorded in Table 2.

Table 2. Dependence of the oxidation rate on [Amino Acid].

[Amino Acid](M)	0.01	0.025	0.05	0.10	0.20
$10^4 x k_1, s^{-1}$ for:					
Glycine	0.98	2.4	5.0	10.0	20.2
Alanine	0.87	2.1	4.4	9.0	18.0
Leucine	1.31	3.2	6.6	13.5	26.5
Valine	0.81	1.9	4.0	8.2	16.0
Phenylalanine	2.1	5.3	10.8	21.8	43.0

[QDC] = 0.001M, $[H_2SO_4]$ = 4.0M, μ = 0.5M, T = 348K.

Plots of k_1, the pseudo first order rate constant against a twenty-fold range of concentration of substrate gave straight lines passing through the origin (Fig.1), indicating that the rate of oxidation was dependent on the first power of the concentration of the substrate. This was further seen by the constant values of k_2, the second order rate constant.

Under pseudo first order conditions, individual kinetic runs were first order with respect to the oxidant, (QDC). Further, the pseudo first order rate constants (k_1) were independent of the initial concentration of the oxidant (QDC). When a constant concentration of substrate (large excess) was used, k_1 did not show any appreciable variation with the change in concentration of the oxidant, indicating

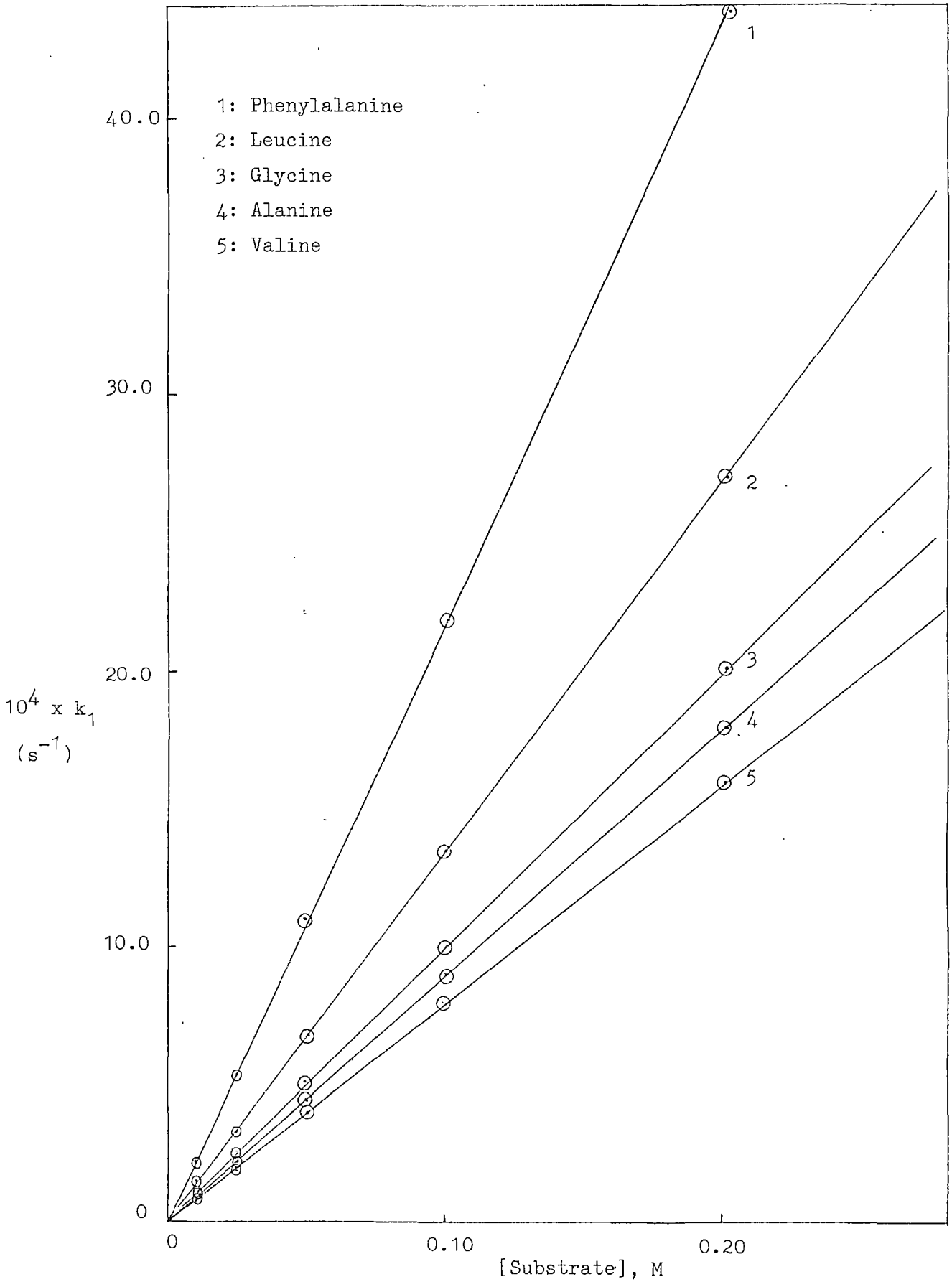


Fig.1: Plot of k_1 against the concentration of substrate

a first order dependence of the reaction on the concentration of the oxidant (Table 3).

Table 3. Dependence of the oxidation rate on [QDC].

10^4[QDC](M)	1.0	2.5	5.0	7.5	10.0
$10^4 xk_1$, s^{-1} for:					
Glycine	0.98	0.92	0.95	0.90	0.98
Alanine	0.85	0.88	0.85	0.81	0.87
Leucine	1.30	1.18	1.24	1.20	1.31
Valine	0.82	0.87	0.82	0.86	0.81
Phenylalanine	2.0	1.8	1.9	2.0	2.1

[Amino Acid] = 0.01M, $[H_2SO_4]$ = 4.0M, μ = 0.5M, T = 348K.

Effect of acid

The reaction was susceptible to changes in acid concentration, and the rate was observed to increase with an increase in the concentration of the acid (Table 4).

Table 4. Dependence of the oxidation rate on [Acid].

$[H_2SO_4]$(M)	1.0	2.0	3.0	4.0	5.0
$10^4 xk_1$, s^{-1} for:					
Glycine	0.24	0.50	0.72	0.98	1.25
Alanine	0.20	0.43	0.62	0.87	1.10
Leucine	0.33	0.65	1.02	1.31	1.70
Valine	0.18	0.39	0.55	0.81	0.93
Phenylalanine	0.56	1.10	1.67	2.10	2.85

[Amino Acid] = 0.01M, [QDC] = 0.001M, μ = 0.5M, T = 348K.

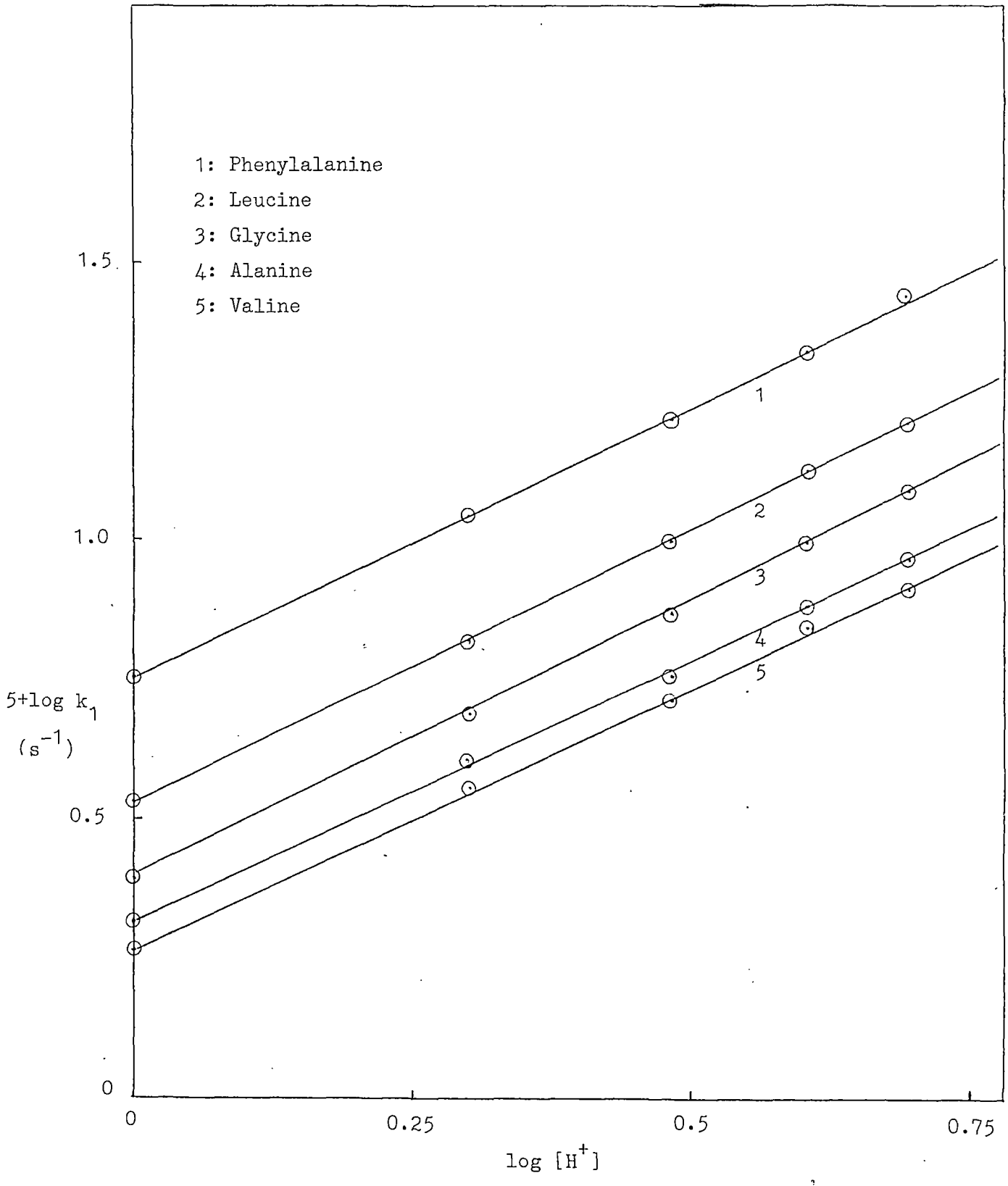


Fig.2: Plot of log k_1 against log $[H^+]$

Plots of log k_1 against log $[H^+]$ were linear with slopes equal to unity (Fig.2), indicating that the rate of the reaction was dependent on the first power of the concentration of the acid.

The linear increase in the oxidation rate with acidity suggested the involvement of a protonated Cr(VI) species in the rate determining step. There have been earlier reports of the involvement of such Cr(VI) species in chromic acid oxidations(108). Protonated Cr(VI) species have been observed in the presence of p-toluenesulphonic acid in nitrobenzene-dichloromethane mixtures(109).

Rate Law

Under the present experimental conditions, wherein pseudo-first-order conditions have been used for all the kinetic runs, the observed rate law can be expressed as:

$$\text{Rate} = -\frac{d[Cr(VI)]}{dt} = k[\text{substrate}][QDC][H^+] \qquad (2),$$

Effect of solvent

All the kinetic runs were performed in aqueous medium. However, it was thought appropriate to study the effect of a change in the dielectric constant of the medium. Hence, the reactions were performed using acetic acid-water mixtures. Reactions involving an ionic reactant tend to be

influenced by changes in the solvent medium. It is hence to be expected that in the present investigation, the solvent should be playing an important part. In the case of each of the substrates oxidized by quinolinium dichromate, the rate of oxidation was fastest in those solvent mixtures that contained the smallest proportions of water, and increasing proportions of acetic acid resulted in an increase in the rate of oxidation (Table 6). The dielectric constants for acetic acid-water mixtures have been estimated from the dielectric constants of the pure solvents(110).

Table 6. Dependence of the oxidation rate on solvent.

H_2O : HOAc(%,v/v)	100:0	95:5	90:10	85:15	80:20
Dielectric constant(D)	78.54	74.92	71.30	67.68	64.06
$10^4 x k_1$, s^{-1} for:					
Glycine	0.98	1.12	1.24	1.48	1.69
Alanine	0.87	0.96	1.03	1.15	1.24
Leucine	1.31	1.47	1.64	1.88	2.20
Valine	0.81	0.89	1.02	1.12	1.22
Phenylalanine	2.10	2.28	2.63	3.08	3.52

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 4.0M, μ = 0.05M, T = 348K.

In the present investigation, in going from 100% water to 80% water, the polarity decreases. This decrease in the polarity of the medium caused an increase in the rate

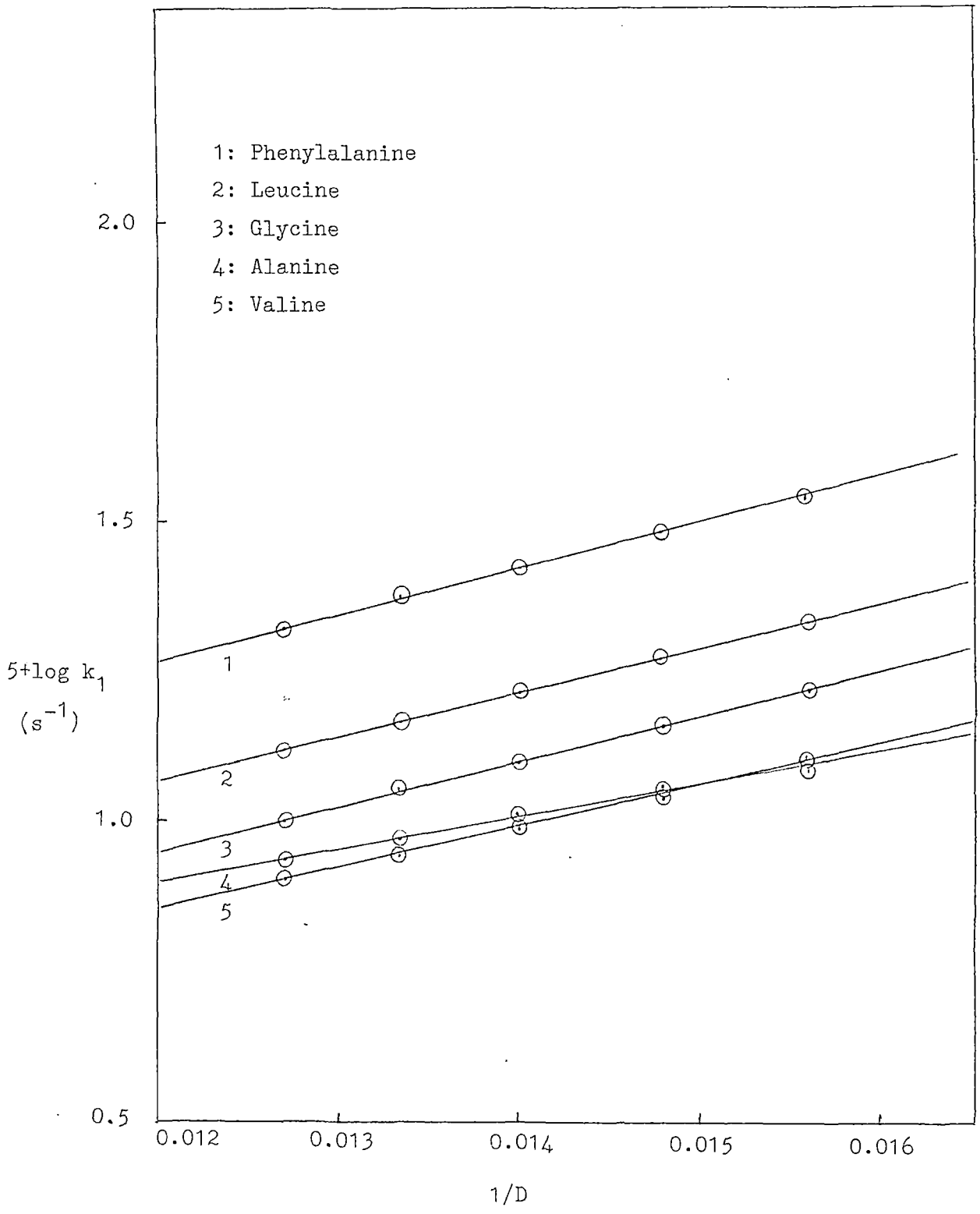


Fig.3: Plot of log k_1 against the reciprocal of dielectric constant

of the reaction (Table 6). Plots of log k_1 against the reciprocal of the dielectric constant were linear (Fig.3), with positive slopes. This suggested an interaction between a positive ion and a dipole(111), and was in consonance with the observation that in the presence of an acid, the rate-determining step involved a protonated Cr(VI) species.

On the basis of the solvating power of the solvent, a correct prediction of a qualitative nature can be made of the rate of the reaction in different solvent media. In the present investigation, the transition state is less polar than the initial state (reactants) because of the increased dispersal of charges in the transition state. This would indicate that the extent of solvation of the transition state was less than that for the reactants, in agreement with the assumptions of Hughes and Ingold(112). Therefore, the decrease in the rate of oxidation on the addition of a more polar solvent, as in the present investigation, is a natural result of the progressive increase in solvation of the reactants more than that of the transition state. The effect of a change in the solvent composition on reaction rates would also depend on factors such as solvent-solute interactions(113,114), and on solvent structure.

Effect of temperature

The rate of the reaction was influenced by changes in temperature (Table 7).

Table 7. Dependence of the oxidation rate on temperature.

Temp (±0.1K)	338	343	348	353	358
$10^4 x k_1$, s^{-1} for:					
Glycine	0.58	0.76	0.98	1.35	1.70
Alanine	0.49	0.70	0.87	1.09	1.52
Leucine	0.77	1.03	1.31	1.70	2.05
Valine	0.52	0.68	0.81	0.96	1.26
Phenylalanine	1.18	1.77	2.10	2.77	3.39

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 4.0M, μ = 0.5M.

Plots of log k_1 against the reciprocal of temperature were linear (Fig.4), suggesting the validity of the Arrhenius equation. The slopes of the plots were used to calculate the activation energies of the reactions (vide "Experimental" Calculations). The other activation parameters were evaluated and have been shown in Table 8.

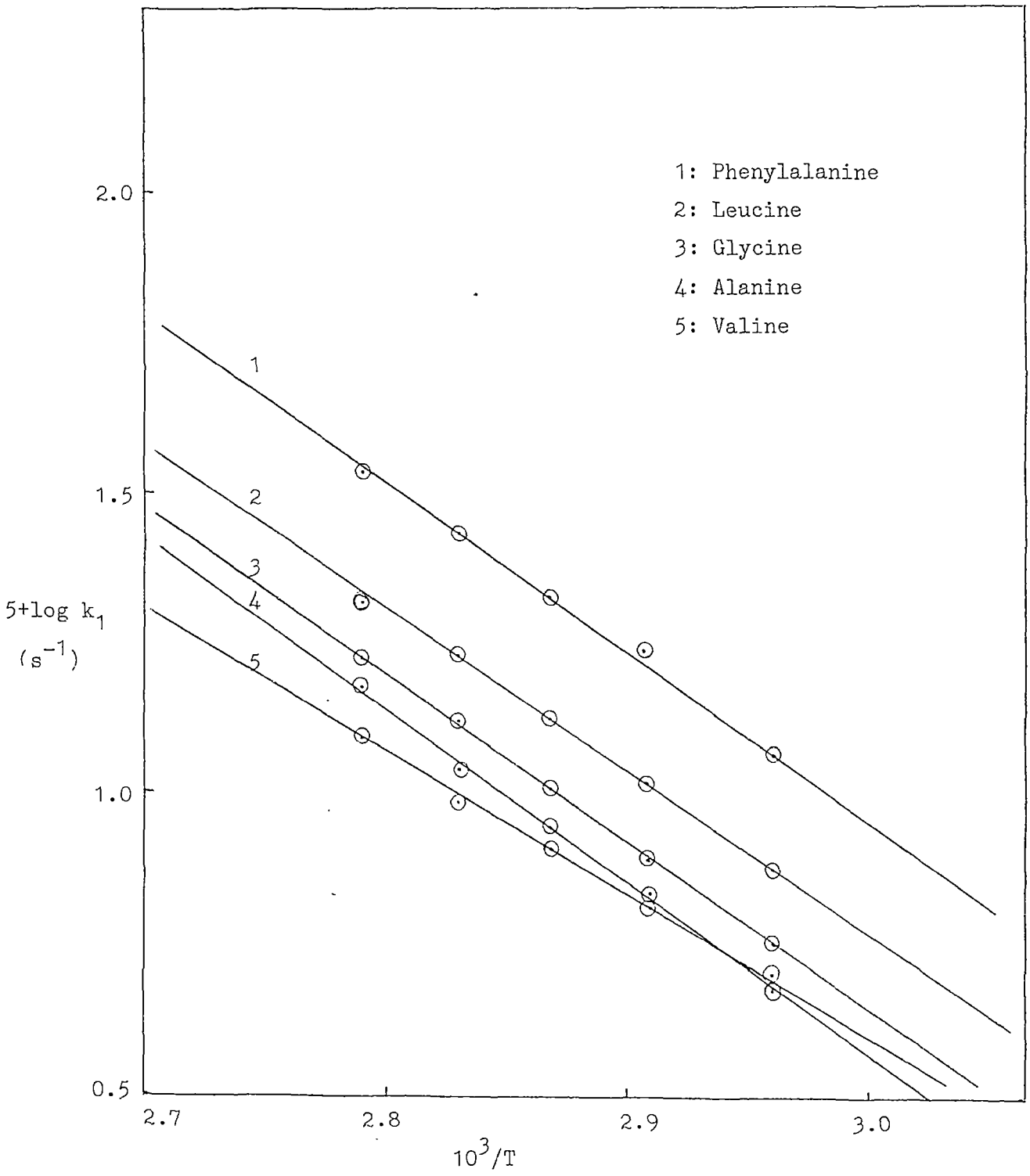


Fig.4: Plot of log k_1 against the reciprocal of temperature

Table 8. Activation Parameters

Amino Acid	E ($kJ\,mol^{-1}$)	$\Delta H^{\neq}$ ($kJ\,mol^{-1}$)	$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	$\Delta G^{\neq}$ ($kJ\,mol^{-1}$)
Glycine	57	54	-168	112
Alanine	57	54	-168	112
Leucine	56	53	-170	112
Valine	59	56	-163	113
Phenylalanine	55	52	-172	112

Error limits: $E \pm 2\ kJmol^{-1}$, $\Delta H^{\neq} \pm 2 kJmol^{-1}$, $\Delta S^{\neq} \pm 3JK^{-1}mol^{-1}$, $\Delta G^{\neq} \pm 2\ kJmol^{-1}$

The oxidations of all the substrates were characterized by negative entropies of activation. This would suggest an ordered transition state, relative to the reactants(115). Differences in solvation of substrates in the ground state and the transition state might also contribute to some extent to the negative entropies of activation(116).

Isokinetic Relationship

The enthalpies and entropies of activation for a reaction are linearly related by the equation

$$\Delta H^{\neq} = \Delta H_o^{\neq} + \beta \Delta S^{\neq} \qquad (3),$$

where β is the isokinetic temperature. For these oxidation reactions, the activation enthalpies and entropies were

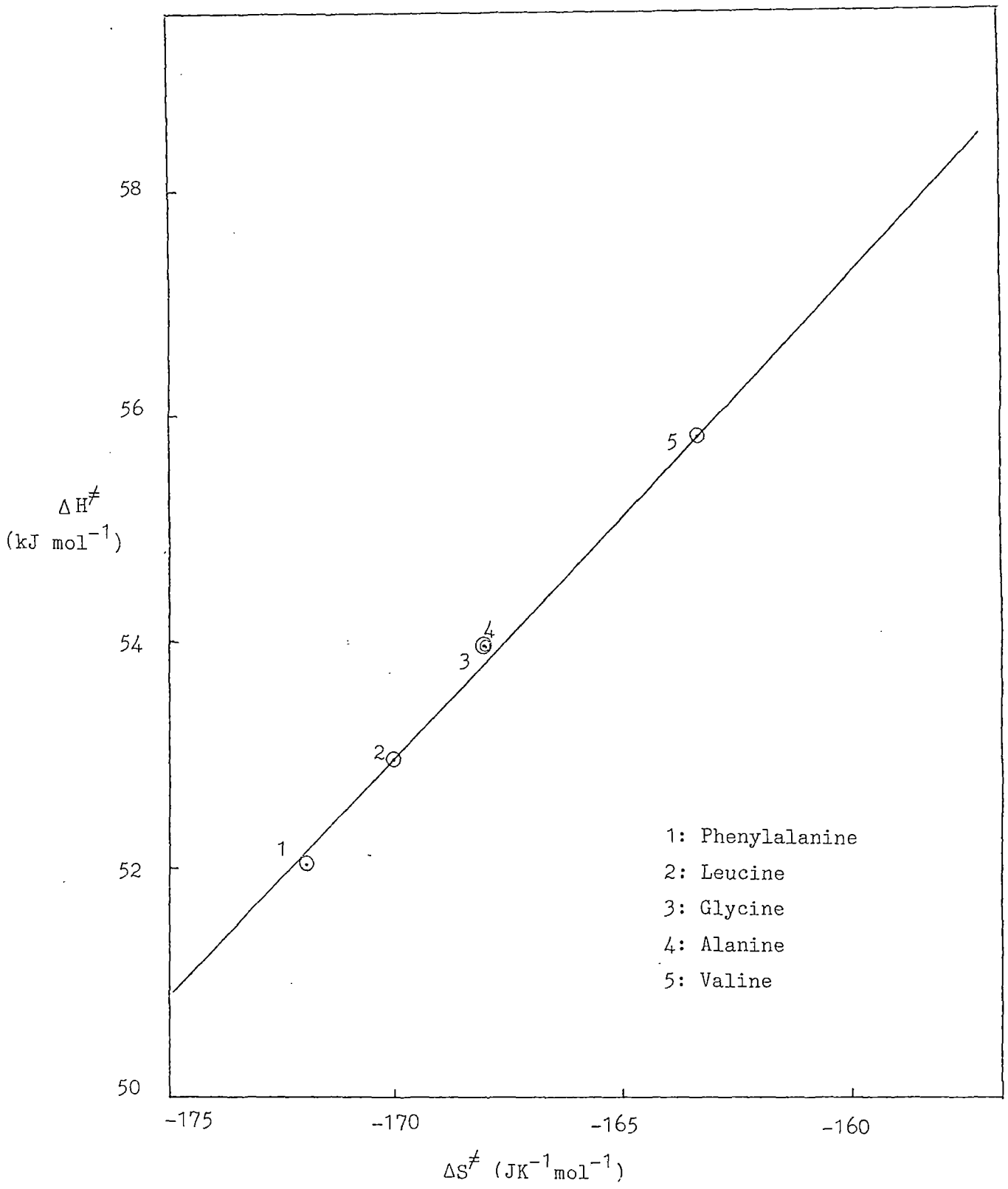


Fig.5: Isokinetic Plot

linearly related. The correlation was tested and found to be valid by applying Exner's criterion(117). The isokinetic temperature, obtained from the plot of $\Delta H^{\neq}$ against $\Delta S^{\neq}$, was 338K (Fig.5). Although current views do not attach much physical significance to isokinetic temperature(118), a linear correlation between $\Delta H^{\neq}$ and $\Delta S^{\neq}$ is usually a necessary condition for the validity of linear free energy relationships. Further, the values for the free energies of activation ($\Delta G^{\neq}$) were nearly constant, indicating that the same mechanism operated for the oxidation of all the substrates studied.

Solvent isotope effect

All the oxidation reactions of these amino acids by quinolinium dichromate, in acid medium, have been carried out in aqueous medium. It was thought appropriate to study the effect of a change in the solvent (from H_2O to D_2O) in order to ascertain the extent of the solvent isotope effect. It was observed that the rates of oxidation of these amino acids were incresed in D_2O medium (Table 9), in agreement with earlier reported observations(119).

Table 9. Solvent isotope effect for the oxidation of amino acids.

Amino Acid	k_{H_2O}	k_{D_2O}	k_{D_2O}/k_{H_2O}
	($10^4 x k_1$, s^{-1})		
Glycine	0.98	1.30	1.33
Alanine	0.87	1.13	1.30
Leucine	1.31	1.84	1.40
Valine	0.81	1.10	1.36
Phenylalanine	2.10	2.84	1.35

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 4.0M, μ = 0.5M, T = 348K

Since D_3O^+ is about three times stronger than H_3O^+ (119,120), the solvent isotope effect, k_{D_2O}/k_{H_2O}, should be greater than unity for acid-catalyzed reactions. If the solvent isotope effect had been less than unity, then this would have indicated a pre-equilibrium proton transfer, followed by a rate-limiting electron-transfer process. Since the solvent isotope effect, k_{D_2O}/k_{H_2O}, was greater than unity (Table 9), this would suggest a proton-catalyzed reaction. This would support the protonation of the oxidant (QDC), an observation which has been reflected in the acid dependence on the rate of the reaction (Table 4).

Effect of ionic strength

Variations in the ionic strength of the medium using sodium perchlorate (μ = 0.01M to 0.50M), did not

influence the rates of these reactions. A ionic reactant (p-51) is expected to be influenced by the ionic strength of the medium (Kinetic effects) !!!

Effect of added salts

The addition of salts such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4 and $MgSO_4$ (concentration range of 1×10^{-4}M to 5×10^{-3}M) did not have any effect on the rates of these reactions. ??

Earlier work on the oxidation of amino acids by alkaline hexacyanoferrate(III) had shown specific cation effects, wherein changing the cation from lithium to cesium had resulted in greater than 100-fold increase in the rate of the reaction(121). In the present investigation, salt effects were not observed. It seems possible that any effects due to the addition of salts, in the concentration range studied, may be compensated for by the high ionic strength of the mèdium and the high acid concentrations employed in this present study, thus vitiating any observed effect due to the addition of salts.

Kinetic isotope effect

The kinetic isotope effect caused by deuterating the α-carbon atom was studied, in order to determine the nature of the intermediate formed in the rate-determining step of these oxidation reactions. The results have been shown in Table 10.

Table 10. Kinetic isotope effect for the oxidation of amino acids.

Amino Acid	$RCH(NH_2)COOH$	$RCD(NH_2)COOH$	k_H/k_D
	($10^4 \times k_1$, s^{-1})		
Glycine	0.98	0.92	1.06
Alanine	0.87	0.83	1.05
Leucine	1.31	1.20	1.08
Valine	0.81	0.78	1.04
Phenylalanine	2.10	2.02	1.04

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 4.0M, μ = 0.5M, T = 348K

The k_H/k_D values were close to unity (Table 10), which indicated that, in the rate-determining step, there was no cleavage of the carbon-hydrogen bond. In the earlier work on the oxidation of amino acids by alkaline hexacyanoferrate(III), a significant kinetic isotope effect had been reported(122). Being a one-electron oxidant, potassium hexacyanoferrate(III) would enable the formation of a radical species, during the course of its reactions with organic substrates, and a kinetic isotope effect at the α-carbon atom would be observed(122). The absence of a kinetic isotope effect, in the present investigation (Table 10), indicated that there was no cleavage of the carbon-hydrogen bond in the rate-determining step of the reaction.

Induced polymerization

In the present investigation, since all the reactions were performed under nitrogen, the possibility of induced polymerization was tested. It was seen that there was no induced polymerization of acrylonitrile or the reduction of mercuric chloride(123). Further, no ESR signals could be detected in these oxidation reactions (E-4, Varian), thus providing no evidence for the formation of radical intermediates. Control experiments were performed, in the absence of the substrate. The concentration of the oxidant (QDC), did not show any appreciable change.

Structure-reactivity relation

The order of reactivities among the substrates was:

L-phenylalanine > L-leucine > L-glycine > L-alanine > L-valine (Table 2).

Based on the reactivities of these substrates, it was observed that the benzyl group in phenylalanine and the $(CH_3)_2CH-CH_2-$ group in leucine behave as strongly electron-releasing groups. It has been found that the rates of oxidation of these amino acids do not correlate satisfactorily with the usual Taft-σ^* (polar substituent constants) or E_s (steric substituent constants) of these groups(124). Hence, the electron-releasing nature, rather than the inductive effect of these polar groups, appears to control

the reactivity. The electron-releasing power of a phenyl group is twice that of a p-methyl group in aromatic substitution. In the Cr(VI) oxidation of primary alcohols, it was found that the maximum increment for a methylene group was -0.1(125). Hence, the computed σ^* value for the benzyl group would be -0.34+(-0.1) = -0.44. The σ^* value of $(CH_3)_2CH-$ has been given as -0.19 (126). Hence the computed σ^* value for $(CH_3)_2CH-CH_2-$ would be -0.29. Using the σ^* value for $C_6H_5-CH_2-$ as -0.44, and σ^* value for $(CH_3)_2CH-CH_2-$ as -0.29, the modified Taft equation:

$$\log k/k_o = \sigma^* \rho^* + \delta E_s \qquad (4)$$

has been used for the structure-reactivity correlation for these amino acids. This was done by plotting ($\log k/k_o - \delta E_s$) against σ^* (where k_o represents the rate constant for glycine, and k represents the rate constants for the other α -amino acids, at 348K, and σ^* and E_s were the polar and steric substituent constants respectively). Solving the simultaneous equation with respect to leucine and phenylalanine, the value of δ (the steric reaction constant) was +0.84, and the value for ρ^* (the polar reaction constant) was -1.48. The above plot was found to be linear, which suggested that both, the steric effect and the polar effect influenced the rate of the reaction. The negative value of ρ^* (-1.48) indicated that the reaction was

facilitated by the high electron density at the reaction site, caused by electron-releasing groups. The positive steric reaction constant ($\delta = 0.84$) indicated that the steric effect was quite pronounced in these oxidation reactions.

Mechanism

The rate of the reaction between the substrate (glycine, alanine, valine, leucine and phenylalanine) and oxidant (quinolinium dichromate, QDC), in acid medium, was observed to be dependent on the first powers of the concentrations of each of the reacting species - substrate, oxidant and acid (Tables 2-4). The linear dependence of the rate of oxidation on acidity (Table 4), suggested the involvement of a protonated Cr(VI) species in the slow step of the reaction. The initial reaction was between the substrate and a protonated Cr(VI) species.

The rate constants for the oxidation of all the amino acids were almost of the same order of magnitude (Table 2). This was because all these amino acids had almost similar pK values. The ionization constants and pH values at the isoelectric points(127) of these amino acids (glycine, alanine, leucine, valine and phenylalanine) at 298K have been given in Table 11.

Table 11. Ionization constants (127) and pH values at the isoelectric points of amino acids at 298K.

Amino Acid	pK_1	pK_2	pH_i
Glycine	2.34	9.60	5.97
Alanine	2.34	9.69	6.01
Leucine	2.36	9.60	5.98
Valine	2.32	9.62	5.96
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.

An amino acid is bifunctional because of the presence of both amino and carboxyl functions. Therefore, its action with acid involves an equilibrium process. The dissociation of amino acids depends upon the pH of the medium. It is well known that amino acids exist as dipolar ions (zwitterions) in aqueous solution. In strongly acidic or alkaline media, the following equilibria exist:

$$RCH(N^+H_3)COOH \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(N^+H_3)COO^- \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(NH_2)COO^- \qquad (5)$$

(cation) (zwitterion) (anion)

In the present investigation, it has been established

that the rate of the reaction was dependent on the first power of the concentration of acid (Table 4). The observed dependence of the rate on [acid] and the experimental conditions employed, in these oxidation reactions, suggested that the amino acids existed overwhelmingly in the cationic form. The conversion of the zwitterion to the cationic form would depend on $[H^+]$, as shown in Eq.(6).

$$RCH(N^+H_3)COO^- + H^+ \rightleftharpoons RCH(N^+H_3)COOH \qquad (6)$$

In acid medium, the zwitterion was converted to the cation $[RCH(N^+H_3)COOH]$, which was the reactive species, under the present experimental conditions.

The effect of changing the solvent composition on the rate of reaction has been discussed in detail by Benson(128), Laidler(129), Frost and Pearson(115), Amis(111), and Entelis and Tiger(130). For the limiting case of zero angle of approach between two dipoles or an ion-dipole system, Amis(111) has shown that a plot of log k_1 (the pseudo-first-order rate constant) against 1/D (the reciprocal of the dielectric constant), gives a straight line, with a negative slope for a reaction between a negative ion and a dipole or between two dipoles, while a positive slope results for a positive ion-dipole interaction. In the present investigation, the effect of a change in solvent composition has been rationalized by changing the dielectric

constant of the medium, using varying proportions of acetic acid and water. It was observed that an increase in the proportion of acetic acid had resulted in an increase in the rate of the reaction, for all the amino acids oxidized by quinolinium dichromate (Table 6). Plots of log k_1 (the pseudo-first-order rate constant) against the reciprocal of the dielectric constant were linear, with positive slopes (Fig.3). This experimental observation was in conformity with the Amis theory(111), and supported the conclusion that, in the present investigation, the rate-determining step involved an interaction between a positive ion and a dipolar species.

The data for the activation parameters (Table 8) indicated that the values obtained for the activation energies(E) were quite similar. The constancy in the values for the free energies of activation ($\Delta G^{\neq}$) suggested a common mechanism of oxidation for all the amino acids studied. The fairly high negative values for the entropies of activation ($\Delta S^{\neq}$) point towards the formation of a more ordered transition state, resulting in a reduction in the degrees of freedom of motion of the molecules. The fairly high positive values of the free energies of activation ($\Delta G^{\neq}$) and the enthalpies of activation ($\Delta H^{\neq}$) indicated that the transition state was highly solvated. The linear trend between the enthalpy of activation ($\Delta H^{\neq}$) and the entropy of activation ($\Delta S^{\neq}$) for all these amino acids

studied showed that the reaction was controlled by both parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$). The isokinetic temperature (β) calculated from the linear plot of $\Delta H^{\neq}$ against $\Delta S^{\neq}$ was 338K.

The observed solvent isotope effects in acid media (Table 9) would support the proposed mechanism of the reaction. It is known that D_3O^+ is about three times stronger than H_3O^+ (119,120) for reactions which are catalyzed by acids. For such reactions, the isotope effect, k_{D_2O}/k_{H_2O}, should be greater than unity. The isotope effects observed (Table 9) conform to the above theory, supporting the protonation of the oxidant(QDC), as evident from the acid dependence on the rate of the reaction (Table 4).

The ionic strength of the medium did not affect the rates. The addition of salts did not reveal any influence on the rates of the reactions. Under these conditions, the direct interaction of the substrate and oxidant, in acid medium, is quite likely to form a reaction intermediate which in turn undergoes further reaction to give the product.

The oxidation of the deuterated amino acids (deuterated at the α-carbon atom) yielded values of the kinetic isotope effect, k_H/k_D, which were close to unity (Table 10). This indicated the absence of a primary kinetic isotope

effect, and confirmed that there was no cleavage of the carbon-hydrogen bond in the rate-determining step of the reaction.

The oxidation of all the amino acids, under nitrogen atmosphere, failed to induce the polymerization of acrylonitrile, or to bring about the reduction of mercuric chloride. No ESR signals were detected in these oxidation reactions, thus providing no evidence for a radical mechanism.

In the oxidation of α-amino acids by N-bromoacetamide, the neutral form of the amino acids was proposed as the reactive species(63,64,131). Kinetic investigations of the oxidation of α-amino acids by N-bromobenzene sulfonamide or bromamine-B(BAB) had indicated the reaction of the neutral form of the amino acid with the oxidant(132). The mechanistic pathway for the oxidation of amino acids by peroxomonosulfate envisaged a reaction involving the zwitterionic form of the amino acid(66).

The oxidation of amino acids by permanganate in concentrated acidic media was postulated to occur via the reaction between the cationic form of the amino acid and permanganic acid(133). At high concentrations of acid, the reaction between the cationic form of the amino acid and the positive halogen source was postulated as the rate-determining step in the oxidation of amino acids by chloramine-T(134). In acid media, amino acids exist as a mixture

of the zwitterionic [$RCH(N^+H_3)COO^-$] and cationic [$RCH(N^+H_3)COOH$] forms. In the present investigation, the observed direct dependence on the concentration of acid (Table 4) would suggest that the amino acids existed overwhelmingly in the cationic form. In acid solution, the zwitterion would be converted to the cation [$RCH(N^+H_3)COOH$], which is the reactive species, under the present experimental conditions.

Structure-reactivity correlations have been used to support the mechanism of the oxidation of amino acids. There have been earlier reports on the use of structure-reactivity correlations in the oxidation of α-amino acids by alkaline hexacyano-ferrate(III) catalysed by Os(VIII) ions, where values of $\rho^* = -0.64$ and $\delta = +1.48$ had been obtained(135). It was suggested that these reactions had a pronounced steric effect, but a negligible polar effect (135). In the oxidation of α-amino acids by N-bromo benzamide, in acid medium, the values obtained were $\rho^* = -0.83$ and $\delta = +0.49$ (79). It was further suggested that, since the reaction centre was not close to the site of substitution, the magnitude of the reaction constant was low(79).

In the present investigation, the values obtained for the reaction constants were $\rho^* = -1.48$ and $\delta = +0.84$. The magnitude of the reaction constants suggested that the oxidation of these amino acids by quinolinium dichromate

in acid medium, would be influenced by both factors, the polar effect and the steric effect. Since the reaction centre was close to the site of substitution, the magnitude of the reaction constants should be fairly high. This has been observed (ρ^* = -1.48 and δ = +0.84) thus supporting the proposed reaction mechanism.

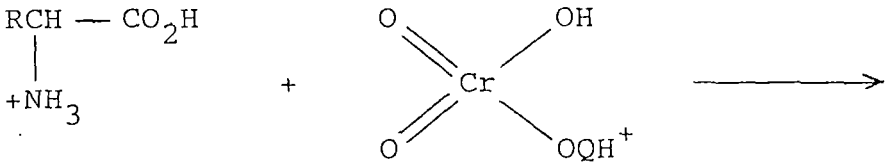
Based on the observed kinetic data, it would be justified to postulate that, in the present study, the oxidation of amino acids by quinolinium dichromate(QDC), in acid medium, would proceed via a direct interaction between the cationic form of the amino acid and the oxidant (QDC) to yield a reaction intermediate, which would then undergo further reaction to give the product. The mechanistic pathway for the oxidation of these amino acids (glycine alanine, valine, leucine and phenylalanine) by quinolinium dichromate (QDC), in acid medium, has been shown in the Scheme.

The major products of these oxidation reactions (vide "Experimental : Product Analysis)were:

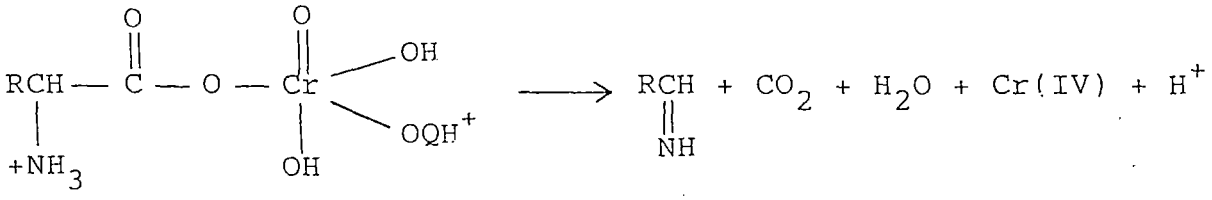
(a) the corresponding nitriles (∿75-80%), which were characterized by chemical and spectral methods; and

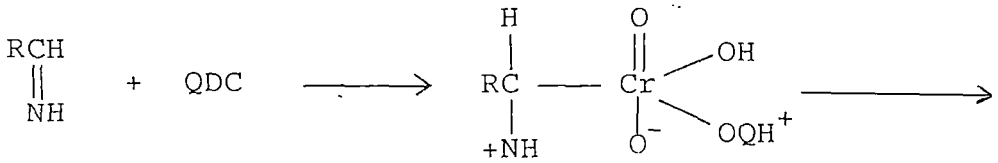
(b) trace amounts of the corresponding aldehydes (∿5-10%) which were characterized by their respective 2,4-dinitrophenylhydrazone derivatives.

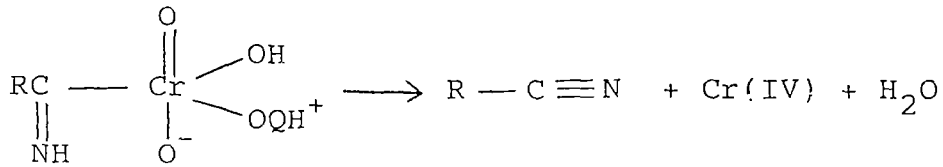
SCHEME



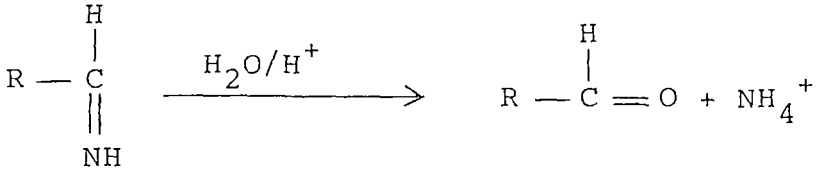
(Amino Acid) (QDC)







(Nitrile)



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KINETICS OF OXIDATION OF SERINE, THREONINE AND TYROSINE

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 (3.0Å), 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 a 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).

Protoalkaloids related to tyrosine are important precursors of various groups of alkaloids(17,18). Tracer experiments with barley have demonstrated the origin of tyramine and hordenine from tyrosine(19-21). Isoquinoline alkaloids have been derived by the condensation of a carbonyl compound with a derivative of tyrosine(22-24). 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(25-28). Tyrosine has been shown to be a precursor of betanidin, an alkaloid of the imidazole group(29).

The kinetics of oxidation of these amino acids (serine, threonine and tyrosine) have become important because of their biological significance. Serine has been oxidized by a variety of oxidizing agents such as chloramine-T(30-32), N-bromosuccinimide(33,34), peroxydisulfate(35), Fenton's reagent(36), ceric ions(37,38), chloramine-B(39), chromic acid(40), N-bromoacetamide(41,42), and by periodate in acid medium(43). Threonine has been oxidized by N-bromosuccinimide(33), peroxydisulfate(35), ceric ions(37), cobalt(III) ions(44), acidic $KMnO_4$ (45), chloramine-T (46-50), peroxomonosulfate(51), Mn(III) sulfate(52), potassium hexacyanoferrate(III) in alkaline medium(53), and by 1-chloro-

benzotriazole(54). Tyrosine has been oxidized by Cu^{2+}/H_2O_2 (55), peroxidase(56-58), chymotrypsin(59), and by potassium hexacyanoferrate(III) in alkaline medium(60).

PRESENT WORK

The present work is a kinetic investigation of the oxidation of serine, threonine and tyrosine by quinolinium dichromate in acid medium, at constant ionic strength, under a nitrogen atmosphere.

Stoichiometry (vide "Experimental")

The stoichiometry of each of the reactions was determined. A stoichiometric ratio Δ [QDC]/ Δ [Substrate] of 1.08 was obtained (Table 1), corresponding to the overall equation:

$$RCH(NH_2)COOH + 2Cr^{VI} \longrightarrow RCN + CO_2 + 2Cr^{IV} + 4H^+ \qquad (1)$$

Here, R = $-CH_2OH$(serine), $-CHOHCH_3$(Threonine),

$-CH_2$-Ph-OH (Tyrosine)

Table 1. Stoichiometry of the oxidation of amino acids; [Substrate]= 0.005M, T = 343K.

$[H_2SO_4]$(M)	1.0	3.0	4.0	5.0
10^2[QDC](M)	2.50	2.60	2.70	2.80
Δ [QDC]/ Δ[Serine]	1.05	1.08	1.10	1.12
Δ [QDC]/ Δ [Threonine]	1.08	1.05	1.11	1.06
Δ [QDC]/ Δ[Tyrosine]	1.07	1.12	1.04	1.08

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 (Tables2-3).

Table 2. Rate data for the oxidation of serine and theonine.

[Substrate] (10^2xM)	[QDC] (10^3xM)	Serine ($10^4 \times k_1$, s^{-1})	Threonine
1.0	1.0	1.6	0.6
2.5	1.0	4.0	1.5
5.0	1.0	8.2	3.1
10.0	1.0	16.5	6.3
20.0	1.0	33.0	12.5
1.0	0.75	1.5	0.6
1.0	0.50	1.6	0.5
1.0	0.25	1.5	0.7
1.0	0.10	1.7	0.6

$[H_2SO_4]$ = 3.0M, μ = 0.5M, T = 343K.

Table 3. Rate data for the oxidation of tyrosine

[Substrate] (10^2xM)	[QDC] (10^3xM)	Tyrosine ($10^4 \times k_1$, s^{-1})
1.0	1.0	2.5
2.5	1.0	6.3
5.0	1.0	12.5
10.0	1.0	25.3
20.0	1.0	51.0
1.0	0.75	2.3
1.0	0.50	2.2
1.0	0.25	2.5
1.0	0.10	2.6

$[H_2SO_4]$ = 2.0M, μ = 0.5M, T = 313K

Plots of k_1, the pseudo-first-order rate constant, against a twenty-fold range of concentration of substrate were linear passing through the origin, indicating that the rate of oxidation was dependent on the first power of the concentration of the substrates. The values of k_2 (second order rate constant) were fairly constant, confirming the first order dependence of the rate on the concentrations of the substrate (Tables 2-3).

When the concentration of the substrate was kept constant (taken in large excess), the pseudo-first-order rate constant (k_1) did not show any appreciable variation with changing concentrations of the oxidant (Tables 2-3), indicating that the rate of the reaction was dependent on the first power of the concentration of the oxidant (QDC).

Effect of acid

The rate of the reaction was observed to be dependent on the first power of the concentration of the acid (Table 4).

Table 4. Dependence of the oxidation rate on [Acid]

$[H_2SO_4]$(M)	1.0	2.0	3.0	4.0	5.0
$10^4 \times k_1$, s^{-1} for:					
Serine	0.5	1.0	1.6	2.1	2.6
Threonine	0.2	0.4	0.6	0.9	1.1
Tyrosine	1.2	2.5	3.7	5.0	6.1

[Amino Acid]=0.01M, [QDC] = 0.001M, μ= 0.5M, T = 343K (for serine and threonine) and 313K (for tyrosine).

Plots of log k_1 against log $[H^+]$ were linear with unit slopes, showing that the reaction was dependent on the first power of the concentration of the acid.

Rate law

The linear increase in the oxidation rate with acidity suggested the involvement of a protonated Cr(VI) species in the rate-determining step. The involvement of such protonated species has been well established in chromic acid oxidation(61).

Under the present experimental conditions, wherein pseudo-first-order conditions have been used for all the kinetic runs, the rate law can be expressed as:

$$\text{Rate} = -\frac{d[Cr(VI)]}{dt} = k\,[\text{Substrate}][QDC][H^+] \qquad (2)$$

Effect of solvent

The kinetic runs were carried out in acetic-water mixtures, in order to study the effect of a change in the dielectric constant of the medium. The dielectric constants for acetic acid-water mixtures have been estimated from the dielectric constants of the pure solvents(62). The estimated dielectric constants of the solvent mixtures used have been shown in Table 5.

The rate of the reaction was susceptible to changes

in the polarity of the solvent medium, with varying proportions of acetic acid and water. With an increase in dielectric constant of the medium, there was a decrease in the rate of the reaction (Table 5). This was in consonance with the observation that the use of more polar solvents required larger reaction times(63).

Table 5. Dependence of the oxidation rate on solvent.

H_2O:HOAc (%, v/v)	100:00	95:5	90:10	85:15	80:20
Dielectric constant(D)	78.54	74.92	71.30	67.68	64.06
$10^4 x k_1$, s^{-1} for:					
Serine	1.6	1.8	2.1	2.7	3.2
Threonine	0.6	0.64	0.7	0.8	0.9
Tyrosine	2.5	3.0	3.4	3.9	5.0

[Amino Acid] = 0.01M, [QDC] = 0.001M, μ = 0.5M, [H_2SO_4] = 3.0M (for serine and threonine) and 2.0M (for tyrosine), T = 343K (for serine and threonine) and 313K (for tyrosine).

Plots of log k_1 against the reciprocal of dielectric constants were linear with positive slopes (Fig.1). This suggested an interaction between a positive ion and a dipole (64), and confirmed that the rate-determining step, in the presence of acid, involved a protonated Cr(VI) species. The effect of a change in solvent composition on the reaction rate would also depend on factors such as the solvating power of the solvent(65), solute-solvent interactions (66,67), and solvent structure.

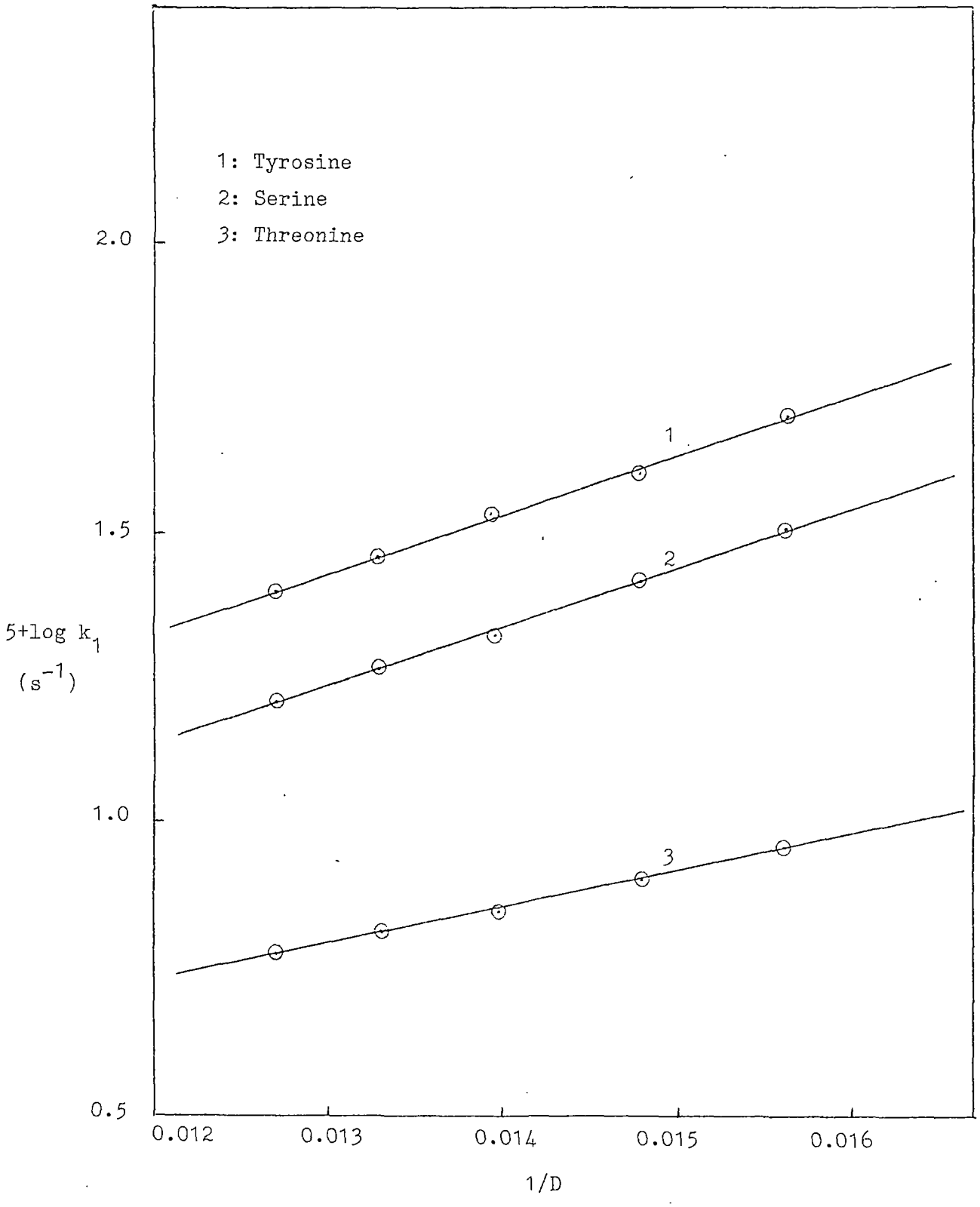


Fig.1: Plot of log k_1 against the reciprocal of dielectric constant

Effect of temperature

The rates of the reactions were observed to be susceptible to changes in temperature (Tables 6-7).

Table 6. Dependence of the oxidation rate on temperature.

Temp (±0.1K)	333	338	343	348	353
$10^4 x k_1$, s^{-1} for:					
Serine	1.0	1.3	1.6	1.9	2.4
Threonine	0.3	0.4	0.6	0.8	1.1

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 3.0M, μ = 0.5M

Table 7. Dependence of the oxidation rate on temperature (Tyrosine)

Temp (±0.1K)	303	308	313	318	323
$10^4 \times k_1$, s^{-1}	1.5	1.9	2.5	3.1	4.2

[Tyrosine] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 2.0M, μ = 0.5M

Plots of log k_1 against the reciprocal of temperature were linear (Fig.2). The slopes of the plots were used to calculate the activation energies of the reactions. The other activation parameters were calculated, and have been shown in Table 8.

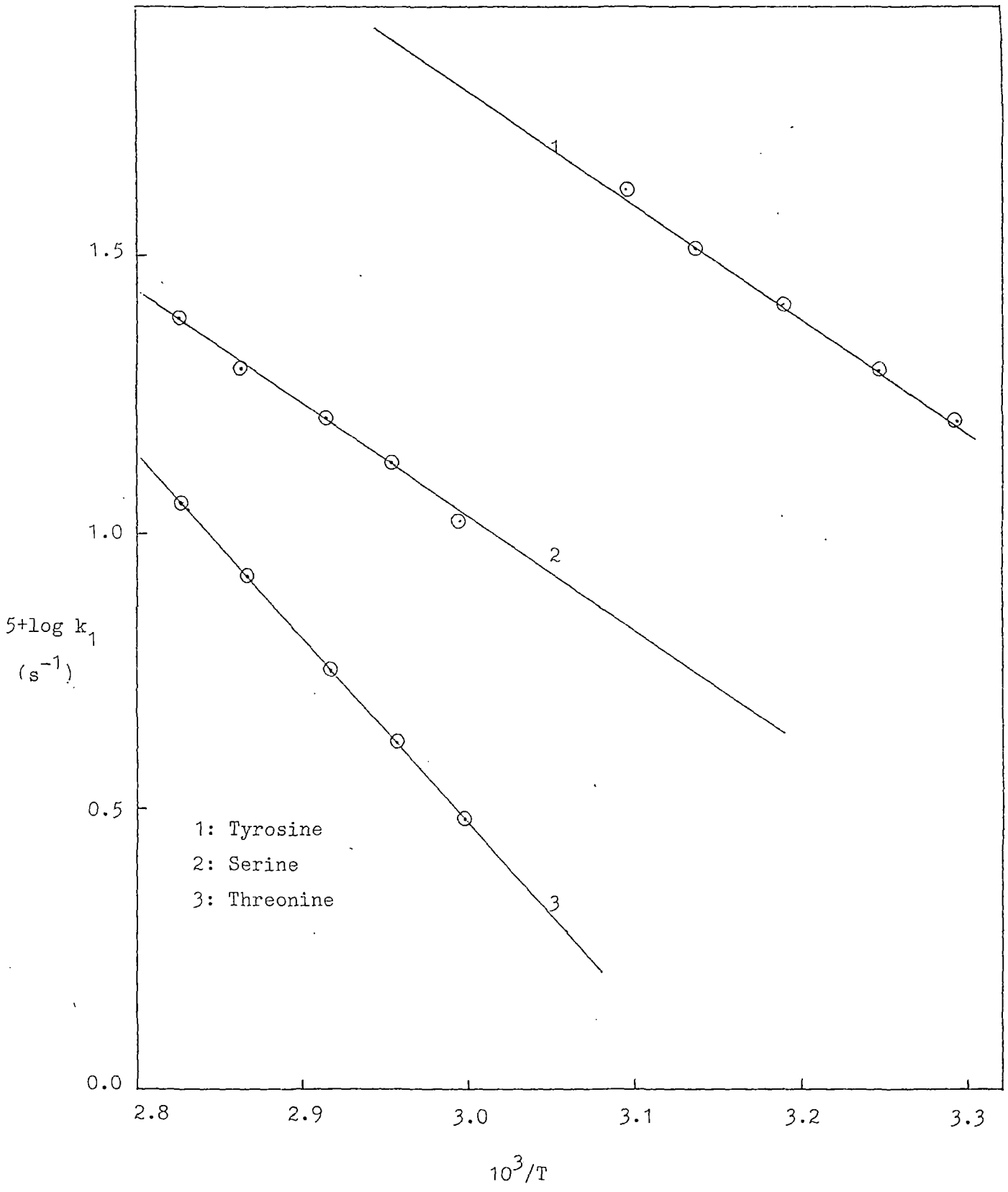


Fig.2: Plot of log k_1 against the reciprocal of temperature

Table 8. Activation parameters.

Amino Acid	E ($kJ\ mol^{-1}$)	$\Delta H^{\neq}$ ($kJ\ mol^{-1}$)	$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	$\Delta G^{\neq}$ ($kJ\ mol^{-1}$)
Serine	47	44	-191	110
Threonine	60	57	-160	112
Tyrosine	44	41	-210	107

Error limits: $E \pm 2kJmol^{-1}$, $\Delta H^{\neq} \pm 2kJmol^{-1}$, $\Delta S^{\neq} \pm 3JK^{-1}mol^{-1}$, $\Delta G^{\neq} \pm 2\ kJmol^{-1}$

Isokinetic Relationship

For these oxidation reactions, the activation enthalpies and entropies were linearly related. The correlation was tested by applying Exner's criterion(68), and found to be valid. The isokinetic temperature, obtained from the plot of $\Delta H^{\neq}$ against $\Delta S^{\neq}$, was 320K. Without attaching too much physical significance to isokinetic temperature, it could be postulated that a linear correlation between $\Delta H^{\neq}$ and $\Delta S^{\neq}$ would be a necessary condition for the validity of linear free energy relationships. The values obtained for the free energies of activation ($\Delta G^{\neq}$) were fairly constant, suggesting that the oxidation of all the substrates studied (serine, threonine and tyrosine) proceeded via similar mechanistic pathways.

Solvent isotope effect

All the oxidation reactions of these amino acids by quinolinium dichromate, in acid medium, were carried out in aqueous medium. The effect of a change in the solvent (from H_2O to D_2O) was studied. The rates of oxidation of these amino acids were increased in D_2O medium (Table 9).

Table 9. Solvent isotope effect for the oxidation of amino acids.

Amino Acid	k_{H_2O} ($10^4 x k_1$, s^{-1})	k_{D_2O}	k_{D_2O}/k_{H_2O}
Serine	1.6	2.2	1.38
Threonine	0.6	0.8	1.33
Tyrosine	2.5	3.4	1.36

[Amino Acid] = 0.01M, [QDC] = 0.001M, μ = 0.5M, [H_2SO_4] = 3.0M (for serine and threonine) and 2.0M (for tyrosine), T = 343K (for serine and threonine) and 313K (for tyrosine).

The data in Table 9 showed that the k_{D_2O}/k_{H_2O} values were greater than unity, which was in agreement with earlier reported observations(69). If the solvent isotope effect, k_{D_2O}/k_{H_2O}, had been less than unity, then this would have indicated a pre-equilibrium proton-transfer, followed by a rate-determining electron-transfer process. Since the solvent isotope effect, k_{D_2O}/k_{H_2O}, was greater than unity (Table 9), this would support a proton-catalyzed reaction of the oxidant (QDC), an observation which has been

confirmed by the acid dependence on the rate of the reaction (Table 4).

Effect of ionic strength

Variations in the ionic strength of the medium using *sodium perchlorate (μ=0.01M to 0.50M) did not influence* the rates of these reactions.

Effect of added salts

The addition of salts like NaCl, $NaNO_3$, KNO_3, Na_2SO_4 and $MgSO_4$ (concentration range of $1x10^{-4}$M to $5x10^{-3}$M) did not have any influence on the rates of these reactions. It would be appropriate to postulate that any effects due to the addition of salts, in the concentration range studied, could be compensated by the high ionic strength of the medium and the high acid concentrations used in the present study, thus vitiating any observed effect due to the addition of salts.

Kinetic isotope effect

The kinetic isotope effect caused by deuterating the α-carbon atom was studied (Table 10).

Table 10. Kinetic isotope effect for the oxidation of amino acids.

Amino Acid	$RCH(NH_2)COOH$	$RCD(NH_2)COOH$	k_H/k_D
	($10^4 \times k_1$, s^{-1})		
Serine	1.6	1.57	1.02
Threonine	0.6	0.58	1.03
Tyrosine	2.5	2.4	1.04

[Amino Acid] = 0.01M, [QDC] = 0.001M, μ = 0.5M, $[H_2SO_4]$ = 3.0M (for serine and threonine) and 2.0M (for tyrosine), T = 343K (for serine and threonine) and 313K (for tyrosine).

The k_H/k_D values were close to unity (Table 10), which indicated that, in the rate-determining step, there was no cleavage of the carbon-hydrogen bond.

Induced polymerization

In the present investigation, it was observed that there was no induced polymerization of acrylonitrile or the reduction of mercuric chloride(70). Further, no ESR signals could be detected in these oxidation reactions (E-4, Varian), indicating the absence of radical intermediates. Control experiments were carried out, in the absence of the substrate. The concentration of the oxidant (QDC), did not show any appreciable change.

Mechanism

The rate of the reaction between the substrate (serine, threonine and tyrosine) and oxidant (quinolinium dichromate, QDC), in acid medium, was dependent on the first powers of the concentrations of each —— substrate, oxidant and acid (Tables 2-4). The first order dependence of the rate of oxidation on acidity suggested the participation of a protonated Cr(VI) species in the rate-determining step of the reaction. The initial reaction was that between the substrate and a protonated Cr(VI) species.

The effect of a change in the dielectric constant of the medium showed that the polarity of the medium played an important part in the oxidation of these amino acids by quinolinium dichromate (QDC). An increase in the dielectric constant of the medium had resulted in a decrease in the rate of the reaction (Table 5). Linearity in the plots of log k_1 (the pseudo-first-order rate constant) against the inverse of the dielectric constant, gave positive slopes. This indicated an interaction between a positive ion and a dipolar species, in agreement with the Amis theory(64).

The activation parameters obtained (Table 8) would also support the mechanism of the reaction. The near constancy of the $\Delta G^{\neq}$ values indicated a common mechanism for

the oxidation of all the amino acids studied. Large negative values for the entropies of activation ($\Delta S^{\neq}$) in the present oxidation suggested that the transition state was more ordered, resulting in the loss of freedom of motion. The linearity observed between the enthalpy of activation ($\Delta H^{\neq}$) and the entropy of activation ($\Delta S^{\neq}$) showed that the oxidation reactions were controlled by both these parameters, $\Delta H^{\neq}$ and $\Delta S^{\neq}$. The isokinetic temperature calculated from the linear plot was 320K.

The solvent isotope effect, k_{D_2O}/k_{H_2O}, was greater than unity (Table 9). This observation was in conformity with the theory that, for acid-catalyzed reactions, k_{D_2O}/k_{H_2O} values should be greater than unity. The solvent isotope effect observed (Table 9), supported the protonation of the oxidant (QDC), as seen from the acid dependence on the rate of the reaction (Table 4).

Variations in the ionic strength of the medium and the addition of salts did not exert any influence on the rates of these reactions. This indicated a direct reaction between the substrate and oxidant, in acid medium, to give an intermediate which undergoes further reaction to yield the product.

The oxidation of the deuterated amino acids (deuterated at the α-carbon atom) gave values of k_H/k_D which

were close to unity (Table 10). The absence of a primary kinetic isotope effect was thus inferred, which confirmed that there was no cleavage of the carbon-hydrogen bond in the rate-determining step of the reaction.

There was no induced polymerization or the reduction of mercuric chloride(70) in any of these oxidation reactions. Further, no ESR signals were observed in the oxidation of these amino acids, indicating the absence of radical intermediates.

The dissociation of amino acids depends upon the pH of the medium. In aqueous solution, amino acids exist as dipolar ions (zwitterions). The ionization constants and pH values at the isoelectric points(71) for these amino acids (serine, threonine and tyrosine) are given in Table 11.

Table 11. Ionization constants(71) and pH values at the isoelectric points of amino acids at 298K.

Amino Acid	pK_1	pK_2	pH_i
Serine	2.21	9.15	5.68
Threonine	2.74	9.62	6.16
Tyrosine	2.18	9.21	5.69

For these amino acids,

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

In strongly acidic or alkaline media, the following equilibria exist:

$$\underset{\text{(cation)}}{RCH(N^{+}H_3)COOH} \underset{H^{+}}{\overset{OH^{-}}{\rightleftharpoons}} \underset{\text{(zwitterion)}}{RCH(N^{+}H_3)COO^{-}} \underset{H^{+}}{\overset{OH^{-}}{\rightleftharpoons}} \underset{\text{(anion)}}{RCH(NH_2)COO^{-}} \qquad (3)$$

In acid solution, amino acids exist as a mixture of the zwitterionic [$RCH(N^{+}H_3)COO^{-}$] and cationic [$RCH(N^{+}H_3)COOH$] forms. In the present investigation, the reactions were carried out in acid media. In acid media, the zwitterion would be converted to the cation [$RCH(N^{+}H_3)COOH$], which is the reactive species, under the present experimental conditions. Earlier investigations have established that, in acidic media, the reactions between amino acids and oxidants occurred via the cationic form of the amino acids(72,73). It has been shown that in strongly acid media, threonine existed in the cationic form(74).

Based on the observed experimental observations, it can be postulated that, in the present study, the oxidation of amino acids (serine, threonine and tyrosine) by quinolinium dichromate(QDC), in acid medium, occurred via a direct interaction between the cationic form of the amino acid and the oxidant(QDC) to yield an intermediate which would then undergo further reaction to give the product.

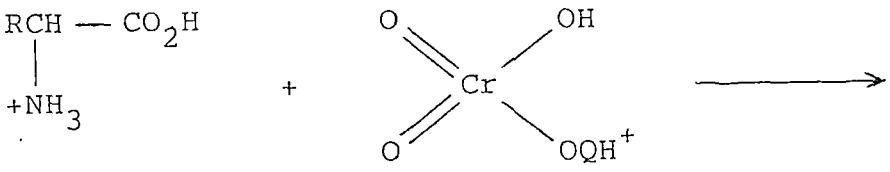
The mechanistic pathway for the oxidation of these amino acids (serine, threonine and tyrosine) by quinolinium dichromate(QDC), in acid medium, has been shown in the Scheme.

The major products of these oxidation reactions (vide "Experimental": Product Analysis) were:

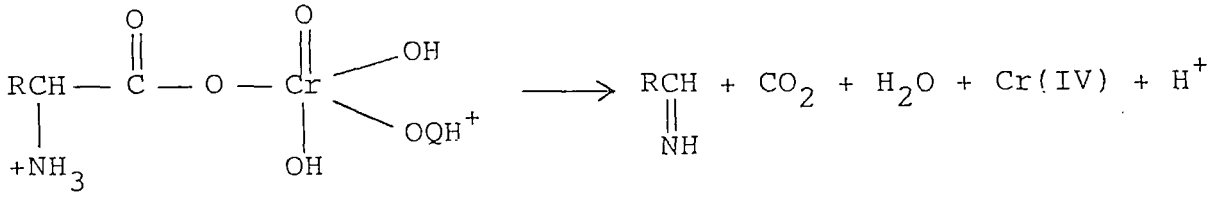
(a) the corresponding nitriles ($\sim$75-80%), which were characterized by chemical and spectral methods; and

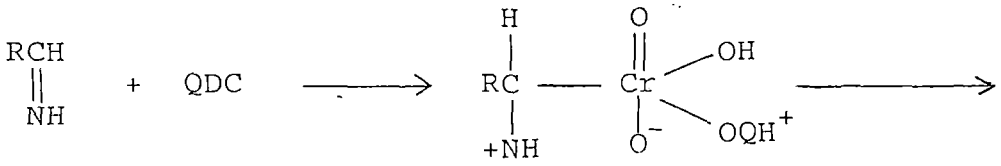
(b) trace amounts of the corresponding aldehydes ($\sim$5-10%), which were characterized by their respective 2,4-dinitrophenylhydrazone derivatives.

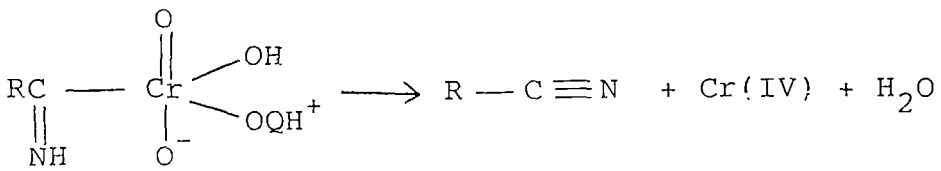
SCHEME



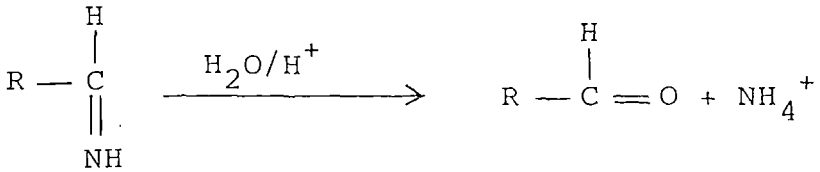
(Amino Acid) (QDC)







(Nitrile)



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KINETICS OF OXIDATION OF METHIONINE AND CYSTEINE

Pulse radiolysis studies have shown the presence of transient species when thiols were irradiated at a pH where some ionization of the thiol group had occurred. These species had an absorption band from approximately 350 to 500nm, with a maximum at 400 to 450nm 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-$\dot{\bar{S}}$-R which resulted from the reaction of the thiyl radical with the thiolate ion(1).

$$R\dot{S} + RS^- \rightleftharpoons RS\dot{\bar{S}}R$$

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

$$R\dot{S} + R\dot{S} \rightleftharpoons RSSR,$$

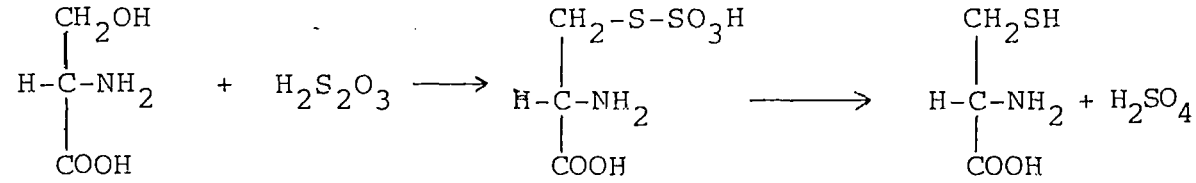
as observed in the case of cysteine(2). In the case of cysteine, the extinction coefficient at 420nm was of the order $9x10^3$ l $mol^{-1}cm^{-1}$(3).

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, wherein the extra stability of the

tertiary radical was attributed to the abstraction from the β-carbon atom with respect to sulfur(4).

ESR studies on single crystals of cysteine hydrochloride monohydrate gave an isotropic doublet as the main radical species with a high, but axial symmetric g factor, attributed to the $\dot{S}CH_2CH(COOH)N^+H_3Cl^-$ radical(5).

The direct H_2S-H_2O interchange enzyme, serine sulfhydrase, is responsible for the sulfuration or desulfuration of cysteine(6). Cysteine formation through the addition of thiosulfate to serine may be important in the sulfur metabolism of some organisms, with S-sulfocysteine serving as an intermediate,



(Serine) (S-sulfocysteine) (Cysteine)

The only thiol oxidation reaction to oxy-derivatives of general biochemical significance is that of cysteine to cysteine sulfinic acid(6,7). This is thought to be the initial reaction in the main pathway for the utilization of cysteine sulfur for sulfate production(6,7).

Cysteine can donate its sulfur 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 peptide bond formation, as in the synthesis of *biotin by microorganisms*(8).

Cysteine is the pivotal compound in thiol metabolism. Sulfate and other oxidized forms of sulfur are reduced to the level of sulfide which enters the organic linkage as cysteine. All biological thiols and their derivatives such as disulfides, thioesters, thioethers and sulfonium salts derive their sulfur through cysteine. This is accomplished either by trans-sulfuration or by incorporation of the cysteine structure directly.

Electrochemical methods have been used for the quantitative analysis of thiol and disulfide groups in organic compounds as well as in proteins(9-14). The polarography of thiols was characterized by a well-defined anodic wave (15-18). In the case of cysteine, the shape of the polarogram depended strongly on the pH and buffer used(19), the anodic wave being due to the formation of mercurous mercaptide(19). It was observed that cysteine was oxidized by a one-electron process to cystine, which on further oxidation gave cysteic acid(20-21).

Methionine is one of the twenty amino acids utilized for protein synthesis. N-formyl methionine is an important chain initiator in protein synthesis(22). Methionine reacts with adenosine triphosphate(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(23,24), gramine(25), sedamine(26), nicotine(27), isoquinoline alkaloids(28), indole alkaloids(29), quinoline alkaloids(30,31), purine alkaloids(32), and diterpenoids(33).

Thiols have been oxidized to disulfides by peroxidic compounds(34), DMSO(35), halogens(36), diethyl azodicarboxylate(37), nitro and nitroso compounds(38), iodosobenzene(39) transition metal ions(40-45), lead tetracetate(46), metal oxides(47,48), flavine derivatives(49), and by photooxidation(50).

The oxidation of cysteine has been carried out by various oxidizing agents such as DMSO(51), autooxidation in the presence of Cu^{2+}ions(52,53), chromic acid(54,55), permanganate(56), radiolytic oxidation(57), bromide and iodide radical anions(58), superoxide ion(59), 12-tungsto

cobaltate(III) ions(60), potassium hexacyanoferrate(III) in alkaline medium(61), vanadium(V) in acid medium(62), and by Fe(III) under anaerobic conditions both in acidic and alkaline media(63).

Methionine has been oxidized by the chromate ion(64), Au^{3+}ion(65), halogen radical anions(66), autooxidation in the presence of ascorbic acid(67), periodate(68), hydrogen peroxide(69), chloramine-B(70,71), chloramine-T(72) bromamine-T(73), potassium hexacyanoferrate(III) in alkaline medium(74), iodoxybenzene(75), and by aqueous chromium(VI) ions(76).

PRESENT WORK

The present work reports the kinetic features of the oxidation of sulfur containing amino acids (methionine and cysteine) by quinolinium dichromate(QDC), in acid medium at constant ionic strength, under a nitrogen atmosphere.

Stoichiometry (vide "Experimental")

The stoichiometry of each of the reactions was determined. Stoichiometric ratios $\Delta[QDC]/\Delta[Methionine]$ of 0.70, and $\Delta[QDC]/\Delta[Cysteine]$ of 1.08 (Table 1) were obtained, which conformed to the overall equations:

$$3Mt + 2Cr^{VI} + 3H_2O \longrightarrow 3Mt\text{-sulfoxide} + 2Cr^{III} + 6H^+ \quad (1)$$

(Mt : Methionine)

$$2RSH + Cr^{VI} \longrightarrow RSSR + Cr^{IV} + 2H^+ \quad (2)$$

(Cysteine)

Table 1. Stoichiometry of the oxidation of amino acids; [Amino Acid] = 0.005M, T = 313K.

$[H_2SO_4](M)$	0.10	0.20	0.25	0.50
$10^2[QDC](M)$	2.50	2.60	2.70	2.80
$\Delta[QDC]/\Delta[Methionine]$	0.65	0.70	0.72	0.75
$\Delta[QDC]/\Delta[Cysteine]$	1.10	1.04	1.14	1.04

Effect of substrate and oxidant

Under pseudo-first-order conditions, individual kinetic runs were observed to be first order in oxidant (quinolinium dichromate, QDC). The pseudo-first-order rate constants were independent of the initial concentration of the oxidant (Table 2).

Table 2. Dependence of the oxidation rate on [QDC].

10^4[QDC](M)	0.5	1.0	2.5	5.0	7.5	10.0
$10^4 xk_1$, s^{-1} for:						
Methionine	2.46	2.55	2.40	2.62	2.37	2.50
Cysteine	2.52	2.73	2.68	2.55	2.64	2.60

[Amino Acid] = 0.01M, $[H_2SO_4]$ = 1.25M, μ = 0.5M, T = 313K.

The order of the reaction with respect to substrate was determined by changing the amino acid concentration and observing the effect on the rate, at constant [QDC] and $[H_2SO_4]$. The results are recorded in Table 3.

Table 3. Dependence of the oxidation rate on [Amino Acid]

10^2[Amino Acid](M)	1.0	2.5	5.0	10.0	25.0
$10^4 xk_1$, s^{-1} for:					
Methionine	2.5	6.3	12.5	25.7	64.1
Cysteine	2.6	6.8	13.6	27.1	69.2

[QDC] = 0.001M, $[H_2SO_4]$ = 1.25M, μ = 0.5M, T = 313K.

It was observed that the reactions showed a first order dependence on the concentration of the substrate. Linear plots of k_1 (the pseudo-first-order rate constant) against the concentration of the substrate were obtained, passing through the origin, which indicated a first order dependence of the reaction on the concentration of the substrate. The second order rate constants (k_2) were found to remain constant as the concentration of the substrate was increased, confirming that the reaction was first order with respect to the substrate concentration (Table 3).

Effect of acid

The rate of the reaction showed an increase with increasing concentrations of acid (Table 4).

Table 4. Dependence of the oxidation rate on [Acid]

$[H_2SO_4](M)$	0.25	0.50	0.75	1.0	1.25	1.50
$10^4 x k_1$, s^{-1} for:						
Methionine	0.5	1.0	1.5	2.0	2.5	3.1
Cysteine	0.6	1.2	1.7	2.2	2.6	3.4

[Amino Acid] = 0.01M, [QDC] = 0.001M, μ = 0.5M, T = 313K.

Plots of log k_1 against log$[H^+]$ were linear, with slopes equal to unity, indicating that the reaction was dependent on the first power of the concentration of the acid. The linear increase in the rate of oxidation with acidity

indicated the involvement of a protonated Cr(VI) species in the rate determining step. The involvement of such species is prevalent in chromic acid oxidation reactions(77).

Rate Law

Under the present experimental conditions, wherein pseudo-first-order conditions have been used for all the kinetic determinations, the rate law could be expressed as

$$\text{Rate} = -\frac{d[Cr(VI)]}{dt} = k[\text{substrate}][QDC][H^+] \qquad (3)$$

Effect of solvent

Changes in the dielectric constant of the medium were brought about by varying the composition of the solvent mixtures, using methanol and water. The dielectric constants for methanol-water mixtures were estimated from the dielectric constants of the pure solvents. Changes in the composition of solvent mixtures affected the rates of these reactions. The essential features concerning the effect of solvent on the rate of oxidation of cysteine and methionine by QDC could be summarized as follows:

(a) Increasing proportions of methanol resulted in an increase in the rate of the reaction (Table 5).

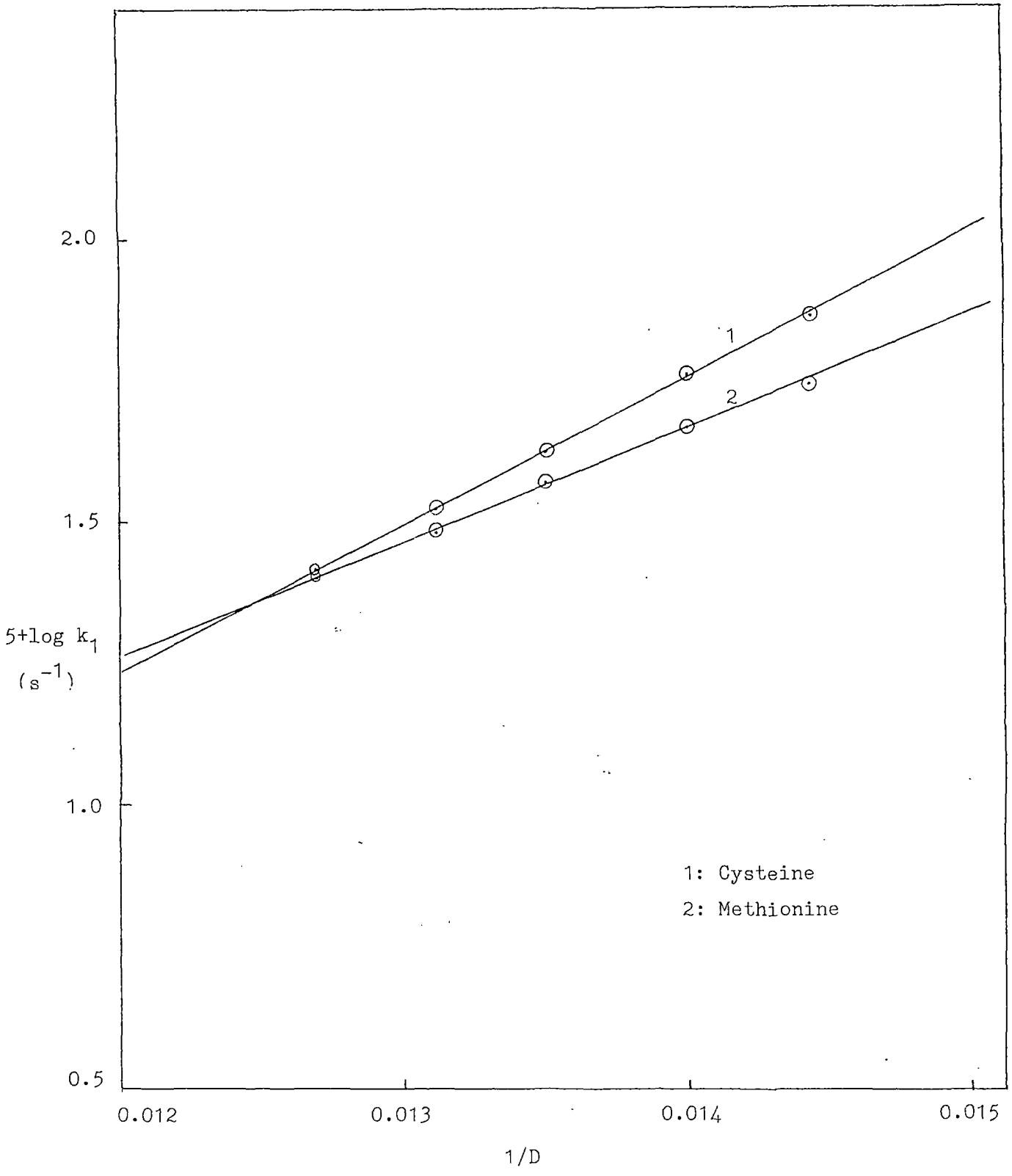


Fig.1: Plot of log k_1 against the reciprocal of dielectric constant

Table 5. Dependence of the oxidation rate on solvent.

H_2O : methanol(%,v/v)	100:0	95:5	90:10	85:15	80:20
Dielectric constant(D)	78.54	76.24	73.95	71.65	69.35
$10^4 x k_1$, s^{-1} for:					
Methionine	2.5	3.1	3.5	4.7	5.4
Cysteine	2.6	3.3	4.2	5.5.	7.4

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 1.25M, μ = 0.5M, T = 313K.

This was in accordance with the observation that larger reaction times were required for more polar solvents(78). This was brought about by a lowering of the dielectric constant of the medium, which would result in a less polar transition state compared to more polar reactants.

(b) Plots of log k_1 against the reciprocal of dielectric constant were linear, with positive slopes (Fig.1), indicating that the reaction was of the ion-dipole type(79). This also confirmed the participation of a protonated Cr(VI) species in the rate-determining step of the reaction.

(c) Solvent effects on the rates of reaction could also be due to factors such as the solvating power of solvents (80), solute-solvent interactions(81,82), and solvent structures.

Effect of temperature

The rates of the reactions were increased with an increase in temperature (Table 6).

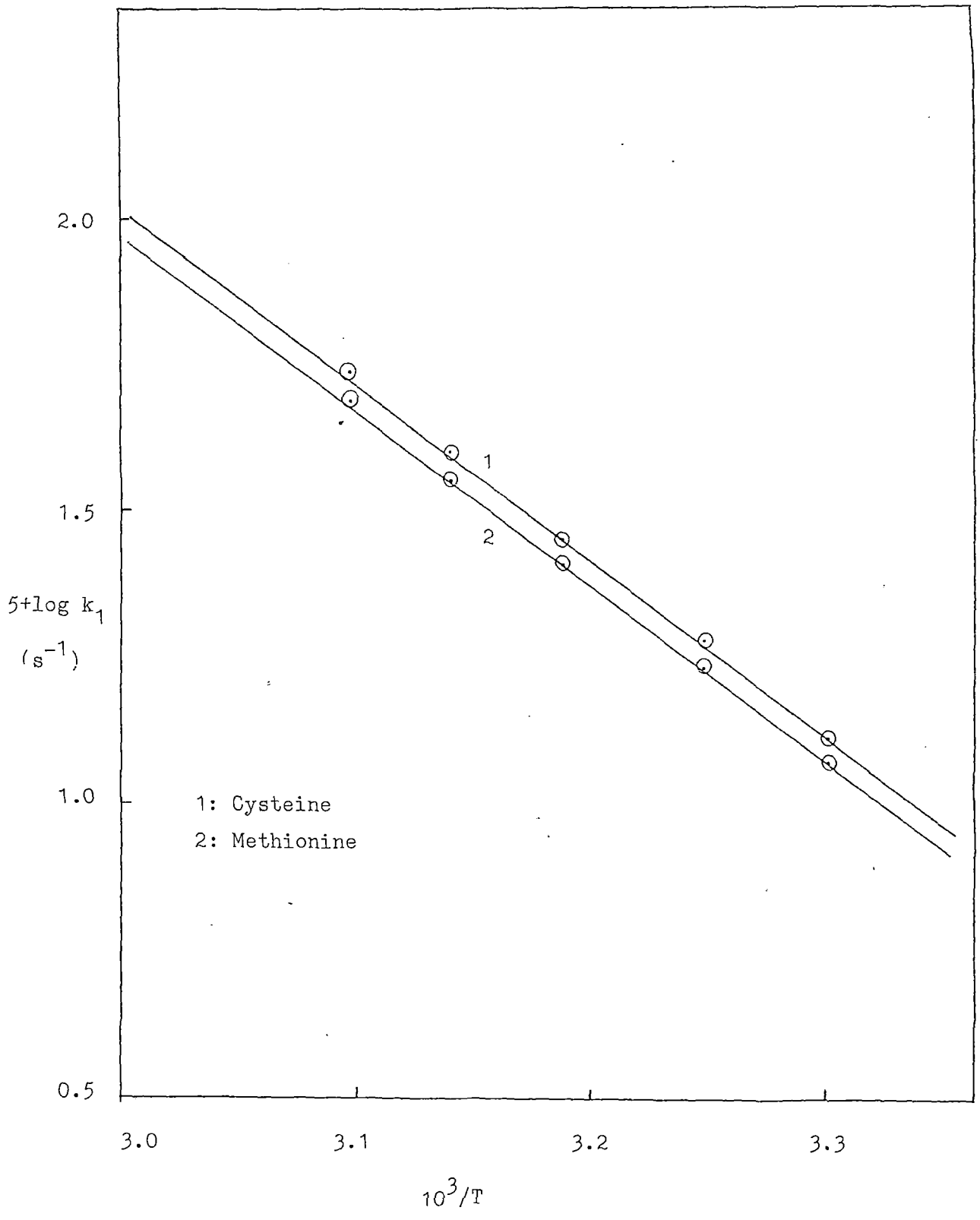


Fig.2: Plot of log k_1 against the reciprocal of temperature

Table 6. Dependence of the oxidation rate on temperature.

Temp (±0.1K)	303	308	313	318	323
$10^4 x k_1$, s^{-1} for:					
Methionine	1.2	1.7	2.5	3.5	5.1
Cysteine	1.3	1.8	2.6	3.9	5.8

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 1.25M, μ = 0.5M.

Plots of log k_1 against the reciprocal of temperature were linear (Fig.2), and the slopes of these plots were used to calculate the activation energies. The other activation parameters were evaluated and have been shown in Table 7.

Table 7. Activation parameters.

Amino Acid	E ($kJ\ mol^{-1}$)	$\Delta H^{\neq}$ ($kJmol^{-1}$)	$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	$\Delta G^{\neq}$ ($kJ\ mol^{-1}$)
Methionine	47	44	-172	98
Cysteine	44	41	-180	97

Error limits: E ± $2kJmol^{-1}$, $\Delta H^{\neq}$ ± $2kJmol^{-1}$, $\Delta S^{\neq}$ ± $4JK^{-1}mol^{-1}$, $\Delta G^{\neq}$ ± $2kJmol^{-1}$

Solvent isotope effect

The oxidation reactions were studied in aqueous medium. When D_2O was used, it was observed that the rates of oxidation of the amino acids were increased in D_2O medium (Table 8), in agreement with earlier reported observations(83).

Table 8. Solvent isotope effects for the oxidation of amino acids.

Amino Acid	k_{H_2O}	k_{D_2O}	k_{D_2O}/k_{H_2O}
	($10^4 \times k_1$, s^{-1})		
Methionine	2.5	3.7	1.45
Cysteine	2.6	4.1	1.58

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 1.25M, μ = 0.5M, T = 313K.

If the solvent isotope effect, k_{D_2O}/k_{H_2O}, had been observed to be less than unity, then this would have indicated a pre-equilibrium proton-transfer followed by a rate-limiting electron-transfer process. Since the solvent isotope effect was greater than unity (Table 8), this would imply a proton-catalyzed reaction, and would support the protonation of the QDC species, an observation which has been confirmed by the acid dependence on the rate of the reaction (Table 4).

Kinetic isotope effect

The kinetic isotope effect caused by deuterating the methyl group of methionine by using L-methionine-methyl-d_3 (Aldrich) was studied. The results are shown in Table 9.

Table 9. Kinetic isotope effect for the oxidation of methionine.

Substrate	$10^4 \times k_1$, s^{-1}
Methionine	2.5
Methionine-methyl-d_3	2.6

[Substrate] = 0.01M, [QDC] = 0.001M, [H_2SO_4] = 1.25M, μ = 0.5M, T = 313K.

The data in Table 9 shows the absence of a primary kinetic isotope effect. This confirmed that the carbon-hydrogen bond (of the methyl group in methionine) was not cleaved in the rate-determining step of the reaction.

Effect of ionic strength

Changes in the ionic strength of the medium using sodium perchlorate (μ = 0.01M to 0.50M) did not have any effect on the rates of these oxidation reactions.

Induced polymerization

The possibility of induced polymerization was tested. It was seen that there was no induced polymerization of acrylonitrile or the reduction of mercuric chloride(84). Further, no ESR signals could be detected in these oxidation reactions (E-4, Varian), thus providing no evidence for the formation of radical intermediates. Control experiments were performed, in the absence of the substrate.

The concentration of the oxidant(QDC) did not show any appreciable change.

Mechanism

The rate of the reaction between the substrate (methionine and cysteine) and oxidant (quinolinium dichromate, QDC), in acid medium, was observed to be dependent on the first power of the concentrations of each reacting species ——— substrate, oxidant and acid (Tables 2-4). The linear increase in the rate of oxidation with acidity (Table 4) indicated the involvement of a protonated Cr(VI) species in the rate-determining step. The initial reaction would be between the substrate and the protonated Cr(VI) species.

The observed increase in the rate of the reaction with a decrease in the polarity of the solvent medium (Table 5), indicated that the transition state was much less polar than the reactants. Linear plots of log k_1 against the reciprocal of the dielectric constant (Fig.1) had yielded positive slopes. This suggested an interaction between a positive ion and a dipole(79), and was in consonance with the observation that, in the presence of an acid, the rate-determining step involved a protonated Cr(VI) species.

The oxidation reactions were characterized by negative entropies of activation (Table 7). This would suggest a more ordered transition state as compared to the reactants(85). Differences in solvation of substrates in the ground state and transition state might also contribute to some extent to the negative entropies of activation(86). The values for the free energies of activation ($\Delta G^{\neq}$) were nearly constant, indicating that similar mechanisms operated for the oxidation of these substrates (methionine and cysteine).

The solvent isotope effect, k_{D_2O}/k_{H_2O}, was greater than unity (Table 8). This observation suggested a proton-catalyzed reaction, thus supporting the protonation of the oxidant (QDC), as seen from the acid dependence on the rate of the reaction (Table 4).

The oxidation of deuterated methionine (by deuterating the methyl group of methionine) did not show a primary kinetic isotope effect (Table 9), which indicated that the carbon-hydrogen bond (of the methyl group in methionine) was not cleaved in the rate-determining step.

The absence of the induced polymerization of acrylonitrile, the absence of the reduction of mercuric chloride (84), and the absence of any ESR signals all indicated

that no radicals could be detected during the course of the oxidation reactions of methionine and cysteine.

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:

$$\underset{\text{(cation)}}{RCH(N^+H_3)COOH} \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} \underset{\text{(zwitterion)}}{RCH(N^+H_3)COO^-} \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} \underset{\text{(anion)}}{RCH(NH_2)COO^-} \qquad (4)$$

In acidic solution, the zwitterion was converted into the cation, $[RCH(N^+H_3)COOH]$, which was the reactive species, under the present experimental conditions.

The pK_a value for cysteine (-SH group) has been reported to be 10.28(87). It can be assumed that cysteine existed mostly in the ionized form in aqueous medium. Since disulfide was the final product of the oxidation reaction, the sulfhydryl group (-SH) provides the site of attack.

The pK_a value for methionine is 5.71(87). Since sulfoxide was the final product of the oxidation reaction, the S-methyl group ($-S-CH_3$) provides the site of attack.

Considering the mechanism of oxidation of these amino acids (methionine and cysteine) by quinolinium dichromate (QDC), in acid medium, the attack may occur either

at nitrogen or at sulfur. The products obtained from the oxidation of these amino acids (methionine sulfoxide from methionine, and the disulfide from cysteine), would suggest that the oxidant (QDC) attacks the sulfur group. Further, sulfur is more nucleophilic than nitrogen, and the sulfhydryl group (-SH) can donate electrons more easily than the amino group ($-NH_2$). Therefore, the mechanistic pathway is via an electron-pair donation by sulfur present in methionine (and cysteine) to the Cr=O bond of the oxidant, as in the case of olefin oxidation(88).

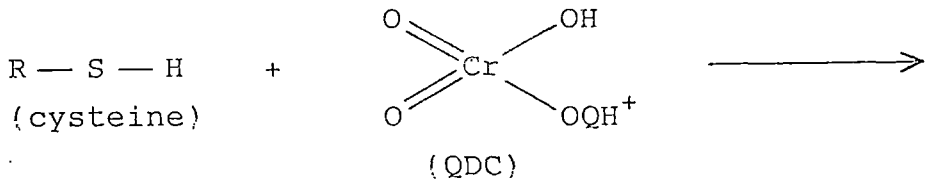
The reaction sequence has been shown in Schemes 1 and 2.

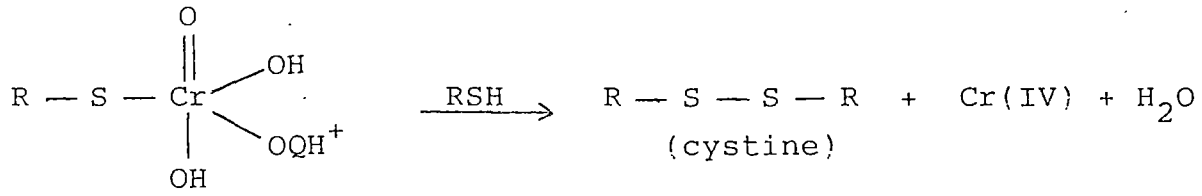
The products of these oxidation reactions (vide "Experimental" : Product Analysis) were:

(a) methionine sulfoxide (from methionine) which was characterized as N-benzoyl methionine sulfoxide ($\sim$75%); and

(b) cystine (from cysteine), which was characterized by chemical methods ($\sim$70-80%).

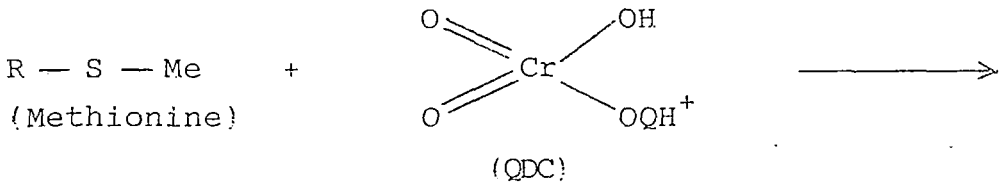
SCHEME 1

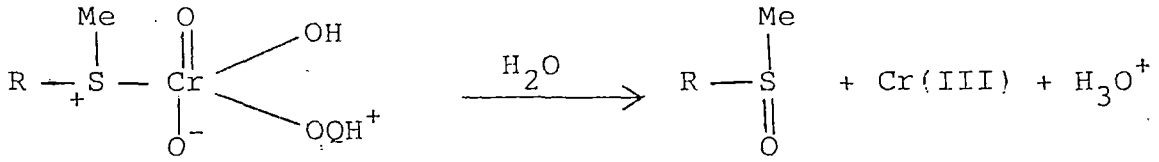




[R = $-CH_2 - CH(NH_2)COOH$]

SCHEME 2





(Methionine sulfoxide)

[R =-$(CH_2)_2$-CH(NH_2)COOH, Me = CH_3-]

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KINETICS OF OXIDATION OF GLUTAMIC ACID AND ASPARTIC ACID

Glutamic acid has been used as a precursor in the biosynthesis of the pyrollidine and piperidine rings of nicotine and anabasine(1-3). Glutamic acid has been found to be a constituent of various lupine alkaloids(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 anerobic 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 aspartic 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 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 oxidized by a variety of oxidizing agents such as ceric ions(16,17), Chloramine-T(18-20), bromamine-B(21), chloramine-B(22), peroxydisulfate catalysed by Cu^{2+} ion(23), $KMnO_4$ in acid medium(24), hexacyanoferrate(III) catalysed by Ru(VI) ion(25), peroxydisulfate ion(26,27), N-bromoacetamide(28,29) periodate(30), acidic permanganate ion(31), Fenton's reagent(32), Fe^{2+} ion(33), potassium hexacyanoferrate(III) in alkaline medium(34) and by 1-chlorobenzotriazole(35).

PRESENT WORK

The present work is a detailed kinetic study of the oxidation of glutamic acid and aspartic acid by quinolinium dichromate(QDC), in acid medium, at constant ionic strength, under a nitrogen atmosphere.

Stoichiometry (vide "Experimental")

The stoichiometry of each reaction was determined. A stoichiometric ratio, Δ[QDC]/ Δ[Amino Acid], of 1.08 was obtained (Table 1).

Table 1. Stoichiometry of the oxidation of amino acids; [Amino Acid]= 0.005M, T=348K.

$[H_2SO_4]$(M)	1.0	3.0	4.0	5.0
10^2[QDC](M)	2.50	2.60	2.70	2.80
Δ[QDC]/ Δ[Glutamic Acid]	1.03	1.10	1.08	1.12
Δ[QDC]/ Δ[Aspartic Acid]	1.05	1.08	1.12	1.05

The stoichiometry conformed to the overall equation:

$$RCH(NH_2)COOH + 2Cr^{VI} \longrightarrow RCN + CO_2 + 2Cr^{IV} + 4H^+ \quad (1)$$

Here R = $-CH_2-CH_2COOH$ (glutamic acid),

$-CH_2COOH$ (aspartic acid).

Effect of substrate and oxidant

The rate of the reaction was observed to be dependent on the first powers of the concentrations of each, substrate and oxidant(QDC). The order of the reaction with respect to substrate was obtained by changing the substrate concentration and observing the effect on the rate, at constant [QDC] and [Acid]. The results have been recorded in Table 2.

Table 2. Rate data for the oxidation of amino acids.

[Substrate] (10^2 x M)	[QDC] (10^3 x M)	Glutamic acid (10^4 x k_1, s^{-1})	Aspartic Acid
1.0	1.0	0.84	0.44
2.5	1.0	2.05	1.06
5.0	1.0	4.16	2.18
10.0	1.0	8.30	4.40
20.0	1.0	16.50	8.85
1.0	0.75	0.80	0.45
1.0	0.50	0.85	0.48
1.0	0.25	0.85	0.42
1.0	0.10	0.88	0.44

$[H_2SO_4]$ = 4.0M, μ = 0.5M, T = 348K.

Plots of k_1, the pseudo-first-order rate constant, against the concentrations of the substrate were linear, passing through the origin, indicating that the rate of oxidation was dependent on the first power of the concentration of the substrate. This was further confirmed by the constant values of k_2, the second order rate constant.

When a constant concentration of substrate (large excess) was employed, k_1 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 2).

Effect of acid

The reaction was dependent on the concentration of the acid, and the rate was observed to increase with an increase in the concentration of the acid (Table 3).

Table 3. Dependence of the oxidation rate on [Acid]

$[H_2SO_4]$(M)	1.0	2.0	3.0	4.0	5.0
$10^4 \times k_1$, for:					
Glutamic acid	0.19	0.42	0.65	0.84	1.05
Aspartic acid	0.10	0.22	0.35	0.44	0.58

[Amino Acid] = 0.01M, [QDC] = 0.001M; μ = 0.5M, T = 348K.

Plots of log k_1 against log[H^+] were linear, with unit slopes, indicating that the rate of the reaction was first order with respect to the concentration of the acid.

The linear increase in the rate of oxidation with acidity suggested the involvement of a protonated Cr(VI) species in the rate-determining step. Earlier reports of the involvement of such Cr(VI) species in chromic acid oxidations have been confirmed(36). Protonated Cr(VI) species have been observed in the presence of p-toluenesulphonic acid in nitrobenzene-dichloromethane mixtures(37).

Rate law

Under the present experimental conditions, wherein pseudo-first-order conditions have been employed for all the kinetic determinations, the rate law can be expressed as:

$$\text{Rate} = -\frac{d[\text{Cr(VI)}]}{dt} = k[\text{Substrate}][\text{QDC}][H^+] \qquad (2)$$

Effect of solvent

Reactions involving an ionic reactant are influenced by changes in the solvent composition of the medium. It is therefore to be expected that the solvent assumes an important role in such reactions. Changes in solvent

composition were brought about by using varying proportions of acetic acid and water. In the case of each of these amino acids oxidized by quinolinium dichromate, the rate of oxidation was observed to be slowest in those solvent mixtures that contained the largest proportions of water, and increasing proportions of acetic acid resulted in an increase in the rate of oxidation. The dielectric constants for acetic acid-water mixtures were estimated from the dielectric constants of the pure solvents(38). The data have been recorded in Table 4.

Table 4. Dependence of the oxidation rate on solvent.

H_2O:HOAc (%, v/v)	100:00	95:5	90:10	85:15	80:20
Dielectric constant(D)	78.54	74.92	71.30	67.68	64.06
$10^5 \times k_1$, s^{-1} for:					
Glutamic acid	8.4	9.7	11.4	13.5	16.8
Aspartic acid	4.4	5.0	5.6	6.5	8.0

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$=4.0M, μ = 0.5M, T=348K.

In the present investigation, increasing proportions of acetic acid resulted in a decrease in the polarity of the medium. The decrease in the polarity of the medium caused an increase in the rate of the reaction (Table 4). Plots of log k_1 against the reciprocal of the dielectric constant

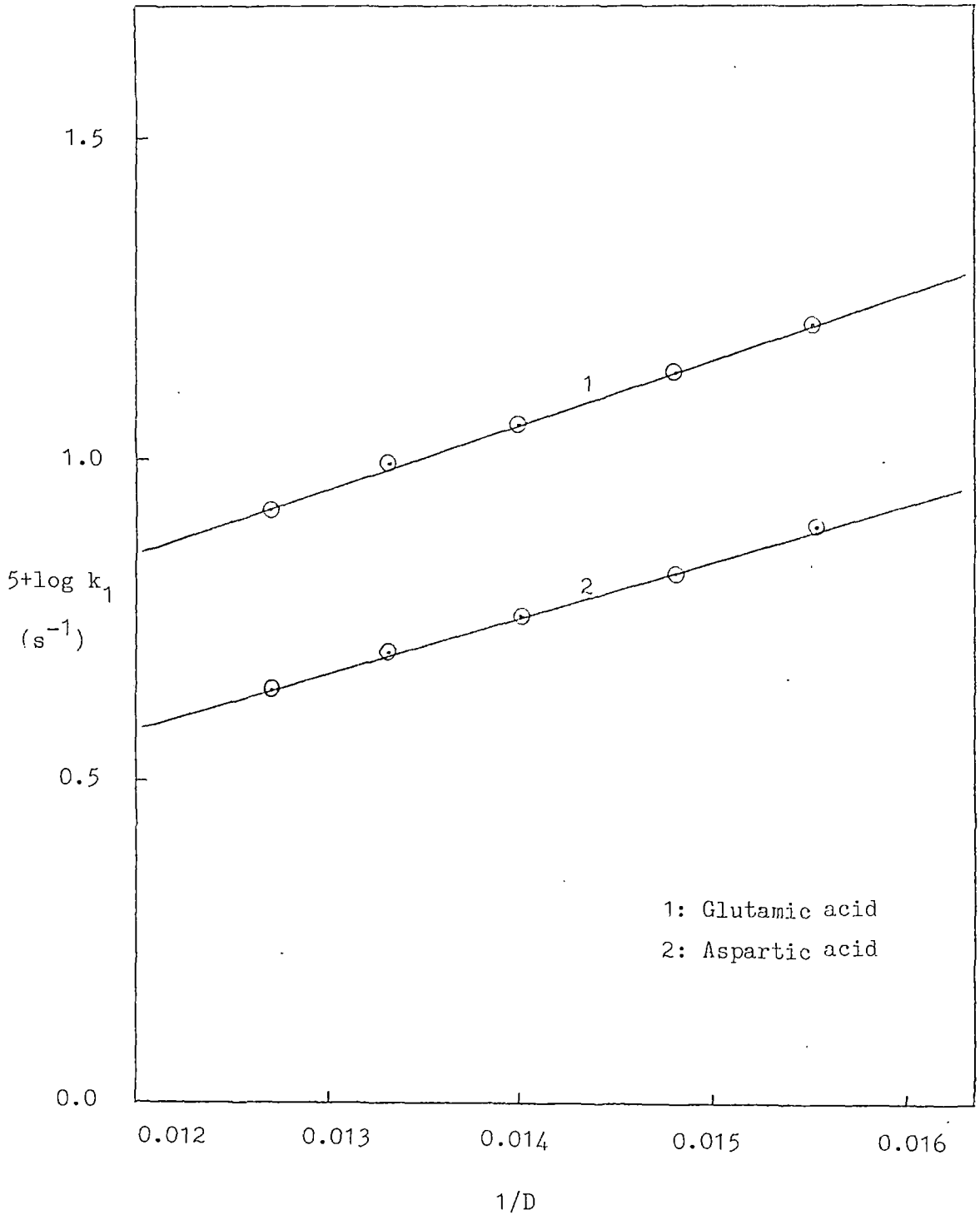


Fig.1: Plot of log k_1 against the reciprocal of dielectric constant

were linear, with positive slopes (Fig.1). This indicated an interaction between a positive ion and a dipole(39), and was in conformity with the observation that in the presence of an acid, the rate determining step involved a protonated Cr(VI) species. The effect of changes in the solvent composition on reaction rates, in general, would also depend on factors such as the solvating power of the solvent(40), solute-solvent interactions(41,42), and solvent structure.

Effect of temperature

The reactions were influenced by changes in temperature, and an increase in the temperature resulted in an increase in the rate of the reaction (Table 5).

Table 5. Dependence of the oxidation rate on temperature

Temp (±0.1K)	338	343	348	353	358
$10^5 \times k_1$, s^{-1} for:					
Glutamic acid	5.1	6.7	8.4	10.3	13.7
Aspartic acid	2.7	3.5	4.4	5.5	6.5

[Amino Acid] = 0.01M, [QDC] = 0.001M, $[H_2SO_4]$ = 4.0M, μ = 0.5M.

Plots of log k_1 against the reciprocal of temperature were linear (Fig.2), suggesting the validity of the Arrhenius equation. The slopes of the plots were used to calculate

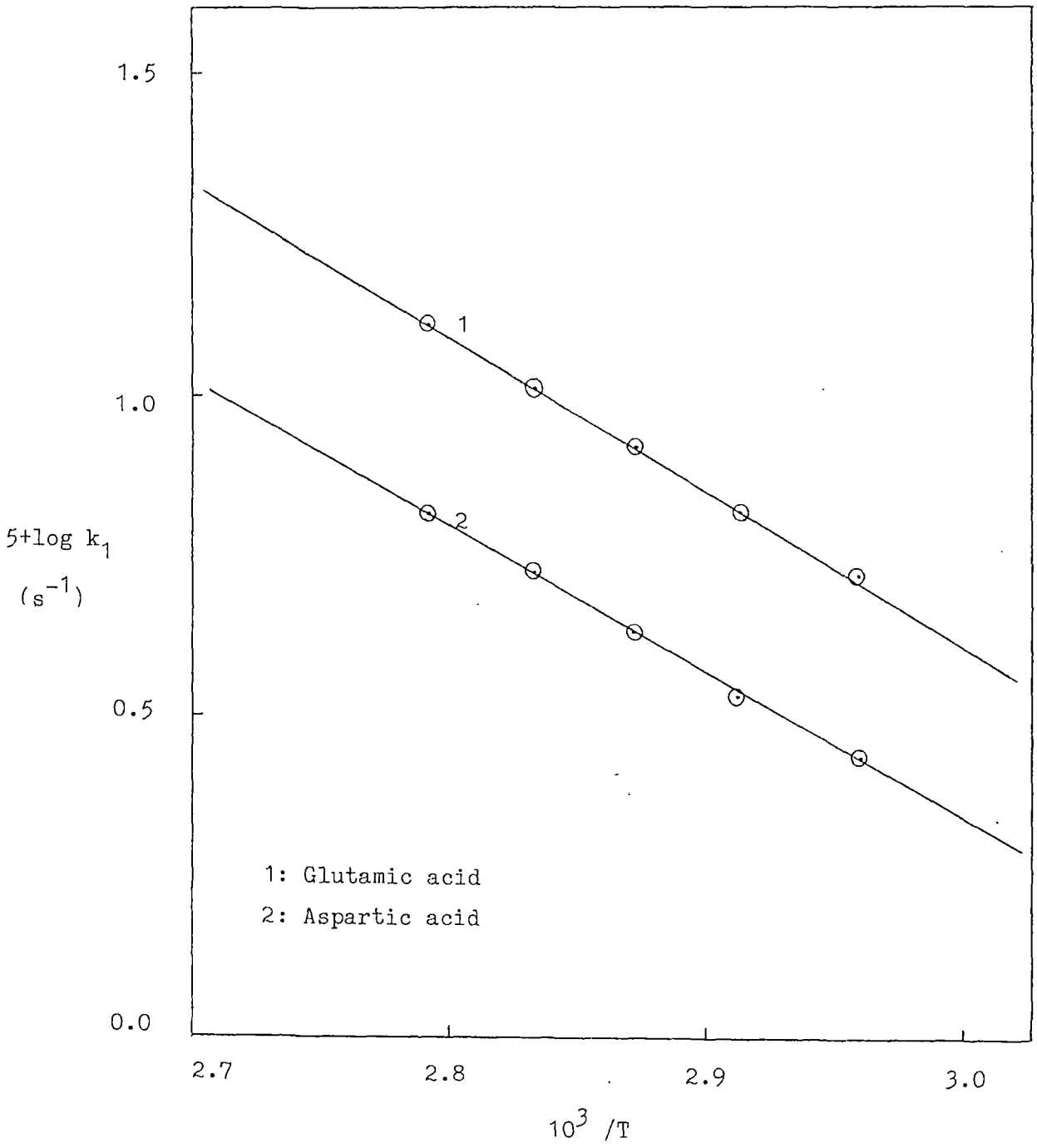


Fig.2: Plot of log k_1 against the reciprocal of temperature

the activation energies of the reactions. The other activation parameters were calculated and have been shown in Table 6.

Table 6. Activation parameters.

Amino Acid	E ($kJ\ mol^{-1}$)	$\Delta H^{\neq}$ ($kJ\ mol^{-1}$)	$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	$\Delta G^{\neq}$ ($kJ\ mol^{-1}$)
Glutamic acid	45	42	-206	113
Aspartic acid	46	43	-203	114

Error limits: $E \pm 2kJmol^{-1}$, $\Delta H^{\neq} \pm 2kJmol^{-1}$, $\Delta S^{\neq} \pm 4JK^{-1}mol^{-1}$, $\Delta G^{\neq} \pm 4JK\ mol^{-1}$

Solvent isotope effect

When the oxidation reactions were carried out in D_2O medium, it was observed that k_{D_2O}/k_{H_2O} values were greater than unity (Table 7), in agreement with observations reported earlier(43).

Table 7. Solvent isotope effect for the oxidation of amino acids.

Amino Acid	k_{H_2O}	k_{D_2O}	k_{D_2O}/k_{H_2O}
	($10^5 \times k_1$, s^{-1})		
Glutamic acid	8.4	11.4	1.36
Aspartic acid	4.4	6.1	1.39

[Amino Acid]=0.01M, [QDC]=0.001M, $[H_2SO_4]$=4.0M, μ = 0.5M, T=348K.

If the solvent isotope effect, k_{D_2O}/k_{H_2O}, had been less than unity, then this would have indicated a pre-equilibrium proton transfer, followed by a rate-determining electron-transfer process. Since the solvent isotope effect, k_{D_2O}/k_{H_2O}, was greater than unity (Table 7), this would suggest a proton-catalyzed reaction, an observation which has been reflected in the acid dependence on the rate of the reaction (Table 3).

Effect of ionic strength

Variations in the ionic strength of the medium using sodium perchlorate (μ = 0.01M to 0.50M), did not affect the rates of these oxidation 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 $1x10^{-4}$M to $5x10^{-3}$M) did not exert any influence on the rates of these oxidation reactions.

Kinetic isotope effect

The kinetic isotope effect caused by deuterating the α-carbon atom was studied. The results are shown in Table 8.

Table 8. Kinetic isotope effect for the oxidation of amino acids.

Amino Acid	$RCH(NH_2)COOH$	$RCD(NH_2)COOH$	k_H/k_D
	($10^5 \times k_1$, s^{-1})		
Glutamic acid	8.4	8.2	1.02
Aspartic acid	4.4	4.2	1.05

[Amino Acid]=0.01M, [QDC]=0.001M, $[H_2SO_4]$=4.0M, μ = 0.5M, T = 348K.

The k_H/k_D values were close to unity (Table 8), which indicated that, in the rate-determining step of the reaction, there was no cleavage of the carbon-hydrogen bond.

Induced polymerization

The possibility of induced polymerization was tested. It was observed that there was no induced polymerization of acrylonitrile or the reduction of mercuric chloride(44). There were no ESR signals detected in these oxidation reactions (E-4, Varian), thus providing no evidence for the formation of radical intermediates. Control experiments were performed, in the absence of the substrate. The concentration of the oxidant (QDC) did not show any appreciable change.

Mechanism

Based on the stoichiometry of the oxidation reactions, and the observed experimental data, the mechanistic pathway of the reaction has to be considered. Some of the kinetic observations which must be taken into account are the following:

(i) The rate of the reaction was observed to be dependent on the first power of the concentrations of each - substrate, oxidant and acid (Table 2-3). The first order dependence of the rate on the concentration of the acid (Table 3) indicated that the rate-determining step involved a reaction between the substrate and a protonated Cr(VI) species.

(ii) A decrease in the polarity of the solvent medium (water-acetic acid, v/v) resulted in an increase in the rate of reaction (Table 4). Linear plots of log k_1 against the reciprocal of the dielectric constant yielded positive slopes, which indicated an ion-dipole type of interaction (Fig.1). This was in agreement with the Amis theory(39). This also supported the involvement of a protonated Cr(VI) species in the rate-determining step of the reaction.

(iii) These oxidation reactions were characterized by large negative values for the entropies of activation (Table 6), which suggested that the transition state was

more ordered, relative to the reactants(45). These negative entropies of activation could also be accounted for, by considering the differences in solvation of substrates in the ground state and in the transition state(46). The near constancy of the free energies of activation (Table 6) showed that the same mechanism was operative for the oxidation of these amino acids.

(iv) The solvent isotope effect, k_{D_2O}/k_{H_2O} was greater than unity (Table 7), which suggested a proton-catalyzed reaction. This would imply the participation of a protonated Cr(VI) species in the rate-determining step, which has been supported by the acid dependence on the rate of the reaction (Table 3).

(v) The kinetic isotope effect for the oxidation of these amino acids (obtained by deuterating the α-carbon atom), had yielded k_H/k_D values close to unity (Table 8), which indicated that in the rate-determining step there was no cleavage of the carbon-hydrogen bond.

(vi) The absence of induced polymerization of acrylonitrile, the absence of the reduction of mercuric chloride (44), and the absence of any ESR signals all indicated that there were no radical species formed during the course of the reaction.

(vii) Variations in the ionic strength of the medium, and the addition of salts, had no influence on the rates of these reactions. This suggested that there was a direct reaction between the substrate and oxidant, in acid medium, to give an intermediate which underwent further reaction to give the product.

The dissociation of amino acids depends upon the pH of the medium. Amino acids exist as zwitterions in aqueous solution. The ionization constants and the pH values at the isoelectric points(47) for these amino acids (glutamic acid and aspartic acid) are given in Table 9.

Table 9. Ionization constants(47) and pH values at the isoelectric points of amino acids at 298K.

Amino Acid	pK_1	pK_2	pK_3	pH_i
Glutamic acid	2.16	4.32	9.96	3.24
Aspartic acid	1.99	3.87	10.0	2.93

For these amino acids,

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

In strongly acidic or alkaline media, the following equilibria exist:

$$\underset{\text{(cation)}}{RCH(N^+H_3)COOH} \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} \underset{\text{(zwitterion)}}{RCH(N^+H_3)COO^-} \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} \underset{\text{(anion)}}{RCH(NH_2)COO^-} \qquad (3)$$

In the present investigation, the reactions were performed in acid media. In acidic solution, the zwitterion would be converted to the cation [$RCH(N^+H_3)COOH$], which was the reactive species, under the present experimental conditions. Earlier workers have shown that, in acid media, the reactions between amino acids and oxidants had occurred via the cationic form of the amino acids(48,49).

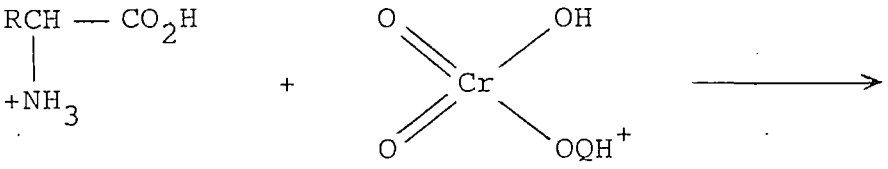
Based on the observed kinetic data, it can be postulated that the oxidation of these amino acids (glutamic acid and aspartic acid) by quinolinium dichromate (QDC), in acid medium, was via a direct reaction between the cationic form of the amino acid and the oxidant (QDC). The intermediate formed then underwent further reaction to yield the product. The mechanism for the oxidation of these amino acids (glutamic acid and aspartic acid) by quinolinium dichromate, in acid medium, has been shown in the Scheme.

The major products of these oxidation reactions (vide "Experimental": Product Analysis) were:

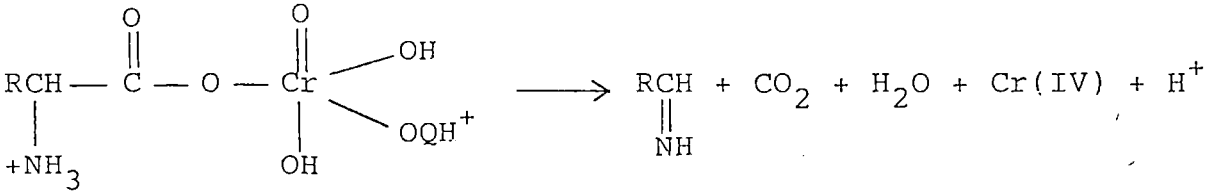
(a) the corresponding nitriles ($\sim$ 75-80%), which were characterized by chemical and spectral methods; and

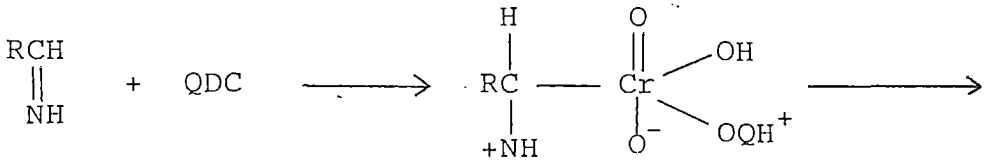
(b) trace amounts of the corresponding aldehydes ($\sim$ 5-10%), which were characterized by their respective 2,4-dinitrophenyl hydrazone derivatives.

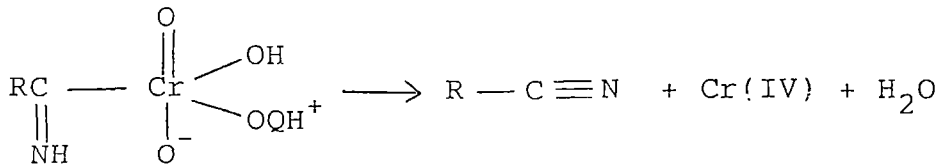
SCHEME



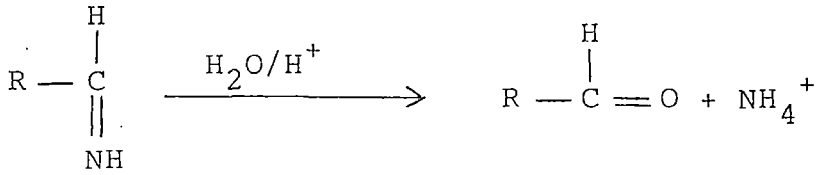
(Amino Acid) (QDC)







(Nitrile)



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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). Lysine has been used in the syntheses of sedamine(6), anabasine(7), and 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 heterocycles 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 the 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 proteins, 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 arginine and histidine have been oxidized by chloramine-T(30-35), hexacyanoferrate(III) catalysed by Os(VIII) ion(36,37), potassium permanganate in acid medium(38), N-bromosuccinimide in aqueous perchloric acid medium(39), photooxidation(40), aquapentacyanoferrate(II) ion(41), diethyl pyrocarbonate in aqueous solution(42), N-bromoacetamide in acid medium(43) and in alkaline medium (44), potassium hexacyanoferrate(III) in alkaline medium (45), N-chlorobenzamide in aqueous methanol(46) and by 1-chlorobenzotriazole(47).

PRESENT WORK

The present work reports the kinetic features of the oxidation of basic amino acids (lysine, arginine, histidine) by quinolinium dichromate(QDC) in acid medium, at constant ionic strength, under a nitrogen atmosphere.

Stoichiometry (vide "Experimental")

The stoichiometry of each oxidation reaction was determined. The stoichiometric ratio Δ[QDC]/Δ[Substrate] of 1.08 was obtained (Table 1).

Table 1. Stoichiometry of the oxidation of amino acids; [Amino acid]= 0.005M, T = 333K.

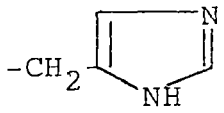
$[H_2SO_4]$(M)	0.10	0.20	0.25	0.50
10^2[QDC](M)	2.50	2.60	2.70	2.80
Δ [QDC]/ Δ[Lysine]	1.10	1.04	1.15	1.02
Δ [QDC]/ Δ[Arginine]	1.04	1.09	1.14	1.03
Δ [QDC]/ Δ[Histidine]	1.13	1.05	1.03	1.12

The stoichiometry conformed to the overall equation:

$$RCH(NH_2)COOH + 2Cr^{VI} \longrightarrow RCN + CO_2 + 2Cr^{IV} + 4H^+ \quad (1)$$

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

$-(CH_2)_3-NH-C(=NH)-NH_2$ (arginine),


(histidine)

Effect of substrate and oxidant

Under pseudo-first-order conditions, individual kinetic runs were observed to be first order with respect to the oxidant(QDC). The pseudo-first-order rate constants were found to be independent of the initial concentration of the oxidant (Table 2).

Table 2. Dependence of the oxidation rate on [QDC]

10^4[QDC](M)	1.0	2.5	5.0	7.5	10.0
10^5 x k_1, s^{-1} for:					
Lysine	23.0	20.0	24.0	20.0	21.0
Arginine	12.0	13.0	11.0	12.0	12.0
Histidine	2.0	2.5	2.0	2.7	2.2

[Amino Acid]=0.01M, $[H_2SO_4]$=1.5M, μ = 0.5M, T=333K.

The order of the reaction with respect to substrate concentration was determined by changing the amino acid concentration, and observing the effect on the rate, at

constant [QDC] and $[H^+]$. The results are reported in Table 3. The values of k_1/[Amino Acid] were fairly constant, for each substrate, which confirmed that the reaction was first order with respect to the concentration of amino acid.

Table 3. Dependence of the oxidation rate on [Amino Acid]

[Amino Acid](M)	0.01	0.025	0.05	0.10	0.20
$10^4 \times k_1$, s^{-1} for:					
Lysine	2.1	5.2	10.4	20.5	41.0
Arginine	1.2	2.9	5.8	12.2	24.0
Histidine	0.22	0.54	1.1	2.3	4.8

[QDC] =0.001M; $[H_2SO_4]$=1.5M, μ =0.5M, T=333K.

Effect of acid

The rate of the reaction was observed to be dependent on the first power of the concentration of the acid (Table 4).

Table 4. Dependence of the oxidation rate on [Acid]

$[H_2SO_4]$(M)	0.50	0.75	1.0	1.50	3.0
$10^5 \times k_1(s^{-1})$ for:					
Lysine	7.0	10.0	15.0	21.0	43.0
Arginine	4.0	6.0	9.0	12.0	26.0
Histidine	0.7	1.1	1.4	2.2	4.5

[Amino Acid]=0.01M, [QDC]=0.001M, μ=0.5M, T=333K.

Plots of log k_1 against log[H^+] were linear, with slopes equal to unity, showing that the reaction was dependent on the first power of the concentration of the acid.

The linear increase in the rate of oxidation with an increase in the concentration of the acid suggested the involvement of a protonated Cr(VI) species in the rate-determining step of the reaction. The involvement of such protonated Cr(VI) species has been well established in chromic acid oxidation reactions(48). Protonated Cr(VI) species have been observed in the presence of p-toluene sulphonic acid in dichloromethane-nitrobenzene mixtures(49).

Rate law

Under the present experimental conditions, wherein pseudo-first-order conditions have been used for all the kinetic runs, the rate law can be expressed as

$$\text{Rate} = -\frac{d[\text{Cr(VI)}]}{dt} = k[\text{Substrate}][\text{QDC}][H^+] \qquad (2)$$

Effect of solvent

The solvent composition of the reaction medium was changed by using varying proportions of acetic acid and water. The dielectric constants for acetic acid-water mixtures were estimated from the dielectric constants of the pure solvents(50).

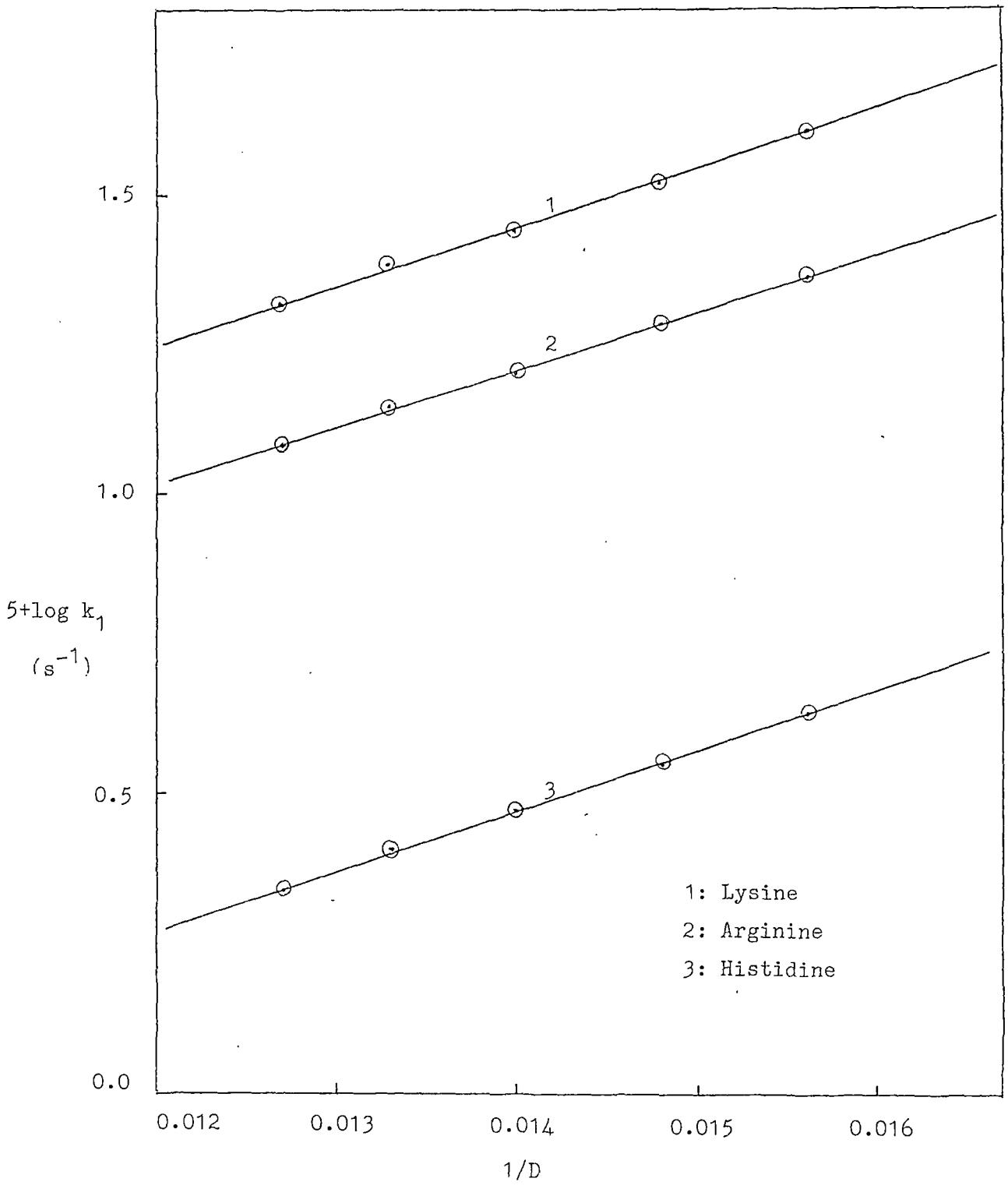


Fig.1: Plot of log k_1 against the reciprocal of dielectric constant

The rate of the reaction was susceptible to changes in the polarity of the solvent medium. With an increase in the dielectric constant of the medium, there was a decrease in the rate of the reaction. This was in consonance with the observation that the use of more polar solvents required larger reaction times(51). The data has been recorded in Table 5.

Table 5. Dependence of the oxidation rate on solvent.

H_2O:HOAc(%, v/v)	100:0	95:5	90:10	85:15	80:20
Dielectric constant(D)	78.54	74.92	71.30	67.68	64.06
$10^4 \times k_1$, s^{-1} for:					
Lysine	2.1	2.4	2.7	3.3	4.0
Arginine	1.2	1.4	1.6	1.9	2.3
Histidine	0.22	0.26	0.30	0.35	0.43

[Amino Acid]=0.01M, [QDC]=0.001M,[H_2SO_4] =1.5M, μ=0.5M, T=333K.

Plots of log k_1 against the reciprocal of dielectric constant were linear with positive slopes (Fig.1). This suggested an interaction between a positive ion and a dipole(52), and confirmed that the rate-determining step, in the presence of acid, involved a protonated Cr(VI) species. The effect of a change in solvent composition on reaction rates would also depend on factors such as the solvating power of the solvent(53), solute-solvent interactions(54,55),

and solvent structure.

Effect of temperature

The rate of the reaction was influenced by changes in temperature (Table 6).

Table 6. Dependence of the oxidation rate on temperature.

Temp (±0.1K)	Lysine	Arginine	Histidine
	(10^5 x k_1, s^{-1})		
323	12.0	5.0	1.1
328	15.4	7.7	1.5
333	21.0	12.0	2.2
338	26.9	18.6	3.1
343	34.7	25.7	4.3
348	46.8	35.0	5.8

Plots of log k_1 against the reciprocal of temperature were linear (Fig.2). The slopes of the plots were used to calculate the activation energies of the reactions. The other activation parameters were calculated and have been shown in Table 7.

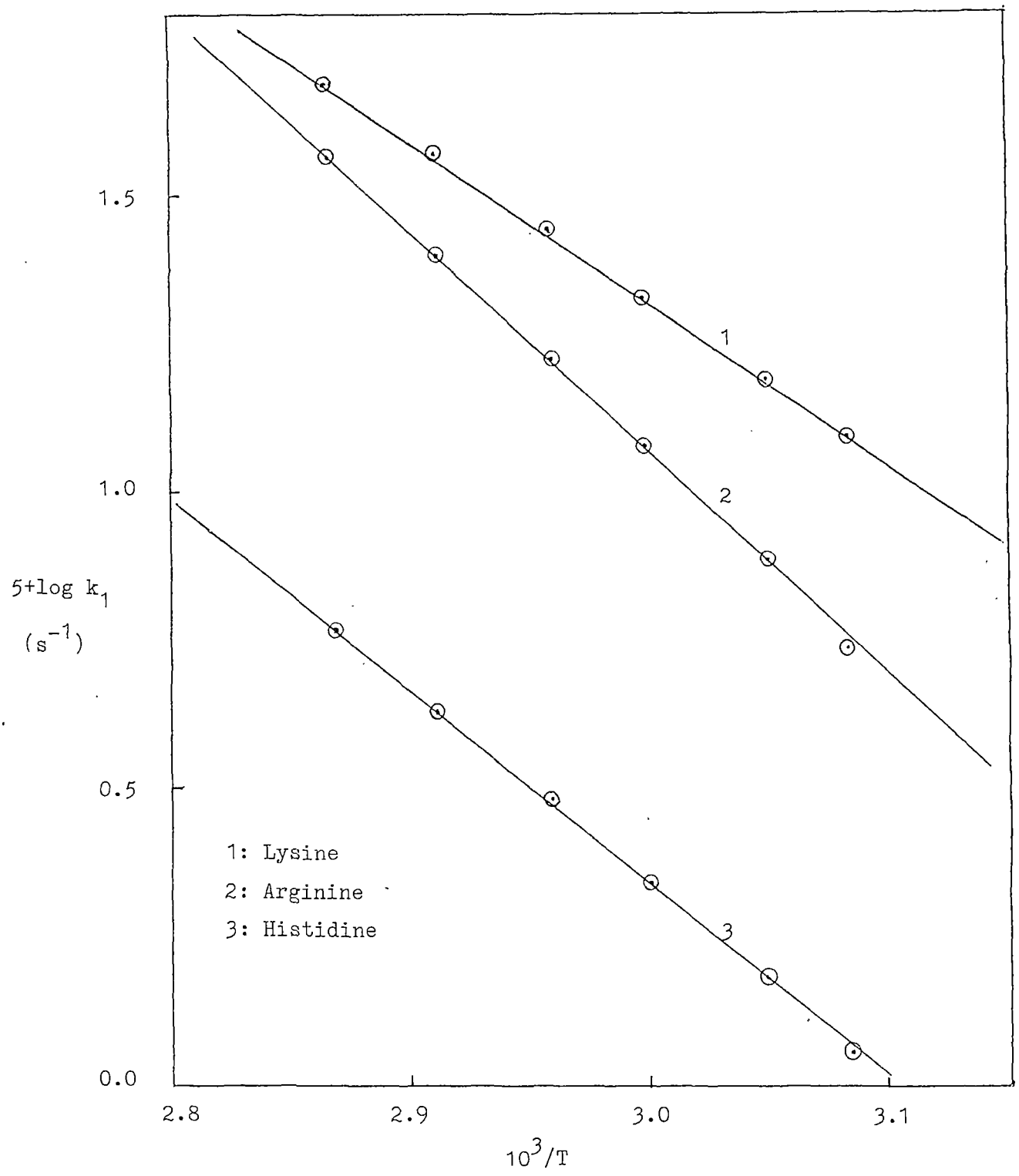


Fig.2: Plot of log k_1 against the reciprocal of temperature

Table 7. Activation parameters.

Parameter	Lysine	Arginine	Histidine
$E(kJmol^{-1})$	48	54	63
$\Delta H^{\neq}(kJmol^{-1})$	45	51	60
$\Delta S^{\neq}(JK^{-1}mol^{-1})$	-181	-169	-151
$\Delta G^{\neq}(kJmol^{-1})$	105	107	110

Error limits: $E \pm 2kJmol^{-1}$, $\Delta H^{\neq} \pm 2kJmol^{-1}$, $\Delta S^{\neq} \pm 4JK^{-1}mol^{-1}$, $\Delta G^{\neq} \pm 2kJmol^{-1}$.

The oxidations of all substrates were characterised by negative values of the entropies of activation. This would suggest an ordered transition state, relative to the reactants(56). Differences in solvation of substrates in the ground state and in the transition state might also contribute, to some extent, to the negative entropies of activation(57).

Isokinetic Relationship

For the oxidation of basic amino acids (lysine, arginine and histidine) by quinolinium dichromate, in acid medium, it was observed that the activation enthalpies and entropies were linearly related. For such a correlation, the applicability of Exner's criterion(58) was found to be valid. The isokinetic temperature was obtained by using the relationship:

$$\Delta H^{\neq} = \Delta H_o^{\neq} + \beta \Delta S^{\neq} \qquad (3)$$

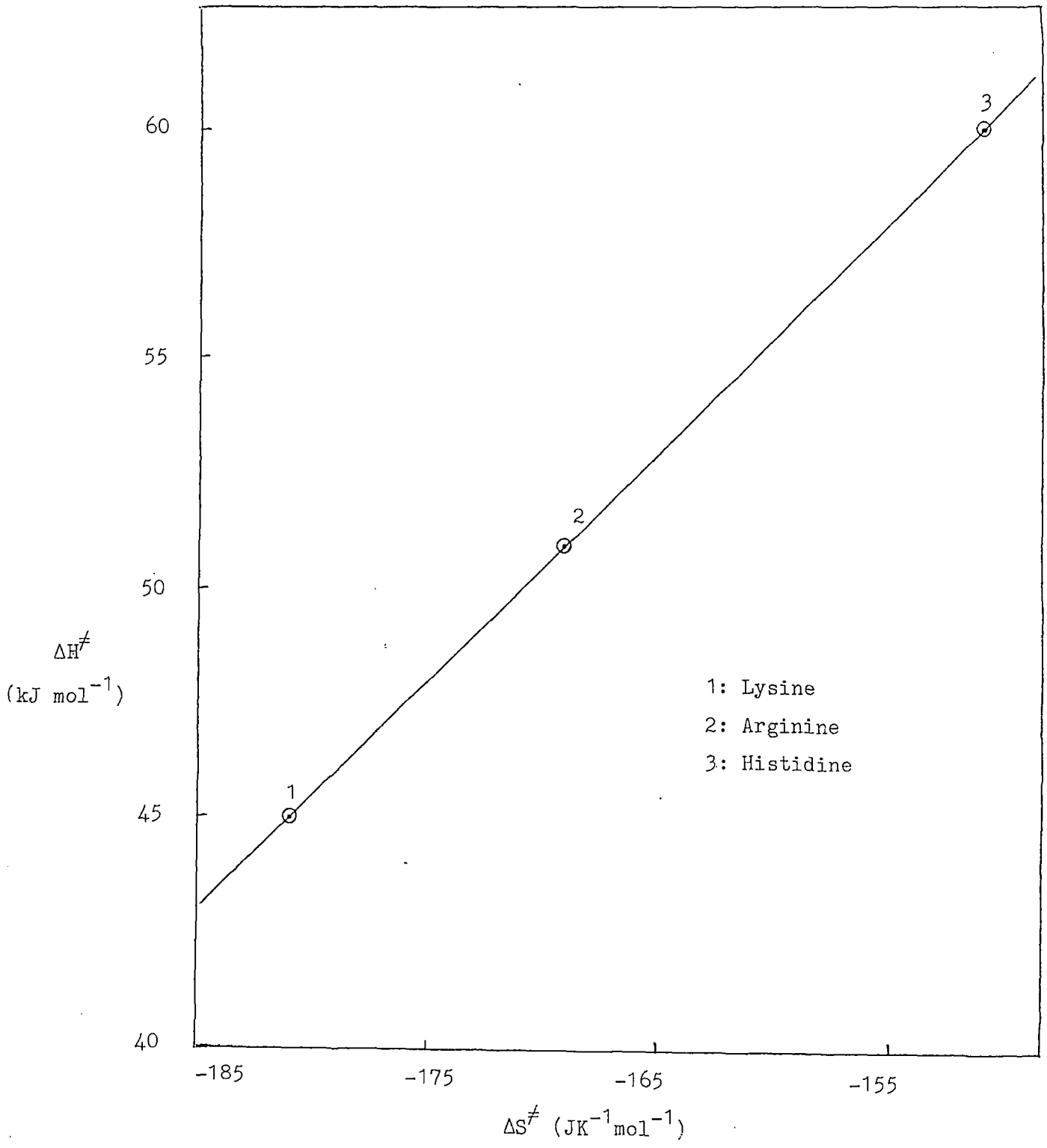


Fig.3: Isokinetic Plot

where β was the isokinetic temperature. From the linear plot of $\Delta H^{\neq}$ against $\Delta S^{\neq}$, the isokinetic temperature was found to be 438K (Fig.3). Although there is not much physical significance attached to isokinetic temperatures(59), a linear correlation between $\Delta H^{\neq}$ and $\Delta S^{\neq}$ is usually a necessary condition for the validity of linear free energy relationships. Further, the value for the free energy of activation ($\Delta G^{\neq}$) were fairly constant, indicating that the same mechanism operated for the oxidation of all the basic amino acids (lysine, arginine and histidine).

Solvent isotope effect

The rates of oxidation of these amino acids were increased in D_2O medium (Table 8), in agreement with earlier reported work(60).

Table 8. Solvent isotope effect for the oxidation of amino acids.

Amino Acid	k_{H_2O}	k_{D_2O}	k_{D_2O}/k_{H_2O}
	($10^5 \times k_1$, s^{-1})		
Lysine	21.0	28.3	1.35
Arginine	12.0	15.3	1.28
Histidine	2.2	2.9	1.32

[Amino Acid]=0.01M, [QDC]=0.001M, [H_2SO_4]=1.5M, μ =0.5M, T=333K.

The solvent isotope effect, k_{D_2O}/k_{H_2O}, was greater than unity (Table 8). This would imply a proton-catalyzed reaction, and would support the protonation of the oxidant (QDC), an observation which finds support from the acid dependence on the rate of the reaction (Table 4).

Effect of ionic strength

Variations in the ionic strength of the medium using sodium perchlorate (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 and $MgSO_4$ (concentration range of $1x10^{-4}$M to $1x10^{-3}$M) did not have any influence on the rates of these oxidation reactions.

Kinetic isotope effect

The kinetic isotope effect was studied by deuterating the α-carbon atom. The k_H/k_D values were close to unity (Table 9).

Table 9. Kinetic isotope effects for the oxidation of amino acids.

Amino Acid	$RCH(NH_2)COOH$	$RCD(NH_2)COOH$	k_H/k_D
	($10^5 \times k_1$, s^{-1})		
Lysine	21.0	20.5	1.02
Arginine	12.0	11.7	1.03
Histidine	2.2	2.1	1.05

[Amino Acid]=0.01M, [QDC]=0.001M, $[H_2SO_4]$=1.5M, μ=0.5M, T=333K

The data in Table 9 indicated that there was no cleavage of the carbon-hydrogen bond in the rate-determining step of the reaction.

Induced polymerization

The possibility of induced polymerization was checked. It was seen that there was no induced polymerization of acrylonitrile or the reduction of mercuric chloride (61). No ESR signals could be detected in these oxidation reactions, thus providing no evidence for the formation of radical intermediates. Control experiments were performed in the absence of the substrate. The concentration of the oxidant (QDC) did not show any appreciable change.

Mechanism

For the oxidation of the basic amino acids (lysine, arginine and histidine) by quinolinium dichromate (QDC) in acid medium, the observed kinetic data could be summarized as follows:

(i) The rate of the reaction was dependent on the first power of the concentration of each — substrate, oxidant and acid (Tables 2-4). The linear increase in the rate of oxidation with acidity (Table 4) indicated the involvement of a protonated Cr(VI) species in the slow step of the reaction. The initial reaction would be between the substrate and the protonated Cr(VI) species.

(ii) The observed increase in the rate of the reaction, with a decrease in the polarity of the solvent medium (water-acetic acid, v/v), as seen from the data in Table 5, indicated that the transition state was much less polar than the reactants. Linear plots of log k_1 against the reciprocal of the dielectric constant yielded positive slopes (Fig.1). This suggested an interaction between a positive ion and a dipole(52), and was in consonance with the observation that, in the presence of acid, the rate determining step involved a protonated Cr(VI) species.

(iii) Negative values for the entropies of activation (Table 7) suggested that the transition state was more

ordered than the reactants(56). The values for the free energies of activation ($\Delta G^{\neq}$) were nearly constant, which indicated that all these amino acids (lysine, arginine and histidine) underwent oxidation by similar pathways. Linearity in the plot between the enthalpy of activation ($\Delta H^{\neq}$) and the entropy of activation ($\Delta S^{\neq}$) suggested that these oxidation reactions were controlled by both parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$). The isokinetic temperature was 438K.

(iv) The solvent isotope effect, k_{D_2O}/k_{H_2O}, was greater than unity (Table 8), which implied a proton-catalysed reaction. This would support the protonation of the oxidant (QDC), as seen from the acid dependence on the rate of the reaction (Table 4).

(v) The kinetic isotope effect for the oxidation of these amino acids (obtained by deuterating the α-carbon atom) yielded k_H/k_D values close to unity (Table 9). This would indicate the absence of a carbon-hydrogen bond cleavage in the rate-determining step of the reaction.

(vi) Changes in the ionic strength of the medium and the addition of salts did not exert any influence on the rates of oxidation of these amino acids (lysine, arginine and histidine). This indicated a direct interaction between the substrate and oxidant, in acid medium, to give an

intermediate which could further react to yield the product.

(vii) There was no induced polymerization of acrylonitrile or reduction of mercuric chloride(61), which indicated the absence of radical species during the course of the reaction.

The rate constants for the oxidation of all these amino acids (lysine, arginine and histidine) were almost of the same order of magnitude. This was because these amino acids have almost similar pK_1 values. The ionization constants and pH values at the isoelectric points(62) of these amino acids (lysine, arginine and histidine) at 298K have been given in Table 10.

Table 10. Ionization constants(62) and pH values at the isoelectric points of amino acids at 298K.

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.

The dissociation of amino acids depends upon the pH of the medium. It is known that amino acids exist as dipolar ions (zwitterions) in aqueous solution. In strongly

acidic or alkaline media, the following equilibria exist:

$$\underset{\text{(cation)}}{RCH(N^{+}H_3)COOH} \underset{H^{+}}{\overset{OH^{-}}{\rightleftharpoons}} \underset{\text{(zwitterion)}}{RCH(N^{+}H_3)COO^{-}} \underset{H^{+}}{\overset{OH^{-}}{\rightleftharpoons}} \underset{\text{(anion)}}{RCH(NH_2)COO^{-}} \qquad (3)$$

In the present study, the reactions were carried out in acid media. In acidic solution, the zwitterion would be converted to the cation [$RCH(N^{+}H_3)COOH$], which was the reactive species, under the present experimental conditions.

It has been established that the oxidation of amino acids by various oxidants, in concentrated acidic media, had occurred via the reaction between the cationic form of the amino acid and the oxidant(63,64).

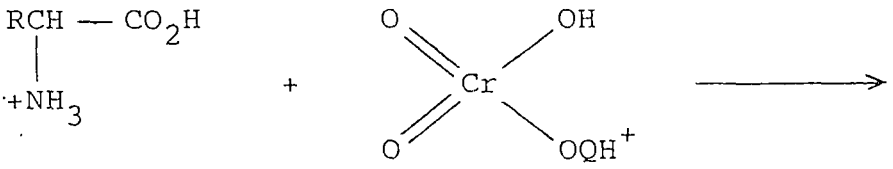
Based on the observed experimental data, the mechanistic pathway for the oxidation of these basic amino acids (lysine, arginine and histidine) by quinolinium dichromate (QDC), in acid medium, would proceed by means of a direct reaction between the cationic form of the amino acid and the oxidant(QDC), to yield an intermediate which would then undergo further reaction to give the product. The sequence of reactions for the oxidation of these amino acids (lysine, arginine and histidine) by quinolinium dichromate (QDC), in acid medium, has been shown in the Scheme.

The major products of these oxidation reactions (vide "Experimental": Product Analysis) were:

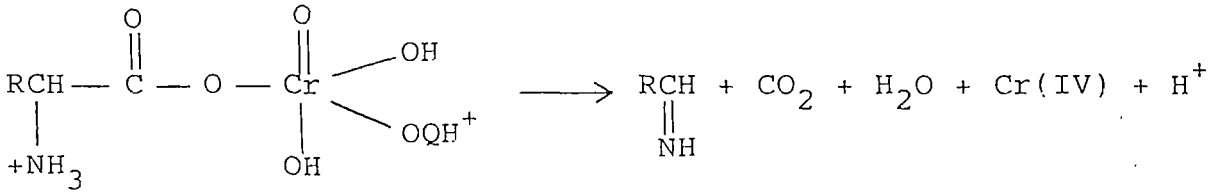
(a) the corresponding nitriles (∿75-80%), which were characterized by chemical and spectral methods; and

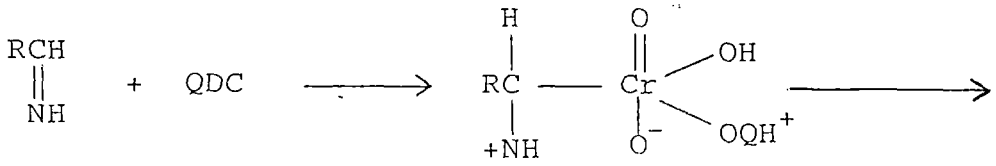
(b) trace amounts of the corresponding aldehydes (∿5-10%), which were characterized by their respective 2,4-dinitrophenylhydrazone derivatives.

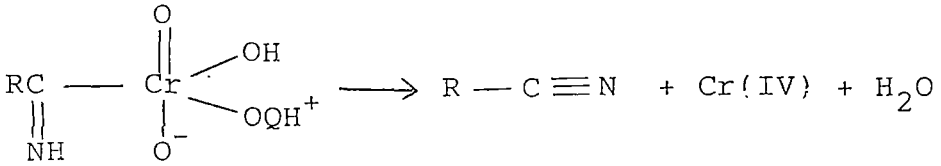
SCHEME



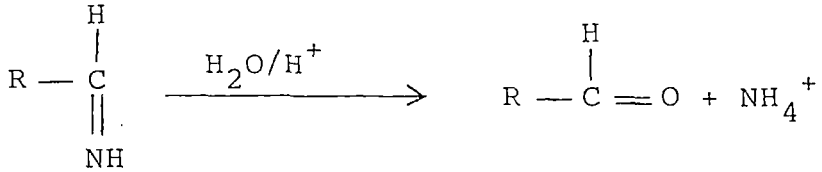
(Amino Acid) (QDC)







(Nitrile)



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SUMMARY

Hexavalent chromium compounds have been widely used as oxidizing agents, reacting with diverse kinds of organic substrates. The mechanism of oxidation varies with the nature of the chromium(VI) species and the solvent used. The development of newer chromium(VI) reagents for the oxidation of organic substrates continues to be a subject of interest. A number of novel chromium(VI) oxidizing agents have been introduced, especially for complex or highly sensitive substances where great selectivity and effectiveness, coupled with mildness of conditions, are prerequisites for success.

Some of the chromium(VI) reagents which have been used as efficient oxidizing agents are:
chromium trioxide; chromyl chloride; Jones reagent —— a solution of Cr(VI) oxide in concentrated sulfuric acid(1); Collins' reagent —— dipyridinium Cr(VI) oxide in dichloromethane(2); Corey's reagent —— pyridinium chlorochromate(3); pyridine oxodiperoxy chromium(VI) reagent(4); pyridinium dichromate(5); bis tetrabutylammonium dichromate(6); Chaudhuri's reagent —— pyridinium fluorochromate(7); 4-(dimethylamino)-pyridinium chlorochromate(8); Cr(VI) oxide diperoxide(9); chlorotrimethylsilane-Chromium trioxide (10); chromium peroxide complexes(11); imidazolium dichro-

mate(12); pyridinium bromochromate(13); biphosphonium dichromate(14); and 3-carboxy pyridinium dichromate(15).

New procedures have been emerging involving non-aqueous chromium(VI) reagents with the general idea that anhydrous conditions are more conducive to mild oxidation.

The reagent employed in the present investigation, quinolinium dichromate(QDC) . $(C_9H_7N^+H)_2Cr_2O_7^{2-}$, has emerged as a very useful and versatile oxidant(16), which is clearly deserving of widespread application.

The oxidation of amino acids has become important, both from a chemical point of view and in trying to explore the various transformations involved in the metabolism of amino acids. Owing to the differing nature of the hydrocarbon portion, amino acids can undergo various kinds of reactions depending on whether the particular amino acids contain non-polar groups, polar substituents, acidic or basic substituents.

In the present investigation, the kinetics of oxidation of amino acids by quinolinium dichromate (QDC), in acid medium, at constant ionic strength have been studied. The amino acids which have been used for the purposes of oxidation have included :

1. Glycine, alanine, valine, leucine and phenylalanine: Chapter 1.
2. Serine, threonine and tyrosine: Chapter 2.
3. Methionine and cysteine: Chapter 3.
4. Aspartic acid and glutamic acid: Chapter 4.
5. Lysine, arginine and histidine: Chapter 5.

All the oxidation reactions were performed under a nitrogen atmosphere. The stoichiometries of the individual kinetic reactions were determined. For all the kinetic runs, the progress of the oxidation reaction was followed by monitoring the disappearance of Cr(VI) at 440nm, spectrophotometrically. The rates of all the reactions were found to be dependent on the first power of the concentrations of each —— substrate, oxidant and acid. The first order dependence of the rate on acid concentration indicated that a protonated Cr(VI) species was involved in the rate determining step of the reaction.

The rate of the reaction showed an increase, with increasing proportions of acetic acid. Plots of log k_1 (the pseudo-first-order rate constant) against the reciprocal of the dielectric constant were linear, with positive slopes, indicating an ion-dipole type of reaction. This was in consonance with the observation that the use of more polar solvents required larger reaction times. This

also indicated that, in the presence of an acid, the rate determining step involved a protonated Cr(VI) species.

The effect of changes in temperature on the rates of the reactions has been studied, and the activation parameters have been evaluated. The reactions were characterized by negative entropies of activation ($\Delta S^{\neq}$). This suggested a highly ordered transition state, relative to the reactants. Although current views do not attach much physical significance to isokinetic temperatures, a linear correlation between $\Delta H^{\neq}$ and $\Delta S^{\neq}$ has been considered a necessary condition for the validity of linear free energy relationships. Plots of $\Delta H^{\neq}$ vs $\Delta S^{\neq}$ were linear, indicating that the oxidation reactions of amino acids were controlled by both these parameters. The isokinetic temperatures(β) obtained were 338K (for glycine, alanine, valine, leucine and phenylalanine); 320K (for serine, threonine and tyrosine); 438K (for lysine, arginine and histidine). Further, the free energies of activation ($\Delta G^{\neq}$) were nearly constant, indicating that the same mechanism operated for the oxidation of all these amino acids.

The kinetic rates of oxidation were in accordance with the theory of electronic substituent effects. Structure-reactivity correlations were carried out for some amino acids (glycine, alanine, valine, leucine and phenylalanine). It was observed that the Taft equation, which

could be applied to these amino acids, was of the form:

$$\log k/k_0 = -1.48 \ \sigma^* + 0.84 \ E_s \qquad (1)$$

The validity of this relationship indicated that both, the polar effect ($\rho^* = -1.48$) and the steric effect ($\delta = +0.84$), influenced the rate of the reaction. Since the reaction centre was near the site of substitution, the magnitudes of the reaction constants were expected to be fairly high. This has been observed in these oxidation reactions.

The solvent isotope effects, k_{D_2O}/k_{H_2O}, in the oxidation of all these amino acids, have been observed to be greater than unity. This indicated that the reactions were catalyzed by acid. This would support the protonation of the oxidant (QDC), as seen from the acid dependence on the rate of the reaction.

The oxidation of the deuterated amino acids (deuterated at the α-carbon atom) yielded values of the kinetic isotope effect, k_H/k_D, which were close to unity. The absence of a primary kinetic isotope effect indicated that, in the rate determining step of the reaction, there was no cleavage of the carbon-hydrogen bond. The oxidation of deuterated methionine (by deuterating the methyl group of methionine) did not show a primary kinetic isotope effect. This indicated that the carbon-hydrogen bond (of the methyl

group in methionine) was not cleaved in the rate-determining step of the reaction. During the course of the reaction, there was no induced polymerization of acrylonitile, no reduction of mercuric chloride, and no ESR signals could be detected. This showed the absence of radical species.

Variations in the ionic strength of the medium, and the addition of salts had no effect on the rates of these reactions. This indicated a direct reaction between the substrate and oxidant, in acid medium, to give an intermediate which on further reaction gave the product.

The dissociation of amino acids depends upon the pH of the medium. In aqueous solution, amino acids exist as dipolar ions (zwitterions). In strongly acidic or alkaline media, the following equilibria exist:

$$RCH(N^+H_3)COOH \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(N^+H_3)COO^- \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} RCH(NH_2)COO^- \qquad (2)$$

(cation) (zwitterion) (anion)

In acid solution, amino acids exist as a mixture of the zwitterionic [$RCH(N^+H_3)COO^-$] and cationic [$RCH(N^+H_3)COOH$] forms. In the present investigation, the reactions were carried out in acid media. The zwitterion would be converted to the cation [$RCH(N^+H_3)COOH$], which was the reactive species, under the present experimental conditions.

Based on the observed kinetic data, the mechanistic pathway for the oxidation of these amino acids by quinolinium dichromate (QDC), in acid medium, would proceed via a direct interaction between the cationic form of the amino acid and the oxidant to yield an intermediate, which would then undergo further reaction to yield the product.

For the oxidation of the sulfur containing amino acids (cysteine and methionine) by quinolinium dichromate, in acid medium, since disulfide was the final product of oxidation (from cysteine), and sulfoxide was the final product of oxidation (from methionine), the sulfhydryl group (-SH) of cysteine and the S-methyl group ($S-CH_3$) of methionine would provide the site of attack. Considering the mechanism of oxidation of cysteine and methionine, the attack could occur either at nitrogen or at sulfur. The products obtained from the oxidation of these amino acids (the disulfide from cysteine, and methionine sulfoxide from methionine), would suggest that the oxidant (QDC) attacks the sulfur group. Further, sulfur is more nucleophilic than nitrogen, and the sulfhydryl group (-SH) could donate electrons more readily than the amino group ($-NH_2$). Therefore, the mechanism was via an electron-pair donation by sulfur present in methionine (and cysteine) to the Cr=O bond of the oxidant.

The major products obtained in these oxidation reactions in good yield (∿75-80%) were:

a) the corresponding nitriles, which were characterized by chemical and spectral methods;

b) trace amounts of the corresponding aldehydes, which were characterized by their respective 2,4-dinitrophenyl-hydrazone derivatives;

c) methionine sulfoxide (from methionine) which was characterized as N-benzoyl methionine sulfoxide;

d) cystine (from cysteine), which was characterized by chemical methods.

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1. Kinetics of oxidation of sulfur containing amino acids by quinolinium dichromate

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