SYNTHESIS AND CHEMISTRY

OF HYDRAZINO ACIDS

ABDUL WAHID

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Abdul Wahid

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INTRODUCTION

CHAPTER -1

INTRODUCTION

Of all the theories put forth to explain the α -amino acids oxidation, no one accounts for the experimental data exactly. Keeping in mind the special nature and characteristic properties of the hydrazo acids, evident from their proposed structure, these were foresought as possible unstable intermediates in the chemical oxidation of α -amino acids. Before reviewing the synthesis and chemistry of α - hydrazo and α-hydrazino acids, a short review of α -amino acids oxidation is in order.

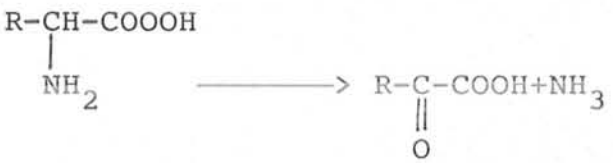
Oxidative Degradation of α -Amino Acids

Facile oxidation of α -amino acids can be brought about by both Biological and Chemical means. In biological systems, in the course of metabolism α -amino acids are converted to the corresponding α -keto acids. Chemical oxidations of α -amino acids were carried out to illuminate the biochemical studies, but the products of oxidation were different. It was therefore recognized that the biological and chemical oxidations proceed by entirely different routes and that the conclusions drawn for observations made under

chemical conditions had little bearing on the bio-chemical mechanism of amino acid oxidation.

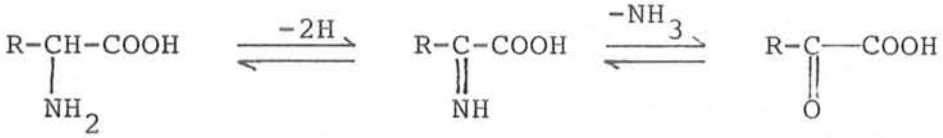
Enzymatic Oxidation Under Biological Conditions:

In metabolic pathway α-amino acids undergo oxidative deamination and give the corresponding α- keto acids[1]. This primary product of oxidation enters into further transformations depending upon the conditions and environment.

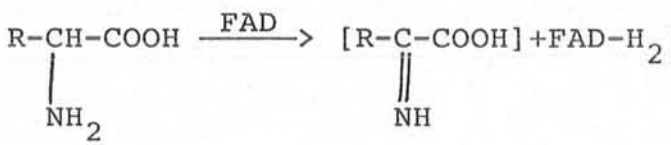


Knoop[2] proposed a mechanism involving an hypothetical intermediate, α-imino acid. The presence of α-imino acid has been demonstrated in a few cases[3]. Knoop[1,4] administered α-keto- γ-phenylbutyric acid to a dog subcutaneously and isolated d, α-acetylamino- γ-phenylbutyric acid. It was revealved by this experiment that living organism is capable of producing α-amino acid from α-keto acid and ammonia. The first step in the oxidation of α-amino was therefore postulated as reversible.

Kotake[5-8] obtained the same results.



The reversibility of this step was proved further when a number of ∝-amino acids were synthesized by hydrogenating a mixture of the corresponding ∝-keto acids and ammonia. ∝-Imino acid was suggested as unstable intermediate.[9] Krebs[10] carried out studies on kidney and liver of many animals showing that there were enzymes which were able to produce ammonia from ∝-amino acid with concomitant uptake of oxygen. The mechanism of oxidative deamination became much clear when in a quantitative study of oxidative deamination of alanine to pyruvic acid, by enzyme preparations from liver and kidney of a wide variety of animal species, Krebs observed that approximately one mole of oxygen was cosumed for every two moles of ammonia formed. The reaction generally is represented as a dehydrogenation of an ∝-amino acid by a flavoenzyme to yield reduced flavoenzyme and the corresponding ∝-amino aicd[11].



The proposed intermediate (α -imino acids) would be expected to be unstable and to hydrolyse rapidly to the corresponding α -keto acid and ammonia.

$$[R\text{-}\underset{\underset{NH}{\|}}{C}\text{-}COOH] + H_2O \longrightarrow R\text{-}\underset{\underset{O}{\|}}{C}\text{-}COOH + NH_3$$

Another assumption was made by Dakin[12], he proposed an alternative β -oxidation of the type well established for normal fatty acids and suggested that an α - β - unsaturated α -amino acid of the type

$R\text{-}CH\text{=}C(NH_2)\text{-}COOH$ might exist in equilibrium with the corresponding α -imino acid. Thus the hypothetical changes which an α -amino acid undergoes according to the ideas outlined can be represented as follows:

$$R\text{-}CH_2\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH \rightleftharpoons [R\text{-}CH_2\text{-}\underset{\underset{NH}{\|}}{C}\text{-}COOH] \overset{H_2O}{\rightleftharpoons} RCH_2\text{-}\underset{\underset{O}{\|}}{C}\text{-}COOH + NH_3$$

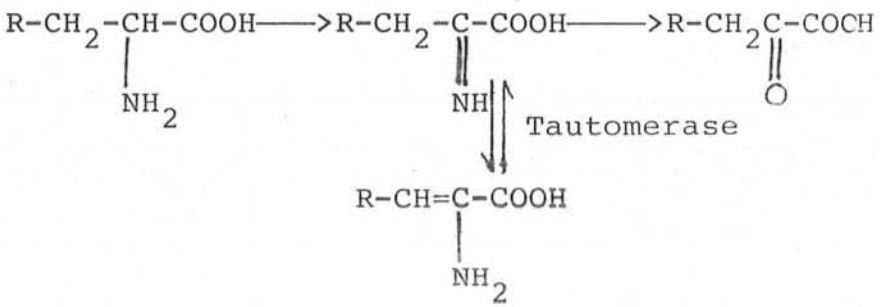
$$R\text{-}CH_2\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH \rightleftharpoons [R\text{-}\underset{\underset{NH_2}{|}}{CH}\text{=}C\text{-}COOH] \overset{H_2O}{\rightleftharpoons} R\text{-}CH_2\text{-}\underset{\underset{O}{\|}}{C}\text{-}COOH + NH_3$$

$$[R\text{-}CH_2\text{-}\underset{\underset{NH}{\|}}{C}\text{-}COOH] \overset{H_2O}{\rightleftharpoons} NH_3 + R\text{-}CH_2\text{-}\underset{\underset{O}{\|}}{C}\text{-}COOH$$

The imino acid hypothesis has been supported indirectly by the evidence made against α- β -unsaturation in the course of α-amino acid oxidase reaction. It was found that the four isomers of isoleucine are converted by amino acid oxidases to the corresponding optically active α -keto- β -methylvaleric acid[13,14]. Similarly the (L) isomers of β -phenylserine were transformed by L-amino acid oxidase to the respective optical isomers of mandelic acid[15].

Further evidence was provided by the susceptibility of D-and L-isomers of α-amino phenylacetic acid to the action of amino acid oxidases, despite the fact that they do not possess a β -hydrogen[10,16]. Additional evidence excluding the possibility of unsaturation has arisen from studies on the oxidation of L-leucine in the presence of D_2O by L-amino acid oxidase; the isolated α-ketoisocaproate did not contain appreciable amounts of deutrium[17]. Although the intermediate formation of α-imino acids was consistant with the available data, the first direct experimental evidence that amino acid oxidation occurs through this much earlier postulated intermediate was presented by Pitt[2]. He suggested that an amino acid and its enamine

tautomer, an α - β -unsaturated amino acid, can be considered as analogues, respectively, of the keto and enol forms of an α-keto acid. In the presence of an excess of enol-keto tautomerase, amino acid oxidation gave rise temporary accumulation of an intermediate identified as the enamine derivative



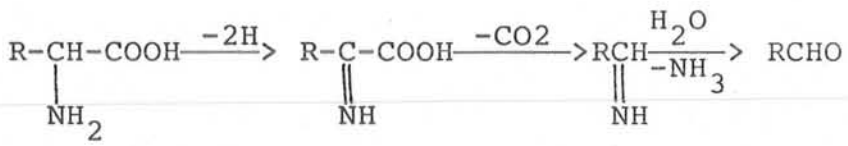
This was assumed to arise from the more labile and hitherto unknown α -imino acid by the action of the tautomerase. It has been emphasized that the keo tautomer of α-keto acid is the ultiamte product of the L-amino acid oxidase reaction. Since tautomerase was essential for the formation of enamine and of keto acid enolate, it was concluded that the imino tautomer was the initial α -amino acid oxidation product. This experimental finding refuted unequivocally the suggestion of Dakin[12] and Taborsky[18], that enamine was the normal intermediate. There is thus little doubt that

the oxidative conversion of α-amino to α-keto acids in vivo takes place by way of an intermediate α-imino acid.

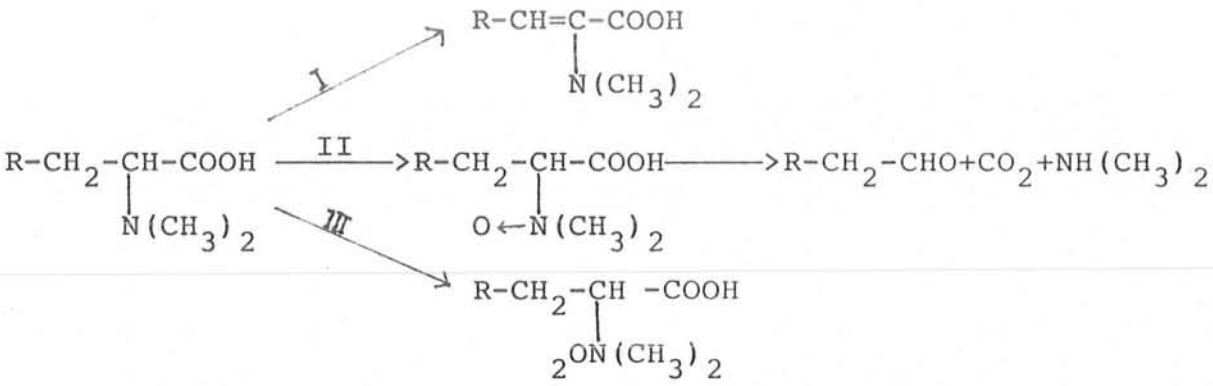
Chemical Oxidation of α-Amino Acids

It has been described earlier that the chemical oxidation of amino acids resulted in products different from that were produced in biological oxidation . It was Streeker[19] who first showed the oxidative decarboxylation in alanine by the action of alloxan. The products were acetaldehyde and carbon dioxide. Other oxidizing agents like ozone[20] and hydrogen peroxide[21,22] have since been shown to convert α-amino acids to the corresponding aldehydes, ammonia and carbon dioxide. The studies carried out by Wieland and Bergel[22] on the non-biological oxidation of α-amino acids with molecular oxygen in the presence of activated charcoal as catalyst showed that the products of degradation were different from thsoe obtained in biological oxidation. The usual products in the biological oxidation are an α-Keto acid and ammonia, while the products in this case were ammonia, carbon dioxide and aldehydes with one carbon less than the parent amino acid.

The following mechanism was postulated for the non-biological oxidation of α-amino acids on the basis of these results.



The α-amino acid suffers dehydrogenation to an α-imino acid which is decarboxylated to give the aldehyde imine which undergoes hydrolysis to yield aldehyde and ammonia. This sequence received some temporary support when it was found that α-amino isobutyric acid which lacks hydrogen atom on the α-carbon atom could not be oxidized with oxygen in the presence of charcoal udner these codnitions. Comparison of the rate of degradation of alanine and leucine by Bergel and Bolz[23] showed that the α-amino acid with the longer side chain reacted faster. They also observed that mono and particularly di-N-alkylated α-amino acids underwent degradation much faster than α-amino acids, but that substitution of the nitrogen to the betaine stage stablized the compounds towards oxidizing agents. The mechanism previously proposed[22] could not explain these results and a new scheme representing these possible sequences was put forward.



Sequence (I) was rejected when it was shown that dimethyl amino acetic acid was easily degraded, inspite of the fact that there did not exist any possibility of α- β -dehydrogenation[24]. Sequence (II) was considered unacceptable, as it postulates the formation of amine oxides which are quite stable under the conditions employed for oxidative decarboxylation of α-amino acids[20]. The third sequence which envisaged the formation of a peroxy intermediate received some consideration, since some active oxygen was found in the oxgenated α-amino acid obtained by breif treatment of α-amino acids with oxygen in the presence of charcoal. It was also supported by the work of Graffrons[25] who had previously shown the formation of peroxy compounds of amines through photo oxidation, using dyes as sensitizers. The failure of kidney enzyme to degrade the di-N-alkylated amino acid was explained by considering the enzyme as a typical dehydrogen-

ase, whereas charcoal was thought to act either like a dehydrogenase or an oxidase, depending upon the nature of the substrate in question.

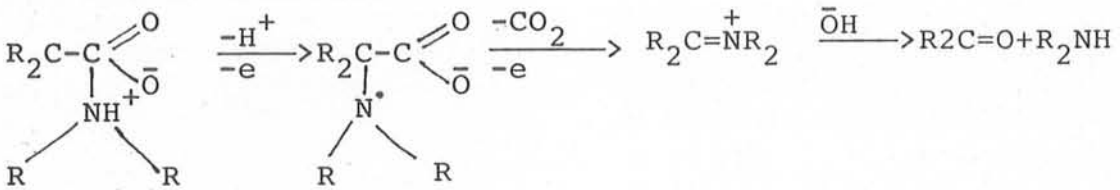
A few weeks later, Bergal and Bolz[23] published the results of the decomposition of α-N,N-dimethylaminoisobutyric acid and α-aminoisobutyric aicd. They observed that, although α-aminoisobutyric acid did not exhibit any sign of degradation by the oxygen-charcoal system, α-N,N-dimethylaminoisobutyric acid was rapidly and quantitatively transformed to carbon dioxide, dimethylamine and acetone under these codnitions. The possibility of dehydrogenation was eliminated by these results. The formation of peroxide was not detected. Therefore it was postulated that oxygen is capable of addition to the nitrogen atom of α-amino acids to form a labile intermediate which instantly decomposes to give the final products.

The work of Bergel and Bolz[23] received confirmation from the investigation of Herbst and Clarke[24], who used silver oxide as the oxidizing agent in aqueous solutions. Silver oxide had earlier been used to study the oxidation of glycine[25,26]. Herbst and Clarke gave the following

equation to explain the action of silver oxide on N,N-dimethyl glycine.

$$(CH_3)_2N\text{-}CH_2\text{-}COOH + 3Ag_2O \longrightarrow (CH_3)_2NH + CO_2 + H_2O + 6Ag$$

They summarized their conclusion as oxidation was suppressed by the prsence of either mineral acid or alkali. It was therefore reasonable to suppose that the primary reaction was a function of an amphion $R\text{-}CH(NH_3)^+CO\bar{O}$. Both of the hydrogen atoms of the α-carbon atom in glycine were replaced by alkyl groups without diminishing the reducing power of amino acids towards silver oxide. It was further cofnirmed that methylation of amino group led to enhanced reactivity due to increase in the basicity of the basic group. Replace-ment of all the hydrogen atoms on the nitrogen, as in betaine, completely abolished the reactions. Herbest and Clarke suggested an alternative general scheme for the oxidation of all types of α-amino acids.

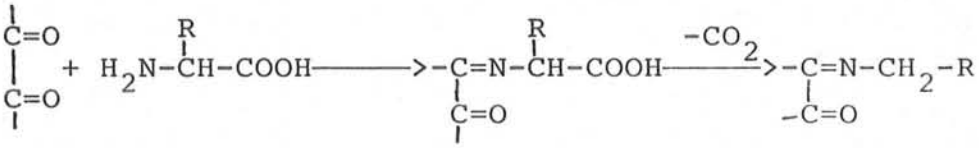


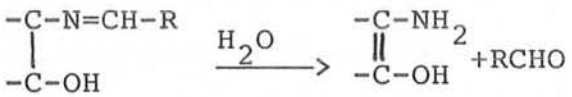
In this scheme oxidation is linked with the initial loss of

a single hydrogen atom from the nitrogen atom in conrast to the dehydrogenation as represented by the mechanism of Wieland and Bergel[22] which was incapable of explaining the oxidation of α-aminoisobutyric acid. The action of silver oxide does not seem to have any analogy with the oxidation of α-amino acids in living tissues, since administration of α-aminoisobutyric acid to dogs led to excretion of unchanged material and not of acetone and increased amounts of urea[27]. Herbst and Clarke for the first time stressed the necessity of the presence of a dissociated carboxylic group in this reaction. α-Amino acids are degraded by a very large number of ketones, ninhydrin being the most important of these. This reagent has been used for the quantitative estimation of α-amino acids[28].

Much controversy has remained about this reaction, a number of mechanisms have been proposed but the nature of this reaction has been understood only recently. D. J. McCaldin reviewed this subject and examined the earlier theories critically[29]. A group of workers[30] showed that certain carbonyl compounds other than ninhydrin, that have the general structure $-CO-(CH=CH)_n-CO$, where n may be zero or an integer and one of the -C=O may be aldehydic or ketonic, bring about the degradation of α-amino acids. In light of

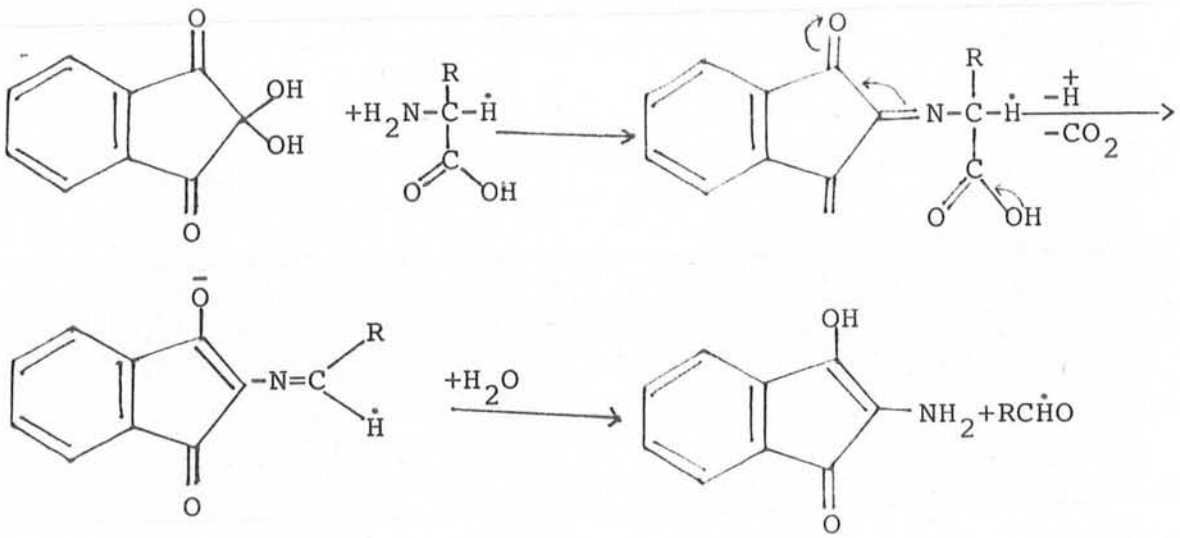
their observations they suggested that the presence of substituted amino group was essential for such a degradation by ketonic substances. Replacement of hydrogen atom on α-carbon atom has no effect on degradation. Mechanism for degradation with ketonic substances is



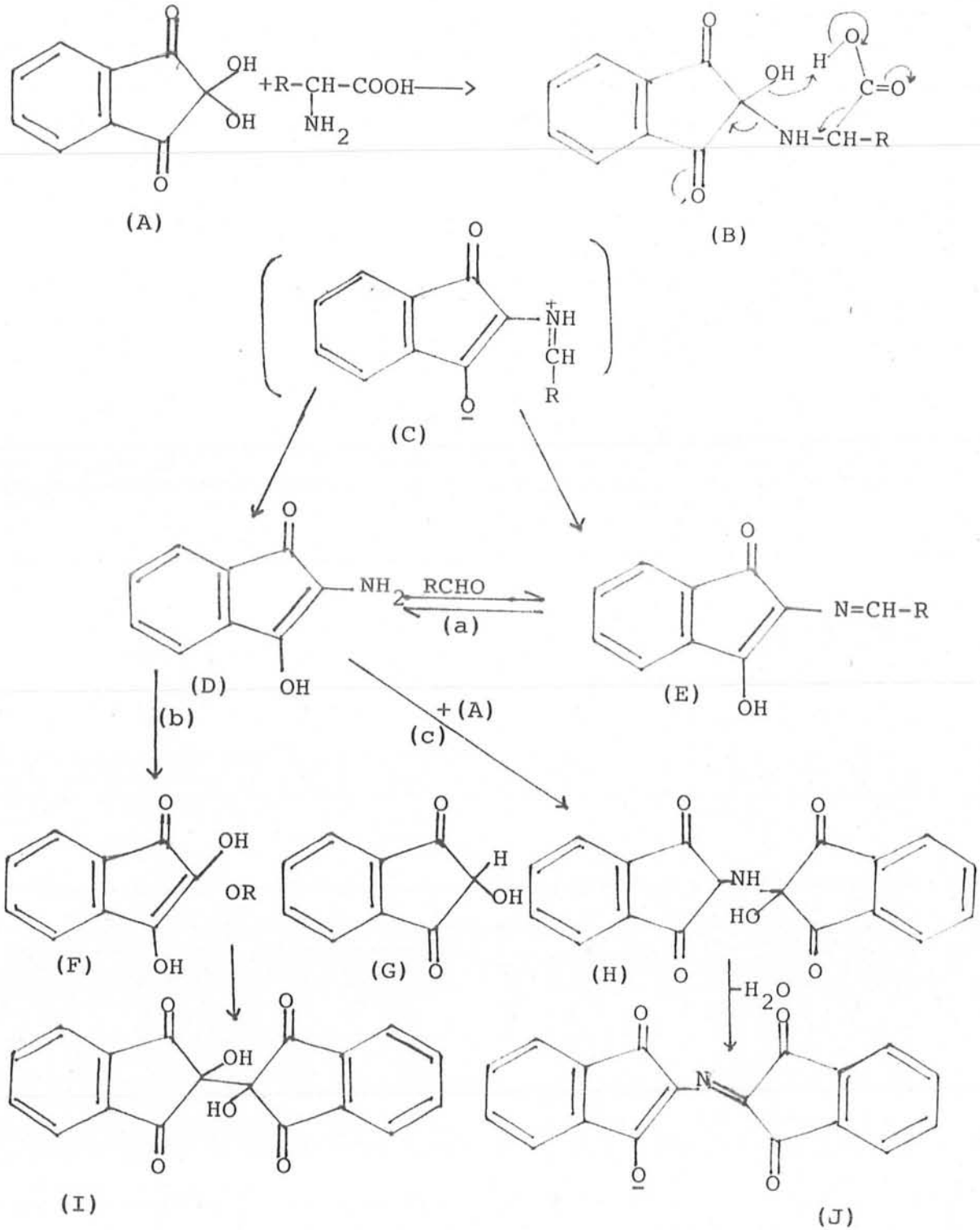


Kay and Rowland[31] argued that if the Schonberg's mechanism involving a tautomerization was correct, the recoil labelled DL-alanine would have exchanged and lost tritium on α-carbon during reaction with ninhydrin. The radioactivity of the resulting acetaldehyde (or derivative) would then serve as a measure of the original α-hydrogen radioactivity. However, in actual experiments the results showed only a minor loss of tritium activity during the decarboxylation with ninhydrin of recoil labelled DL-alanine to acetaldehyde. This confirmed the earlier findings[32]. It was also found that when unlabelled DL-alanine was oxidized with ninhydrin in

the presence of tritiated water of high radioactivity, the resulting derivative of acetaldehyde was almost non-radioactive. These experiments, therefore, clearly demonstrated that α-hydrogen is non-labile throughout the entire oxidation sequence, and that under these reaction conditions, the mechanism of the ninhydrin oxidation could not involve tautomeric forms. Kay and Rowland suggested mechanism (I) which was consistent with their observations. A general mechanism (II) for the reaction of ninhydrin with amines, amino acids and imino acids was put forward by Mc Caldin[29]. This emerged from the result of the reaction between ninhydrin and cyclic α-imino acids[33] and is based on the mechanism of Strecker degradation and explains the formation of "Ruhemann's purple" and hydrindantin in the reaction with α-amino acids.



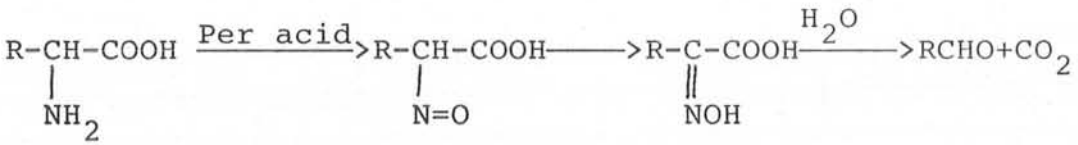
Mechanism(I)(Kay & Rowland,1959)



Mechanism II (McCaldin, 1960)

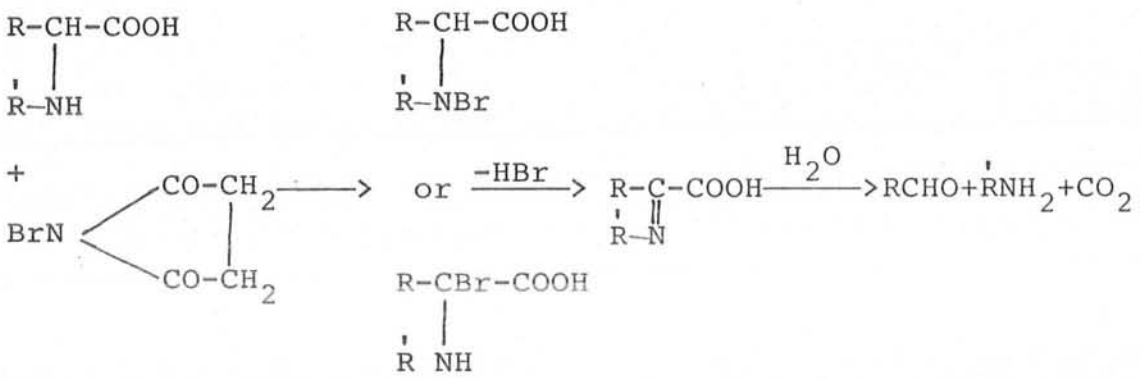
Contrary to the postulations of Moubasher and Abrahim[34] which required the enamine-vinylamine shift this mechanism involves a concerted series of electron transfers. The indications for the formation of the intermediate (B) is strong since this reaction is the initial step of the Strecker degradation, and analogous substances have been isoalted from the reaction mixtures of nihydrin with cyclic bases. The next step is the formation of zwitterionic species (C) which results through dehydration and decarboxylation. This intermediate (C) then undergoes either hydrolysis or rearrangement to give amine (D) which was isoalted by Ruhemann. On further hydrolysis (D) gives (F) or its tautomer (G). In last, an additional molecule of nihydrin condenses with (D) to give rise the Ruhemann's Purple (J). At pH(1-2.5), the rection proceeds via route(b) and ammonia is evolved quantitatively without the production of "Ruhemann's Purple", whereas at pH 5, path(c) must be open as under these conditions, colour formation is the basis of analytical method[36]. The formation of the ddifferent products, like aldehyde, carbon dioxide,ammonia, hydrindantin and "Ruhemann's Purple", is clearly explained by this scheme. It also suggests the common path way for the reaction of ninhydrin with amines, imino acids and amino acids,and throws light on why this reaction proceeds more rapidly with substances which contain

carboxyl group adjacent to the nitrogen atom. The appearance of different colour shades with different α-amino acids is perhaps due to the presence of varying quantities of different Schiff bases of type (E) which are formed via routes (a) and (b). The mechanism for the oxidative degradation of α-amino acids with the reagents like perbenzoic acid and bezoyl peroxide was also put foward by Schonberg and Moubasher[37]



This mechanism was purely hypothetical and was not based on the isolation of intermediates. It is shown to be untenable. Aqueous solutions of α-keto acid oximes are stable at room temeprature but decompose rapidly at higher temperatures to give the corresponding nitriles, carbon dioxide and water in quantitative amounts; the action of peroxides, such as hydrogen peroxide, under appropriate conditions results in the transofrmation of α-Keto acid oximes to the corresponding hydroxamic acids[38]. Under these conditions, therefore, aldehydes are not obtained either by hydrolysis or by oxidation of α-oximino acids.

N-Bromosuccinimide has also been used for the quantitative decarboxylation of -amino acids, proteins and peptides. Decarboxylation occurs and carbon dioxide which is evolved is measured manometrically[39]. The action of N-Bromosuccinimide on α-amino acids was first reported by Schonberg and co-workers[40]. They suggested the following mechanism for the degradation of α-amino acids with N-Bromosuccinimide.



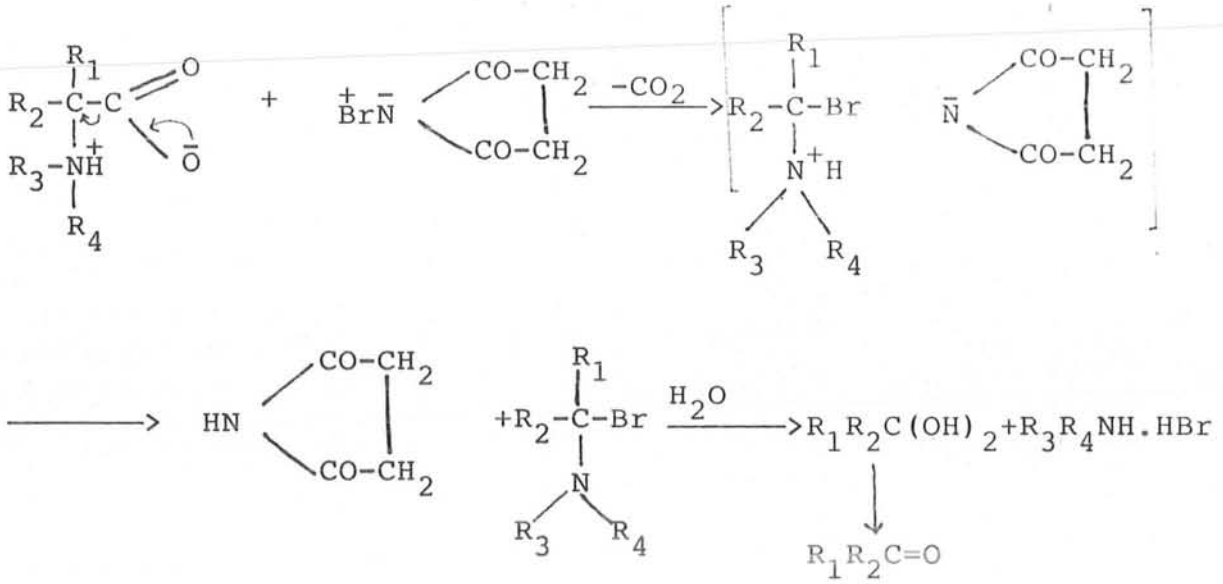
Since this sequence was based on limited experimental work and none of the proposed intermediates has been isoalted, the reaction was reinvestigated by Heyns and Stange[41] whose findings may be summarized as follows:

Unsubstituted α-amino acids, N-alkylated α-amino acids and α-amino acids with an unsubstituted amino group but a tertiary α-carbon atom undergo normal oxidation with N-bromosuccinimide to give the corresponding carbonyl comp -

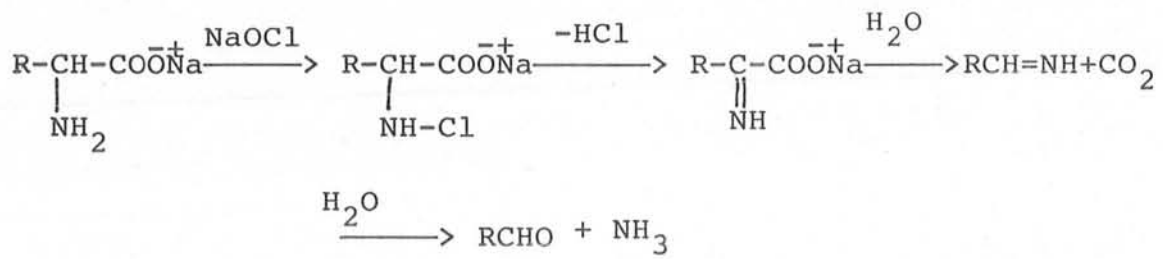
ounds. Fully substituted α-amino acids (e.g.N,N-dimethyl α - aminoisobutyric acids) do not react with N-bromosuccinimide, but their silver slats undergo degradation in poor yield. N-Acyl- α -amino acids are resistant towards N-bromosuccinimide,but their silver salts are smoothly oxidized to give the corresponding aldehydes, carbondioxide and acetamide in ehtyl acetate-methanol solution. In aqueous solution, the yield of degradation products was lower. N-Acyl- α -amino acids react with N-bromosuccinimide in ethyl acetate in the presence of added silver acetate, a better yield of aldehyde being obtained when the quantity of silver acetate and N-bromosuccinimide is increased.

Heyns and Stange interpreted these results by suggesting a new mechanism for α -amino acid oxidation with N-bromosuccinimide as the oxidizing agent. Br^+ was represented as the active oxidizing species. This scheme explained all known facts of amino acid oxidation with N-bromosuccinimide and eliminated the earlier proposed mechanism[40]. The stability of N-acyl-α -amino acids towards N-bromosuccinimide was explained as due to their inability to acquire a betaine type structure. Further support for this ionic mechanism was obtained when it was shown that while oxidation of

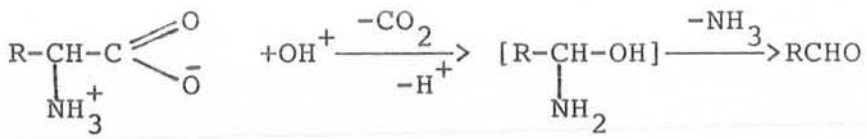
α -amino acids with N-bromosuccinimide proceeded well in water and other polar solvents, little or no reaction took place in non-polar solvents.



The observation that decarboxylation was facilitated when the amino nitrogen was positively charged was additional evidence in favour of the ionic mechanism. Various othe reagents have been employed to study the oxidation of -amino acids but they are of less importance in connection with the study of mechanism. Langheld[42] used sodium hypochlorite and proposed the formation of unstable α-imino acid intermediate.

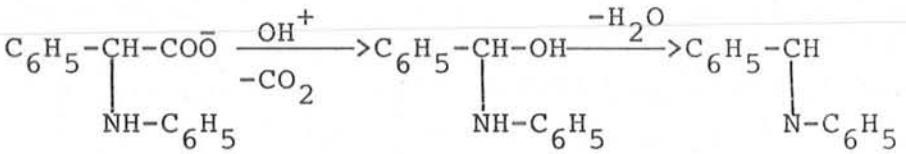


This mechanism is also untenable since it is now shown that under oxidizing conditions α-imino acids are converted to amides[43]. The approach of Spenser, Crawhall and Smyth[32] to the problem of the mechanism of oxidative decarboxylation of α-amino acids was of general nature. They first showed that $\alpha\beta$-$^3H_2$ valine and $\alpha\beta$-$^3H_2$ phenylalanine when oxidized with various oxidizing agents gave 1:2-$^3H_2$ isobutyraldehyde and 1,2-$^3H_2$ phenylacetaldehyde of specific activities identical with those of the parent amino acids, thereby confirming the earlier evidence[23-25] that the hydrogen on the α-carbon does not participate in the reaction. Their suggested mechanism is as follows:

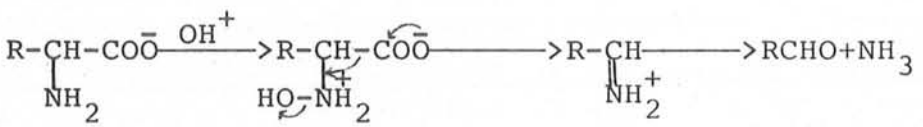


In this scheme an oxidizing agent (formally represented as OH^+) attacks at the α-carbon atom as an electrophile with the concurrent loss of carbon dioxide to give the carbinolamine as a primary unstable intermediate which on further rapid decomposition gives rise to aldehyde and ammonia. In order to confirm the occurence of carbinolamine as an unstable intermediate they oxidized, α,N-diphenylglycine (α-anilinophenylacetic acid) under alkaline conditions and seperated in good quantity the expected benzalaniline

formed from the corresponding unstable carbinolamine by spontaneous dehydration. This experiment, thus substantially proved, the intermediacy of carbinolamine:



Another reaction pathway to represent the already known effects of oxidizing agents on α-amino acids was postulated by Sweeley and Horning[44]. They observed that ferric ion catalysed the decomposition of N,N-dimethylglycine Oxide and put forward a mechanism for α-amino acid oxidation involving formation of an N-oxide, followed by decomposition of the latter by a concerted β-elimination of CO_2 and hydroxide ion through the agency of a ferric ion amino acid complex.



The amino acid N-Oxide intermediate of this mechanism may be considered as the zwitterionic form of α-hydroxyamino acids, which had earlier been postulated as intermediates in α-imino acids oxidation[45].

The general mechanism of oxidation put forward by Spenser, Crawhall and Smyth differs from that of Sweeley and Horning in two respects: The first entails $\overset{+}{O}H$ attack on the α-carbon and simultaneous decarboxylation, leading to an unstable carbinolamine. This is a generalization of the pathway proposed in the special case of oxidation by N-bromosuccinimide when Br^+ was taken to be the electrophilic species. The second mechanism requires OH^+ attack at the nitrogen to yield an amino acid N-oxide (i.e., the amphoteric form of a hydroxyamino acid), which decarboxylates in subsequent step. This is a generalization of the reaction sequence put foward by Langheld[42] for the oxidation of α-amino acids by hypochlorites.

Contrary to the predictions of Sweeley and Horning's which demands the decomposition of α-hydroxyamino acid to aldehyde, carbon dioxide and ammonia, α-hydroxyamino acids (proposed intermediates in α-amino acid oxidation) were found to udnergo spontaneous disproportionation reaction in an aqueous medium, slowly at room temperature and rapidly at high temperature to yield almost equimolar quantities of the corresponding α-amino acid and α-oximino acids as the primary products[46]. The stiochiometry of the oxidation of

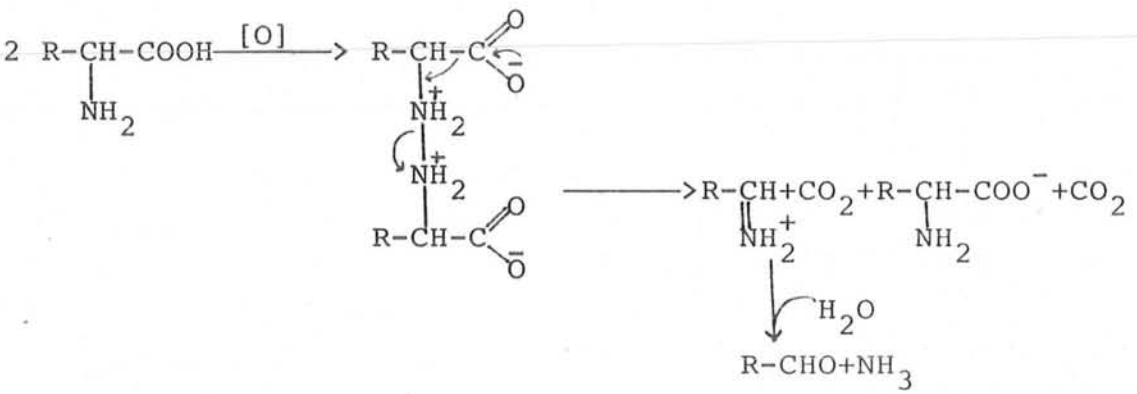
an amino acid is such that one mole of an amino acid on treatment with two equivalents of an oxidizing agent undergoes complete converstion to aldehyde, carbondioxide and an ammonia Transformation of an α-amino acid to the corresponding α-hydroxyamino acid also requires two equivalents of an oxidizing agent. Since as is now known that amino acid is regenerated spontaneously from α-hydroxyamino acids, more than two equivalents of an oxidizing agent would be needed, to transform one mole of an amino acid to the final products, if this conversion was to proceed by way of an α-hydroxyamino acid intermediate. This argument, therefore, eliminates the mechasnism put forward by Sweeley and Horning[44] and leaves the mechanism of Spenser, Crawhall and Smyth[32] for further consideration.

HYDRAZO ACIDS:POSSIBLE INTERMEDIATE IN AMINO ACID OXIDATION

The aim of the present work is to synthesize α-hydrazo acids and to study the products of chemical oxidation. The idea about hydrazo acids being possible intermediates in amino acid oxidation came from the fact that hydrazine is industrially prepared by the oxidation of ammonia[47].

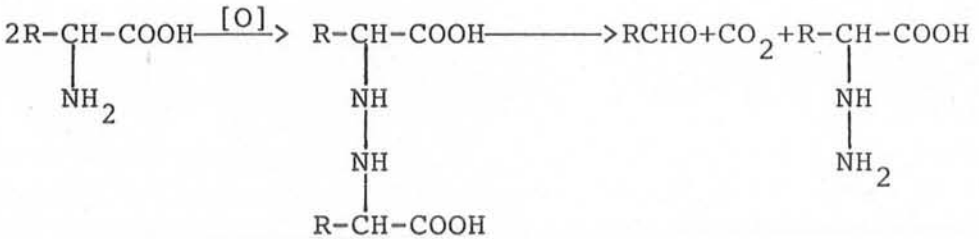
$$2NH_3 \xrightarrow{[O]} NH_2{-}NH_2 + H_2O$$

On the basis of this oxidation an alternate possible mechanism of α-amino acid oxidation through the intermediacy of hydrazo acid can be considered



This mechanism involves the utilization of two equivalents of an oxidizing agent per molecule of an α-amino acid to give one mole of the corresponding aldehyde, carbon dioxide and ammonia.

Another mechanism requires the formation of hydrazino acids as intermediate



The defect in this mechanism is that one mole of an α-

amino acid requires more than two equivalent for complete oxidation. However, it will be interesting to find the products of oxidation of α-Hydrazino acids.

Thiele and Heuser synthesized 2:2́ - Hydrazoisobutyric acid starting from acetone by the Strecker synthesis scheme[48-49] Thiele and Baily reported the synthesis of 2:2́ -Hydrazo dipropionic acid from acetaldehyde[50]. Some other hydrazo acids are also referred in the organic chemistry literature. Hydrazophenylacetic acid prepared from phenylglyoxylic acid on its reaction with hydrazine in alcohol. The resulting hydrazine phenylglyoxylic acid hydrazone was mixed with HCl to give azine. This azine on reduction with 2.3% Na-Hg gives hydrazophenylacetic acid[51].

August Darapsky et al have prepared hydrazo-p-tolylacetic acid from p-tolylglyoxylic acid, hydrazo γ-phenylbutyric acid from benzylpyruvic acid and hydrazo α-naphthylacetic acid from α-naphthylglyoxylic acid. The procedure was same as for hydrazophenylacetic acid[52].

We have considered the synthesis of an hydrazo acid by the reduction of a mixture of hydrazino acid and α-keto acid. The advantage of this method is to obtain hydrazo acid with mixed substituents instead of the same substituent. The chemistry of hyrazino acids

has been studied in detail. Traube et al synthesized α-hydrazinobutyric acid[53]. Theile and Heuser reported the formation of α-hydrazino- α- methylpropionic acid and its derivatives [54]. He also synthesized α-hydrazinopropionic acid from acetaldehyde[55]. Traube described the preparation of a series of α-hydrazino aliphatic acids by the reduction of the isonitramino acids[56]. August Darapsky et al prepared a number of acid by the reduction of α-keto acid hydrazone. They also prepared a series of α-hydrazino acids from α-halo acids and hydrazine either in alcohol or in water [57]. Bailey[58] and Berger[59] similarly used α-halo acids for the synthesis of these acids.

A.Carmi et al improved the Darapsky's method using the ion-exchange resins for the isolation and purification of the hydrazino acids[60]. They extended the reaction to the preparation of α-(1-methyl hydrazino)-aliphatic acids. These workers prepared the hydrazone derivatives of α-hydrazino acids with aromatic aldehydes. E.J.Glamkowski et al[61] prepared these acids from the reduction of a hydrazone of an α-keto acid, M. Sletziner et al synthesized α-hydrazino acids by the functionalisation of the carbonyl compounds in a Strecker-like Synthesis[62]. Optically active α-hydrazino acids were synthesized from the reaction of hydrazino with α -halo acids by Darapsky[63]. Harmut Niedrich[64] Sandor

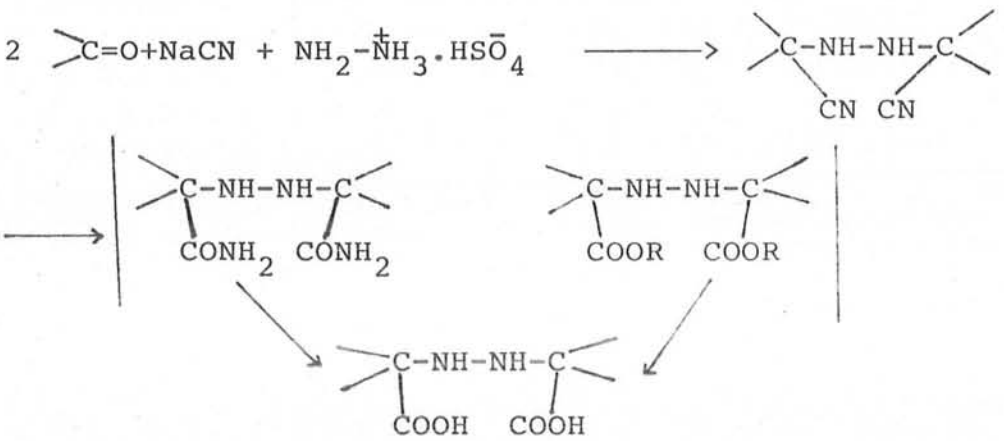
Karaday et al prepared optically active α- (3, 4 dihydroxy benzyl) α-hydrazinopropionic acid from dl- α-hydrazino-α-(4-hydroxy-3-methoxybenzyl) propionitrile that was resolved by l-menthoxyacetylation[65]. Kazuo Ahiwa and Shun-ichi-Yamada prpeared L- α- hydrazino acids from L- α-amino acids by nitroamination and subsequent reduction[66]. α-Hydrazino acids have also been isolated from natural source[67].

α-Hydrazino acids have aroused interest widely in recent years mainly because of their biological activity. A.Carmi et al[60] have investigated them as potential antimetablites. A number of research workers have described these compounds as inhibtors of amino acid decarboxylase [61,68-74].

A. Anraku and coworkers reported these compounds as inhibitors of growth of cell and the transport activity[75].

Different Possible Routes by which Hydrazo Acids can be Synthesized.

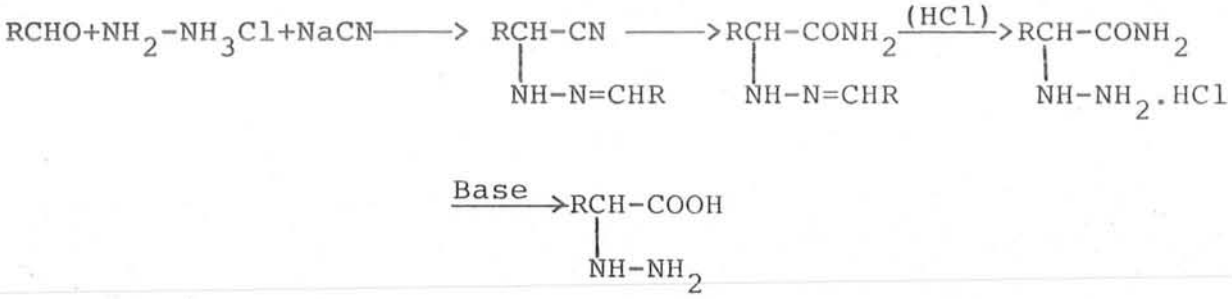
I. By the hydrolysis of 1,2-Di-1(1-cyano) alkylhydrazine. Following the Strecker synthesis scheme, 1-2-Di-1(1-cyano) alkylhydrazines are easily prepared from NaCN, Hydrazine Sulphate and the respective ketone. The synthesis scheme through amide and ester is given below:



II. Synthesis via α-Hydrazino acids.

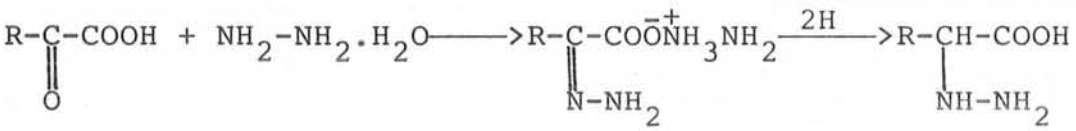
The hydrazino acids with the general structure R-CH(NHNH$_2$)-COOH, have been prepared by four methods:

(i) α-hydrazino acids by the hydrolysis of α-hydrazino nitriles that are prepared through Strecker synthesis and subsequently hydrolysed to acid through amide



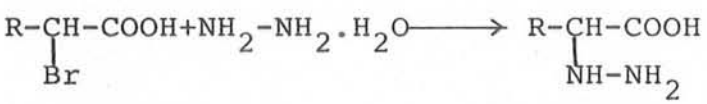
(ii) α-Hydrazino acids from α-keto acids.

α-keto acids react with $NH_2-NH_2.H_2O$ in alcohol to give hydrazine keto acid hydrazone which on reduction forms hydrazino acid.



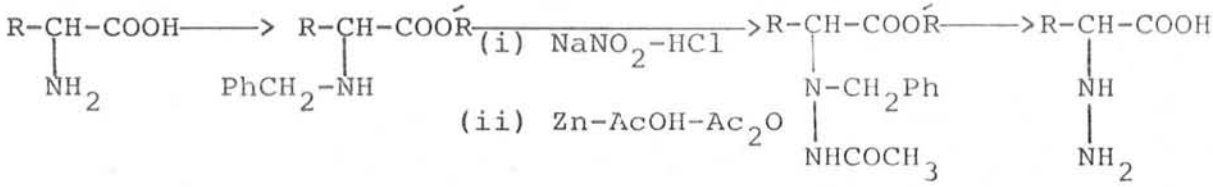
(iii) From α- halo acid.

Excess of Hydrazine on reaction with α-halo acids give α-hydrazino acids

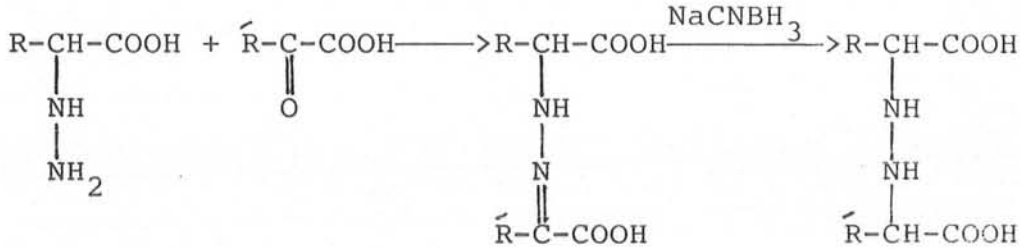


(iv) From α-amino acids.

α-Amino acids can be converted to α-hydrazino acids by the followig scheme.

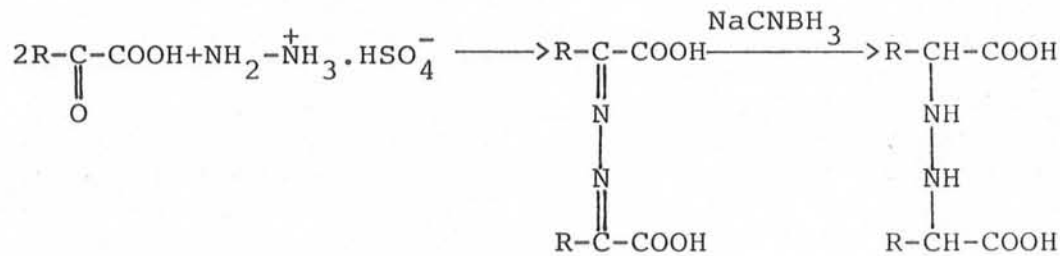


In second step the α-hydrazino acids can be condensed with α -keto acids and reduced by $NaCNBH_3$ to give various hydrazo acids.



The significance of this procedure lies in the variable nature of R - and R' -.

III. From the reduction of Azine produced from -keto acid and hydrazine sulphate. The scheme is outlined below:



EXPERIMENTAL

I

A. Synthesis of Nitriles

1. 1, 2-Di-1-(1-cyano) cyclohexyl hydrazine.

In a screw capped bottle were placed 15.4g (0.32 mole) of sodium cyanide, 20.5 g. (0.16 mole) of hydrazine sulphate and 400 ml of ice water. The bottle was capped to prevent loss of hydrogen cyanide and was kept in an ice bath for 15 minutes. To the cooled mixture was added 29.4 g. (0.3 mole) of cyclohexanone. The bottle was recapped and cooled for an additional fifteen minutes. The bottle was then shaken intermittently over a period of six hours and allowed to stand an additional 14 hours. The bottle was cooled before opening. The suspension was filtered by means of suction and the cake was washed with 250 ml. of ice water thoroughly. After the crude product had been pressed dry on filter, it was transferred to a 500 ml. conical flask and 150 ml. of boiling 95% ethanol was added. Brought the suspension as quickly as possible into solution by warming on hot plate and filtered through a prewarmed Buchner funnel. Washed the filter with an additional 10 ml. hot ethanol to redissolve any organic residue. Combined filtrates were warmed to dissolve any precipitate. The solution was allowed to stand undisturbed for 6 hours in

an ice box. The product was collected on a Buchner funnel, washed with 15 ml. of cold 95% ethanol and dried over solid $CaCl_2$ in a vacuum dessicator.

Yield = 24 g (64.6%), M.P= 147 -149 ^{o}C

IR $\bar{\nu}$ max cm^{-1} = 3262 (s), 3004 (m), 2956 (s), 2926 (s), 2230 (s), 1455(s), 1515 (m).

2. 1,2-Di-1-(1-cyano) Cyclopentylhydrazine

In a screw capped bottle were placed 15.4 g (0.32 mole) of sodium cyanide, 20.5 g (0.16 mole) of hydrazine sulphate and 400 ml of ice-water. Working the same procedure as in (1) added 25.2 g (0.3 mole) cyclopentanone.

Yield= 17.66g(54%), M.P.= 84 - 85 ^{o}C

3. 1,2-Di-1,(1-Cyano) Butylhydrazine

20.5 g hydrazine sulphate (0.16 mole), 15.4 g. (0.32 mole) sodium cyanide was placed in a screw -capped bottle containing 400 ml. ice water. Capped the bottle to prevent the loss of hydrogen cyanide and cooled in an ice bath for fifteen minutes. 21-6 g (0.3 mole) of n-butyraldehyde was added to the cooled solution and bottle was recapped and cooled for additonal 15 minutes in an ice bath.

The reaction mixture was shaken over a period of 6 hours intermittently and allowed to stand for an additional 14 hours. Then cooled the bottle in ice bath for some time and opened. Oily layer was separated in a separatory funnel and the aqueous layer further extracted with ether. Mixed the ethereal extract with oily layer and washed with water. Dried over anhydrous Na_2SO_4 and evaporated the ether on r.v.evaporator yellowish syrupy liquid was obtained.

Yeild =17.5 g(60%)

IR: $\sqrt{}$ max cm^{-1} =3280 (s), 2969 (s), 2233 (m), 1520(w) 1466 (s)

4. 1,2-Di-2(2-Cyano) Propylhydrazine

In a screw capped bottle were placed 15.4 g (0.32 mole) of sodium cyanide, 20.5 g (0.16 mole) of hydrazine sulfate and 400 ml of ice-water. The bottle was capped to prevent the loss of hydrogen cyanide and was kept in an ice bath for 15 minutes. To the cooled mixture was added 17.4 g (0.3 mole) of acetone. The bottle was recapped and cooled for an additional 15 minutes. The bottle was then shaken over a period of 6 hours intermittently

and allowed to stand an additional 14 hours time. The bottle was cooled again before opening. Filtered and washed the suspension with 250 ml. of ice water. Pressed the product dry on filter and then recrystallized from diethyl ether.

Yield = 17.4 g (70%), M.P. =90 - 92 ^{o}C

IR: $\bar{\nu}$ max. cm^{-1}= 3278 (s), 2996 (s), 2234 (s), 1526 (m), 1469 (s), 1202 (s) and 836 (s)

5. <u>1,2-Di-2-(2-Cyano) butlhydrazine</u>

In a screw capped bottle containing 400 ml. ice water were placed 15.4 g. (0.32 mole) of sodium cyanide and 20.5 g. (0.16 mole) of hydrazine sulphate. Cooled the bottle for 15 minutes in ice bath. 21.6 g (0.3 mole) of ethyl methyl ketone were added and the bottle after being recapped was cooled for additional fifteen minutes. Worked up as in the preparation of (3). Syrupy liquid was obtained.

Yield = 18.3 g (63 %)

IR: $\bar{\nu}$ max. cm^{-1}= 3280 (s), 2950 (s), 2236 (m), 1518(m), 1473 (s) and 888 (s)

6. <u>1,2-Di-2(2-Cyano)Pentylhydrazine</u>

15.4 g (0.32 mole) of sodium cyanide, 20.5 g

(0.16 mole) of hydrazine sulphate and 400 ml ice water were taken in a screw capped bottle. Cooled the bottle in an ice bath. After fifteen minutes 25.8 g. (0.3 mole) of methyl propyl ketone were added to the bottle, recapped the bottle and cooled in ice bath for additional fifteen minutes. Proceeded further as in (3).

Yeild = 22 g (66%)

IR: $\bar{\nu}$ max cm^{-1}=3280 (s), 2955 (s), 2236 (m), 1515 (m), 1470(s) and 889 (s).

7. <u>1,2-Di-3-(3-Cyano)Pentylhydrazine</u>

Sodium cyanide 15.4 g (0.32 mole), hydrazine sulphate (0.16 mole) and 400 cc. of ice water were placed in a screw capped bottle. The bottle was capped to prevent the escape of hydrogen cyanide and placed in an ice bath for 15 minutes. After this time 25.4 g. (0.3 mole) of diethyl ketone was added. Recapped the bottle and cooled for an additional 15 minutes. Proceeded further as in (3).

Yield = 21.5 g (65%)

IR: $\bar{\nu}$ max.cm^{-1}= 3280(s), 2974(s), 2236(m), 1518(w), 1464(s) and 888 (s).

B. Conversion of Nitrile to Amide

1. In a 250 ml. reagent bottle was placed 40ml. con. sulphuric acid [sp. gr. 1.84]. The bottle was cooled in refrigerator. To the chilled sulphuric acid was added previously cooled powder of 1,2-Di-1(1-cyano) cyclohexylhydrazine and the bottle was agitated to completely mix the powder with cold acid. When the solution became clear, the reagent bottle was kept for two months in the cold. After that time the mixture was added dropwise in cracked ice. The container was cooled at the same time in an ice-salt mixture. The mixture was thus diluted to about 250 ml while keeping it cool. Neutralized the acid with 25% NH_3 while keeping the solution temperature below 10 ^{o}C. When the solution became neutral it was allowed to stand for some time in ice-bath. White ppt of the 1,2-Di-1(1-carbamido) cyclohexylhydrazine settled down in the flask. It was collected on a Buchner funnel. Recrytallized from ethanol.

Yield = 2.5 g. (44%), M.P= 194 - 98 ^{o}C (decom.)

IR: $\bar{\nu}$ max. cm^{-1}= 3430-3376 (s.doublet), 3280 (s), 3178 (s), 2938 (s) 2860 (s), 1665 - 1653 (s.b), 1557 (w), 1467 (s), 1392 (s) and 963 (s).

C. Esterification of Nitriles

1. 1,2-Di-2-(2carbmethoxy) Propylhydrazine

Anhydrous HCl was passed through a stirred suspension of powdered hydrazoisobutyronitrile 8.3 g (0.05 mole) in 65 ml. methanol cooled in ice-bath. When enough HCl has been passed to saturate the reaction mxiture, the flow was stopped and the reaction mixture was stirred at ambient temperature untill there was obtained a clear solution. This solution was kept at 5 ^{o}C for eighteen hours. As no solid appeared in the flask, it was evaporated on rotary vacuum evaporator at 30 ^{o}C to dryness. The dry residue was then added portionwise to stirred ice-cooled 12% HCl(100 ml). Allowed the solution to stand at room temperature for three hour and then evaporated on rotary vacuum evaporator on 45 ^{o}C. The diester was obtained as sticky solid. Repeated purification by recrystallization from pet - ether gave prism like cystals.

Yield = 7.2 g (62%), M.P. =53 - 4 ^{o}C.

I.R, $\bar{\nu}$ max. cm^{-1}= 3280(s), 2994 (s), 1687 (s), 1526 (m) 1493 (s) and 885 (s)

2. 1,2-Di-2-(2-carbmethoxy)Butylhydrazine.

3. 1,2-Di-2(2-carbmethoxy) Pentylhydrazine.

4. 1,2-Di-3(3-carbmethoxy) Pentylhydrazine.

0.05 mole of each nitrile was taken in 65 ml. methanol. According to the procedure (1)the esters were prepared. In all the three cases the esters were oily liquids.

Yields=

2	7.8 g. (60%)
3	8.9 g. (62%)
4	9.0 g. (63%)

D. Saponification of Esters

1. 2;2 Hydrazoisobutyric Acid.

4 g. (0.1 mole) sodium hydroxide was dissolved in 20 ml. of water. The powdered ester 7.2 g (0.03 mole) was added to it. Refluxed for half an hour. Cooled to the room temperature and acidified with HCl to pH 4. Evaporated the water on rotary vacuum evaporator. Dissolved the residue in excess of absolute ethanol separated the NaCl by filtration. On concentrating the solution and subsequent cooling, crystals appeared. Filtered and washed with absolute ethanol. Dried in dessicator over phosphorus pentoxide.

Yield = 2.04 g. (20% based on the starting Nitrile).

MP. 220 ^{o}C ;(lit. 223 ^{o}C)

IR: $\bar{\nu}$ max. cm^{-1}=3278 (s), 3086 (s), 2990 (s), 2950 - 2500 (b), 1704 (s), 1605 (m), 1497 (s), 1477 (s), 1222 (m) and 885 (s)

II. SYNTHESES OF α-HYDRAZINO ACIDS

1. α-Hydrazinoacetic acid: To 50 g. (0.5 mole) of 32% aqueous hydrazine was added gradually 9.45 g. (0.1 mole) monochloroacetic acid. After forty eight hours 8.5 g. (approximately 0.2 mole) sodium hydroxide was added and the solution distilled in vacuo to dryness. The residue was treated with 65 ml. ethanolic HCl (approx. 30% w/v), refluxed gently, cooled and saturated with gaseous HCl for two hours. The solution was boiled with 50 ml. absolute ethanol and filtered hot. The ester hydrochloride crystallized yielding 10.3 g. (66.5%) melting point 150 - 152 °C.

A solution of 4.65 g. (0.03 mole) ethyl hydrazino acetate hydrochloride in 35 ml. water was refluxed with stirring in the presence of 1.5 g. Amberlite CG 120 for three hours. Filtered solution was passed through Amberlite CG 400. . The effluent was concentrated to 5 g. in vacuo and added portionwise to 25 ml. absolute ethanol. The crude acid was recrystallized from hot water.

Yield = 45% , M.P = 148 - 151 °C

2. α-Hydrazinobutanoic acid: 8.35 g. (0.05 mole) brómobutanoic acid was added gradually with cooling to 32 g.

(0.5 mole) of 50% aqueous hydrzine. After the addition was complete the reaction mixture was allowed to stand for 48 hours. The excess hydrazine was distilled on rotary vacuum evaporator. The dried residue was dissolved in 100 ml. water and passed through Amberlite CG 120 (strong acid form). Column was first washed with distilled water, acidic effluent came out. On evaporation a crystalline solid was obtained. It was identified as α-hydroxybutanoic acid. After washing the column with water to neutrality, 400 ml. 4% ammonia was passed and the elute concentrated to dryness. The resulting solid residue was disolved in the least quantity of water and then absolute ethanol was added gradually. 30ml ethanol was required. 2.36 g (40%) α-hydrazinobutanoic acid was obtained. M.P = 200 - 205 ^{o}C.

IR $\bar{\nu}$ max. cm^{-1} = 3262 (s), 2974 (s), 2722 (b), 2206 (m), 1626 (s), 1587 (s), 1516 (s), 1413 (s), 1236 (s)

3. <u>α-Hydrazino - β -methylbutanoic Acid</u>: 9.05 g. (0.05 mole) α - Bromo- β -methylbutanoic acid was added dropwise with cooling to 32 g. (0.5 mole) of 50% aqueous hydrazine. The reaction mixture was worked up as described under α-hydrazinobutanoic acid. Evaporation of the alkaline eluate gave 3.1 g. (47%) α-hydrazino- β -methyl butanoic acid.

Recrystallized from water. M.P = 230-5 ^{o}C.

IR: $\bar{\nu}$ max. cm^{-1} = 3268 (s), 2968 (s), 2872 - 2722 (b), 2212 (s), 1683 (w), 1626 (s), 1597 (m), 1506 (s), 1413 (s), 1242 (s), 1209 (m).

4. α-Hydrazinohexanoic Acid. 9.75 g. (0.05 mole) α-Bromohexanoic acid was added dropwise with cooling to 32 g. (0.5 mole) of 50% aqueous hydrazine. After the addition was compelte the reaction mixture was allowed to stand for 48 hours. The reaction mixture was worked up as described under α-hydrazinobutanoic acid. From the alkaline eluate 3.06 g. (42 %) product was isolated.
M.P = 197 - 200 ^{o}C.
IR: $\bar{\nu}$ max. cm^{-1} = 3262 (s), 2956 (s), 2866 - 2722 (b), 2212 (m), 1629 (s), 1587 (s), 1530 (s), 1506 (s), 1413 (s), 1341 (s).

5. α-Hydrazino Phenylacetic Acid. 4.30 g. (0.02 mole) of α-Bromophenylacetic acid was added in small portions with cooling to 12.8 g. (0.2 mole) of 50% aqueous hydrazine. After the addition had been complete the reaction mixture was allowed to stand for 48 hours. The excess hydrazine was removed on rotary vacuum evaporator. The dried residue was dissolved in water. Acidified with HCl

and extracted with ether to remove, any residual mandelic acid. Some ethanol was added and the reaction mixture was allowed to stand. Colourless crystals of the α-hydrazino phenylacetic acid settled on the bottom. The crystals were collected on a Büchner funnel washed with 95% ethanol and dried in dessicator. 1.49 g. =(45%)yield. M.P = 189 ^{o}C.

III. SYNTHESES OF α-HYDRAZO ACIDS

1. α - Hydrazo Phenylacetic Acid:

6 g. (0.04 mole) of phenylglyoxylic acid were taken in a 250 ml. round bottom flask in 50 ml. absolute ethanol. To the solution with stirring was added excess of anhydrous hydrazine. The solution became hot and turned yellow. Stirring was continued for one hour. After that time the reaction mixture was evaporated to dryness on rotary vacuum evaporator. The hydrazine phenylglyoxylic acid hydrazone was formed. Its melting point was checked. It melted on 160 - 1 ^{o}C. A small portion of this compound was reduced with $NaCNBH_3$ to give phenylhydrazinoacetic acid. M.P = 188 ^{o}C. The remaining hydrazine phenylglyoxylic hydrazone was treated with HCl. Immediately after mixing with HCl the yellow azine precipitated down. The azine was collected on Büchner funnel and repeatedly washed with distilled water and dried. M.P = 150-2 ^{o}C.

2.96 g (0.01 mole) of azine was taken in 30 ml distilled water. To the stirred slurry was added 1.26 g. (0.2 mole) of $NaCNBH_3$. the pH of the mixture was adjusted on 5-6. After 24 hours the stirring was stopped, brought the pH to 2-3 and filtered after one hour.Washed the filter repeatedly with distilled water and dried.

M.P = 160 -1 ^{o}C. Yield = 2.85 g (95%).

I.R: $\bar{\nu}$ max. cm^{-1} = 3274 (s), 3244 (s), 3130 (s), 3004 (w), 2800 - 2608 (b), 1719 (s - broad), 1605 (m), 1500 (m), 1458 (s).

2. <u>α - Hydrazo (p)- Tolyl acetic Acid</u>: p-Tolylglyoxylic acid 4.92 (0.03 mole) was dissolved in 50ml. absolute methanol. To this solution was added an excess of $NH_2NH_2H_2O$ (7.5 ml. about 0.15 mole) slowly with cooling. After the addition of hydrazine the reaction mixture was kept at 70^{o}C for an hour. Diluted with water and added dilute HCl to precipitate out the azine. Filtered the azine and washed with distilled water. Dried in dessicator.

Yield = 4.374 g. (90%).

4.374 g. azine was taken in a conical flask. Dissolved in methanol. Added 3.789 (0.06 mole) $NaCNBH_3$ to it and stirred for 24 hours. Maintained the pH of reaction mixture at 5-6. At the end the pH was brought to 2-3. Filtered after an hour,- washed with cold water repeatedly. Dried. Yield = 4.1 g. (83.3%), M.P = 148 - 150 ^{o}C.

3. <u>α -Hydrazo Naphthylacetic Acid</u>. 4 g. (0.02 mole) α -naphthylglyoxylic acid was dissolved in (30 ml.) absolute ethanol. To this solution was added an excess of

NH_2-NH_2.H_2O (6 ml.), slowly with cooling. After one hour, evaporated the ethanol on rotary vacuum evaporator and dissolved the hydrazine salt of napthyl glyoxylic acid hydrazone in water. The azine was precipitated by the addition of dil HCl. Filtered the azine, washed with water. The azine was dissolved in methanol. 2.52 g. (0.04 mole) $NaCNBH_3$ was added and stirred the mixture with adjusting the pH at 5-6 for 24 hours. After that time the pH was brought to 2-3 with the addition of dil. HCl. Filtered after one hour, washed repeatedly with cold water, dried in dessicator over $CaCl_2$. Yield = 1.72 g.(43%), M.P = 185 - 7 ^{o}C.

IV. Attempt to synthesise α-hydrazo acids from α-keto acid and α-hydrazino acids.

i. 1.18 g (0.01 mole) α-hydrazino butyric acid was taken in a round bottom flask (100 ml). To this was added 1.5 g (0.01 mole) phenylglyoxylic acid in 30 ml ethanol. The mixture was stirred for 24 hours and then 0.65 g (0.01 mole) sodium cyanoborohydride was added. The pH of the system was adjusted on 5-6. The stirring was continued for further 24 hours. Evaporated the solvent on rotary vacuum evaporator. The solid residue was treated with dil hydrochloric acid to destroy any remaining sodium cyanoborohydride. Extracted with ether. The ether extract on

evaporation gave unreacted phenylglyoxylic acid and mandelic acid. The remaining solution was evaporated to dryness and again dissolved in minimum quantity of distilled water to have any hydrazo acid. No solid product could be isolated. The solution was checked for the hydrazino acid by running a paper chromatogram with reference hydrazino acid. Only one spot appeared in the dissolved residue and that had an equal Rf value with the reference (α -hydrazino butyric) acid.

2. By the above given procedure hydrazo acid could not be obtained from α - hydrazino- β -methylbutyric acid.

3. Hydrazo acid could not be obtained from α-hydrazino hexanoic acid.

V. Oxidation Studies of Hydrazo and Hydrazino Acids.

1. Oxidation of Hydrazophenyl acetic acid with two equivalents of pottassium permangnate.

300 mg (0.001 mole) phenylhydrazo acetic acid was taken in a 50 ml two neck r.b. flask. The compound was dissolved in 10 ml of 0.2N sodium hydroxide solution. The flask was fitted with a dropping funnel containing 10ml 0.2N(0.002 eq) pottassium permanganate solution. Nitrogen gas inlet was also fitted to the funnel. To the second neck of flask was fitted a reflux water condensor,attacked to a delivery tube at the top that passed through the trap containing barium hydroxide solution. Another trap with Nessler's reagent to test the presence of ammonia was attached next. Dropped the pottassium permanganate solution, stirred the reaction mixture with the continuous supply of nitrogen gas.White ppt of barium carbonate was formed in the first trap. The Nessler's reagent did not change colour. Barium carbonate was filtered,washed with distilled water, dried and weighed,0.188 g (approx.0.001 mole). The reaction mixture was filtered. Extracted the filtrate twice with ether. Evaporated the ether at room temperature. To the oily residue, a solution of 2, 4-dinitrophenylhydrazine was added. Orange coloured ppt

was filtered, washed with dilute HCl and distilled water. Dried and weighed, 0.236 g (approx. 0.001 mole).

M.P= 230 - 234 ^{o}C (benzaldehyde 2,4-Dinitrophenyl hydrazone. M.P=237 ^{o}C).

2. Oxidation of hydrazophenyl acetic acid with an excess of pottassium permanganate.

300 mg (0.001 mole) hydrazophenylacetic acid was taken in a 50ml two neck r.b.flask. Dissolved in 10 ml 0.2N sodium hydroxide solution. The set up of apparatus was identical to that used in (V-1). Pottassium permanganate was used in excess. The reaction mixture was warmed to 70 ^{o}C. Cooled and destroyed the excess of permanganate with the addition of ethanol. Filtered and acidified with dil hydrochloric acid. Extracted with ether. Evaporation of ether gave powdered material,weight=221 mg (approx.0.002 mole). Recrystallized from water,MP=120 ^{o}C. Mixed M.P=120 ^{o}C. It was identified as benzoic acid. Barium carbonate was filtered and dried. It weighed 371 mg (approx. 0.002 mole). Ammonia could not be detected.

3. Successive oxidation of hydrazo phenylacetic acid.

300 mg (0.001 mole) hydrazo phenyl acetic acid

was taken in a 50 ml conical flask. The acid was dissolved by the addition of 10 ml 0.2N sodium hydroxide solution. 10 ml 0.2N (0.002 eq) pottassium permanganate solution was added to the above mentioned solution in the following manner.

The additions were made in five portions, 2ml portion each and after each addition the reaction was allowed to complete till the disappearance of pinkish colour of permanganate and the appearance of manganese dioxide ppt. After each addition a very small portion was drawn by pipette ,filtered and then a spot was applied on a precoated (cellulose) TLC plate. The reference spots of phenylglycine and hydrazophenyl acetic acid were also applied. The plate was developed in BAW (4:1:5). The spots applied from the oxidation mixture showed bluish colour after spray with ninhydrin solution at distance comparable with phenylglycine. The spot that was applied after the 2 ml pottassium permanganate solution has been added was rather intense.

4. Oxidation of α-hydrazino butanoic acid.

118 mg (0.001 mole) α-hydrazino butanoic acid was taken in a 50 ml two neck r.b. flask. Dissolved the

acid by the addition of 10 ml 0.2N sodium hydroxide solution. The apparatus was set up as in (V-1), with the only difference that before the barium hydroxide trap, a trap of the acidic solution of 2,4-Dinitrophenylhydrazine was added. 10 ml 0.2N (0.002 eq) pottassium permanganate solution was added through dropping funnel. After the completion of reaction, connected the neck of r.b. flask directly with the first trap and warmed the reaction mixture above 50 ^{o}C. Orange ppt was formed in the first trap. In the barium hydroxide trap barium carbonate was formed. Filtered both the precipitates, washed with the distilled water and dried. The expected propanal 2,4-Dinitrophenyl hydrazone weighed 201 mg (approx. 0.001 mole). Barium carbonate weighed 171 mg (approx. 0.001 mole).

VI. Decomposition Studies

1. Decomposition of α-hydrazophenyl acetic acid in aqueous solution.

75 mg (0.25 mmole) hydrazophenyl acetic acid was taken in 50 ml r.b. flask. Added 30 ml distilled water to it and fitted a reflux water condensor. From the top of the condensor, a delivery tube was attached that led to a trap containing barium hydroxide solution as in (V-1). Heated in a boiling water bath for several hours. Cooled and extracted with ether. The ether was evaporated at room temperature and the residue treated with 2, 4-Dinitrophenylhydrazine solution. Orange ppt of benzal - 2,4-Dinitrophenylhydrazone appeared, filtered, washed with dil hydrochloric acid and distilled water. Dried and weighed, 60 mg (approx. 0.25 mmol). M.P= 233 ^{o}C. The barium carbonate from the trap was filtered,wahsed with distilled water, dried and weighed. 41 mg (0.25 mmole).

2. Decomposition of α-hydrazophenylacetic acid in acidic solution.

75 mg (0.25 mole) hydrazophenylacetic acid was taken in 50 ml r.b. flask. Added 30 ml distilled water and

4 -5 drops of concentrated hydrochloric acid. Fitted a reflux water condensor. From the top of the condensor a delivery tube led to a trap containing barrium hydroxide solution as in (V-1). Warmed for an hour at 95 ^{o}C. Cooled and extracted with ether. Evaporated the ether and treated the residue with acidic solution of 2,4-Dinitrophenylhydrazine. Orange coloured ppt was filtered, washed with dil. hydrochloric acid and distilled water, dried and wighed, 63 mg (approx. 0.25 mmole). M.P=233-234 ^{o}C. Barium carbonate was filtered, washed with distilled water and dried. Weight = 40 mg (approximately 0.25 mmole).

SUMMARY
(TABLES)

I——A. Syntheses of Nitriles

S.No.	Aldehyde or Ketone $R_1R_2C=O$	Akkyl (Cyano) Hydrazines $R_1R_2C(CN)-NH-NH-CR_1R_2(CN)$	Yield	M.P. ^{O}C
1.	$R_1,R_2=-(CH_2)_5-$	$R_1R_2=-(CH_2)_5-$	64.6%	147-149
2.	$R_1,R_2=-(CH_2)_4-$	$R_1R_2=-(CH_2)_4-$	54%	84 - 85
3.	R_1=Pr, R_2=H	R_1=Pr, R_2=H	60%	Viscous oil
4.	R_1,R_2=Me	R_1,R_2=Me	70%	90 - 92
5.	R_1=Et, R_2=Me	R_1=Et, R_2=Me	63%	Viscous oil
6.	R_1=Pr, R_2=Me	R_1=Pr, R_2=Me	66%	Viscous oil
7.	R_1,R_2=Et	R_1,R_2=Et	65%	Viscous oil

I——B. Conversion of Nitriles to Amides

S.No.	Alkyl (Cyano) Hydrazines $R_1R_2C(CN)-NH-NH-C(CN)R_1R_2$	Amide $R_1R_2C(CONH_2)-NH-NH-C(CONH_2)R_1R_2$	Yield	M.P. ^oC
1.	$R_1,R_2 = -(CH_2)_5-$	$R_1,R_2 = -(CH_2)_5-$	44%(i)	194-198(d)
2.	$R_1R_2 = -(CH_2)_4-$	$R_1,R_2 = -(CH_2)_4-$	Failed to give corresponding amides*.	
3.	R_1=Pr, R_2=H	R_1=Pr, R_2=H		
4.	R_1,R_2=Me	R_1R_2=Me		
5.	R_1=Et, R_2=Me	R_1=Et, R_2=Me		
6.	R_1=Pr, R_2=Me	R_1=Pr, R_2=Me		
7.	R_1,R_2=Et	R_1,R_2=Et		

*Following methods were used for the conversion of nitriles to amides.

i. Dissolved in cold H_2SO_4 (98%).[77]
ii. Treatment with KOH in ter-Bu-OH.[78]
iii. In Formic acid/HCl.[79]
iv. Poly phosphoric acid.[80]
v. Alkali hydroxide in diethylene glycol.[81]
vi. Using Cu^o catalyst.[82]
vii. Using weakly alkaline Boric acid salts.[83]

I——C. Nitriles to Esters

S.No.	Alkyl (Cyano) hydrazines $R_1R_2C(CN)-NH-NH-CR_1R_2(CN)$	Esters $R_1R_2C(COOMe)-NH-NH-CR_1R_2(COOMe)$	Yield	M.P. °C
1.	$R_1R_2-(CH_2)_5-$	$R_1R_2=-(CH_2)_5-$	——*	
2.	R_1,R_2=Me	R_1,R_2=Me	62.1%	53-54
3.	R_1=Et,R_2=Me	R_1=Et,R_2=Me	60%	Viscous oil
4.	R_1=Pr,R_2=Me	R_1=Pr,R_2=Me	62%	-do-
5.	R_1,R_2=Et	R_1,R_2=Et	63%	

*Ester could not be obtained under these reaction conditions.[84]

I———D. Hydrazo Acids from Esters and Amides

Nature of Comp.	$R_1R_2C(COOMe)-NH-NH-CR_1R_2(COOMe)$ and $R_1R_2C(CONH_2)-NH-NH-CR_1R_2(CONH_2)$	Acid $R_1R_2C(COOH)-NH-NH-CR_1R_2(COOH)$	Yield	M.P °C
Ester	R_1, R_2=Me	R_1, R_2=Me	20%	220
Ester	R_1=Et, R_2=Me	———	(a)	
Ester	R_1=Pr, R_2=Me	———	(a)	
Ester	R_1, R_2=Et	———	(a)	
Amide	R_1, R_2=$-(CH_2)_5-$	———	(b)	

a. Acids could not be obtained on saponification.

b. The conversion of amide to acid failed using the following methods:

i. Simple alkaline hydrolysis

ii. Acid hydrolysis

iii. Diazotization.[86]

II. Syntheses of Hydrazino Acids

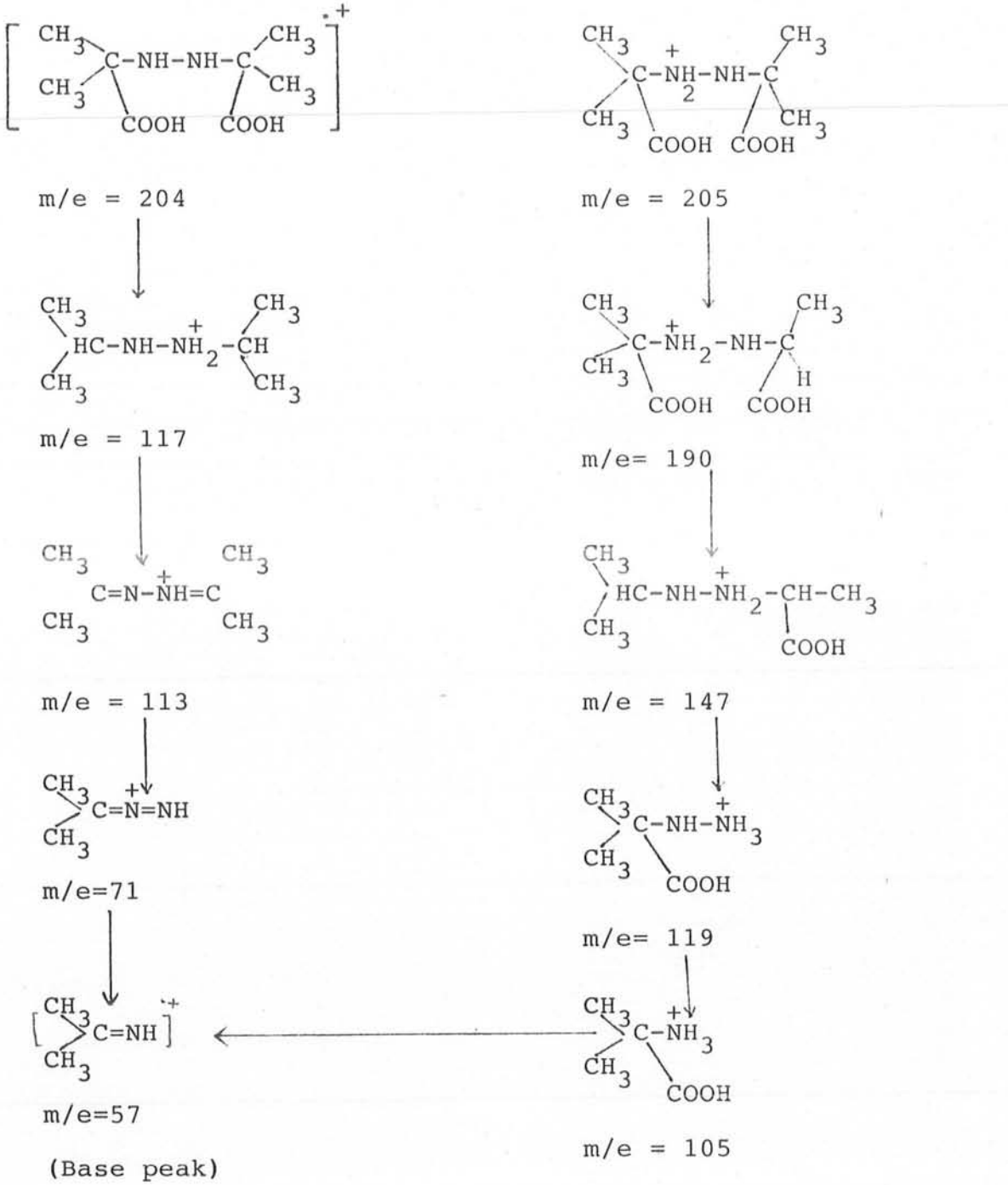
S.No.	Hydrazino Acid $R-\underset{\underset{NH-NH_2}{|}}{CH}-COOH$	Method of Prepn.	Yield	M.P ^oC
1.	R=H	-Bromo acid $+NH_2-NH_2$	45%	148-151
2.	R=Et	-do-	40%	200-205
3.	R=Iso-Pr	-do-	47%	230-235
4.	R=n-Bu	-do-	42%	197-200
5.	$R=C_6H_5$	i. -do-	49%	189
		ii. -keto acid + NH_2-NH_2	39%	188-189
		iii. Nitrile hydroly -sed	42%	188-189

III. Syntheses of α-Hydrazo Acids.

S.No.	Hydrazo acid $R_1R_2C(COOH)-NH-NH-C(COOH)R_1R_2$	Preparation methods	Yield	M.P °C
1.	$R_1=C_6H_5, R_2=H$	Reduction of azine prepared from the corr-sponding α-keto acid.	69%	160-1
2.	$R_1=p-(CH_3)C_6H_4$ $R_2=H$	-do-	83.3%	148-150
3.	R_1=naphthyl, $R_2=H$	-do-	43%	185-7

Chart

Fragmentation Pattern of 2:2 Hydrazoisobutyric Acid

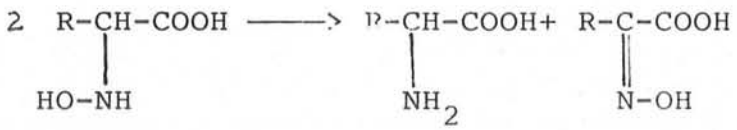


DISCUSSION AND CONCLUSION

In the introduction to this thesis the various general mechanisms which had been postulated for the oxidation of α-amino acids were outlined. Of these two were compatible with the experimental data when this work was begun. One of these two mechanisms was proposed by Spenser, Crawhall and Smyth[32] and demanded on electrophilic OH^+ attack on the α-carbon atom of an amino acid with concerted elimination of carbon dioxide and the formation of a carbinolamine intermediate, the latter substance being unstable and undergoing further decomposition to give an aldehyde and an amine. This oxidation mechanism may be considered as a generalization of the pathway potulated earlier by Heyns and Stange[41] in the special case of oxidation by N-bromosuccinimide in which Br^+ was tkaen to be the electrophilic species. The second mechanism which is due to Sweeley and Horning,[44] on the other hand, required OH^+ attack at the nitrogen atom of an amino acid to yield an amino acid N-oxide, which underwent decarboxylation in a subsequent step. This mechanism appears to be a generalization of the reaction sequence suggested by Langheld[42] for the oxidation of α-amino acids by hypochlorite.

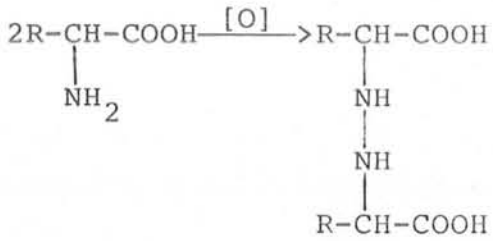
As a means of differentiating between these two

views, in investigation of the role of α-hydroxyamino acids as possible intermediates in the oxidation of α-amino acids has already indicated that α-hydroxy amino acids cannot be considered as intermediates in the oxidation of α-amino acids because they were found to undergo disproportionation reactions to give the corresponding α-amino acids and α-oximino acids

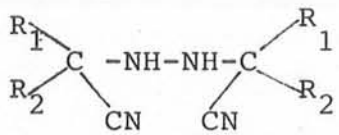


This disproportionation means that the stoichiometry of the oxidation of an α-amino acid which requires two equivalents of an oxidizing agent to perform complete conversion to aldehyde, carbon dioxide and an amine is violated. The oxidation of α-amino acid via α-hydroxy amino acid will require more than two equivalents of an oxidizing agent per one mole of an α-amino acid. This result left the mechanism proposed by Spenser, Crawhall and Smyth for further consideration.

In the present work we have considered the possibility of the hydrazo acids as intermediates in the oxidation of α-amino acids.



In order to propose formally the hydrazo acids as intermediates in the oxidation of α-amino acids it was necessary to synthesize these compounds and study their chemical properties, particularly, their behaviour in acidic and neutral solutions. The results of our studies have shown that these compounds are very difficult to synthesize, as their synthetic intermediates undergo decomposition under mild hydrolysis conditions.

The syntheses of several alkyl (cyano) hydrazines with general structure  were carried out in order to proceed next for the preparation of the corresponding α-hydrazo acids. The behaviour of these alkyl (cyano) hydrazines as cited in literature and as we observed is such that they decompose slowly at room temperature both in the solid state and in solutions. This decomposition is accelerated at higher temperatures. The decomposition products are, the corresponding azines

and HCN. The azines on further decomposition give hydrazine and the corresponding aldehyde/ketone. The net result is the reversal of the reaction to give back the starting materials. Keeping the unstable nature of these alkyl (cyano) hydrazines in mind, a number of methods were applied to hydrolyse these compounds to the corresponding acids. Under mild conditions only one nitrile could be converted to the corresponding amide and other attempts[77-83] to achieve this conversion were unsuccessful. The route for the preparation of hydrazo acids from nitriles via the corresponding di-esters was also tried. Under the mild esterification condtions[84] only the esters of aliphatic alkyl (cyano) hydrazines could be obtained. The hydrolysis of only one of the four di-esters was succesful.

2:2́ hydrazo isobutyric acid obtained from the hydrolysis of the 1,2-Di-2-(2-cyano) propyl hydrazine was characterized by IR and mass spectroscopy. Mass spectrograph shows $M^{\cdot+}$ at m/e 204, compatible with the M. formula $C_8H_{16}O_4N_2$. Base peak at m/e 57 is explained by the formation of the fragment $(CH_3)_2C{=}NH$. Other framentation patterns are shown in the chart (page-60).

Synthesis of α-hydrazo acids through the intermediacy of α-hydrazino acids did not give the desired resu-

lts. Hydrazino acid and α-keto acid mixture could not be reduced with sodium cyanoborohydride to give hydrazo acid.

α- Hydrazo acids with the aryl group on α-carbon atom were synthesized in good yields by the reaction of α-keto acids in ethanol with anhydrous hydrazine and subsequent reduction after the formation of azine. The stability of these acids under normal conditions can be attributed to the presence of α-aryl substitution. However, these acids also decompose in aqueous and acidic solutions at high temperatures.

The products of decomposition were approximately one mole carbon dioxide and one mole corresponding aldehyde per mole of hydrazo acid. In the oxidation study of hydrazo acid when two equivalents of oxidant were used per mole of hydrazo acid, the products of oxidation were aldehyde and carbon dioxide.

In the successive oxidation of the hydrazo acid, two equivalents oxidizing agent was added in five portions to one mole of hydrazo acid. After each addition a small portion of the reaction mixture was drawn and applied on

precoated TLC plate. Reference spots were also applied. The results showed the formation of the corresponding α-amino acid during the course of incomplete oxidation.

Oxidation of one mole hydrazo acid with an excess of oxidizing agent resulted in the formation of carbon dioxide and the corresponding aromatic acid. Aromatic acid is obviously formed from the further oxidation of the initial aldehyde produced.

The decomposition of α-hydrazo acids to give the corresponding aldehyde, carbon dioxide and a mole of α-amino acid, leads to the conclusion that α-hydrazo acids can be considered as possible intermediates in the oxidation of α-amino acids through the mechanism given in the introduction. The stoichometry of oxidation of α-amino acids is fully satisfied. The draw back of this mechanism is that the decomposition is not spontaneous as it requires acidic conditions and higher temperatures for accelerated decomposition.

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