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CHEMISTRY OF THE α-HYDROXYLAMINO ACIDS

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By

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SCOPE AND CONTENTS:

The chemistry of α-hydroxylamino acids is critically reviewed.

An attempt has been made to establish a general synthesis of α-hydroxylamino acids from α-bromo acids. A comparison of the use of cation and anion exchange columns as a means of purification has been included.

The results of paper and thin layer chromatography of α-hydroxylamino acids have been reported.

α-Hydroxylamino acids have been shown to be intermediates in the conversion of α-bromo acids to α-oximino acids with excess hydroxylamine.

In addition, a new base-catalyzed decomposition of α-hydroxylamino acids to α-keto acids and ammonia is described.

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CHAPTER 1

INTRODUCTION

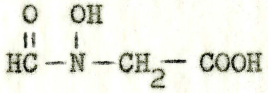
The chemistry of the naturally occurring α-amino acids is well documented and the importance of these compounds in biochemical systems is fully recognized. Comparatively little is known, however, concerning an analogous series of compounds, the α-hydroxylamino acids. Although a number of α-hydroxylamino acids have been synthesized and hydroxylamino analogues of several amino acids are known to occur in various natural products, a thorough study of their chemical and physical properties is lacking and large discrepancies in their reported properties are to be found in the literature.

The present thesis embodies a critical review of the existing knowledge of α-hydroxylamino acids and the results of an investigation into several aspects of their chemistry.

Naturally occurring hydroxylamino acids

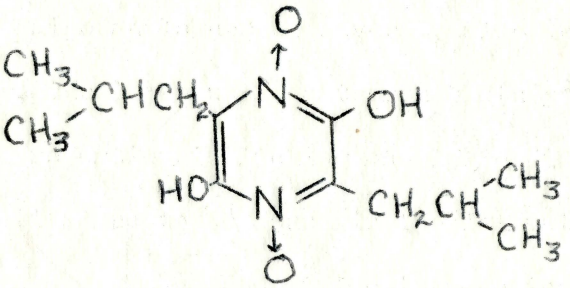
Several hydroxylamino acids, corresponding in structure to protein amino acids, have been found to occur in nature as components of more complex products isolated from a number of micro organisms.

The fermentation broth of *Penicillium frequentans* Westling was found to contain an active antitumor agent, hadacidin (I), which was shown to be the N-formyl derivative of α-hydroxylaminoacetic acid, the analogue of glycine (Kaczka, Gitterman, Dulaney, and Folkers, 1962).



I

The structure of pulcherriminic acid established by MacDonald (1963) as 2,5-diisobutyl-3,6-dihydroxypyrazine-1,4-dioxide (II) contains the structural units of two α-hydroxylaminoisocaproic acid moieties, the analogue of leucine.



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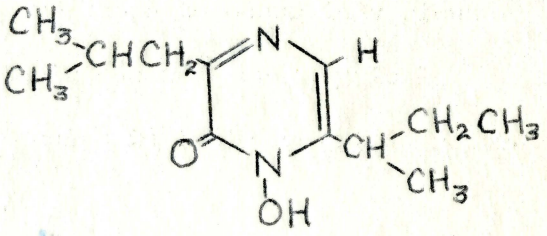
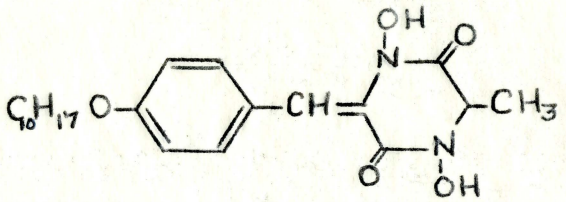


III

Aspergillic acid (III), an antibiotic derived from certain strains of Aspergillus flavus, has been shown to have a related structure (Dutcher, 1958), containing the structural units of leucine and of the isoleucine analogue, α-hydroxylamino-β-methylvaleric acid.

Mycelianamide (IV), isolated from the mycelium of strains of Penicillium griseofulvum Dierckx, contains the structural units of α-hydroxylaminopropionic acid, the alanine analogue, and of α-hydroxylamino-β-(p-hydroxy)-phenylpropionic acid, the analogue of tyrosine (Birch, Massy-Westropp, and Rickards, 1956).



IV

Two ω-hydroxylamino analogues of basic amino acids have also been isolated. Snow (1954) reported the formation of α-amino-ω-hydroxylaminohexanoic acid, the analogue of lysine, on hydrolysis of mycobactin, a growth factor for Mycobacterium johnei. The decarboxylation product of the above acid, 1-amino-5-hydroxylaminopentane, has been found in both ferrioxamine B and ferrimycin A (Bickel, Fechtig, Hall, Keller-Schlierfien, Prelog, and Vischer, 1960).

α-Amino-ω-hydroxylaminovaleric acid, the analogue of ornithine, has been found in the antibiotic albomycin (Turková, Mikeš, and Šorm, 1962) as well as in the cyclic hexapeptides, ferrichrome and ferrichrome A (Emery and Neilands, 1961).

Synthesis of α-hydroxylamino acids

Long before α-hydroxylamino acids were recognized as components of natural products the synthesis of a number of these compounds was described. Several methods have been used.

The earliest of these methods, modelled on the Strecker synthesis of α-amino acids, consisted of the conversion of aldehydes to the corresponding hydroxylaminonitriles, followed by hydrolysis of the nitrile group. The α-hydroxylamino nitriles were prepared either by

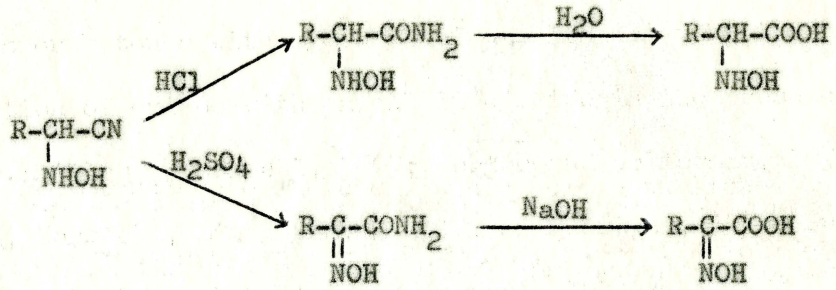
addition of dry hydrogen cyanide to oximes, or by the reaction of aldehydes or ketones with hydroxylamine salts and alkali cyanide, or by the reaction of oximes with an alkali cyanide in the presence of bisulphite (v. Miller and Plöchl, 1893; Porter and Hellerman, 1939, 1944; Lillevik, Hossfeld, Lindstrom, Arnold, and Gortner, 1942; Hurd and Longfellow, 1951; Neelakantan and Hartung, 1958).

$$R\text{-}CH{=}NOH + HCN \longrightarrow R\text{-}CH(NHOH)CN$$

$$R\text{-}CHO + NH_3OH.Cl + NaCN \longrightarrow R\text{-}CH(NHOH)CN + NaCl$$

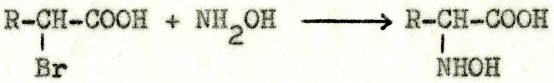
$$R\text{-}CH{=}NOH \xrightarrow{NaHSO_3} R\text{-}CH\begin{matrix}SO_3Na\\NHOH\end{matrix} \xrightarrow{CN^-} R\text{-}CH(NHOH)CN$$

Hydrolysis of the hydroxylamino nitriles with concentrated hydrochloric acid (v. Miller and Plöchl, 1893; Hurd and Longfellow, 1951; Snow, 1954; Neelakantan and Hartung, 1958; Ahmad, 1960) or concentrated sulphuric acid (v. Miller and Plöchl, 1893) yielded the desired products. The use of concentrated sulphuric acid led to the formation of considerable amounts of the corresponding α-oximino acid amides, which on treatment with aqueous sodium hydroxide were hydrolyzed to the α-oximino acids



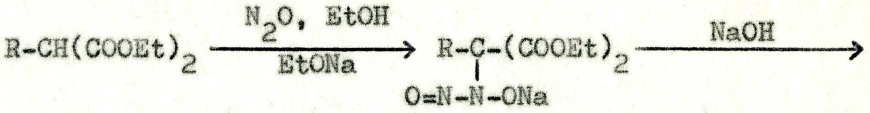
Although hydrolysis of the nitriles with hydrochloric acid gave satisfactory yields of α-hydroxylamino acids, various side reactions were found to occur, especially at elevated temperatures or on prolonged standing. When α-hydroxylamino nitriles possessing a hydrogen atom on the α-carbon atom were refluxed with concentrated hydrochloric acid the reaction mixture contained not only the α-hydroxylamino acid, but also the corresponding α-amino acid, the corresponding α-oximino acid, the lower homologous aldehyde, and the lower homologous aliphatic acid (Ahmad, 1960). With α-hydroxylamino nitriles possessing no hydrogen atom on the α-carbon atom refluxing with concentrated hydrochloric acid produced, in addition to the α-hydroxylamino acid, the corresponding α-amino acid and the lower homologous ketone. The ketone was thought to arise from the decomposition of the corresponding nitroso compound. Nitroso compounds in the presence of hydrochloric acid are known to rearrange to ketoximes which on hydrolysis yield ketones (Müller and Metzgen, 1955; Müller, Fries and Metzgen, 1955). Treatment of these nitriles at room temperature with concentrated hydrochloric acid gave rise to a transient blue colour, typical of nitroso compounds.

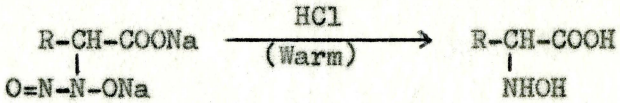
In a second method for the synthesis of α-hydroxylamino acids (Cook and Slater, 1956), α-bromo acids were treated with stoichiometric amounts of methanolic hydroxylamine.



Much earlier it had been found (Hantzsch and Wild, 1896) that reaction of α-halo acids with excess hydroxylamine yielded not α-hydroxylamino acids but the corresponding α-oximino acids. This reaction will be discussed later in this chapter.

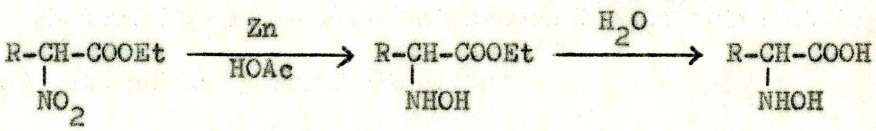
The third method leading to α-hydroxylamino acids entailed treatment of an alkylacetoacetate or an alkylmalonic ester with nitric oxide in absolute alcohol in the presence of one equivalent of sodium ethoxide. Treatment of the intermediate isonitramino product with aqueous sodium hydroxide, followed by treatment with hydrochloric acid, gave the desired α-hydroxylamino acid (Traube, 1895).





The fourth and most recent method utilizes the reduction of the corresponding α-nitro esters with zinc dust in aqueous acetic acid, followed by hydrolysis of the ester (Rodgers and Neilands, 1963;

Neilands and Azari, 1963).



It was found that although the yields were low (15 - 20%) the method was useful since the starting α-nitro esters are generally easily obtained (Kornblum, Chalmers, and Daniels, 1955; Kornblum, 1962; Finkbeiner and Stiles, 1963).

Earlier attempts to obtain α-hydroxylamino acids by reduction of the corresponding α-oximino acids had failed. Reduction, even under carefully controlled conditions, did not stop at the desired stage, but led to α-amino acids in every case (Hamlin and Hartung, 1942).

Even though several α-hydroxylamino acids have been prepared by each of these four methods, all of them have limitations. The first method, involving hydrolysis of the corresponding α-hydroxylamino nitriles, is limited in a number of ways. As was mentioned previously, the reaction mixture was found to contain a number of products including the corresponding α-amino acids (Ahmad, 1960). It is the presence of these compounds which leads to the great difficulty in the preparation of pure α-hydroxylamino acids. It was only by careful column chromatography that Ahmad (1960) was able to overcome this separation problem. In fact, it is mainly the presence of α-amino acids which has led to discrepancies in the reported properties of α-hydroxylamino acids. This point will be treated more fully later in this chapter. In addition this method is limited only to the preparation of the purely aliphatic α-hydroxylamino acids. All attempts to prepare the α-hydroxylamino nitriles from

benzaldehyde, phenylacetaldehyde, hydrocinnamaldehyde, and acetophenone were unsuccessful (Neelakantan and Hartung, 1958).

The second method, starting with the α-bromo acids, appears the most straightforward of all the methods. However, this method had only been used to prepare three aliphatic α-hydroxylamino acids (Cook and Slater, 1956). A more complete study of this method was thus made and the results will be discussed in chapter two of this thesis.

The method of Traube (1893) like the first method gives rise to a mixture of products including α-amino acids and hence a serious separation problem is involved in the use of this method.

The fourth method, involving reduction of α-nitro esters (Neilands and Azari, 1963), was published very recently. It appears promising even though it has been found to give the desired product in poor yields only. Detailed experimental data have not been reported, however, and the final hydrolysis step may well result in the formation of α-amino acids as impurities giving rise to the separation problem previously mentioned.

Physical properties

α-Hydroxylamino acids have been found to be colourless, crystalline compounds, soluble in water, slightly soluble in absolute alcohol, and insoluble in ether and other organic solvents.

Conflicting reports concern the acid-base properties of the α-hydroxylamino acids. Early workers (v. Miller and Plöchl, 1893) reported them as giving strongly acidic solutions. More recent work, however, has

shown them to be amphoteric substances, soluble in both dilute acids and dilute bases. The α-hydroxylamino acids were reported (Neelakantan and Hartung, 1958) as having isoelectric points between pH 6 and pH 7, but later work (Ahmad, 1960) has shown the isoelectric points to lie between pH 3.6 and pH 4.1 (see Table 1).

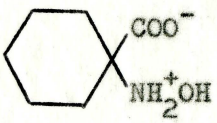
When α-hydroxylamino acids were titrated with sodium hydroxide in the presence of formaldehyde there was a considerable decrease in the apparent pK_2. The titration curve with hydrochloric acid, however, did not change significantly in the presence of formaldehyde. Since it must have been the hydroxylamine group rather than the carboxyl group which reacted with the formaldehyde and since it was the sodium hydroxide curve which was affected by the formaldehyde, the pK_2 was shown to be that connected with the dissociation $R\text{-}\overset{+}{N}H_2OH \longrightarrow R\text{-}NHOH + H^+$. This adaptation of the Sorensen 'formol' titration method proved that the α-hydroxylamino acids, like the analogous α-amino acids, exist as zwitterionic species at their isoelectric points, having the structure $^-OOC.CHR.\overset{+}{N}H_2OH$. Confirmation of this dipolar structure is found in the infra-red absorption spectra of the α-hydroxylamino acids, which includes bands characteristic of $-COO^-$ (1600 - 1500 cm^{-1}) and of $-NH_2^+$ groups (1620 - 1560 cm^{-1} and 800 cm^{-1}).

Reduction and oxidation

Reduction of the α-hydroxylamino acids in ethanol solutions with palladium charcoal in the presence of ammonia gave the corresponding α-amino acids (Neelakantan and Hartung, 1958). When the reduction was carried out with zinc in the presence of acetic acid, a small amount of

TABLE 1

pK_1, pK_2, and Isoelectric points of α-Hydroxylamino acids (Ahmad, 1960):

α-Hydroxylamino acids	pK_1	pK_2	Isoelectric point $(pK_1 + pK_2)/2$
1. $CH_3.CH(NH_2^+OH)-COO^-$	2.05	5.75	3.90
2. $CH_3.CH_2.CH(NH_2^+OH)-COO^-$	2.20	5.65	3.92
3. $CH_3.CH_2.CH_2.CH(NH_2^+OH)-COO^-$	2.35	5.40	3.88
4. $(CH_3)_2CH.CH(NH_2^+OH)-COO^-$	2.30	5.80	4.05
5. $C_6H_5.CH_2.CH(NH_2^+OH)-COO^-$	2.15	5.20	3.68
6. $(CH_3)_2-C(NH_2^+OH)-COO^-$	2.08	5.95	4.01
7. 	2.45	5.60	4.03

the dioxopiperazines was formed along with the corresponding α-amino acids (Cook and Slater, 1956).

α-Hydroxylamino acids are strong reducing agents and will react with ammoniacal silver nitrate as well as with Fehling's solution in the cold.

On oxidation the acids yield carbon dioxide, the lower homologous aldehyde, and oxides of nitrogen (Snow, 1954; Cook and Slater, 1956; Neilands and Azari, 1963). Cook and Slater (1956) studied the oxidation with potassium permanganate and isolated the lower homologous aldehyde. Oxidation with both ninhydrin and periodic acid (Snow, 1954) yielded aldehydes, and one mole of carbon dioxide, the hydroxylamino group being presumably converted to nitrogen oxides. In the latter case it was found that 2 - 2.5 moles of periodate were consumed. Recently Neilands and Azari (1963) reinvestigated the oxidation of α-hydroxylamino acids with periodic acid. They found the products to be carbon dioxide, nitrous oxide, and the lower homologous aldehyde. The reaction was shown to involve the uptake of 2 moles of periodate.

$$R.CH(NHOH)COOH + 2HIO_4 \longrightarrow RCHO + CO_2 + 1/2N_2O + 3/2H_2O + 2HIO_3$$

In contrast, the N-acyl esters of the α-hydroxylamino acids were found to react with periodic acid in a different manner, forming the corresponding α-oximino esters.

$$\begin{array}{l} R\text{-}CH\text{-}COOEt \\ \quad\;\; | \\ \quad\;\; N\text{-}OH \\ \quad\;\; | \\ C_6H_5\text{-}C{=}O \end{array} + HIO_4 \longrightarrow \begin{array}{l} R\text{-}C\text{-}COOEt \\ \quad\;\; \| \\ \quad\;\; N\text{-}OH \end{array} + C_6H_5COOH + HIO_3$$

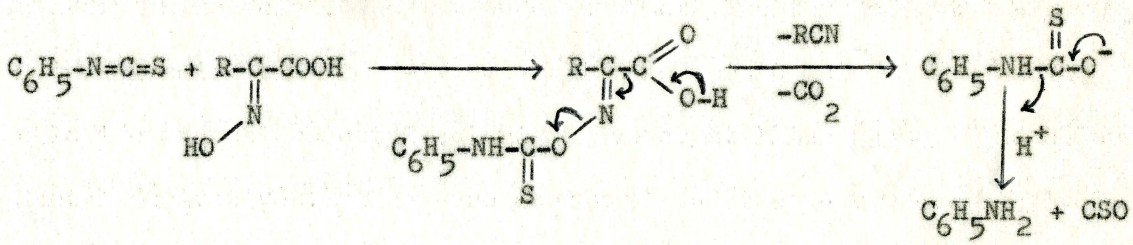
Derivatives

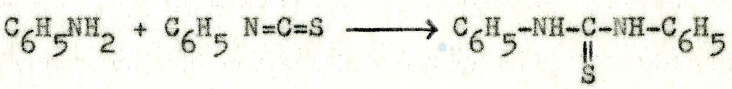
A number of derivatives of α-hydroxylamino acids have been prepared. With diazomethane esterification of α-hydroxylaminoisocaproic acid was found to be accompanied by N-methylation, giving rise to methyl α-(N-hydroxy-N-methylamino)-isocaproate (Cook and Slater, 1956). These same workers reported the formation of various acyl derivatives of α-hydroxylamino acids. Acylation of the methyl esters of both α-hydroxylaminohexanoic acid and α-hydroxylaminoisocaproic acid with benzoyl chloride and chloroacetyl chloride gave crystalline benzoyl and chloroacetyl derivatives. The 2-bromohexanoyl and the α-bromo-γ-methylvaleryl derivatives of the above two α-hydroxylamino acids were also prepared. These derivatives were obtained as high boiling syrups. All of these acyl derivatives gave red colours when treated with methanolic ferric chloride, indicating that N-acylation rather than O-acylation had taken place. The formation of the benzoyl derivatives of ethyl α-hydroxylaminopropionate and ethyl α-hydroxylamino-β-phenylpropionate have also been reported (Neilands and Azari, 1963). These were obtained as crystalline solids in yields of about 25%. The N-formyl derivative of α-hydroxylaminoacetic acid has also been prepared (Kaczka, Gitterman, Dulaney, and Folkers, 1962). This derivative was shown to be a naturally occurring substance, hadacidin, as was mentioned earlier in this chapter.

Attempts to prepare the p-bromophenacyl, p-nitrobenzyl, and p-phenylphenacyl derivatives were unsuccessful (Cook and Slater, 1956).

An attempt was also made to prepare the phenylisothiocyanate derivatives (Neelakantan and Hartung, 1958). It was found that the only crystalline product formed was diphenylthiourea and no explanation was

offered for this observation. Later work (Ahmad, 1960) showed that, under similar conditions, pure α-hydroxylamino acids did not react with phenylisothiocyanate and that the diphenylthiourea was probably formed by reaction of phenylisothiocyanate with α-oximino acids, present as impurities. Reaction of α-oximino acids with phenylisothiocyanate has been shown to release carbon dioxide with the formation of diphenylthiourea.





It should be noted that at elevated temperature small amounts of diphenylthiourea were isolated from the reaction of α-hydroxylamino acids with phenylisothiocyanate. α-Hydroxylamino acids were thought to undergo a disproportionation reaction under these conditions, giving rise to α-oximino acids and α-amino acids. It was the α-oximino acids thus formed which were thought to react with the phenylisothiocyanate. The nature of the disproportionation reaction will be more fully treated later in this chapter.

Other Reactions

Pyrolysis of α-hydroxylamino acids has been reported to yield carbon dioxide, ammonia, the lower aldehydes, the corresponding α-amino

acids, and other unidentified products such as pyridine-like bases (v. Miller and Plochl, 1893; Cook and Slater, 1956).

Another aspect of the chemistry of α-hydroxylamino acids in which conflicting reports have been made is the reaction with ninhydrin. Neelakantan and Hartung (1958) reported that α-hydroxylamino acids gave a purple colour, typical of α-amino acids, when treated with ninhydrin. On the other hand it was found that three α-hydroxylamino acids (α-hydroxylaminoacetic, α-hydroxylamino-β-phenylpropionic acid, and α-hydroxylaminohexanoic acid) gave weak yellow or orange colours with ninhydrin (Snow, 1954). Spenser and Ahmad (1961) confirmed that pure α-hydroxylamino acids did not yield a purple colour with ninhydrin and expressed the view that the results of Neelakantan and Hartung had been due to the presence of α-oximino acids as contaminants in the α-hydroxylamino acid samples.

Paper chromatography of the α-hydroxylamino acids appeared to give anomalous results (Spenser and Ahmad, 1961). When chromatograms of the acids, developed with n-butanol-acetic acid-water (40:10:50 by volume) as the solvent, were visualized with ninhydrin, strong purple spots with long tails appeared. These spots were shown to have Rf values identical with those of authentic samples of the corresponding α-amino acids. Spraying with ammoniacal silver nitrate or triphenyltetrazolium chloride under the appropriate conditions produced black or pink spots only for α-hydroxylaminopropionic acid and α-hydroxylaminoisobutyric acid. The reducing spots of these two acids had higher Rf values than the corresponding α-amino acids (see Table 2). It was con-

cluded that the ninhydrin positive products in these experiments, as in those of Neelakantan and Hartung (1958), were due to α-amino acids derived from α-hydroxylamino acids rather than due to the α-hydroxylamino acids themselves.

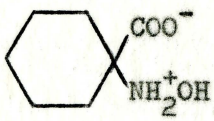
To explain this spontaneous reduction of the α-hydroxylamino acids to α-amino acids without the presence of a reducing agent, Spenser and Ahmad (1961) proposed a disproportionation reaction in which the products would be the corresponding α-amino acids and α-nitroso acids. Since α-nitroso compounds are unstable, the second product would, where possible, be the corresponding tautomeric form - the α-oximino acid. Such a scheme receives support from the fact, mentioned earlier, that in the hydrolysis of α-hydroxylamino nitriles α-amino acids and α-oximino acids were isolated from the reaction mixture. It was also noted that when tautomerization of α-nitroso acids to α-oximino acids could not occur the formation of α-nitroso compounds was indicated by the transient appearance of characteristic blue colours.

In order to gain proof of the proposed scheme, a quantitative study was undertaken. The disproportionation reaction required that one mole of α-hydroxylamino acid should give rise to one half mole each of α-amino and α-oximino acid.

In a series of experiments known amounts of α-hydroxylamino acids were allowed to reflux under nitrogen for twenty-four hours. The α-amino and α-oximino acids which formed, as well as the unreacted α-hydroxylamino acid, were determined quantitatively in the reaction mixture.

TABLE 2

Rf Values of α-Hydroxylamino and α-Amino acids in n-BuOH-AcOH-H_2O Solvent System (Ahmad, 1960):

α-Hydroxylamino acids (HL-AA)	Rf Values of (HL-AA)	Rf Values of Amino acids derived from (HL-AA)	Rf Values of authentic α-amino acids
1. $CH_3.\underset{\displaystyle NH_2^+OH}{\overset{}{\underset{|}{CH}}}-COO^-$	0.35	0.23	0.23
2. $CH_3.CH_2.\underset{\displaystyle NH_2^+OH}{\overset{}{\underset{|}{CH}}}-COO^-$	-	0.36	0.36
3. $CH_3.CH_2.CH_2.\underset{\displaystyle NH_2^+OH}{\overset{}{\underset{|}{CH}}}-COO^-$	-	0.51	0.51
4. $(CH_3)_2CH.\underset{\displaystyle NH_2^+OH}{\overset{}{\underset{|}{CH}}}-COO^-$	-	0.44	0.44
5. $C_6H_5.CH_2.\underset{\displaystyle NH_2^+OH}{\overset{}{\underset{|}{CH}}}-COO^-$	-	0.39	0.39
6. $CH_3\diagdown\underset{\displaystyle CH_3\quad NH_2^+OH}{\overset{}{\underset{|}{C}}}-COO^-$	0.46	0.59	0.59
7. 	-	0.60	0.60

The estimation of α-oximino acid was indirect. It was based on the finding that in aqueous solutions at pH values below pH 7, α-oximino acids decompose quantitatively to carbon dioxide and nitrile, according to the following equation (Ahmad and Spenser, 1960).

$$R\text{-}\underset{\underset{NOH}{\|}}{C}\text{-}COOH \longrightarrow R\text{-}CN + CO_2 + H_2O$$

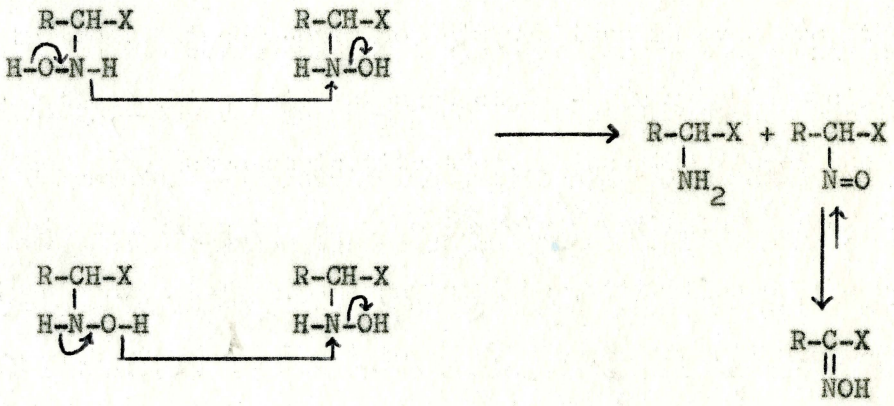
Carbon dioxide swept out of the reaction mixture was estimated gravimetrically as barium carbonate and served as a measure of α-oximino acid concentration. Since α-oximino acid was thus removed from the reaction mixture, the α-amino acid and unreacted α-hydroxylamino acid could be determined by electrometric titration. Since the pK_2 of the α-hydroxylamino acid was known to be approximately 6 while the pK_2 of the α-amino acid is approximately 3.5 pK units higher, titration of the reaction mixture with standard alkali to pH 7.5 serves to estimate α-hydroxylamino acid concentration, giving complete reaction with the hydroxylammonium group of the α-hydroxylamino acid but no reaction with the ammonium group of the α-amino acid. Further titration to pH 9 after addition of formaldehyde, which lowers the apparent pK_2 of the α-amino acid, determines the concentration of α-amino acid.

This experiment was carried out on seven α-hydroxylamino acids and with the exception of α-hydroxylaminoisovaleric acid and α-hydroxylamino-β-phenylpropionic acid equimolar quantities of α-amino and α-oximino acid (determined as carbon dioxide) were formed. The yields were in general lower than theoretically expected for 100% disproportionation. It was felt, however, that the formation of equimolar quantities of

α-amino and α-oximino acids was substantial evidence in favour of a disproportionation reaction. In the case of α-hydroxylamino-β-phenyl-propionic acid, the yield of carbon dioxide was higher than expected for 100% disproportionation and it was felt that some other decomposition process had taken place in addition to the disproportionation.

Two possible mechanisms based on hydride shifts were proposed to explain the disproportionation reaction of α-hydroxylamino compounds in general.

(a)

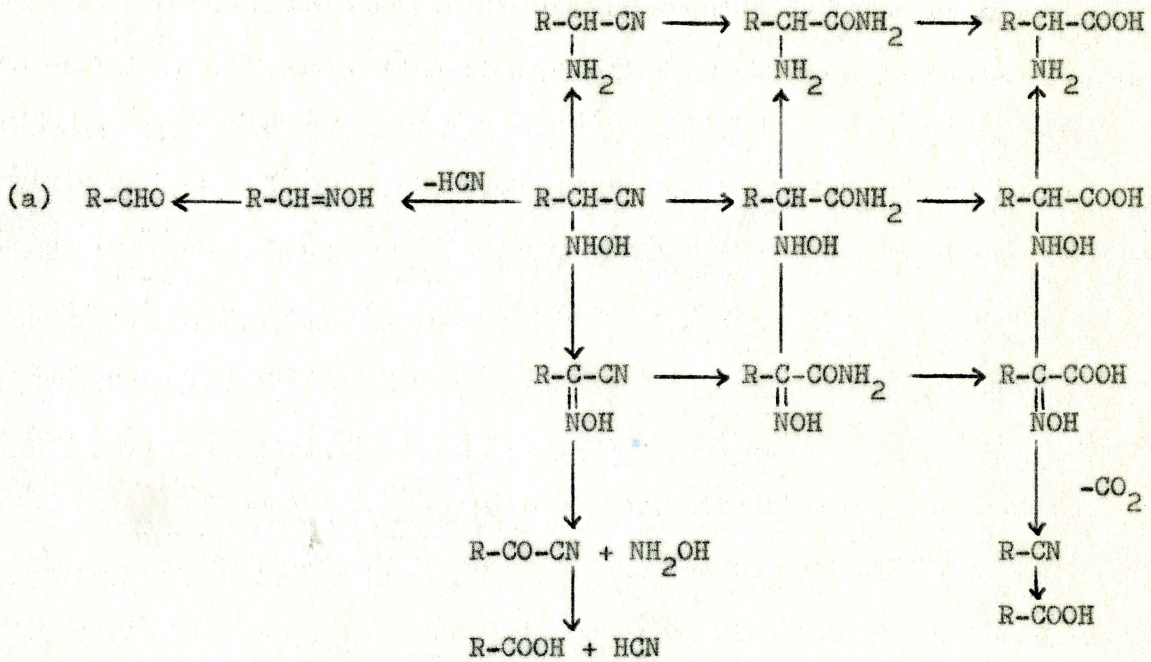


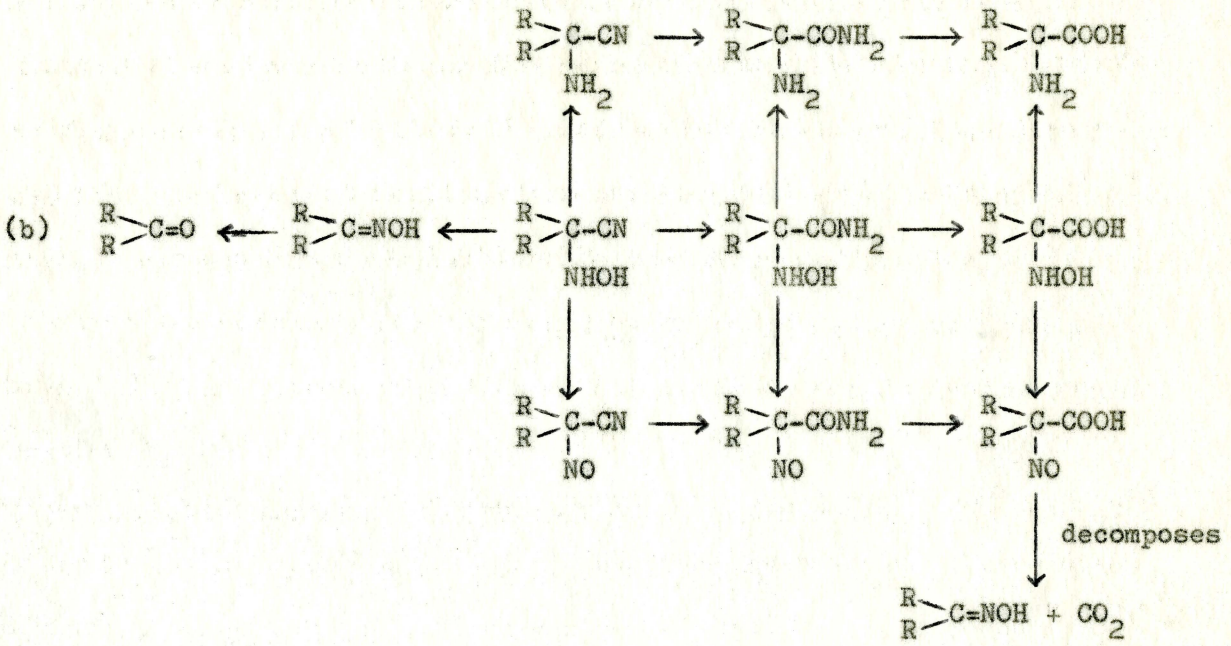
(b)

The mechanism (b) was preferred since it had been shown that O-H .... N and N-H .... O types of hydrogen bonding were preferentially present in solid hydroxylamine (Meyers and Lipscomb, 1955).

A similar disproportionation reaction would account for the presence of various side products formed in the preparation of α-hydroxylamino acids and would explain those results reported by Neelakantan and Hartung (1958) which are in conflict with other work. On the basis of such a disproportionation reaction Ahmad (1960) attempted to rationalize the presence of the side products obtained on hydrolysis

of α-hydroxylamino nitriles. Scheme (a) refers to the compounds possessing a hydrogen atom on the α-carbon atom while scheme (b) refers to those compounds which lack a hydrogen atom on the α-carbon atom. In the latter case the blue nitroso compounds formed would decompose slowly in the presence of hydrochloric acid.





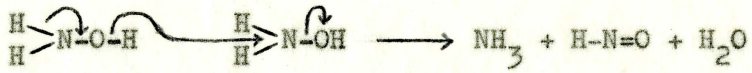
In connection with the disproportionation reaction proposed by Spenser and Ahmad (1961), it is interesting to note that hydroxylamine, N-methylhydroxylamine, and N-ethylhydroxylamine have been reported to decompose to products which can be explained in terms of a disproportionation reaction (Kjellin, 1921).

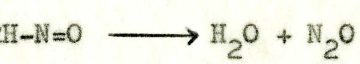
Kjellin found that on heating hydroxylamine decomposes to give ammonia, nitrous oxide, nitrogen, and water according to the following two equations.

$$3NH_2OH \longrightarrow 3H_2O + NH_3 + N_2$$

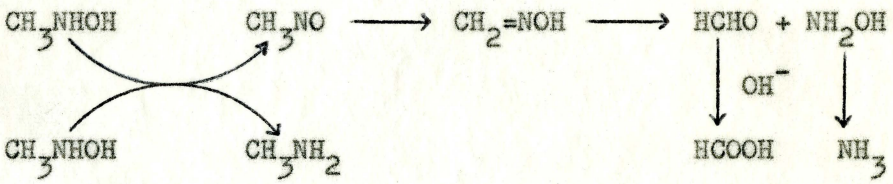
$$4NH_2OH \longrightarrow 3H_2O + 2NH_3 + N_2O$$

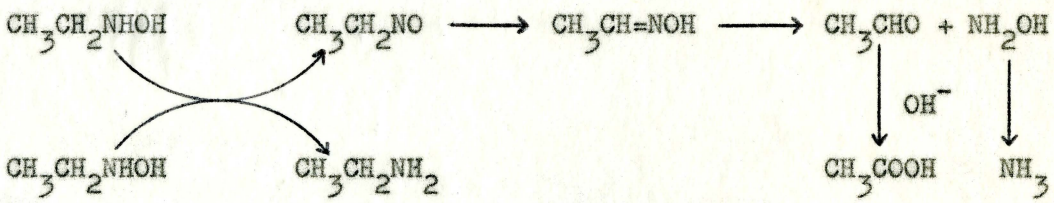
The second equation can be depicted as a disproportionation reaction in the following manner.



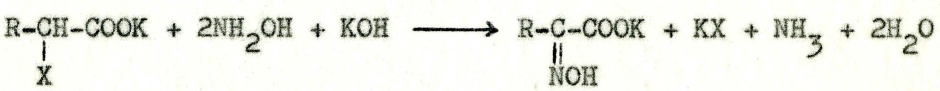


Kjellin also found that N-methylhydroxylamine when distilled in the presence of alkali gave methylamine, ammonia, and formic acid, while N-ethylhydroxylamine gave ethylamine, ammonia, acetic acid, and acetaldehyde. These products can be rationalized by disproportionation reactions in the following manner.

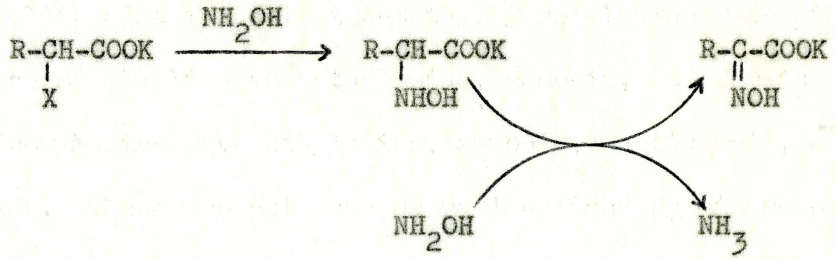




One further reaction, which can be explained in terms of a disproportionation reaction, is the conversion of α-halo acids to α-oximino acids by means of excess hydroxylamine, to which reference has been made earlier. Hantzsch and Wild (1896) reported the preparation of α-oximino acids according to the following equation.



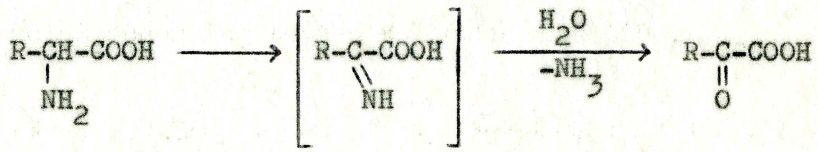
They proposed that the reaction proceeded via the intermediate α-hydroxylamino acid which would undergo immediate oxidation at the expense of the hydroxylamine to afford the α-oximino acid.



Their proposal is supported by the work of Cook and Slater (1956) who, as stated earlier, prepared α-hydroxylamino acids by the treatment of α-bromo acids with equimolar amounts of hydroxylamine. This scheme corresponds, of course, to the disproportionation reaction proposed by Spenser and Ahmad (1961) in which the hydroxylamine moiety replaces the one α-hydroxylamino acid moiety undergoing reduction.

α-Hydroxylamino acids as intermediates in α-amino oxidation

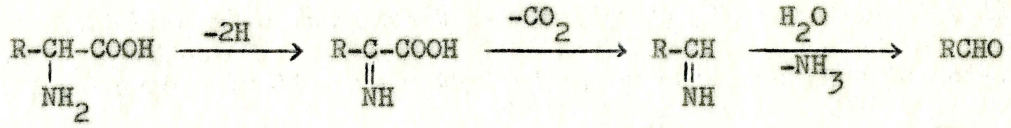
The chemistry of the α-hydroxylamino acids is of interest in connection with the study of the mechanism of the chemical oxidation of α-amino acids. The biochemical oxidation of α-amino acids to α-keto acids has been thoroughly investigated and a mechanism involving α-imino acids as intermediates has been established (Pitt, 1958).



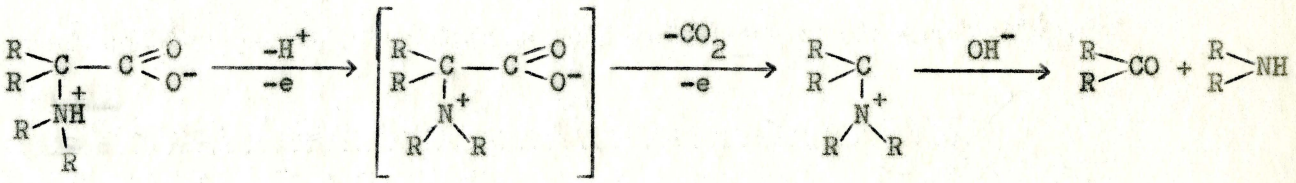
The mechanism of the chemical oxidation of α-amino acids, to aldehydes, ammonia, and carbon dioxide, on the other hand, has not yet

been clarified even though the reaction has been repeatedly studied.

The problem was first investigated by Wieland and Bergel (1924) and a mechanism involving dehydrogenation was proposed.

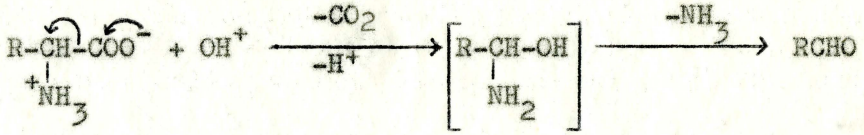


This mechanism was disproved by later work (Bergel and Bolz, 1933) which showed that α-N,N-dimethylaminoisobutyric acid, a compound lacking hydrogen atoms on both the α-carbon atom and the nitrogen atom, was easily oxidized to acetone, dimethylamine, and carbon dioxide. Confirmation of this work came from Herbst and Clarke (1934) who proposed a new mechanism.

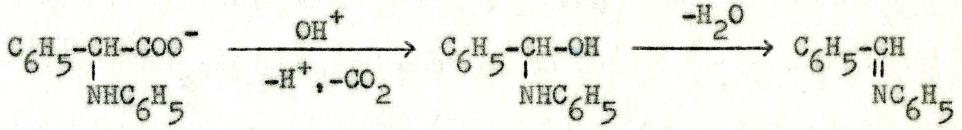


The fact that the hydrogen atom on the α-carbon atom is not involved in the oxidative decarboxylation of α-amino acids was again verified by the work of Spenser, Crawhall, and Smyth (1956). They found that the oxidation of α, β-$^{3}H_2$ valine and α-β-$^{3}H_2$ phenylalanine took place without loss of tritium forming aldehydes with specific activities identical with those of the parent amino acids. They proposed a new mechanism for the reaction in which the oxidizing agent attacks the

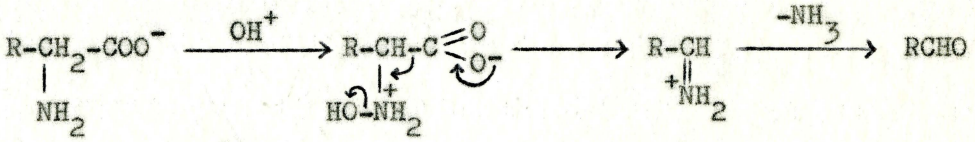
α-carbon atom as an electrophile with a concurrent loss of carbon dioxide. The unstable intermediate carbinolamine would then on rapid decomposition give rise to aldehyde and ammonia.



Support for this mechanism came from the oxidation of α, N-diphenylglycine under alkaline conditions. The unstable intermediate carbinolamine gave, by spontaneous dehydration, the expected benzalaniline rather than decomposition to the aldehyde and amine.



Another mechanism which was compatible with the known facts but which did not call for a carbinolamine intermediate was proposed by Sweeley and Horning (1957). They found that N,N-dimethylglycine oxide decomposed in the presence of ferric ions to give dimethylamine, formaldehyde and carbon dioxide. Their mechanism involved the formation of an N-oxide which, aided by formation of a ferric ion-amino acid complex, decomposed by β-elimination of carbon dioxide and hydroxide ion.



The amino acid N-oxide of this mechanism is actually the zwitterionic form of an α-hydroxylamino acid. The role of α-hydroxylamino acids as intermediates in the oxidation of α-amino acids had been proposed earlier by Steiger (1944).

It has been shown, in at least two cases, that the oxidation of α-amino acids takes place with the stoichiometric amount of oxidizing agent (Fichter and Kuhn, 1924; Herbst and Clarke, 1934). This fact thus implies that if the α-hydroxylamino acid were indeed the intermediate in the oxidation, as proposed in the mechanism of Sweeley and Horning (1957), it must decompose spontaneously to products in aqueous solution at pH 8, even in the presence of excess oxidizing agent rather than reacting with a further mole of the latter. In fact it has been shown that the α-hydroxylamino acids do react readily with oxidizing agents (Neilands and Azari, 1963). The products were found to be carbon dioxide, aldehyde, and nitrous oxide. Ammonia, the normal product of the oxidation of α-amino acids, was not isolated. Ahmad (1960) studied the stability of the α-hydroxylamino acids in aqueous solution at pH 8 in the presence of ferric ions and found them to be fairly stable. The α-hydroxylamino acids were also known to disproportionate in aqueous solution, slowly at room temperature and rapidly at reflux temperature, forming α-amino acids and α-oximino acids. This regeneration of α-amino acid would then require more than one equivalent of oxidizing agent per mole of α-amino acid, a fact which has previously been shown not to hold true in at least two cases. Furthermore the hydrolysis of the α-oximino acids, the second product of the disproportionation reaction, is known to lead to nitriles while oxidation of α-oximino acids leads to hydroxamic

acids. All these facts thus rule out completely the mechanism proposed by Sweeley and Horning (1957).

The foregoing review clearly indicates that several gaps exist in our knowledge of the chemistry of the α-hydroxylamino acids. Improvement of a method of preparation and further investigation of several of the aspects of the disproportionation reaction appeared particularly desirable.

CHAPTER 2

INTRODUCTION

As was illustrated in the previous chapter, the present state of knowledge of the α-hydroxylamino acids leaves much room for further study. The following describes the results of an investigation into several aspects of their chemistry. In particular, the stability of the α-hydroxylamino acids in alkaline solution, which had not been studied previously, was investigated. In addition an attempt was made to establish a generalized method of preparation of the α-hydroxylamino acids.

Synthesis of the α-hydroxylamino acids

Of the various methods for preparing α-hydroxylamino acids reviewed in Chapter One, that involving treatment of α-bromo acids with equivalent amounts of methanolic hydroxylamine (Cook and Slater, 1956) appeared to be most suited as a general method. Although only three aliphatic α-hydroxylamino acids had been synthesized in this way, it was felt that the method could serve as a general one, particularly because of its simplicity and the ready availability of the α-bromo acids.

Seven α-hydroxylamino acids have now been prepared by this method, those corresponding to glycine, alanine, α-aminobutyric acid, valine, leucine, isoleucine, and phenylalanine. Attempts to prepare the analogues of aspartic acid and tyrosine failed.

The α-bromo acids used as starting materials were either obtained commercially or synthesized by one of two methods. A number of aliphatic α-bromo acids were prepared in good yields by the conventional Hell-Volhard-Zelinsky method (Marvel, 1940).

$$R\text{-}CH_2\text{-}COOH \xrightarrow[PCl_3]{Br_2} R\text{-}\underset{\displaystyle Br}{\underset{|}{CH}}\text{-}COOH + HBr$$

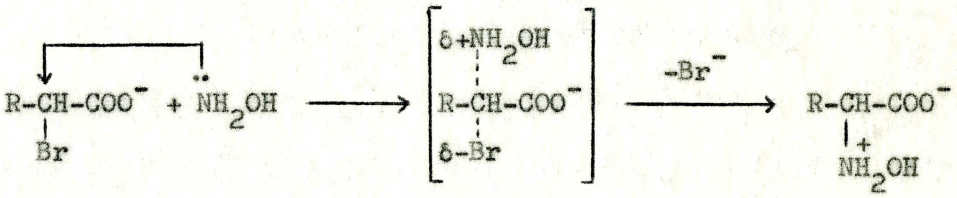
The α-bromo acid required for the synthesis of the analogue of phenylalanine was prepared in 50% yield from phenylalanine itself. The amino acid was treated with nitrous acid at low temperature in the presence of excess bromide ion (Rivers and Lerman, 1948).

$$C_6H_5.CH_2.\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH \xrightarrow[Br^-]{HNO_2} C_6H_5.CH_2.\underset{\displaystyle Br}{\underset{|}{CH}}\text{-}COOH$$

This method was also attempted with tyrosine but the only product formed was a high boiling, dark red oil, even at reduced temperatures. The presence of the hydroxy group may possibly give rise to nitrosation. Phenols are known to undergo substitution with nitrous acid yielding either ortho or para substituted compounds. No further attempt was made to prepare α-bromo-β-(p-hydroxyphenyl)-propionic acid.

The α-bromo acids were neutralized in the cold with standard sodium methoxide and refluxed overnight with one equivalent of methanolic hydroxylamine. The reaction, analogous to the conversion of α-halo acids to α-amino acids (Greenstein and Winitz, 1961),

presumably proceeds with inversion of configuration by an S_N2 mechanism involving nucleophilic attack of the hydroxylamine nitrogen on the α-carbon atom and resulting in the loss of a bromide ion and the formation of the zwitterionic species of α-hydroxylamino acids which they have been shown to form at neutral pH values.



Reaction took place readily with α-bromoacetic acid, α-bromopropionic acid, α-bromobutyric acid, α-bromoisocaproic acid, α-bromoisovaleric acid, α-bromo-β-methylvaleric acid, and α-bromo-β-phenylpropionic acid.

An attempt to prepare α-hydroxylaminosuccinnic acid, the analogue of aspartic acid, was unsuccessful due to the insolubility of the sodium salt of α-bromosuccinnic acid. Various polar non-aqueous solvents (absolute methanol, absolute ethanol, dioxane, and N,N-dimethylformamide) were tried without success. Non-aqueous solvents were required since α-hydroxylamino acids have been shown to disproportionate rapidly in refluxing aqueous solutions (Spenser and Ahmad, 1961).

The utility of this method for the preparation of α-hydroxylamino acids is thus established. In the course of condensation small amounts of the corresponding α-amino acids invariably form, due to the

disproportionation of the products or their further reaction with hydroxylamine.

As was shown in Chapter One the final purification of the products, and in particular the removal of contaminating α-amino acids, poses the biggest problem in the preparation of pure α-hydroxylamino acids. An important aspect of the present work was thus to investigate the experimental conditions by which purification of α-hydroxylamino acids prepared from α-bromo acids could be achieved. The use of ion exchange columns for this purpose appeared promising.

When, in fact, the experimental work was carried out, it was found that in five cases (α-hydroxylaminobutyric acid, α-hydroxylaminoisovaleric acid, α-hydroxylamino-β-methylvaleric acid, α-hydroxylaminoisocaproic acid, and α-hydroxylamino-β-phenylpropionic acid) no separation problem was encountered since these five acids crystallized from the reaction mixture in high purity on cooling, even though only moderate yields (20 - 45%) were obtained.

However, in two other cases (α-hydroxylaminoacetic acid and α-hydroxylaminopropionic acid) the acids did not crystallize and recourse was made to the technique of column chromatography. In this way purification of these two acids was achieved, but in yields which were considerably lower (see Table 3).

In the case of α-hydroxylaminoacetic acid, the reaction mixture was larger in volume than in the other cases due to the lower solubility of the sodium salt of the α-bromoacetic acid. This solution was evaporated to one half volume under reduced pressure to give a light

TABLE 3

α-Hydroxylamino Acids Prepared from α-Bromo Acids:

α-Hydroxylamino acids	Yield (%)	m.p. (decomp.)
1. $CH_2(NHOH)$-COOH	9	139.5 - 42.5°
2. $CH_3.CH(NHOH)$-COOH	11	148.4 - 49.8°
3. $CH_3.CH_2.CH(NHOH)$-COOH	36	172.0 - 73.8°
4. $(CH_3)_2CH.CH(NHOH)$-COOH	19	182.8 - 84.0°
5. $(CH_3)_2CH.CH_2.CH(NHOH)$-COOH	23	173.0 - 74.0°
6. $CH_3.CH_2.CH(CH_3).CH(NHOH)$-COOH	21	171.5 - 72.5°
7. $C_6H_5.CH_2.CH(NHOH)$-COOH	44	158.2 - 59.2°

pink coloured precipitate of impure α-hydroxylaminoacetic acid. The remaining solution on evaporation to dryness gave a mixture of sodium bromide and α-hydroxylaminoacetic acid. The first residue of impure α-hydroxylaminoacetic acid was dissolved in a minimum amount of water and applied to a cation exchange column (Dowex 50W-X4, 200-400 mesh) in the hydrogen ion form and then eluted with 2% ammonia. Only a small fraction could be obtained which was reducing and did not give a positive ninhydrin test. Evaporation of this fraction under reduced pressure at room temperature gave α-hydroxylaminoacetic acid in low yield.

With the α-hydroxylaminopropionic acid attempts to separate the α-hydroxylamino acid by crystallization from the sodium bromide failed since both compounds appeared to have similar solubilities. The reaction mixture in this case was evaporated to dryness under reduced pressure. The residue was dissolved in a minimum amount of water and passed onto a cation exchange column in the hydrogen ion form. A large excess of resin was required as the sodium ions were preferentially attached to the column. The column was washed well with water to remove all traces of bromide ions and then eluted with 2% ammonia. As in the case of α-hydroxylaminoacetic acid only a small fraction of α-hydroxylamino-propionic acid free from α-amino acid impurities could be obtained.

Closer investigation into the nature of the chemistry involved in the separation of zwitterionic species by ion exchange columns revealed the solution to the above separation problem. It has been shown (Partridge and Brimley, 1952) that elution of amino acids from

cation exchange columns is mainly controlled by the pK_1 rather than the pK_2 values. Elution in this case occurs in order of increasing pK_1 values. With anion exchange columns, however, elution depends on the pK_2 with elution occurring in decreasing order of the pK_2 values.

Now, as was shown in Chapter One, α-hydroxylamino acids and their corresponding α-amino acid analogues have approximately the same pK_1 values. Thus, as was found experimentally above, poor separation of the α-hydroxylamino acid from the corresponding α-amino acid is to be expected. The pK_2 values of the two are, however, quite different, that of the α-amino acid being approximately 3.5 pK units higher than that of the α-hydroxylamino acid. One would thus expect good separation by the use of anion exchange columns.

This prediction was tested experimentally with known mixtures of α-hydroxylamino acids and the corresponding α-amino acids. The mixture was applied to an anion exchange column (Dowex 2-X8, 200-400 mesh) in the hydroxide ion form, and elution was carried out with 0.1 N acetic acid or 0.1 N hydrochloric acid. A clean separation was obtained, the α-amino acid leaving the column first, followed by the α-hydroxylamino acid as expected. This procedure was then used to purify the α-hydroxylamino analogues of glycine and alanine.

The method of preparing α-hydroxylamino acids from α-bromo acids coupled with the use of anion exchange columns as a means of final purification of the product, where necessary, appears to be of general application.

Chromatography of the α-hydroxylamino acids

Both paper chromatography and thin layer chromatography techniques were used to assay the purity of the α-hydroxylamino acids. The α-hydroxylamino acids were detected by their reducing action on ammoniacal silver nitrate or triphenyltetrazolium chloride (Snow, 1954). The α-amino acids were detected in the usual way with ninhydrin.

The paper chromatography work gave rise to somewhat different results than those found in earlier work (Ahmad, 1960). Ahmad found that, of the seven α-hydroxylamino acids studied, only α-hydroxylaminopropionic acid and α-hydroxylaminoisobutyric acid gave reducing spots when the α-hydroxylamino acids were chromatographed in the solvent system n-BuOH:HOAc:H_2O (40:10:50 by volume). In each case, however, positive ninhydrin spots appeared which were found to have Rf values identical to those of the corresponding α-amino acids, the Rf values being lower than those of the α-hydroxylamino acids. It was concluded that most of the α-hydroxylamino acids disproportionated completely in the course of chromatography.

In the work here reported it was found, however, that when fresh ethanol solutions of the α-hydroxylamino acids were spotted at room temperature on paper and immediately chromatographed in the same solvent system used by Ahmad, reducing spots were found in all cases. In addition, ninhydrin positive spots with Rf values identical with those of the reducing spots were also observed in each case. The ninhydrin spots appeared slowly on heating at 110° for 5 - 10 minutes or on

TABLE 4

Rf Values of α-Hydroxylamino and α-amino acids in n-BuOH:HOAc:H_2O

Solvent System:

α-Hydroxylamino acids	Rf Values of the α-Hydroxylamino Acids	Rf Values of the corresponding authentic α-amino acids
1. $CH_2(NH_2^+OH)\text{-}COO^-$	0.70	0.20
2. $CH_3.CH(NH_2^+OH)\text{-}COO^-$	0.49	0.33
3. $CH_3.CH_2.CH(NH_2^+OH)\text{-}COO^-$	0.57	0.43
4. $(CH_3)_2CH.CH(NH_2^+OH)\text{-}COO^-$	0.50	0.45
5. $(CH_3)_2CH.CH_2.CH(NH_2^+OH)\text{-}COO^-$	0.79	0.74
6. $CH_3.CH_2.CH(CH_3).CH(NH_2^+OH)\text{-}COO^-$	0.77	0.72
7. $C_6H_5.CH_2.CH(NH_2^+OH)\text{-}COO^-$	0.71	0.65

prolonged standing at room temperature. These spots often appeared orange at first, slowly undergoing a colour change to purple. As mentioned earlier, Snow (1954) reported that α-hydroxylamino acids gave weak yellow or orange colours with ninhydrin.

When the α-hydroxylamino acid solutions were allowed to stand for a prolonged period of time before chromatography, two ninhydrin positive spots were found in some cases. One of these corresponded in Rf value to an equivalent reducing spot while the other had the same Rf value as the corresponding α-amino acid. These same results were also found by spotting fresh solutions on paper and allowing the paper to stand at room temperature overnight prior to chromatographing. Only in a few cases did the double ninhydrin spots appear and in every case positive reducing spots were still detected.

These results indicate that pure α-hydroxylamino acids can be chromatographed in the usual way and visualized either directly with an oxidizing agent or, after standing or heating, with ninhydrin. The fact that these ninhydrin spots have long tails indicates that some disproportionation is also occurring during chromatography.

Thus, when the ethanol solutions of α-hydroxylamino acids or the spotted chromatograms were allowed to stand, some disproportionation takes place prior to development, giving rise to the appearance of double ninhydrin spots, the lower one being due to the corresponding α-amino acid arising from the disproportionation reaction before the chromatograms are developed.

The earlier results (Ahmad, 1960), in which no reducing spots and only ninhydrin spots corresponding to authentic α-amino acids were detected, were presumably due to partial or complete disproportionation prior to chromatography. Ahmad had, in fact, used heat to dry the spotted α-hydroxylamino acid solutions on the chromatograms before development.

In order to substantiate this interpretation of Ahmad's results, spotted chromatograms of several α-hydroxylamino acids were heated prior to chromatography. In each case no reducing spots were detected and only single ninhydrin spots, corresponding to the authentic α-amino acids, were observed. It thus appears that Ahmad had actually chromatographed α-amino acids, formed from the α-hydroxylamino acids by the disproportionation reaction, rather than the α-hydroxylamino acids themselves.

The technique of thin layer chromatography was also applied to the α-hydroxylamino acids. Best results were obtained using Silica Gel G as the supporting substance and n-propanol:water (50:50 by volume) as solvent. Other solvents such as chloroform:methanol:17% ammonia (2:2:1 by volume) and n-propanol:water (70:30 by volume) were also used. The α-hydroxylamino acids and α-amino acids were detected as in the paper chromatography work described earlier. As expected, the reducing spots and the corresponding ninhydrin spot, the latter forming slowly after development, had higher Rf values than the ninhydrin spots of the authentic α-amino acids.

Reactions of α-hydroxylamino acids:

(1) Reaction of α-hydroxylamino acids with hydroxylamine:

In Chapter One reference was made to the preparation of α-oximino acids by the treatment of α-halo acids with excess hydroxylamine under alkaline conditions (Hantzsch and Wild, 1896). The reaction was thought to proceed by way of an α-hydroxylamino acid intermediate and this proposal received support from the fact that the treatment of α-bromo acids with stoichiometric amounts of hydroxylamine yields α-hydroxylamino acids. Such a mechanism requires that reaction of the intermediate α-hydroxylamino acid with excess hydroxylamine should yield α-oximino acids and ammonia in a reaction analogous to the disproportionation reaction now established for α-hydroxylamino acids (Spenser and Ahmad, 1961).

In order to gain some evidence for the above proposal, experiments were carried out in which a known quantity of α-hydroxylamino acid was treated in aqueous solution with one equivalent of hydroxylamine and a one mole excess of alkali. The reaction mixture was refluxed gently under nitrogen for 20 hours and the ammonia released from the basic solution was trapped in a 10% boric acid solution containing methyl red-methylene blue indicator. The ammonia produced could thus be determined by titration with standard acid. α-Oximino acid was isolated by acidification of the reaction mixture and extraction with ether.

This reaction was performed on three α-hydroxylamino acids (α-hydroxylamino-β-phenylpropionic acid, α-hydroxylaminobutyric acid,

and α-hydroxylaminoisocaproic acid) and in each case ammonia and α-oximino acid were formed. The yields of ammonia varied from 92% for the first case to 78% for the last. The corresponding yields of the α-oximino acids were somewhat lower, ranging from 53% to 47%.

These results would thus seem to indicate that α-hydroxylamino acids do react with hydroxylamine in an oxidation-reduction reaction analogous to the disproportionation reaction and that the reaction of α-halo acids with excess hydroxylamine under basic conditions does indeed involve an α-hydroxylamino acid intermediate.

The α-oximino acids might, of course, have been formed from α-hydroxylamino acids in the normal disproportionation reaction. This, however, would result in a maximum yield of 50% since one mole of α-hydroxylamino acid would give rise to only one half mole each of α-oximino acid and α-amino acid. Since in all three cases studied only small amounts of α-amino acids were detected while the α-oximino acids were isolated in approximately 50% yields, it seemed doubtful that the α-oximino acids had formed by the disproportionation reaction. If this were the case, treatment of α-hydroxylamino acids with a one mole excess of alkali in the absence of hydroxylamine should yield α-oximino acids and α-amino acids in equal amounts. As will be shown shortly no α-oximino acids were isolated from such a reaction and only faint ninhydrin tests were obtained.

As mentioned earlier, the yields of ammonia were much higher than the corresponding yields of α-oximino acids and the possibility

existed that the ammonia arose from other reactions. This might have been expected since hydroxylamine is known to decompose in basic solution to ammonia among other products. In order to gain some idea of the importance of these other effects, various control reactions were also performed. When equivalent amounts of hydroxylamine were treated with base, under the conditions of the earlier reaction, ammonia was formed but the yield was never greater than 17%.

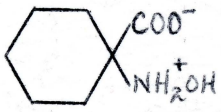
Control reactions in which α-hydroxylamino acids were treated with a one mole excess of alkali were also run. Ammonia was also released in these cases, and in some experiments in quite high yields (see Table 5). In no case was α-oximino acid isolated. Instead the corresponding α-keto acids were isolated.

The normal disproportionation reaction does not, therefore, occur under alkaline conditions. However, the possibility existed that the disproportionation reaction was taking place and that α-oximino acid so formed underwent immediate hydrolysis to α-keto acid and hydroxylamine and that the latter was converted to ammonia in the presence of base. Such a sequence might account for the products which were isolated. This possibility was, however, eliminated by a further control reaction. Treatment of an α-oximino acid with alkali produced no ammonia and no α-keto acid, and the major part of the α-oximino acid was recovered on acidification and extraction with ether. α-Oximino acids are thus stable under alkaline conditions.

These results clearly indicate that the normal disproportionation reaction of the α-hydroxylamino acids, occurring in neutral or acidic

TABLE 5

Decomposition of α-Hydroxylamino acids in the presence of one mole excess alkali:

α-Hydroxylamino acids (HL-AA)	(HL-AA) taken (moles)	α-Keto acids formed		Ammonia formed	
		Amount (moles)	% of theory	Amount (moles)	% of theory
1. $CH_3.CH(NH_2OH^+)-COO^-$	8.7×10^{-4}	2.9×10^{-4}*	33	4.1×10^{-4}	47
2. $CH_3.CH_2.CH(NH_2OH^+)-COO^-$	4.1×10^{-3}	2.0×10^{-3}*	49	2.5×10^{-3}	59
3. $(CH_3)_2CH.CH(NH_2OH^+)-COO^-$	7.5×10^{-4}	1.1×10^{-4}*	15	1.5×10^{-4}	19
4. $CH_3.CH_2.CH(CH_3).CH(NH_2OH^+)-COO^-$	1.3×10^{-3}	3.2×10^{-4}*	25	5.3×10^{-4}	41
5. $C_6H_5.CH_2.CH(NH_2OH^+)-COO^-$	1.4×10^{-3}	9.7×10^{-4}	69	1.0×10^{-3}	75
6. $(CH_3)_2-C(NH_2OH^+)-COO^-$	8.4×10^{-4}	0	0	0	0
7. 	6.3×10^{-4}	1.3×10^{-5}*	2	2.7×10^{-5}	4

* Determined as the 2,4-dinitrophenylhydrazone derivatives

solutions, does not take place under alkaline conditions, and that a base-catalyzed decomposition to α-keto acids and ammonia does occur. These facts, coupled with the fact that α-hydroxylamino acids react with hydroxylamine under alkaline conditions forming α-oximino acids and ammonia, verify the earlier proposal (Hantzsch and Wild, 1896) that the conversion of α-halo acids to α-oximino acids with excess hydroxylamine under alkaline conditions takes place through an α-hydroxylamino acid intermediate.

(2) Conversion of α-hydroxylamino acids to α-keto acids:

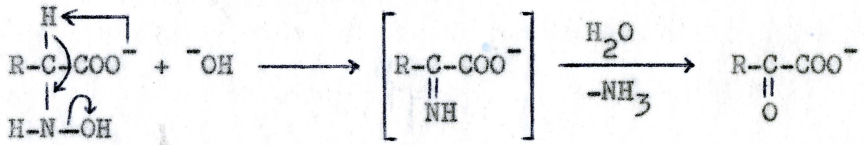
As was shown in the previous section, α-hydroxylamino acids decompose in alkaline solution to α-keto acids and ammonia rather than disproportionating to α-amino acids and α-oximino acids. This reaction was a new aspect of the chemistry of the α-hydroxylamino acids and was thus investigated further. A quantitative study of the reaction of various α-hydroxylamino acids with alkali was undertaken.

Only in the case of α-hydroxylamino-β-phenylpropionic acid was the α-keto acid, phenylpyruvic acid, isolated as such. Phenylpyruvic acid is a high melting solid and very soluble in ether while aliphatic α-keto acids are low melting solids or liquids, very soluble in water as well as ether. In these cases the α-keto acids (pyruvic acid, α-ketobutyric acid, α-ketoisovaleric acid, and α-keto-β-methylvaleric acid) were isolated as their 2,4-dinitrophenylhydrazone derivatives. The yields in this series of reactions were quite varied (see Table 5).

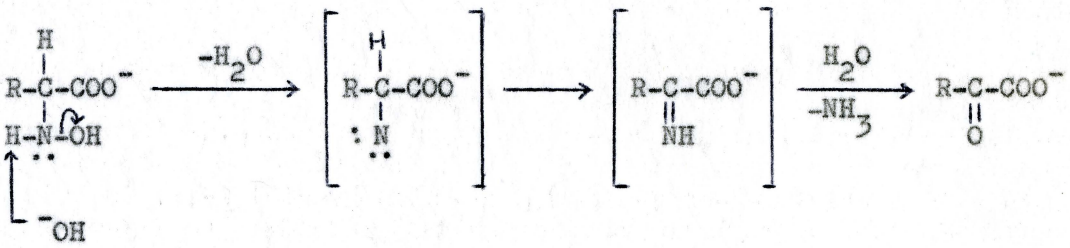
In the two cases where no hydrogen atom was present on the α-carbon (α-hydroxylaminoisobutyric acid and 1-hydroxylaminocyclohexane-

1-carboxylic acid) no ammonia was produced. The small amount of cyclohexanone obtained from the cyclohexyl derivative would be expected to arise from the decomposition of the α-nitroso compound formed in the normal disproportionation reaction. Indeed, a faintly positive ninhydrin test, indicative of the formation of some α-amino acid, was observed.

It is interesting to speculate on the mechanism of this base-catalyzed decomposition of α-hydroxylamino acids to α-keto acids. One possible mechanism involves the abstraction of the hydrogen atom on the α-carbon atom with concurrent loss of hydroxide ion from the nitrogen. This would result in the formation of an imine intermediate which would rapidly hydrolyze to ammonia and α-keto acid.



Another possibility involves the formation of a nitrene intermediate which would by rearrangement give the imine, which on hydrolysis would yield ammonia and α-keto acid.



This base-catalyzed decomposition of α-hydroxylamino acids is also of interest in connection with the mechanism of the chemical oxidation of α-amino acids. As stated in Chapter One, the mechanism

of Sweeley and Horning (1957) involving an α-hydroxylamino acid intermediate required the α-hydroxylamino acid to decompose under alkaline conditions to aldehyde, carbon dioxide, and ammonia. The above work, in which alkaline decomposition has been shown to yield α-keto acids and ammonia, thus reaffirms the previous arguments against the mechanism of Sweeley and Horning.

(3) Conclusion:

Whereas in neutral and acidic solutions α-hydroxylamino acids decompose to equal amounts of α-amino and α-oximino acids by means of a disproportionation reaction, it has now been shown that in alkaline solutions this disproportionation reaction is no longer favoured. A base-catalyzed decomposition to ammonia and α-keto acids occurs.

This reaction would appear to be a general synthetic route to α-keto acids. The various general methods of preparing α-keto acids have been reviewed (Waters, 1947). The above method appears to be a useful addition to these previous methods and is worthy of further investigation.

CHAPTER 3

EXPERIMENTAL

Separation of a known mixture of α-hydroxylamino acid and α-amino acid on an anion exchange column:

α-Hydroxylaminobutyric acid (0.10 g, 0.00084 mole) and α-aminobutyric acid (0.10 g, 0.00096 mole) were dissolved in 100 ml of water and applied to an anion exchange column (Dowex 2-X8, 200-400 mesh, OH^- form).

The anion exchange column was prepared by suspending the resin (6 g), in the Cl^- form, in water and depositing it in a column of 20 mm diameter. The resin was washed with 500 ml carbonate-free water and then converted to the OH^- form by treatment with 2N carbonate-free sodium hydroxide (CO_2-free alkali was prepared by adding 10 g $Ba(OH)_2 . 8H_2O$ to 1 litre of 2N NaOH and filtering immediately before use) until the effluent was basic. After washing with carbonate-free water, the resin was treated with 0.1N hydrochloric acid and then with 2N carbonate-free sodium hydroxide in order to rid the column of impurities. The resin was washed well with carbonate-free water after each treatment.

The known mixture was then applied to the column, followed by 100 ml of carbonate-free water. The column was then eluted with 0.1N hydrochloric acid and the effluent collected in 10 ml fractions. The first twenty fractions (200 ml) gave a positive ninhydrin test and a negative reducing test. On evaporation to dryness under reduced pressure these gave α-aminobutyric acid (0.086 g, 86%).

The next five fractions (50 ml), which gave both a positive ninhydrin test and a positive reducing test, contained a mixture of α-aminobutyric acid and α-hydroxylaminobutyric acid. This was discarded.

The next fifteen fractions (150 ml) gave a negative ninhydrin test and a positive reducing test. This on evaporation to dryness under reduced pressure gave α-hydroxylaminobutyric acid (0.074 g, 74%).

The final six fractions (60 ml), which gave a positive reducing test and a positive chloride ion test, contained the hydrochloride of α-hydroxylaminobutyric acid in the presence of hydrochloric acid. This was discarded.

The same procedure was used in the workup of the reaction mixtures obtained in the preparation of α-hydroxylaminoacetic acid and α-hydroxylaminopropionic acid.

α-Hydroxylaminoacetic acid:

α-Bromoacetic acid (10.0 g, 0.072 mole), dissolved in 350 ml anhydrous methanol, was neutralized at 0° C with 3 M sodium methoxide. Similarly, hydroxylamine hydrochloride (5.0 g, 0.072 mole), dissolved in 100 ml anhydrous methanol, was neutralized in the cold with 3 M sodium methoxide. The two solutions were mixed and the precipitated sodium chloride was removed by filtration.

The reaction mixture was refluxed for 16 hours and then evaporated to one half volume. The light pink solid, which separated from the solution, was removed by filtration. This impure residue of

α-hydroxylaminoacetic acid was purified on an anion exchange column in the manner described previously in this chapter. In this way pure α-hydroxylaminoacetic acid (0.61 g, 9.3%), with melting point 139.5 - 42.5° C (decomp.), was obtained.

Infra-red absorption frequencies in nujol mull (cm^{-1}):695(w), 725(w), 844(w), 885(w), 956(w), 1220 (strong and broad under the tip of the peak), 1350 (shoulder), 1710 (strong and broad under the tip of the peak), 2200-2700 region (medium intensity absorption), 3420(m).

Reported melting points: 135° (decomp.), (Traube, 1895); 145° (decomp.), (Kaczka, Gitterman, Dulaney, and Folkers, 1962).

α-Hydroxylaminopropionic acid:

α-Bromopropionic acid (22 g, 0.144 mole), dissolved in 100 ml dry methanol, was neutralized at 0° C with 3 M sodium methoxide. Similarly, hydroxylamine hydrochloride (10 g, 0.144 mole), dissolved in 150 ml dry methanol, was neutralized in the cold with 3 M sodium methoxide. The two solutions were mixed and the precipitated sodium chloride was removed by filtration.

The reaction mixture was refluxed for 16 hours and then evaporated to dryness under reduced pressure. The residue was dissolved in 100 ml of water and applied to a cation exchange column (Dowex 50W-X4, 200-400 mesh, H^+ form).

The cation exchange column was prepared by suspending the resin (12 g), in the H^+ form, in water and depositing in a column of 20 mm diameter. After washing the resin well with water, the impure α-

hydroxylaminopropionic acid solution was applied. The column was then washed with water until the effluent was free of chloride ion. Elution with 2% aqueous ammonia was then carried out and the effluent collected in 30 ml fractions.

The first three fractions (90 ml) gave a negative ninhydrin test and a positive reducing test. This on evaporation to dryness under reduced pressure gave α-hydroxylaminopropionic acid (1.7 g, 11.3%), which on recrystallization from ethanol melted at 148.4 - 49.6° C (decomp.). Analysis: Found: C, 34.48; H, 6.80; N, 13.13: $C_3H_7NO_3$ requires C, 34.29; H, 6.66; N, 13.33. Infra-red absorption frequencies in nujol mull (cm^{-1}): 672(s), 718(w), 770(w), 798(w), 858(m), 905(m), 960(w), 1002(m), 1058(w), 1088(m), 1146(m), 1262(s), 1272 (shoulder), 1302(s), 1510-1600 region (s), 2200-2700 region (medium intensity absorption). Reported melting points: 194 - 95° (decomp.), (Neelakantan and Hartung, 1958); 146 - 47° (decomp.), (Ahmad, 1960).

The next four fractions (120 ml) gave both a positive ninhydrin test and a positive reducing test. These fractions contained a mixture of α-hydroxylaminopropionic acid and alanine and were discarded.

In a separate experiment an impure mixture of α-hydroxylaminopropionic acid, alanine, and sodium bromide of unknown composition was purified by the use of an anion exchange column in the manner described earlier in this chapter. The column was eluted with 0.1 N hydrochloric acid and the effluent collected in 10 ml fractions.

The first twenty-two fractions (220 ml) gave a positive ninhydrin test. These fractions contained alanine and were discarded.

The next four fractions (40 ml) gave a positive ninhydrin test and a positive reducing test. These fractions contained a mixture of alanine and α-hydroxylaminopropionic acid and were discarded.

The final five fractions (50 ml) gave a positive reducing test and on evaporation to dryness under reduced pressure gave pure α-hydroxylaminopropionic acid (0.10 g) which melted at 153.5 - 57.0° C (decomp.).

α-Hydroxylaminobutyric acid:

α-Bromobutyric acid (15 g, 0.09 mole), dissolved in 100 ml dry methanol, was neutralized at 0° C with 3 M sodium methoxide. Similarly, hydroxylamine hydrochloride (6.3 g, 0.09 mole), dissolved in 100 ml dry methanol, was neutralized in the cold with 3 M sodium methoxide. The two solutions were mixed and the precipitated sodium chloride was removed by filtration.

The reaction mixture was refluxed for 16 hours and then set aside to cool. Colourless crystals of α-hydroxylaminobutyric acid (3.3 g), which separated after 24 hours at 0° C, were removed by filtration. The reaction mixture was then evaporated to dryness under reduced pressure and treated with 100 ml of water when a second crop of α-hydroxylaminobutyric acid (0.5 g) was obtained as an insoluble residue.

The two fractions gave a total yield of 3.8 g (35.5%) of α-hydroxylaminobutyric acid, which on recrystallization from ethanol melted at 172.0 - 73.8° C (decomp.). Analysis: Found: C, 41.10; H, 7.81; N, 11.83: $C_4H_9NO_3$ requires: C, 40.34; H, 7.62; N, 11.76. Infra-red absorption frequencies of α-hydroxylaminobutyric acid in nujol mull

(cm^{-1}): 680(w), 750(w), 814(m), 855(s), 935(w), 974(w), 1015(s), 1030 (shoulder), 1078(w), 1109(w), 1150(w), 1250(w), 1285 (shoulder), 1302(s), 1340(m), 1420 (shoulder), 1612 (strong and broad under the tip of the peak), 2400-2800 region (medium intensity absorption), 3030 (shoulder). Reported melting points: 193 - 94° (decomp.), (Neelakantan and Hartung, 1958); 172 - 73° (decomp.), (Ahmad, 1960); 166 - 67° (decomp.), (v. Miller and Plöchl, 1893).

α-Hydroxylaminoisovaleric acid:

α-Bromoisovaleric acid (8.6 g, 0.047 mole), dissolved in 100 ml dry methanol, was neutralized at 0° C with 3 M sodium methoxide. Similarly, hydroxylamine hydrochloride (3.3 g, 0.047 mole), dissolved in 100 ml dry methanol, was neutralized in the cold with 3 M sodium methoxide. The two solutions were mixed and the precipitated sodium chloride was removed by filtration.

The reaction mixture was refluxed for 16 hours and then set aside to cool. Colourless crystals of α-hydroxylaminoisovaleric acid (0.7 g), which separated after 24 hours at 0° C, were removed by filtration. The reaction mixture was then evaporated to dryness under reduced pressure and treated with 100 ml of water when a second crop of α-hydroxylaminoisovaleric acid (0.5 g) was obtained as an insoluble residue.

The two fractions gave a total yield of 1.2 g (19.1%) of α-hydroxylaminoisovaleric acid, which on recrystallization from ethanol melted at 182.8 - 84.0° C (decomp.). Analysis: Found: C, 45.32; H, 8.36; N, 10.60: $C_5H_{11}NO_3$ requires: C, 45.10; H, 8.27; N, 10.53.

Infra-red absorption frequencies of α-hydroxylaminoisovaleric acid in nujol mull (cm^{-1}): 678(w), 720(w), 775(w), 786(w), 810(w), 844(m), 918(w), 933(w), 1010(m), 1048(w), 1158(w), 1180(w), 1260(w), 1310(m), 1330(w), 1608 (strong and broad under the tip of the peak), 2300-2700 region (medium intensity absorption). Reported melting points: 204° (decomp.), (Cook and Slater, 1956); 192 - 93° (decomp.), (Neelakantan and Hartung, 1958); 198° (decomp.), (Ahmad, 1960).

α-Hydroxylaminoisocaproic acid:

α-Bromoisocaproic acid (20 g, 0.10 mole), dissolved in 100 ml dry methanol, was neutralized at 0° C with 3 M sodium methoxide. Similarly, hydroxylamine hydrochloride (6.95 g, 0.10 mole), dissolved in 100 ml dry methanol, was neutralized in the cold with 3 M sodium methoxide. The two solutions were mixed and the precipitated sodium chloride was removed by filtration.

The reaction mixture was refluxed for 16 hours and then set aside to cool. Colourless crystals of α-hydroxylaminoisocaproic acid (3.0 g), which separated after 24 hours at 0° C, were removed by filtration. The reaction mixture was then evaporated to dryness under reduced pressure and treated with 100 ml of water when a second crop of α-hydroxylaminoisocaproic acid (0.4 g) was obtained as an insoluble residue.

The two fractions gave a total yield of 3.4 g (23.1 %) of α-hydroxylaminoisocaproic acid, which on recrystallization from ethanol melted at 173 - 74° C (decomp.). Analysis: Found: C, 48.92; H, 8.96; N, 9.43: $C_6H_{13}NO_3$ requires: C, 48.97; H, 8.90; N, 9.52. Infra-red

absorption frequencies in nujol mull (cm^{-1}): 690(w), 758(w), 764 (shoulder), 855(m), 918(w), 953(w), 970(w), 981(w), 1010(m), 1055 (shoulder), 1075(w), 1122(w), 1140(w), 1168(w), 1275(m), 1285(m), 1320(w), 1612 (strong and broad under the tip of the peak), 2400-2700 region (medium intensity absorption), 3110 (shoulder). Reported melting points: 194 - 95° (decomp.), (Neelakantan and Hartung, 1958); 155° (decomp.), (Cook and Slater, 1956); 151° (decomp.), (v. Miller and Plöchl, 1893).

α-Hydroxylamino-β-methylvaleric acid:

α-Bromo-β-methylvaleric acid (11.9 g, 0.061 mole), dissolved in 100 ml dry methanol, was neutralized at 0° C with 3 M sodium methoxide. Similarly, hydroxylamine hydrochloride (4.3 g, 0.061 mole), dissolved in 100 ml dry methanol, was neutralized in the cold with 3 M sodium methoxide. The two solutions were mixed and the precipitated sodium chloride was removed by filtration.

The reaction mixture was refluxed for 16 hours and then set aside to cool. Colourless crystals of α-hydroxylamino-β-methylvaleric acid (1.7 g), which separated after 24 hours at 0° C, were removed by filtration. The reaction mixture was then evaporated to dryness under reduced pressure and treated with 100 ml of water when a second crop of α-hydroxylamino-β-methylvaleric acid (0.2 g) was obtained as an insoluble residue.

The two fractions gave a total yield of 1.9 g (21.1%) of α-hydroxylamino-β-methylvaleric acid, which on recrystallization from ethanol melted at 171.5 - 72.5° C (decomp.). Analysis: Found: C, 49.06; H, 8.93; N, 9.32: $C_6H_{13}NO_3$ requires: C, 48.97; H, 8.90; N, 9.52.

Infra-red absorption frequencies in nujol mull (cm^{-1}): 720(w), 775(w), 795(w), 844(w), 1010(m), 1020(m), 1060(w), 1076(w), 1160(w), 1250 (shoulder), 1265(w), 1300(m), 1342(m), 1612 (strong and broad under the tip of the peak), 2400-2750 region (medium intensity absorption), 3030 (shoulder).

α-Bromo-β-phenylpropionic acid:

dl-Phenylalanine (16.5 g, 0.10 mole) was dissolved in 165 ml of 3 N sulphuric acid and cooled to 0° C. Finely-divided potassium bromide (40 g, 0.34 mole) was added with constant stirring. A saturated water solution of sodium nitrite (9.0 g, 0.10 mole) was added dropwise at -10° C over a 3-4 hour period. The reaction mixture was then diluted with 100 ml of water and extracted with ether (3 x 200 ml). The ether extract was dried over anhydrous sodium sulphate and then evaporated under reduced pressure to a pale-yellow syrup. This syrup was vacuum distilled and the α-bromo-β-phenylpropionic acid (11.5 g, 50%) fraction boiling at 128 - 36° C at 0.16 mm pressure was collected, which on cooling solidified to a colourless, crystalline solid with melting point 48 - 52° C.

α-Hydroxylamino-β-phenylpropionic acid:

α-Bromo-β-phenylpropionic acid (15 g, 0.065 mole), dissolved in 100 ml dry methanol, was neutralized at 0° C with 3 M sodium methoxide. Similarly, hydroxylamine hydrochloride (4.5 g, 0.065 mole), dissolved in 100 ml dry methanol, was neutralized in the cold with 3 M sodium methoxide. The two solutions were mixed and the precipitated sodium

chloride was removed by filtration.

The reaction mixture was refluxed for 16 hours and then set aside to cool. Colourless crystals of α-hydroxylamino-β-phenylpropionic acid (4.5 g), which separated after 24 hours at 0° C, were removed by filtration. The reaction mixture was evaporated to dryness under reduced pressure and treated with 100 ml of water when a second crop of α-hydroxylamino-β-phenylpropionic acid (0.7 g) was obtained as an insoluble residue.

The two fractions gave a total yield of 5.2 g (44.2%) of α-hydroxylamino-β-phenylpropionic acid, which on recrystallization from ethanol melted at 158.2 - 59.2° C (decomp.). Analysis: Found: C, 59.52; H, 6.23; N, 7.71: $C_9H_{11}NO_3$ requires: C, 59.67; H, 6.08; N, 7.73. Infra-red absorption frequencies in nujol mull (cm^{-1}): 698(s), 720(w), 746(m), 764(w), 873(m), 910(w), 968(w), 990(w), 1020(w), 1058(w), 1075(w), 1120(w), 1280(m), 1290 (shoulder), 1318(w), 1610 (strong and broad under the tip of the peak), 2350-2700 region (medium intensity absorption), 3210 (shoulder). Reported melting points: 164 - 65° (decomp.), (Steiger, 1944); 159 - 60° (decomp.), (Neelakantan and Hartung, 1958); 159° (decomp.), (Ahmad, 1960); 157 - 58° (decomp.), (Traube, 1895).

Attempted synthesis of α-bromo-β-(p-hydroxyphenyl)-propionic acid:

dl-Tyrosine (18.1 g, 0.10 mole) was dissolved in 165 ml of 3 N sulphuric acid and cooled to 0° C. Finely-divided potassium bromide (40 g, 0.34 mole) was added with constant stirring. A saturated water solution of sodium nitrite (9.0 g, 0.10 mole), was added dropwise at

-10° C over a 3-4 hour period. The reaction mixture was then diluted with 100 ml of water and extracted with ether (3 x 200 ml). The ether extract was dried over anhydrous sodium sulphate and then evaporated under reduced pressure to a deep red syrup. All attempts to vacuum distil this syrup failed. On heating the liquid darkened in colour and formed a tarry residue.

The reaction was repeated at -20 to -25° C in a dry ice-methanol bath but gave similar results.

Attempted synthesis of α-hydroxylaminosuccinic acid:

Bromosuccinic acid (10 g, 0.051 mole), dissolved in 100 ml dry methanol, was neutralized in the cold with 3 M sodium methoxide. The sodium salt, which precipitated out of solution, was insoluble in large excesses of solvent. Similar experiments with other non-aqueous solvents (absolute ethanol, dioxane, and N,N-dimethylformamide) were tried but were equally unsuccessful. No further attempts to prepare α-hydroxylaminosuccinic acid were made.

Paper chromatography of α-hydroxylamino acids:

Paper chromatography was carried out by the ascending technique on Whatman No. 1 paper. One dimensional runs were performed using the organic layer of an n-butanol:acetic acid:water (40:10:50 by volume) mixture as the solvent. α-Amino acids were detected by the conventional ninhydrin spray. The reducing α-hydroxylamino acids were detected either by spraying with 1% ammoniacal silver nitrate (1.0 g silver nitrate in 100 ml water containing 15 ml of 10 M ammonia) when α-hydroxylamino acids

appeared as black spots, or by spraying with a solution of triphenyltetrazolium chloride (Snow, 1954). The dried papers were first sprayed with 0.1% triphenyltetrazolium chloride in n-butanol saturated with water, the papers were then dried and resprayed with a solution containing aqueous sodium hydroxide (10 ml of 10 M aqueous sodium hydroxide + 40 ml 95% ethanol + 50 ml n-butanol). The α-hydroxylamino acid could then be detected by the immediate formation of a pink spot. Authentic samples of α-amino acids were applied to the same papers for comparison of the Rf values.

Thin layer chromatography of α-hydroxylamino acids:

Thin layer chromatography was carried out with Silica Gel G as the stationary phase and n-propanol:water (70:30 by volume) as the solvent. Other solvents used were n-propanol:water (50:50 by volume) and chloroform:methanol:17% ammonia (2:2:1 by volume). The α-amino acids were detected by the conventional ninhydrin spray and the α-hydroxylamino acids were detected by the triphenyltetrazolium chloride method described in the previous section. Authentic samples of α-amino acids were applied to the same plates and used to compare Rf values.

Reaction of hydroxylamine with α-hydroxylamino-β-phenylpropionic acid:

α-Hydroxylamino-β-phenylpropionic acid (0.25 g, 0.0014 mole), hydroxylamine hydrochloride (0.097 g, 0.0014 mole), and potassium hydroxide (0.24 g, 0.0042 mole) were dissolved in 30 ml of water in a 50 ml two-necked flask. The reaction mixture was refluxed gently for 19 hours.

Nitrogen was passed through the reaction mixture and then through 200 ml of boric acid-indicator solution (20 g of boric acid, 6 ml of 0.2% methyl red in 95% ethanol, and 3 ml of 0.2% methylene blue in water made up to 1 litre).

On cooling to room temperature, the reaction mixture was acidified with 1 M sulphuric acid and extracted with ether (3 x 50 ml). The ether extract was dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue of α-oximino-β-phenylpropionic acid (0.128 g, 51.2%) melted at 164.5 - 66.0° C (decomp.) and had an infra-red absorption spectrum identical with that of authentic α-oximino-β-phenylpropionic acid. A mixed melting point showed no depression.

Titration of the boric acid-indicator solution with standard 0.1 M hydrochloric acid to the end point (colour change from green to grey) was used to determine the ammonia released (0.022 g, 91.7%).

Reaction of hydroxylamine with α-hydroxylaminobutyric acid:

α-Hydroxylaminobutyric acid (0.50 g, 0.0042 mole), hydroxylamine hydrochloride (0.30 g, 0.0042 mole), and potassium hydroxide (1.00 g, 0.0126 mole) were treated in the same procedure described for α-hydroxylamino-β-phenylpropionic acid. This reaction produced α-oximinobutyric acid (0.260 g, 53%) and ammonia (0.061 g, 86%).

Reaction of hydroxylamine with α-hydroxylaminoisovaleric acid:

α-Hydroxylaminoisovaleric acid (0.25 g, 0.0019 mole), hydroxylamine hydrochloride (0.13 g, 0.0019 mole), and potassium hydroxide (0.34 g, 0.0057 mole) were treated in the same way as described for

TABLE 6

Reaction of hydroxylamine on α-hydroxylamino acids:

α-Hydroxylamino acids (HL-AA)	(HL-AA) taken in (moles)	Hydroxylamine hydrochloride taken in (moles)	Potassium hydroxide taken in (moles)	α-Oximino acid produced Weight (moles)	%	Ammonia released Weight (moles)	%
1. $C_6H_5.CH_2.\underset{\underset{NH_2OH}{|+}}{CH}-COO^-$	1.4×10^{-3}	1.4×10^{-3}	4.2×10^{-3}	7.2×10^{-4}	51	1.3×10^{-3}	92
2. $CH_3.CH_2.\underset{\underset{NH_2OH}{|+}}{CH}-COO^-$	4.2×10^{-3}	4.2×10^{-3}	1.3×10^{-2}	2.2×10^{-3}	53	3.6×10^{-3}	86
3. $(CH_3)_2CH.\underset{\underset{NH_2OH}{|+}}{CH}-COO^-$	1.9×10^{-3}	1.9×10^{-3}	5.7×10^{-3}	8.9×10^{-4}	47	1.6×10^{-3}	78

α-hydroxylamino-β-phenylpropionic acid. This reaction produced α-oximinoisovaleric acid (0.12 g, 47%) and ammonia (0.027 g, 78%).

Action of base on hydroxylamine:

Hydroxylamine hydrochloride (0.097 g, 0.0014 mole) and potassium hydroxide (0.16 g, 0.0028 mole) were treated in the same procedure described for α-hydroxylamino-β-phenylpropionic acid. This reaction produced ammonia (0.004 g, 16.8%).

Conversion of α-hydroxylamino-β-phenylpropionic acid to phenylpyruvic acid:

α-Hydroxylamino-β-phenylpropionic acid (0.25 g, 0.0014 mole) and potassium hydroxide (0.16 g, 0.0028 mole) were dissolved in 30 ml of water and refluxed gently for 19 hours.

Nitrogen was passed through the reaction mixture and through 200 ml of boric acid-indicator solution.

On cooling to room temperature, the reaction mixture was acidified with 1 M sulphuric acid and extracted with ether (3 x 50 ml). The ether extract was dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue of phenylpyruvic acid (0.159 g, 69%) melted at 155.5 - 58.0° C (decomp.) and had an infra-red absorption spectrum and mixed melting point identical with those of authentic phenylpyruvic acid.

Titration of the boric acid-indicator solution with standard 0.1 M hydrochloric acid was used to determine the ammonia released (0.018 g, 75%).

Conversion of α-hydroxylaminobutyric acid to α-ketobutyric acid:

α-Hydroxylaminobutyric acid (0.5 g, 0.0042 mole) and potassium hydroxide (0.50 g, 0.0084 mole) were dissolved in 30 ml of water and refluxed gently for 19 hours.

Nitrogen was passed through the reaction mixture and through 200 ml of boric acid-indicator solution.

On cooling to room temperature, the reaction mixture was acidified with 1 M sulphuric acid. The reaction mixture was then treated with 20 ml of 2,4-dinitrophenylhydrazine reagent (a solution of 3 g of 2,4-dinitrophenylhydrazine in 15 ml of concentrated sulphuric acid was added to 20 ml of water and 70 ml of 95% ethanol). The 2,4-dinitrophenylhydrazone of α-ketobutyric acid (0.57 g, 49%), which separated immediately, was removed by filtration. On recrystallization from ethanol-water it melted at 198.5 - 200.5° C. It had an infra-red absorption spectrum and mixed melting point identical with those of the 2,4-dinitrophenylhydrazone of authentic α-ketobutyric acid.

Titration of the boric acid-indicator solution with standard 0.1 M hydrochloric acid was used to determine the ammonia released (0.042 g, 59%).

Conversion of α-hydroxylaminopropionic acid to pyruvic acid:

α-Hydroxylaminopropionic acid (0.091 g, 0.00087 mole) and potassium hydroxide (0.080 g, 0.00164 mole) were treated in the same procedure described for α-hydroxylaminobutyric acid. This reaction produced ammonia (0.007 g, 47%) and the 2,4-dinitrophenylhydrazone of pyruvic acid (0.078 g, 33%).

Conversion of α-hydroxylaminoisovaleric acid to α-ketoisovaleric acid:

α-Hydroxylaminoisovaleric acid (0.10 g, 0.00075 mole) and potassium hydroxide (0.090 g, 0.0015 mole) were treated in the same procedure described for α-hydroxylaminobutyric acid. This reaction produced ammonia (0.003 g, 19%) and the 2,4-dinitrophenylhydrazone of α-ketoisovaleric acid (0.033 g, 15%).

Conversion of α-hydroxylamino-β-methylvaleric acid to α-keto-β-methylvaleric acid:

α-Hydroxylamino-β-methylvaleric acid (0.19 g, 0.0013 mole) and potassium hydroxide (0.15 g, 0.0026 mole) were treated in the same procedure as described for α-hydroxylaminobutyric acid. This reaction produced ammonia (0.009 g, 41%) and the 2,4-dinitrophenylhydrazone of α-keto-β-methylvaleric acid (0.092 g, 25%).

Action of base on α-hydroxylaminoisobutyric acid:

α-Hydroxylaminoisobutyric acid (0.10 g, 0.00084 mole) and potassium hydroxide (0.099 g, 0.0017 mole) were treated in the same procedure as described for α-hydroxylaminobutyric acid. This reaction produced no ammonia and did not yield a 2,4-dinitrophenylhydrazone.

Action of base on 1-hydroxylaminohexane-1-carboxylic acid:

1-Hydroxylaminohexane-1-carboxylic acid (0.10 g, 0.00063 mole) and potassium hydroxide (0.074 g, 0.0013 mole) were treated in the same procedure as described for α-hydroxylaminobutyric acid. This reaction produced ammonia (0.003 g, 4%) and the 2,4-dinitrophenylhydrazone of cyclohexanone (0.004 g, 2%).

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