The Chemistry of α, *N*-Hydroxyamino Acids

Afzal AHMAD

Department of Chemistry, University of Islamabad, P.O. Box No. 1090, Islamabad, Pakistan

(Received February 4, 1974)

α,*N*-Hydroxyamino acids have been shown to be unstable compounds. They disproportionate into corresponding α-amino acids and α-oximino acids below pH 7. This has been suggested to be a general reaction in all mono-substituted hydroxylamines.

The chemistry of naturally occurring α-amino acids is well established and their biochemical importance is fully understood. Not enough is known, however, about the chemical behaviour of analogous *N*-hydroxyamino acids, which are now found to be present as parts of several natural products.[1-7]

During the course of investigations on the mechanisms of α-amino acid oxidation it was felt necessary to study the chemistry of α,*N*-hydroxyamino acids because of the conflicting reports in the available literature. They have been reported as weak acids without any significant basic properties,[2] and also as compounds giving acidic reaction in aqueous medium.[8] They are also described as amphoteric substances with isoelectric points between pH 6 and 7.[9] With ninhydrin they are reported to give yellow colors,[7] but have also been claimed to give blue or purple colors just like α-amino acids.[9]

Reasons for these discrepancies have now been determined. When α,*N*-hydroxyamino acids are paper chromatographed using the organic layer of *n*-butanol: acetic acid: water (40: 10: 50) as the irrigation solvent and the chromatograms developed with ninhydrin reagent, purple spots with long comet like tails appeared. The R_f values of these spots were found identical with those of the corresponding α-amino acids. When the chromatograms were sprayed with ammoniacal $AgNO_3$ or with triphenyltetrazolium chloride under appropriate conditions, only α,*N*-hydroxyaminopropionic acid and α,*N*-hydroxyaminoisobutyric acid were faintly detected, as black or pink spots. The R_f values of these two hydroxyamino acids were higher than those of the corresponding α-amino acids.

The appearance of long purple streaks on chromatograms and the partial or complete disappearance of α,*N*-hydroxyamino acids, strongly suggested that it was not the α,*N*-hydroxyamino acids which gave blue or purple colours with ninhydrin as reported, but that α,*N*-hydroxyamino acids had been spontaneously transformed to the corresponding α-amino acids during the process of chromatography. The complete identity of the R_f values of the ninhydrin positive products derived from α,*N*-hydroxyamino acids with authentic samples of the corresponding α-amino acids confirmed this transformation. The R_f values of α,*N*-hydroxyamino acids, of α-amino acids derived from the corresponding α,*N*-hydroxyamino acids on the chromatographic paper and of authentic α-amino acids are listed in Table 1. We have now been successful in separating α-amino acids as by-products of the syntheses of α,*N*-hydroxyamino acids.

Since the chromatographic solvent mixture did not contain any reducing agent, the formation of α-amino

Table 1

S. No.	α,*N*-Hydroxyamino acids (HAA)	Potentiometric Titration with 0.1 M NaOH		Paper chromatography in *n*-BuOH:AcOH:H_2O (40:10:50)		
		pK_1 (pK_2)	Isoelectric point $(pK_1+pK_2)/2$	R_f value of HAA	R_f value of α-amino acid derived from HAA	R_f value of authentic α-amino acids
1	CH_3-CH-COO^- NH_2^+OH	2.05 (5.75)	3.90	0.35	0.23	0.23
2	$CH_3 \cdot CH_2$-CH-COO^- NH_2^+OH	2.20 (5.65)	3.92	—	0.36	0.36
3	$CH_3 \cdot CH_2 \cdot CH_2$-CH-$COO^-$ NH_2^+OH	2.35 (5.40)	3.88	—	0.51	0.51
4	$(CH_3)_2$CH-CH-COO^- NH_2^+OH	2.30 (5.80)	4.05	—	0.44	0.44
5	 NH_2^+OH	2.15 (5.20)	3.68	—	0.59	0.59
6	CH_3 CH_3 C-COO^- NH_2^+OH	2.08 (5.95)	4.01	0.46	0.39	0.39
7	 NH_2^+OH	2.45 (5.60)	4.03	—	0.60	0.60

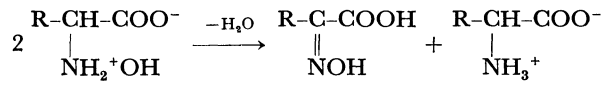


Fig. 1

acids from the corresponding α,*N*-hydroxyamino acids could most easily be explained by taking into consideration the possibility of a disproportionation reaction. The second product, therefore, could either be the corresponding α-nitroso acid or α-oximino acid formed by tautomerization of the nitroso compound (Fig. 1). The presence of α-oximino acids and of blue nitroso compounds on acid hydrolysis of α,*N*-hydroxyamino nitriles have now been demonstrated. The essential requirement of the disproportionation of α,*N*-hydroxyamino acids would be the formation of equimolar quantities of the corresponding α-amino acids and α-oximino acids. Confirmation of this fact came from the quantitative study.

α-Oximino acids, one of the products of disproportionation, have been shown to be unstable compounds in aqueous solutions, especially at higher temperatures and below pH 7. They decompose spontaneously to give lower homologous corresponding nitriles and CO_2[10]

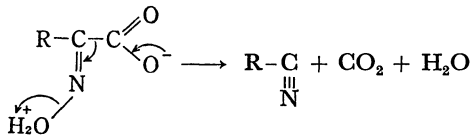


Fig. 2

(Fig. 2). Also since pK_2 ($-N^+H_2OH \rightleftharpoons -NHOH + H^+$) of an α,*N*-hydroxyamino acid is approximately 6 and that of the corresponding α-amino acid is 3—4 pK units higher, it is possible to titrate potentiometrically, a mixture of the two acids with standard alkali to a pH 7.5, which represents a complete titration of hydroxyamonium group of an α,*N*-hydroxyamino acid, whereas at this pH the ammonium group of the amino acid does not react with alkali at all. On addition of formaldehyde at pH 7.5, the pK_2 of the amino acid is sufficiently depressed to make possible the titration of the remaining α-amino acid with a reasonable sharp end point at pH 8.8. One such titration of a mixture of α,*N*-hydroxyaminopropionic acid and the corresponding α-amino acid (alanine) is shown in Fig. 3.

When known amounts of α,*N*-hydroxyamino acids were refluxed in aqueous solution under nitrogen atmosphere, corresponding α-amino acids and α-oximino

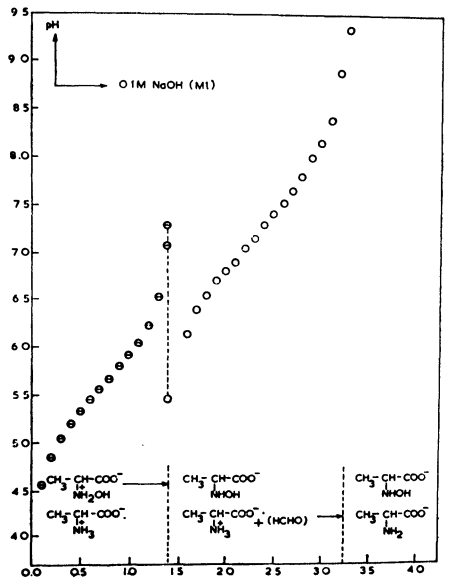


Fig. 3.

acids were obtained as primary products. Under these conditions α-oximino acids were decomposed to give lower nitriles and CO_2. With the exception of α,*N*-hydroxyamino-β-phenylpropionic acid and α,*N*-hydroxyaminoisovaleric acid, all other α,*N*-hydroxyamino acids gave almost equimolar yields of CO_2 (determined as $BaCO_3$) and the corresponding α-amino acids (Table 2). In general, yields of both α-amino acids and CO_2 were than theoretically expected on the basis of 100% disproportionation of the disappeared α,*N*-hydroxyamino acids. In the case of α,*N*-hydroxyaminophenylpropionic acid, the amount of CO_2 evolved was 43% more and phenylalanine formed was 49% less than expected on theoretical consideration based on 100% disproportionation. These differences were perhaps due to some other modes of decomposition in addition to disproportionation.

Since 5 out of 7 α,*N*-hydroxyamino acids studied gave almost equimolar quantities of CO_2 and the corresponding α-amino acids, it is reasonable to infer that two molecules of an α,*N*-hydroxyamino acid undergo mutual oxidation and reduction to give the corresponding α-amino acid and α-oximino acid as the primary products.

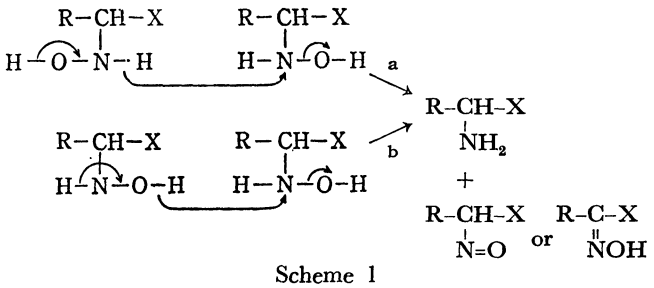
To explain the disproportionation, two mechanisms based on hydride shifts may be considered. Both are essentially alike as in one case nitrogen-linked hydrogen

Table 2

S. No.	α,*N*-Hydroxyamino acids (HAA)	HAA taken mg	HAA reacted mg (%)	α-Amino acid[a] formed mg (%)	$BaCO_3$[b] mg (%)
1	α,*N*-Hydroxyaminopropionic acid	36.00	18.15 (50.4)	8.28 (98.4)	18.0 (96.4)
2	α,*N*-Hydroxyaminobutyric acid	50.00	40.48 (81.0)	14.42 (76.1)	27.0 (74.4)
3	α,*N*-Hydroxyaminovaleric acid	55.00	55.00 (100.0)	18.14 (69.9)	40.0 (91.4)
4	α,*N*-Hydroxyaminoisovaleric acid	50.00	11.43 (22.9)	5.26 (97.6)	9.0 (100.0)
5	α,*N*-Hydroxyamino-β-phenylpropionic acid	50.00	50.00 (100.0)	12.25 (51.0)	41.0 (143.0)
6	α,*N*-Hydroxyaminoisobutyric acid	50.00	20.25 (40.5)	7.21 (76.0)	14.0 (77.0)
7	1-Hydroxyaminocyclohexane-1-carboxylic acid	50.00	40.46 (80.9)	11.45 (59.3)	16.0 (52.6)

a) Percentage of theoretical on the basis of (50/50) disproportionation of the reacted HAA. b) Percentage of theoretical on the basis of quantitative decomposition of α-oximino acid to nitrile and CO_2.

of one molecule of hydroxyamino compound shifts to the nitrogen of the other molecule of hydroxyamino compound as a hydride ion, whereas in the second case a hydrogen from the oxygen rather than the nitrogen of one molecule shifts to nitrogen of the other. (Scheme 1).



Scheme 1

The mechanism (b) seems to be more likely since –OH·····N and N–H·····O; types of hydrogen bonding have been shown to be preferential in solid hydroxylamine.[11]

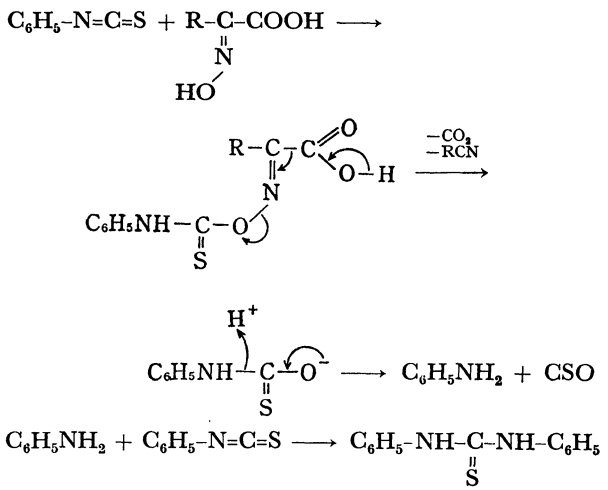
It is now clear that the products obtained by Neelakantan and Hartung[9] and regarded as α,N-hydroxyamino acids and which were described as ninhydrin positive, reducing and amphoteric, were in fact mixtures containing α,N-hydroxyamino, α-amino and α-oximino acids. The reaction products were not carefully analyzed and their true nature was overlooked by them.

When α,N-hydroxyamino-β-phenylpropionic acid was synthesized, a small quantity of α-oximino-β-phenylpropionic acid was actually crystallized out from the reaction mixture. Similarly α-oximino acids in small amounts were found amongst the products of acid hydrolyzates of other α,N-hydroxyamino nitriles. A part of α-oximino acids was possibly lost by decomposition to lower homologous nitriles and CO_2 under the conditions. Amino acids were also found in every case. They were isolated by column chromatography of the crude mixtures by making use of cation and anion exchange resins.

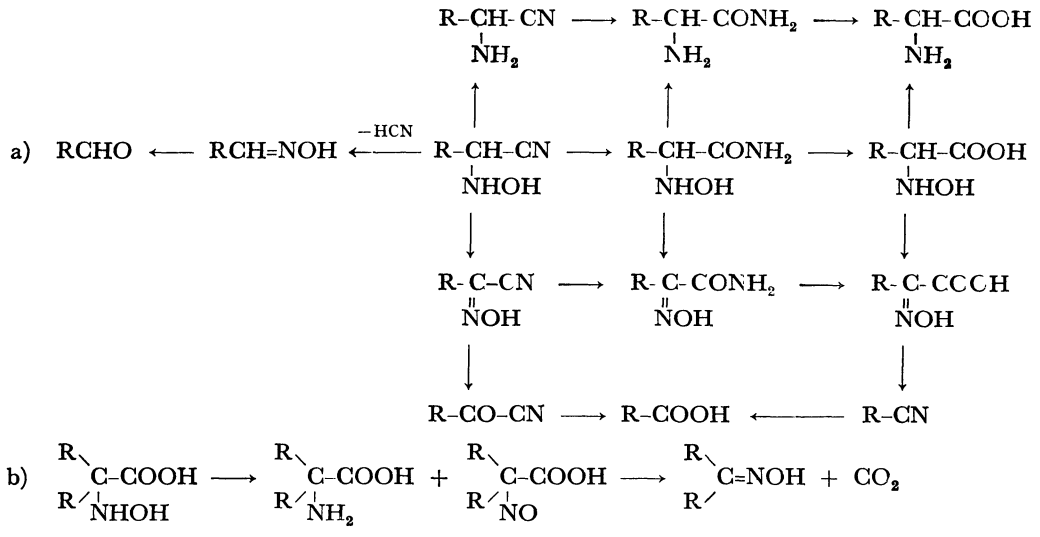
It has now become possible to offer an explanation for the formation of α-amino acid, α-oximino acid, the lower homologous aldehydes and nitriles and the corresponding aliphatic acids as by-products of acid hydrolysis of α,N-hydroxyamino nitriles to give α,N-hydroxyamino acids. This is shown in Scheme 2. Two molecules of α,N-hydroxyamino compound undergo mutual oxidation and reduction to give one molecule each of an α-amino compound and an α-oximino compound. In case of α,N-hydroxyaminoisobutyronitrile and 1-hydroxyamino-1-cyanocyclohexane, which do not possess any hydrogen on α-carbon atoms, similar changes take place but instead of α-oximino compounds, blue nitroso compounds are generated which undergo gradual decomposition.[12]

It has also been observed that all α-oximino acids are quantitatively decomposed to give diphenylthiourea and CO_2 when refluxed for one minute with phenylisothiocyanate in benzene solution or when kept for one week at 40 °C. The possible mechanism of this reaction is shown in Scheme 3.

Analogous experiments with α,N-hydroxyamino acids, contrary to the earlier report,[9] failed to give any diphenylthiourea. But when the reflux time was increased to 5 min or more, a small quantity of diphenyl-



Scheme 3



Scheme 2

thiourea did separate along with a major portion of another unidentified high melting product. It is likely that diphenylthiourea is formed from α-oximino acid which might have been produced by disproportionation of α,*N*-hydroxyamino acid. α,*N*-Hydroxyamino acids prepared by Neelakantan and Hartung yielding diphenylthiourea gives a further evidence in support of our observation that these products were in fact mixtures.

Treatment of α-halo acids with excess hydroxylamine gives rise to α-oximino acids.[13] With equimolar amounts of hydroxylamine, α,*N*-hydroxyamino acids are obtained in 50% yields.[2] The formation of α-oximino acids from α-halo acids and hydroxylamine, a reaction whose oddity has hitherto been ignored can now be explained on the basis of a disproportionation. It is now almost certain that hydroxyamino acid is a primary product which either disproportionates to give the corresponding α-amino acid and α-oximino acid or undergoes oxidation by a second molecule of hydroxylamine which is reduced to ammonia (Fig. 4).

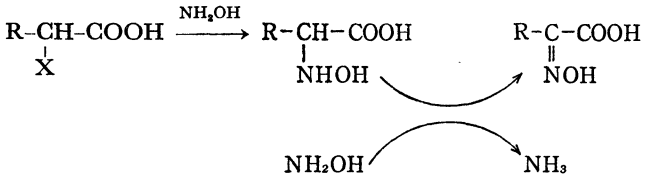


Fig. 4

It is interesting to note that not only α,*N*-hydroxyamino acids, but also hydroxylamine itself, and *N*-methyl and *N*-ethyl hydroxylamines have been reported to decompose in such a fashion[14] to give products whose origin can now be explained easily on the basis of a disproportionation reaction. *N*-Phenylhydroxylamine also gives products compatible with this interpretation.[15]

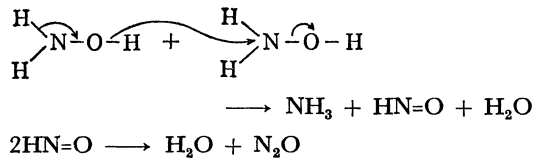


Fig. 5

Hydroxylamine when heated decomposes to give nitrous oxide, ammonia and water. The formation of these products can be explained in the following manner (Fig. 5). *N*-Methylhydroxylamine when distilled in the presence of alkali decomposes to give methylamine, ammonia and formic acid. *N*-Ethylamine under similar conditions gives ethylamine, acetic acid, acetaldehyde and ammonia (Fig. 6).

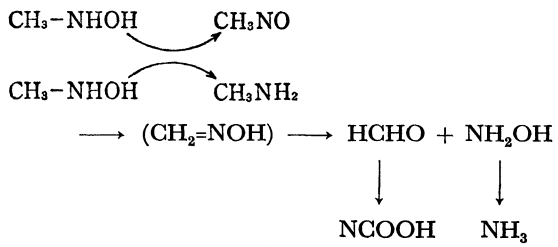


Fig. 6

It is therefore not difficult to infer that not only α,*N*-hydroxyamino acids but also other mono-*N*-substituted hydroxylamines are capable of undergoing disproportionation reactions. This likelihood is at present under investigation and the results will be communicated in a following publication.

Experimental

α,N-*Hydroxyamino Acids.*[16] Six α,*N*-hydroxyamino acids namely (α,*N*-hydroxyaminopropionic acid, α,*N*-hydroxyaminobutyric acid, α,*N*-hydroxyaminoisobutyric acid, α,*N*-hydroxyaminovaleric acid, α,*N*-hydroxyaminoisovaleric acid and 1-hydroxyaminocyclohexane-1-carboxylic acid) were prepared from the corresponding α,*N*-hydroxyaminonitriles by hydrolysis with concentrated hydrochloric acid.[7,9,17,18] When any one of α,*N*-hydroxyaminopropionitrile, α,*N*-hydroxyaminobutyronitrile, α,*N*-hydroxyaminovaleronitrile and α,*N*-hydroxyaminoisovaleronitrile was refluxed with concentrated hydrochloric acid for 5 hr, the reaction mixture was found to contain not only the expected α,*N*-hydroxyamino acid but also the corresponding α-amino acid, α-oximino acid, the lower homologous aldehyde and aliphatic acid. The aldehyde, the aliphatic acid and the oximino acid were separated by ether extraction of the acidic reaction mixture and the α,*N*-hydroxyamino acid was separated from the corresponding α-amino acid by cation and anion exchange resins. The quantity of oximino acid obtained was generally low but increased when the reaction mixture was allowed to stand at room temperature for a longer period before ether extraction.

α,N-*Hydroxyamino-β-phenylpropionic Acid.* α,*N*-Hydroxyamino-β-phenylpropionic acid was obtained from diethylbenzylmalonate.[19] Freshly cut sodium metal (2.3 g, 0.1 mol) was dissolved in absolute ethanol (150 ml) and diethylbenzylmalonate (25.0 g, 0.1 mol) was added, with cooling and stirring. Nitric oxide (generated by dropping conc. H_2SO_4 into a saturated aqueous solution of $NaNO_2$) was bubbled through the ethanolic reaction mixture for one hour. The yellow reaction product was kept overnight at 35 °C and then ethanol removed by evaporating at room temperature under reduced pressure to give a pale yellow slurry which was mixed with 20% aqueous NaOH (60 ml) and kept at 35 °C for 24 hr. The alkaline solution was then extracted with ether to remove any unchanged malonic ester. The solution was then acidified at 5 °C with 30% HCl, resulting in the separation of an oily product, which was extracted into ether. On removal of ether and warming the oily residue with concd HCl (50 ml) for 5 min, phenylpyruvic acid oxime (0.8 g), melting at 169—170 °C (decomp.) crystallized out. This was filtered off, and the filtrate evaporated to dryness. The residue was taken in water (20 ml) and treated with charcoal. The pale yellow clear solution obtained was passed through a column of cation exchange resin (Dowex 50W-X4, 200—400 mesh, H^+ form). After washing the column with water, the products were displaced with 2% aqueous ammonia. The effluent was collected into three fractions a, b, and c. The fraction (a) was strongly reducing towards ammoniacal $AgNO_3$. On evaporation to dryness at room temperature and reduced pressure this fraction yielded α,*N*-hydroxyamino-β-phenylpropionic acid (2.8 g, 15.5%), melting at 159 °C (decomp.) and identical in all respects with an authentic specimen.

The second fraction (b) on evaporation to dryness gave a mixture of α,*N*-hydroxyamino-β-phenylpropionic acid and phenylalanine. The third fraction (c) gave a strong ninhydrin positive reaction and on evaporation to dryness yeilded

phenylalanine (0.13 g), melting at 270..272 °C (decomp.), whose infrared spectrum was identical with an authentic specimen.

Paper Chromatography of α,N-Hydroxyamino Acids. Paper chromatography was carried out by the descending technique on 40 cm long Whatman No. 1 papers. One dimensional runs were performed using the organic layer of *n*-butanol: acetic acid: water (40: 10: 50 by volume) mixture as the solvent. The chromatograms were dried at 35 °C for 16 hr. α-Amino acids formed were detected by the conventional ninhydrin reagent. Reducing α,*N*-hydroxyamino acids were visualized either by spraying with ammoniacal $AgNO_3$ (1.0 g $AgNO_3$ in 100 ml water containing 15 ml 10 M ammonia), when α,*N*-hydroxyamino acids appeared as black spots or by spraying with a solution of triphenyltetrazolium chloride.[16] The dried chromatograms were first sprayed with 0.1% triphenyltetrazolium chloride in *n*-butanol saturated with water, dried once again, and resprayed lightly with a solution containing aqueous NaOH (10 M NaOH (10 ml)+ 95% ethanol (40 ml)+*n*-butanol (50 ml)). The presence of α,*N*-hydroxyamino acids was indicated by the immediate production of pink spots. The R_f values of α-amino acids were compared with those of markers of authentic samples applied to the same papers (Table 1).

Estimation of α-Amino Acid and the Corresponding α,N-Hydroxyamino Acid in the Presence of Each Other. A solution containing α,*N*-hydroxyaminopropionic acid (15.0 mg) and alanine (15.0 mg) in water (2 ml) in a 10 ml beaker, stirred by gently bubbling nitrogen gas, was potentiometrically titrated with 0.1 M NaOH. When the pH of the solution had reached to pH 7.5, the amount of alkali added corresponded to the quantity of the α,*N*-hydroxyaminopropionic acid. At this point 30% aqueous HCHO (1.0 ml), previously titrated with alkali to pH 7.5 was added, and the titration resumed with 0.1 M NaOH until pH rose to pH 8.8. The amount of alkali used in the second titration corresponded to the amount of alanine in the mixture. The plot of pH *versus* alkali added during the titration is shown in Fig. 3. Analogous results were obtained when other pairs of α-amino acids and the corresponding α,*N*-hydroxyamino acids were titrated in the same manner.

Disproportionation of α,N-Hydroxyamino Acids into Corresponding α-Amino Acids and α-Oximino Acids. The α,*N*-hydroxyamino acid (36 to 55 mg) was taken in water (10 ml) in a 25 ml two neck flask, supplied with water cooled condenser and a gas inlet. Nitrogen gas was passed first through a $Ba(OH)_2$ trap and then bubbled through the reaction mixture. CO_2 obtained by the decomposition of α-oximino acid was collected as $BaCO_3$ in a trap containing saturated $Ba(OH)_2$ solution and the reaction train was linked to a further baryta trap. All disproportionation experiments were conducted at reflux temperature over a period of 24 hr. $BaCO_3$ was carefully filtered off, washed with water and methanol before drying to a constant weight. From the weight of $BaCO_3$, the amount of α-oximino acid formed in the course of the reaction was determined.

The aqueous solution in the reaction flask was titrated potentiometrically against 0.1 M NaOH to pH 7.5. This titre was used to calculate the amount of unreacted α,*N*-hydroxyamino acid. At pH 7.5, 30% aqueous HCHO (2 ml, previously adjusted to pH 7.5 with NaOH) was added and the titration carried to pH 8.8. This second titre was taken as a measure of the amount of α-amino acid formed. Results are summarized in Table 2.

Reaction of α,N-Hydroxyamino-β-phenylpropionic Acid with Hydroxylamine. α,*N*-Hydroxyamino-β-phenylpropionic acid (1.0 g, 0.0055 mol) was dissolved in 5% aqueous NaOH (50 ml) containing $NH_2OH \cdot HCl$ (0.385 g, 0.0055 mol) and heated under reflux in a 100 ml reaction flask provided with a gas inlet and a water cooled condenser. Nitrogen gas was bubbled through the reaction mixture and the evolved ammonia was carried along with the nitrogen stream into a trap containing 0.1 M HCl (50 ml). After 12 hr the heating was discontinued and the acid solution in the trap was evaporated to dryness under reduced pressure to give ammonium chloride (0.2649, 91%). The alkaline solution in the reaction flask was cooled in ice and acidified with dil. H_2SO_4. A white material was precipitated out which on filtration, drying and recrystallization from dry benzene, was found identical with phenylpyruvic acid oxime (0.47 g, 46.8%).

Similar results were obtained when α,*N*-hydroxyamino-β-phenylpropionic acid was replaced by other α,*N*-hydroxyamino acids.

Reaction of α,N-Hydroxyamino Acid with Phenylisothiocyanate. α,*N*-Hydroxyaminobutyric acid (1.2 g, 0.01 mol) in dry benzene (50 ml) was mixed with freshly distilled phenylisothiocyanate (2 ml). After keeping at 40 °C for one week there was no sign of any reaction and α,*N*-hydroxyaminobutyric acid was separated unchanged. When the reaction was conducted at the reflux temperature for more than 5 min and the products analyzed, a less than 5% yield of diphenylthiourea was isolated along with an undetermined high melting white substance. Analogous results were obtained when other α,*N*-hydroxyamino acids were employed. α-Oximino acids, when treated with phenylisothiocyanate under identical conditions, almost quantitative yields of diphenylthiourea were obtained.

The author is grateful to Professor I. D. Spenser for helpful discussion during the course of this work.

References

1) E. A. Kaczka, C. O. Gitterman, E. L. Dulaney, and K. Folkers, *Biochemistry*, **1**, 340 (1962).
2) A. H. Cook and C. A. Slater, *J. Chem. Soc.*, **1956**, 4133.
3) J. D. Dutcher, *J. Biol. Chem.*, **171**, 321, 341 (1947).
4) A. J. Birch, R. A. Massy-Westropp, and R. W. Rickards, *J. Chem. Soc.*, **1956**, 3717.
5) T. Emery and J. B. Neilands, *J. Amer. Chem. Soc.*, **83**, 1626 (1961).
6) J. Turkova, O. Mikes, and F. Sorm, *Collect. Czech. Chem. Commun.*, **27**, 591 (1962).
7) G. A. Snow, *J. Chem. Soc.*, **1954**, 2588.
8) Von Miller and Ploche, *Ber.*, **26**, 1545 (1893).
9) L. Neelakantan and W. H. Hartung, *J. Org. Chem.*, **23**, 964 (1958).
10) A. Ahmad and I. D. Spenser *Can. J. Chem.*, **39**, 1340 (1961).
11) E. A. Meyers and W. N. Lipscomb, *Acta Crystallogr.*, **8**, 583 (1955).
12) E. Muller and H. Metzger, *Ber.*, **88**, 165, 1891 (1955).
13) A. Hantzsch and W. Wild, *Ann.*, **289**, 285 (1896).
14) C. Kjellin, *Svensk Kem. Tidskr.*, **33**, 213 (1921); *Chem. Abstr.*, **16**, 211.
15) Bamberger, *Ber.*, **27**, 1548 (1894).
16) A. Ahmad, This Bulletin, **47**, 1819 (1974).
17) W. V. Miller and J. Plochl, *Ber.*, **26**, 1545 (1893).
18) C. D. Hurd and J. M. Longfellow, *J. Org. Chem.*, **16**, 761 (1951).
19) W. Traube, *Ber.*, 2297 (1895).