[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND PHARMACEUTICAL CHEMISTRY, MEDICAL COLLEGE OF VIRGINIA]

α-Hydroxylamino Nitriles and α-Hydroxylamino Acids[1,2]

L. NEELAKANTAN AND WALTER H. HARTUNG

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α-Hydroxylamino nitriles may be synthesized by (a) treating oximes with dry hydrogen cyanide, (b) allowing appropriate aldehydes or ketones to react with hydroxylamine salt and an alkali cyanide, or (c) allowing oximes to react with an alkali cyanide in the presence of bisulfite. The nitriles may be hydrolyzed to the corresponding α-hydroxylamino acids. The α-hydroxylamino acids may also be obtained from diethylmalonates by first preparing with nitric oxide isonitramino intermediates, which may then be hydrolyzed. The hydroxylamino acids may be reduced in the presence of Pd/C catalyst to their corresponding α-amino acids.

The examination of α-amino acids not normally found in nature for possible antimetabolite activity, particularly for any merit in the chemotherapy of cancer, affords a wide field for study. For example, compounds in which the R group of the general formula R—CH(NH$_2$)—COOH varies over the wide limits open to synthetic means offers one direction for such investigation.[3] Another involves the modifications of the amino group or of the amino-bearing carbon atom. Thus Wilson and Irvin[4] have observed that α-oximino- and α-alkyloximino acids, R—C(=NOH)—COOH and R—C(=NOH′)—COOH, respectively, originally prepared as intermediates for the synthesis of α-amino acids and for use in the synthesis of peptides,[1,5] exhibit inhibition of protein synthesis in Ehrlich ascites carcinoma cells.

A further modification appears in the hydroxylamino analogs of α-amino acids, R—CH(NHOH)—COOH. Nothing is known about their biological properties other than that cycloserine may be looked upon as derived from a hydroxylamino acid.

The hydrogenation of α-oximino acids appeared as an attractive and simple route for their synthesis. Unfortunately, thus far no method or procedure has been found by which this may be accomplished. With sodium amalgam and alcohol, even with an amount calculated to furnish one molecule of hydrogen, α-amino acid and unchanged hydroxylamino acid were isolated. With palladium-charcoal catalyst reducing either the oximino acid or the alkyloximino acid, no change in rate of hydrogenation could be detected to suggest that the hydroxylamino acid is formed as an intermediate; and when the hydrogenation was interrupted after one molecule of hydrogen was taken up, the product was a mixture of α-amino acid and oximino acid.

α-Hydroxylamino nitriles may be prepared by the addition of hydrogen cyanide to oximes, either under anhydrous[6] or appropriate conditions.[7] We have employed the reaction whereby anhydrous hydrogen cyanide, reacting with oximes of simple aliphatic aldehydes forms α-hydroxylamino nitriles. The products obtained in this manner are given in Table I.

$$\text{R—CH(NOH)} + \text{HCN} \rightarrow \text{R—CH(NHOH)—CN}$$

Also it is now found that the Strecker synthesis of α-amino nitriles[8] lends itself to the preparation of α-hydroxylamino nitriles: Thus simple aldehydes

$$\text{R—CHO} + \text{NH}_3\text{OH·Cl} + \text{NaCN} \longrightarrow \text{R—CH(NHOH)—CN} + \text{NaCl}$$

and also ketones such as acetone, cyclopentanone, and others react in the manner indicated. The products prepared by this procedure are indicated in Table I. The reaction is not successful with benzaldehyde, phenylacetaldehyde, hydrocinnamal-

(1) Paper No. 17 in Amino Acid series. For No. 16 see L. M. C. Shen and W. H. Hartung, *J. Org. Chem.*, **23**, 96 (1958).

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(3) J. D. Smith, J. Andrako, and W. E. Weaver, Contract No. SA-43-ph-1807 with the Cancer Chemotherapy National Service Center.

(4) J. E. Wilson and J. L. Irvin, unpublished results.

(5) R. H. Barry and W. H. Hartung, *J. Org. Chem.*, **12**, 460 (1947); W. E. Weaver and W. H. Hartung, *J. Org. Chem.*, **15**, 741 (1950); W. H. Hartung, D. N. Kramer, and G. P. Hager, *J. Am. Chem. Soc.*, **76**, 2261 (1954).

(6) (a) W. v. Miller and J. Plöchl, *Ber.*, **25**, 2020 (1892); **26**, 1548 (1893). (b) C. C. Porter and L. Hellerman, *J. Am. Chem. Soc.*, **66**, 1652 (1944).

(7) (a) H. A. Lillevik, R. L. Hossfeld, H. V. Lindstrom, R. T. Arnold, and R. A. Gortner, *J. Org. Chem.*, **7**, 164 (1942). (b) C. C. Porter and L. Hellerman, *J. Am. Chem. Soc.*, **61**, 754 (1939). (c) F. Adickes, *J. prakt. Chem.*, **161**, 279 (1943).

(8) R. E. Steiger, *Org. Syntheses*, **22**, 13 (1942); **24**, 9 (1944).

TABLE I

α-HYDROXYLAMINO NITRILES

Compound	Prepared by Procedure	Yield, %	M.P.	Analysis, N Found	Analysis, N Calcd.
$CH_3-CH(NHOH)-CN$[a]	B C	42 50	96–97	31.12	32.56
$CH_3CH_2-CH(NHOH)-CN$[b]	B C	50 65	86–87	27.61	28.00
$(CH_3)_2=C(NHOH)-CN$[c]	B C	60 45	98–99	27.56	28.00
$CH_3CH_2CH_2-CH(NHOH)-CN$[d]	A B C	65 75 90	103–104	24.30	24.58
$(CH_3)_2CH-CH(NHOH)-CN$	A B C	60 80 90	93–94	24.25	24.58
$CH_3CH_2C(CN)(CH_3)NHOH$	B C	50 35	60–61	24.18	24.58
$CH_3CH_2CH_2CH_2-CH(NHOH)-CN$	A B C	67 85 95	102–103	20.51	20.88
$(CH_3)_2CHCH_2-CH(NHOH)-CN$[e]	A B C	65 85 95	103–104	20.87	20.88
$CH_2(CH_2)_2C(CN)NHOH$	B C	95 95	50–52	21.93	22.22
$CH_2(CH_2CH_2)_2C(CN)NHOH$	B C	98 95	136–137	19.80	19.99

[a] Reported by v. Miller and Plöchl[6a] m.p. 97°. [b] Reported by v. Miller and Plöchl[6a] m.p. 86–87°. [c] Reported by v. Miller and Plöchl[6a] m.p. 98.5°. [d] Reported by v. Miller and Plöchl[6a] m.p. 102°. [e] Reported by v. Miller and Plöchl[6a] m.p. 103–104°. [f] Calcd. for $C_7H_{12}N_2O$: C, 60.00, H, 8.32, N, 19.99. Found: C, 60.64, 60.66; H, 8.84, 8.67; N, 19.80, 19.60.

dehyde, or acetophenone. With these compounds a change in solvents, longer reaction time, or increase in temperature were of no avail; in each instance only the oxime of the carbonyl compound was isolated. Nor could the desired hydroxylamino nitrile be obtained by allowing the cyanohydrin to react in aqueous medium with hydroxylamine.

Pratt and Richtmyer,[9] treating D-allose with sodium cyanide, obtained a nitrile. In our treatment of aldoximes with sodium cyanide no desired product was formed. However, when the oximes were allowed to react with sodium bisulfite, satisfactory yields of α-hydroxylamino nitriles were obtained, and the product was readily isolated. It is presumed that the reaction proceeds *via* the bisulfite addition product, quite analogous to the conversion of carbonyl bisulfite addition products into their corresponding cyanohydrins.[10] The reaction does not take place

$$R-CH{:}NOH \xrightarrow{NaHSO_3} R-CH(SO_3Na)NHOH \xrightarrow{CN^-} R-CH(CN)NHOH$$

with the oximes of benzaldehyde or hydrocinnamaldehyde, not even in the presence of phosphate buffer.

The α-hydroxylamino nitriles are colorless crystalline substances, with camphor-like odor, are soluble in alcohol, ether, acetone and other organic solvents except for the petroleum hydrocarbons; they are somewhat soluble in water, the solubility decreasing with increase in molecular weight. They are stable over periods of several weeks to months, slowly decomposing and discoloring on standing. They reduce Fehling's solution and silver nitrate in the cold. They are soluble in dilute acids and may be recovered from acid solution by treatment with base.

When allowed to stand at room temperature in fuming hydrochloric acid for three days the α-hydroxylamino nitriles are hydrolyzed in satisfactory yields to the corresponding α-hydroxylamino acids. The hydrolysis may also be carried out with sulfuric acid, usually in less time, but is more likely to be accompanied by undesirable side reactions.

Another synthesis is an adaptation of the pro-

(9) J. W. Pratt and N. K. Richtmyer, *J. Am. Chem. Soc.*, **77**, 1906 (1955).

(10) L. Neelakantan and W. H. Hartung, data to be published later.

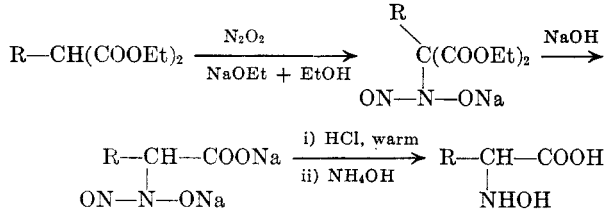
TABLE II

α-HYDROXYLAMINO ACIDS

Compound	Prepared by Procedure	Yield, %	M.P. (dec.)	Analysis N Found	Analysis N Calcd.
$CH_3-CH(NHOH)-COOH$	D	40	194–195	13.00	13.33
$CH_3CH_2-CH(NHOH)-COOH$[a]	D	45	193–194	11.44	11.77
$(CH_3)_2=C(NHOH)-COOH$[b]	D	45	170–171	11.48	11.77
$CH_3CH_2CH_2-CH(NHOH)-COOH$[c]	D E	60 45	194–195	10.34	10.52
$(CH_3)_2CH-CH(NHOH)-COOH$	D	55	192–193	10.32	10.52
$CH_3CH_2-C(CH_3)(NHOH)-COOH$	D	50	140–142	10.28	10.52
$CH_3CH_2CH_2CH_2-CH(NHOH)-COOH$[d]	D E	65 50	194–195	9.90	9.52
$CH_2CH_2-C(CH_2CH_2)(NHOH)-COOH$[e]	D	60	200–202	9.40	9.64
$(CH_3)_2CHCH_2-CH(NHOH)-COOH$	D	60	194–195	9.31	9.92
$C_6H_5CH_2-CH(NHOH)-COOH$[f]	E	65	159–160	7.71	7.73

[a] Reported by v. Miller and Plöchl[6a] m.p. 166–167°. [b] Reported by Munch, *Ber.*, **29**, 64 (1896) m.p. 168°. [c] Reported by v. Miller, *Ber.*, **26**, 1553 (1893) m.p. 156°. [d] Calcd. for $C_6H_{13}NO_3$: C, 48.96%, H, 8.9%, N, 9.57%. Found: C, 49.11, 49.38; H, 8.37, 8.84; N, 9.95, 10.2. [e] Reported by Traube[11] m.p. 157–158°. [f] Calcd. for $C_6H_{11}NO_3$: C, 49.73, H, 7.64, N, 9.65. Found: C, 50.53, 50.83; H, 7.73, 7.77; N, 9.40, 9.20.

cedure of Traube,[11] who treated an alkylacetoacetic ester with nitrogen oxide, N_2O_2, in the presence of sodium ethoxide to form an isonitramino intermediate which on appropriate hydrolysis afforded the sodium salt of the isonitramino acid. It is found that alkylmalonic esters behave simi-



larly. The products obtained by this reaction are included in Table II.

The α-hydroxylamino acids are stable, colorless crystalline solids, soluble in water, and sparingly soluble in alcohol, ether, acetone, and other usual organic solvents. They melt at quite high temperatures with decomposition. Like their α-amino acid analogs, they are amphoteric, soluble at room temperature in both dilute mineral acids and diluet bases. Their isoelectric points lie between *p*H 6 and *p*H 7. They form stable hydrochlorides. They give a positive ninhydrin reaction. In the presence of formaldehyde, they may be titrated by the Sörensen procedure. In ethanolic solution and in the presence of ammonia they may be reduced with palladium-charcoal catalyst to the corresponding α-amino acids, which may then be further characterized by conversion into some suitable derivative, *e.g.*, the *N*-benzoyl or thiourea derivative.

The α-hydroxylamino acids treated with phenylisothiocyanate, after gentle warming, give a vigorous reaction. However, the only crystalline product thus far isolated from this reaction has been diphenylthiourea, for which the mechanism of formation is not understood. The best yields are obtained when equimolar amounts of reagents are employed. Since hydroxylamine does not give such results, it appears that perhaps this reaction may prove useful in distinguishing between amino acids and hydroxylamino acids.

The infrared absorption spectra of the α-hydroxylamino acids show characteristic bands for the $-COO^-$ and the $-NH_2OH^+$ ions. But thus far attempts to prepare *N*-benzoyl or *N*-acetyl derivatives have been unsuccessful. These possibilities are being explored further.

(11) W. Traube, *Ber.*, **28**, 2301 (1895).

EXPERIMENTAL

α-Hydroxylamino nitriles. The data for these compounds are summarized in Table I. Typical syntheses are described.

Procedure A. With 14.0 g. of freshly distilled valeraldoxime (0.139 mole) was mixed 10 ml. of anhydrous hydrogen cyanide (0.2+ mole), and the mixture was allowed to stand for 2 days at room temperature. The solid was sucked dry on a Buchner funnel and washed with petroleum ether; it weighed 12.0 g. (67.7%).

Procedure B. To 21.0 g. of *n*-butyraldehyde (0.3 mole) was added a solution of 23.0 g. of hydroxylamine hydrochloride in 100 ml. water, and then with vigorous stirring was added over a period of 30 min. a solution of 15.2 g. of sodium cyanide (0.31 mole) in 50 ml. water. Stirring was continued for 3 days. The crystalline mass which had formed was sucked dry on a Buchner funnel, washed with a little water, and recrystallized from ether–petroleum ether solvent; 28.5 g. (75%).

Procedure C. Twenty grams of butyraldoxime (0.25 mole) was treated with 12.5 g. of sodium cyanide (0.25 mole) and 72 ml. of a saturated solution of sodium bisulfite; the solution was stirred at room temperature for 3 days. The crystalline mass was filtered off, washed with a little water, and recrystallized from ether–petroleum ether solvent; 25.8 g. (90.4%).

α-Hydroxylamino acids. The data for these are summarized in Table II. Typical experiments for their preparation are given.

Procedure D. A solution prepared from 10 g. of α-hydroxylamino nitrile and 60 ml. of concentrated hydrochloric acid was cooled in an ice bath and saturated with HCl gas, then allowed to stand at 0° for 1 day and at room temperature for 2 more days. The solution was then diluted with 40 ml. of water and refluxed for 6 hr. The water and hydrogen chloride were then removed on a steam bath and at reduced pressure to leave a residue of the α-hydroxylamino acid hydrochloride and ammonium chloride. The desired product was purified by either of two methods.

(1) The residue was taken up in water, decolorized with charcoal, filtered, and the *p*H of the solution was adjusted by the addition of ammonium hydroxide to between 6 and 7; after standing for a day in the refrigerator the solution deposited crystals which were collected on a Buchner funnel, washed with a little cold water, and crystallized from hot water to give colorless product.

(2) The residue was extracted with hot absolute ethanol and filtered; the filtrate was made basic with pyridine and allowed to stand for a day. The crystals were collected and purified as before.

Procedure E. A tenth gram-atom of sodium (2.3 g.) was dissolved in 140 ml. of absolute ethanol, and to the solution was added, with cooling, 5.0 g. of diethyl benzylmalonate (0.1 mole). Nitric oxide (generated by dropping concentrated sulfuric acid on aqueous sodium nitrite) was passed through the solution for 1 hr. and the reaction mixture allowed to stand at room temperature for 1 day. The alcohol was then volatilized at 30° at reduced pressure. To the residue was added 60 ml. of 20% sodium hydroxide solution (approximately 0.3 mole NaOH), allowed to stand at room temperature for a day, and the alkaline solution extracted with ether to remove any unchanged malonic ester. Then, keeping the temperature below 25°, dilute acid was added, and the oil which separated was boiled with 50 ml. concentrated hydrochloric acid for 15 min. The clear solution was cooled and with ammonium hydroxide the *p*H was adjusted to *p*H between 6 and 7. The crystalline colorless precipitate was collected on a Buchner funnel, washed with a little cold water, and then recrystallized from hot water; 10.0 g. (55.4%).

Reduction of α-hydroxylamino acids. Five grams of α-hydroxylaminovaleric acid in 30 ml. concentrated ammonium hydroxide solution, diluted by addition of 10 ml. water, was shaken on a Parr apparatus in hydrogen at four atmospheres pressure with 1 g. of palladium-charcoal catalyst. After 8 hr. the catalyst was filtered off, and the solution was evaporated to dryness; the residue weighed 3.5 g. The *N*-formyl derivative melted at 132–133°; reported 132°.[12]

In a similar manner β-phenyl-α-hydroxylaminopropionic acid was converted to β-phenylalanine, which was identified as its *N*-benzoyl derivative, m.p. 188–189°; reported 187–188°.[13] α-Hydroxylaminocaproic acid was reduced to α-aminocaproic acid, of which the *N*-formyl derivative melted at 115°; reported 113–115°.[14]

1-Hydroxylamino-1-cyclopentanecarboxylic acid was reduced to 1-amino-1-cyclopentanecarboxylic acid, hydrochloride salt, m.p. 222–224° (dec.) agreeing with the value observed for an authentic sample of the hydrochloride.[15] After hydrogenation, the product no longer reduced Fehling's solution or silver nitrate solution.

RICHMOND, VA.

(12) E. Abderhalden, *Chem. Zentr.*, **1921**, III, 296.
(13) E. Fischer and A. Mouneyrat, *Ber.*, **33**, 2383 (1900).
(14) D. Marko, *Ann.*, **362**, 333 (1908).
(15) L. Neelakantan, unpublished result.

[CONTRIBUTION FROM THE UNIVERSITY OF NORTH CAROLINA]

Methyl Ketone Isosters of α-Amino Acids[1]

KENNETH L. HOY[2] AND WALTER H. HARTUNG[3]

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In the many correlations between the structure of organic compounds and their physiological activity the sequence of the four atoms $=N-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-O-$ seems to be of considerable significance, for it appears in many substances exhibiting varied pharmacodynamic properties. Thus, compounds in which the oxygen atom appears as an alcoholic hydroxyl or its derivatives include acetylcholine, procaine, methantheline, epinephrine, chloroamphenicol, diphenhydramine, and the cinchona alkaloids, to name only a few. In the α-amino acids is found the same sequence, but with the terminal carbon as a carboxyl group; these are so numerous and of such importance as to constitute a specialized area in both organic and biological chemistry.

(1) Paper number 18 in the amino acid series. For number 17 see L. Neelakantan and W. H. Hartung, *J. Org. Chem.*, **23**, 964 (1958).
(2) Present address: Union Carbide Chemicals Company, South Charleston, W. Va.
(3) Present address: Medical College of Virginia, Richmond, Va.