ACTA CHEMICA SCANDINAVICA 17 (1963) S 190 – S 194

Synthesis and Reactions of the α-N-Hydroxyamino Acids

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The reduction of α-nitro esters with zinc dust in aqueous acetic acid has been employed as a general synthesis for an important group of naturally occurring substances, the α-N-hydroxyamino acids. The latter are instantly cleaved by periodic acid to CO_2, nitrous oxide and aldehydes. The acyl-N-hydroxamido ester derivatives react with periodic acid to yield the acyl moiety and the corresponding α-oximino ester.

The colored coordination compound, ferric-hydroxamate, has found favor in analytical chemistry for several decades[1,2]; living cells, as master analytical chemists, have apparently employed this complex in iron metabolism for many millions of years[3,4].

$$n\ \begin{matrix} R' - N - OH \\ | \\ R - C = O \end{matrix} + Fe^{III} \rightleftharpoons \left[\begin{matrix} R' - N - O \\ | \\ R - C = O \end{matrix}\right]_n + Fe^{III} + n\,H^+$$

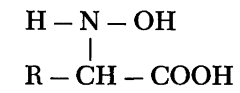
Yeast suspended in nitrite solution have been shown[5] to contain acethydroxamic acid (R = CH_3, R′ = H). Here the substance probably arises by reaction of the formed hydroxylamine with active acyl compounds such as acetyl coenzyme A. All naturally occurring hydroxamic acids apparently belong to the secondary series (R′ = alkyl) and in many instances the carbon skeleton bears a striking resemblance to that of the familiar amino acids. Thus the α-amino-ω-hydroxyamino acid corresponding to L-ornithinine occurs in the ferrichrome compounds[6] and in albomycin[7] whilst the lysine analogue is found in mycobactin[8]. The decarboxylation product of the latter analogue, 1-amino-5-hydroxyamino pentane, is a principal constituent of nocardamin[9]. Similarly, the α-N-hydroxyamino acids appear in hadacidin[10] (glycine), mycelianamide[11] (alanine) and the aspergillic[12] acid family (isoleucine).

The chemical synthesis of the DL-α-amino-ω-hydroxyamino acids has already been reported[13]. Recently, in connection with a projected study on the biosynthe-

sis and structure of proteins, it became necessary to obtain a number of synthetic α-N-hydroxyamino acids and to elucidate the mechanism of periodic acid attack on these compounds and their derivatives.

Synthesis of the α-N-hydroxyamino acids

In the present communication we wish to describe certain synthetic methods and reactions for the α-N-hydroxyamino acids. These compounds,

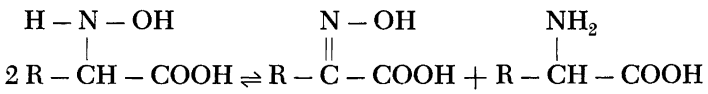


which were known in the last century[14], have been prepared through a variety of routes including the addition of HCN to aldoximes followed by hydrolysis[15], the treatment of α-bromo acids with hydroxylamine[16], the addition of hydroxylamine to α-β unsaturated acids[17], and the reaction of nitrogen oxide with a substituted malonic ester[15].

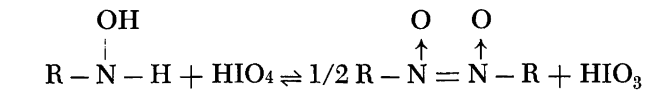
The only general method for introduction of the hydroxyamino group is the reduction of the parent nitro compound with zinc dust. However, in the case of the α-N-hydroxyamino acids, this approach has been used sparingly and in one reported application the product was not extensively characterized[18]. Since α-nitroesters are now quite readily available[19,20], we undertook to investigate the general applicability of this reaction. It was discovered that yields of 15–25 % could be obtained by carrying out the reaction at 0–5°C in glacial acetic-water solution. In spite of the modest yields, the method, which was proven by preparation of a known (N-hydroxyphenylalanine) and a new (N-hydroxyisoleucine) α-N-hydroxyamino acid, is recommended in those instances where the parent nitro compound is readily available.

Reactions of the α-N-hydroxyamino acids

The hydroxyamino group is readily oxidized or reduced, thus affording disproportionation reactions[21] of the type:



Two useful color tests for the hydroxyamino function are the instant reduction of tetrazolium in alkaline solution in the cold[8], and the formation of ferric hydroxamate after acylation and addition of ferric chloride. For simple alkyl-substituted hydroxylamines, treatment with performic acid apparently causes the carbon bearing the hydroxyamino group to be degraded to carboxyl[7]. A notable reaction of the alkyl-hydroxylamines, that with periodic acid[22,23], gives rise to the corresponding *cis*-nitroso dimer:



This reaction appears to be instantaneous and, in view of the substantial absorption of the dimer in the ultraviolet, it has possibilities for analytical application. On oxidation with periodic acid, free hydroxylamine is converted to nitrous oxide, possibly via a nitroxyl dimer intermediate[23].

$$H-\overset{\uparrow}{O}-N=N-\overset{\uparrow}{O}-H \rightleftharpoons N_2O + H_2O$$

Snow[8] reported that α-N-hydroxyamino acids react with periodic acid to give CO_2, aldehydes and possibly "oxides of nitrogen". The experiments to be reported here confirm and extend these observations. Under the conditions used, the following equations probably account for the observed reaction of periodic acid with the α-N-hydroxyamino acids and their derivatives:

$$R-\underset{|}{\overset{H-N-OH}{CH}}\,COOH + 2\ HIO_4 \rightleftharpoons 1/2\ N_2O + 3/2\,H_2O + CO_2 + R-\overset{O}{\overset{\|}{C}}-H + 2\,HIO_3$$

$$C_6H_5-\underset{|}{\overset{O}{\overset{\|}{C}}}=N-OH \qquad\qquad N-OH$$
$$R-\underset{|}{CH}-COOC_2H_5 + HIO_4 \rightleftharpoons C_6H_5COOH + R-\overset{\|}{C}-COOC_2H_5 + HIO_3$$

Thus the periodic acid oxidation of a peptide chain containing an α-N-hydroxyamino acid residue should afford the oxime if the residue is either N-terminal or internal. At the C-terminal position, the aldehyde, CO_2 and nitrous oxide should result.

EXPERIMENTAL

Synthesis of α-N-hydroxyamino acids

α-N-hydroxy-β-phenyl propionic acid. This N-hydroxyamino acid was obtained by reduction and hydrolysis of ethyl-α-nitro-β-phenyl propionate[24].

Diethylnitromalonate (40 g, 195 mmoles) was added with stirring to 120 ml of ethanol containing 4.5 g (195 mmoles) sodium and 34.2 g (200 mmoles) of benzyl bromide was added in small portions to the hot solution. The mixture was refluxed for 48 hours (exclusion of moisture), the ethanol was removed under reduced pressure, the residue was suspended in 200 ml of water and thrice extracted with 100 ml portions of toluene. The combined toluene extract was dried over $MgSO_4$ to yield 42 g of viscous oil. The latter was dissolved in 100 ml ether, the resulting solution was chilled in an ice bath and to it was added, with vigourous stirring, 100 ml of ethanol containing 3.26 g (142 mmoles) sodium. Stirring was continued for five hours in the cold, the solution evaporated to a reddish-brown oil and the latter suspended in 300 ml of water and twice extracted with 100 ml portions of ether. The yellow aqueous phase was chilled to 1°C and acidified to Congo red. The ether extract of this solution was dried over $MgSO_4$ and evaporated to yield 20 g (89 mmoles) of substance with properties corresponding to those of ethyl-α-nitro-β-phenyl propionate prepared by an alternate procedure[24].

The yellow oil (20 g, 89 mmoles) was added to mixture of 100 ml glacial acetic acid and 5 ml water in a 250 ml flask. The flask was immersed in an ice-salt mixture, and to the solution zinc dust (30 g, 460 mmoles) was added in small portions with vigorous stirring over a period of one hour. The temperature was maintained at 5° ± 2°C. The mixture was then filtered and the pH was adjusted to 7 with slow addition of cold concentrated NaOH solution. The reaction

mixture was immediately twice extracted with 250 ml portions of chloroform. The combined chloroform extracts were extracted three times with 100 ml water followed by two extractions with 250 ml of 3 N HCl. The combined aqueous extracts were then refluxed for 7 hours, the volume of the solution was reduced to 50 ml, decolorized with charcoal and filtered. The pH of the clear filtrate was adjusted to 4 with cold concentrated NH_4OH. A white crystalline mass formed upon leaving the mixture in the ice box for 18 hours. The precipitate was filtered, dissolved in 1 N HCl and recrystallized by the addition of concentrated NH_4OH to pH 4, yielding 3 g (16.6 mmoles, 19 %) of white powder.

The product gave a strong tetrazolium test and a neutral equivalent of 182.8 (theoretical, 181.2). This hydroxyamino acid was obtained previously in a series of steps beginning with the treatment of an alkylacetoacetic ester with N_2O_2[15].

Ethyl-α-benzoylhydroxamido-β-phenylpropionate. The solution obtained by filtering off the zinc oxide from the reduction of 20 g (89 mmoles) of ethyl-α-nitro-β-phenylpropionate (see above) was treated with 9.8 ml (12.3, 89 mmoles) of benzoyl chloride. Several volumes of water were added and the hydroxamic acid extracted into ethyl acetate. The latter solution was washed twice each with 100 ml of 0.1 N HCl and cold 0.1 M phosphate buffer pH 7. The ethyl acetate solution was then washed twice with 0.1 N HCl and twice with water. The evaporation of the organic layer under reduced pressure left an oily residue which was twice recrystallized from ethanol-water yielding 7 g (22 mmoles, 25 %) of colorless needles, m.p. 115–118°C (uncorr.). Titration of a sample in 50 % ethanol gave a neutral equivalent of 321.0 (theoretical, 313.3) with a pK_a' of 10.

α-N-Hydroxy-β-methyl valeric acid. This hydroxyamino acid, which apparently has not previously been reported, was obtained by reduction and hydrolysis of the corresponding α-nitroester[24] via the above procedure. The overall yield was 15 % of material with a pK_{a_2}' of 5.9, a strongly positive tetrazolium test and a neutral equivalent of 148.8 (theoretical, 147.2). (Found: C 49.2; H 8.7; N 9.2. Calc. for C_6HNO_3: C 48.9; H 8.9; N 9.5.)

Ethyl-α-benzoylhydroxamido-propionate. This compound was obtained by acylation (see above) of the zinc reduction product of ethyl-α-nitropropionate[19]. The yield was 25 % of almost colorless needles, m.p. 88°C (uncorr.). The pK_a' in 10 % ethanol was 9.5 with neutral equivalent of 242.1 (theoretical, 236.2). An 8.4 mg (35.5 μmole) sample treated with excess ferric chloride in 100 ml of aqueous solution at pH 2.5 displayed an absorption maximum of 500 mμ with an extinction coefficient of 1.07×10^3.

Periodic acid oxidation of free (A) and substituted (B) α-N-hydroxyamino acids

A. Free N-hydroxyamino acids

(1) Hydroxyamino moiety. A 147 mg (1 mmole) quantity of DL-α-hydroxyamino-γ-methyl valeric acid[15] was dissolved in 10 ml of water contained in a manometer. The introduction of 5 ml of aqueous solution containing 912 mg (4 mmoles) H_5IO_6 from a side arm gave rise to instantaneous evolution of gas. The latter was collected and analyzed at room temperature by gas chromatography on a column of silica gel[25] using helium carrier and an Aerograph Model A-100 detector. The column was fitted with a caroxite CO_2-trap and a calibration curve prepared by injecting various volumes of pure nitrous oxide. Injection of an aliquot of the sample demonstrated the presence of a component with the same retention volume as authentic nitrous oxide. The yield, 80 % of theory, was regarded as satisfactory in view of the known substantial water-solubility of nitrous oxide.

(2) Carboxyl moiety. A 33 mg (0.25 mmole) quantity of DL-α-hydroxyamino-β-methyl butyric acid was dissolved in 10 ml of water in a closed system. Excess 0.2 N periodic acid solution was introduced via a dropping funnel and the evolved gas was trapped in a barium hydroxide solution. The precipitate was collected, washed and dried to yield 50.8 mg of $BaCO_3$ (theoretical, 49.4 mg; 102 %).

(3) Carbon skeleton. Aqueous solutions of several different α-N-hydroxyamino acids containing paraffinic side chains were treated with periodic acid and the resulting solution extracted with ether. The ether extracts were analysed by gas chromatography on a firebrick column at 125°C. These experiments showed the presence of components with the same retention volume as authentic samples of the expected[8] aldehydes.

B. Substituted N-hydroxyamino acid

(1) N-Benzoyl-hydroxyamino-alanine ethyl ester. Exactly 240 mg (1 mmole) of ethyl-DL-α-benzoylhydroxamidopropionate was dissolved in 30 ml of warm water and allowed to react with 912 mg (4 mmoles) H_5IO_6 added dropwise in 5 ml of water. There was no obvious evolution of gas and hence the reaction mixture was worked up in the following way. The solution was exhaustively extracted with ether and the aqueous phase discarded. The ether extract was evaporated, the residue dissolved in aqueous alcohol and the solution carefully neutralized with N NaOH. The neutral solution was again exhaustively extracted with ether and the combined ether extracts set aside as Fraction I. The aqueous phase was acidified with dilute HCl and the ether extract of this solution designated as Fraction II.

Fraction I was evaporated and the residue recrystallized from ethanolpetroleum ether to yield needles with m.p. 92–93°C, not depressed on admixture with authentic[26] α-oximinoethyl-propionate. The pK_a' (9.8) and infrared spectrum (KBr pellet) were identical with those of the authentic specimen. The yield was 68 mg (52 %).

Fraction II was evaporated and the residue crystallized from ethanol-water yielding 77 mg (63 %) of benzoic acid, the latter characterized by m.p. (119°C), pK_a' (4.2), neutral equivalent (120), all of which were identical with those of a known sample.

Acknowledgement. Thanks are due to the *Office of Naval Research* (Grant 222-39) and the *United States Public Health Service* (Grant E-4156) for financial assistance. One of us (P.A.) holds post doctoral fellowship No. CPD – 12,355 – C 2 (1961 – 63) from the *U.S. Public Health Service.*

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Received April 1, 1963.