HYDROXYLAMINE DERIVATIVES

COMMUNICATION 6. SYNTHESIS AND SOME REACTIONS OF 3-(AMINOOXY)ALANINE*

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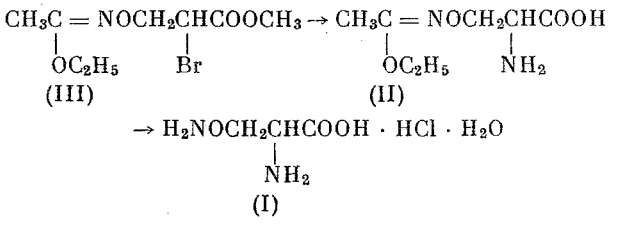
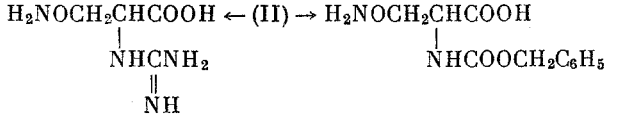
Among the most interesting derivatives of hydroxylamine are amino acids that contain an H_2NO group. We recently described a convenient synthesis for the natural amino acid DL-canaline and studied some of its reactions [2, 3]. The closest homolog of this amino acid—3-(aminooxy)alanine—is of interest on its own account: a cyclic acyl derivative of this is the antibiotic cycloserine. 3-(aminooxy)alanine itself has biological activity, and it inhibits some enzymes containing pyridoxal phosphate [4-6]. In this paper we report the development of a convenient method of preparing 3-(aminooxy)alanine and the study of some of its reactions.

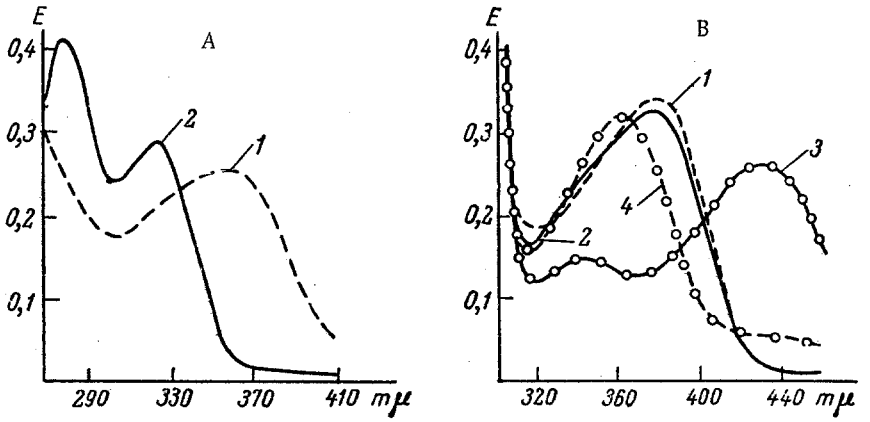


We have prepared the ester (III) previously from ethyl acetohydroxamate and methyl 2,3-dibromopropionate [7]. Amination of the bromo acid obtained by the alkaline hydrolysis of (III) proceeds readily and smoothly under the conditions usual for the amination of α-halo acids. The best yields were obtained by carrying out the reaction in liquid ammonia. It should be mentioned that the bromo acid itself is unstable at room temperature and gradually decomposes with the liberation of ethyl acetate. In acidic aqueous solutions the amino acid (II) is hydrolyzed extremely readily to 3-(aminooxy)alanine (I), which separates as its monohydrochloride. The yield of (I), based on the bromo ester (III), amounts to 70%. The simplicity of the synthesis and the high yields at each stage are attained by the use, as starting substance, of the bromo ester (III), whose molecule contains a specific grouping of a substituted hydroxamic ester, stable in the course of the synthesis, without substantial effect on the mobility of the α-halogen atom, and smoothly convertible in an acid medium into a substituted hydroxylamine. The method developed can obviously be applied in the synthesis of various homologs of (I), for by an analogous procedure we were able to prepare 2-amino-3-(aminooxy)butyric ester. Various 3-(aminooxy) N-substituted alanines were prepared from the amino acid (II):



The selective formation of such derivatives of substance of type (I) in which only the 2-amino group is substituted has presented difficulty until now, because disubstituted derivatives are formed in most reactions and the selective hydrolysis of these cannot usually be effected. In those cases in which compounds with a protected aminooxy group are used for the preparation of derivatives, the removal of the protection is accompanied by the elimination of the substituent in the amino group.

* For Communication 5, see [1].

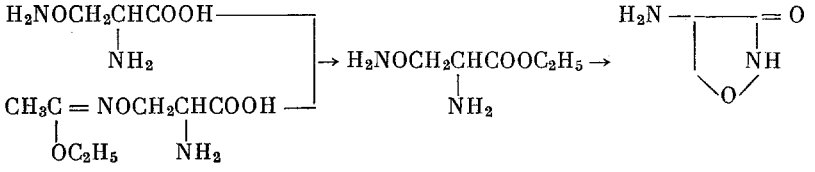


A. Ultraviolet spectra of pyridoxal phosphate (PP) in presence of 3-(aminooxy)-alanine: 1) $5 \cdot 10^{-5}$ M PP + $5 \cdot 10^{-3}$ M 3-(aminooxy)alanine (pH 7); 2) ditto (pH 1). B. Ultraviolet spectra of glutamate-aspartate-transaminase (GAT) in presence of 3-(aminooxy)alanine: 1) GAT (protein content 2 mg/ml) + $1 \cdot 10^{-2}$ M 2-(aminooxy)-alanine (pH 8.2); 2) ditto (pH 4.5); 3) GAT (pH 4.5); 4) GAT (pH 8.2).

A characteristic property of 3-(aminooxy)alanine is its ability to form oximes by reaction with carbonyl compounds:

$$>C=O + H_2NOCH_2\underset{\displaystyle NH_2}{\underset{|}{C}}HCOOH \rightarrow >C=NOCH_2\underset{\displaystyle NH_2}{\underset{|}{C}}HCOOH.$$

Oximes from 3-(aminooxy)alanine are good crystallizing substances and can probably be applied for the identification of aldehydes and ketones. The synthesis of (I) described in this paper is of great practical importance, because the ester of (I) serves as the main starting compound for the preparation of the antibiotic cycloserine (4-amino-3-isoxazolidinone). The ester of (I) is formed in good yield by the esterification not only of (I), but also of the substance from the preceding stage of the synthesis, the amino acid (II):*



We studied the mechanism of inhibition by 3-(aminooxy)alanine of glutamate-aspartate-transaminase, whose co-enzyme is pyridoxal phosphate [4, 9]. 3-(aminooxy)alanine is fixed in the active center of the apoenzyme and forms and oxime link with pyridoxal phosphate, as follows from a comparison of the spectra showing the interaction of (I) with pyridoxal phosphate and with highly purified (above 95%) glutamate-aspartate-transaminase (see the figure). A detailed discussion of the mechanism of the interaction of (I) and its derivatives with the enzyme will be published separately [4].

EXPERIMENTAL

3-(Aminooxy)alanine (I). 27 g of the bromo ester (III) was hydrolyzed at 30-35° with 20% aqueous sodium hydroxide solution. The mixture was cooled to −10° and acidified with hydrochloric acid; the bromo acid was separated rapidly and heated with 75 ml of liquid ammonia for six hours at 65-70°. Ammonia was evaporated, and the crystalline mixture of the amino acid (II) and ammonium bromide was suspended in 100 ml of alcohol; 33 ml of concentrated hydrochloric acid was added, and the mixture was filtered. 30 g of dimethylaniline was added over a period of one hour to the filtrate with stirring, the mixture was cooled to 0°, and after two hours (I) was separated; yield 12.2 g (70%); m.p. 141-142° [10].

* The method that we have developed for the preparation of 3-(aminooxy)alanine and its ester has provided the basis for an industrial synthesis of cycloserine [8].

3-(1-Ethoxyethylideneaminooxy)alanine (II). The crystalline mixture formed in the amination of the bromo ester (III) was washed with methanol and dissolved in concentrated aqueous ammonia. Isopropyl alcohol was added, and after the removal of ammonia in a vacuum we obtained (II), m.p. 179-180° (decomp.). Found: N 14.99%. $C_7H_{14}O_4N_2$. Calculated: N 14.73%.

3-(Aminooxy)alanine Ethyl Ester Dihydrochloride. 1.9 g of 3-(1-ethoxyethylideneaminooxy)alanine was boiled for 2.5 h with 20 ml of absolute ethanol containing 3.6 g of hydrogen chloride. The reaction mixture was vacuum-evaporated to dryness, the residue was dissolved in 4 ml of absolute ethanol, and 20 ml of dry ethyl acetate was added cautiously. After two hours crystalline 3-(aminooxy)alanine ethyl ester dihydrochloride was filtered off; yield 1.5 g (70%); m.p. 153-154° (from alcohol) [10].

3-(Aminooxy)-N-(benzyloxycarbonyl)alanine (IV). 1.7 g of benzyl chloroformate was added with cooling and stirring to a solution of 1.9 g of the amino acid (II) in 2 N aqueous NaOH. At the end of the reaction the reaction mixture was extracted with ether, and the aqueous part was acidified to Congo Red with hydrochloric acid at −10°. Then, at a temperature of −10 to −5°, the mixture was extracted rapidly with methylene chloride; the extract was filtered through magnesium sulfate, and solvent was vacuum-distilled off. The residue was treated with 6 ml of 0.02 N alcoholic HCl. Alcohol was vacuum-distilled off, the residue was dissolved in 4 ml of water, 2 N NaOH was added to bring the pH to 4, and the mixture was left for a few hours at 0°. The yield of (IV) was 60%; m.p. 128-130° (aqueous alcohol). Found: N 11.24%. $C_{11}H_{14}O_5N_2$. Calculated: N 11.02%.

3-(1-Ethoxyethylideneaminooxy)-2-guanidinopropionic Acid (V). 0.01 mole of the amino acid (II) was added to a methanolic solution of 0.02 mole of 2-methylpseudourea. After five days the guanidino acid (V) was filtered off; yield 45-50%; m.p. 210-212° (decomp.). Found: N 24.15%. $C_8H_{16}O_4N_4$. Calculated: N 24.12%.

N-(Benzyloxycarbonyl)-3-[(benzyloxycarbonyl)aminooxy]alanine. 8.4 g of sodium bicarbonate and 5.2 g of magnesium oxide were added to a solution of 9 g of (I) in 100 ml of water. An ethereal solution of 22 g of benzyl chloroformate was then added dropwise with stirring and cooling. After 12 h the mixture was filtered, and a suspension of the precipitate in water was acidified with concentrated hydrochloric acid; when rubbed out with ether, the oil liberated crystallized. The ether layer of the filtrate was rejected, the aqueous layer was acidified with concentrated hydrochloric acid, and the oil liberated crystallized when rubbed out with ether. The two portions of crystals were combined and recrystallized from benzene; yield 30%; m.p. 139°. Found: N 7.21%. $C_{19}H_{20}O_7N_2$. Calculated: N 7.21%.

3-(Benzylideneaminooxy)alanine. 4 N NaOH was added to a solution of 1.7 g of 3-(aminooxy)alanine (I) in 50% alcohol to bring the pH to 5-6, and an alcoholic solution of 1.2 g of benzaldehyde was then added. After 12 h the precipitate was separated and washed with water and alcohol. An almost quantitative yield of 3-(benzylideneaminooxy)alanine was obtained; m.p. 198° (decomp.). Found: N 13.26%. $C_{10}H_{12}O_3N_2$. Calculated: N 13.45%.

2-Amino-3-(1-ethoxyethylideneaminooxy)butyric Acid. 32 g of 20% aqueous NaOH was added to 48 g of ethyl 2-bromo-3-(1-ethoxyethylideneaminooxy)butyrate at 30 ± 2°, and the mixture was stirred for 30 min at room temperature and then acidified to pH 2 with 10% hydrochloric acid (temperature not above −5°). The acid liberated was extracted with ether, ether was vacuum-evaporated from the extract, and the residue was introduced into an autoclave together with 100 ml of liquid ammonia and 0.2 g of ammonium nitrate. The reaction mixture was heated for 12 h at 55-60°. Ammonia was evaporated, the residue was dissolved in absolute ethanol, alcohol was vacuum-distilled off at room temperature, and the residue was treated two more times in the same way. The solid residue was ground with cold water, filtered off, and washed with acetone. The yield of amino acid was 18.0 g (55%); m.p. 177-178°. For analysis the substance was reprecipitated from aqueous ammonia. Found: N 13.70; 13.89% $C_8H_{16}N_2O_4$. Calculated: N 13.72%.

Ethyl 2-Bromo-3-(1-ethoxyethylideneaminooxy)butyrate was prepared like the bromo ester (III) from ethyl 2,3-dibromobutyrate and acetohydroxamic acid; yield 50-55%; b.p. 90° (1-1.5 mm); n_D^{22} 1.4645. Found: N 5.04%. $C_{10}H_{18}BrNO_4$. Calculated: N 4.73%.

Under similar conditions we prepared the corresponding chloro ester from methyl 2,3-dichlorobutyrate; yield 43%; b.p. 84-85° (2 mm); n_D^{20} 1.4501. Found: N 5.54%. $C_9H_{16}O_4NCl$. Calculated: N 5.90%.

Methyl 2-Amino-3-(aminooxy)butyrate Dihydrochloride. 5 g of 2-amino-3-(1-ethoxyethylideneaminooxy)-butyric acid was dissolved in 7 ml of concentrated hydrochloric acid, and the solution was vacuum-evaporated. The residue was dried and dissolved in 70 ml of absolute methanol. Dry hydrogen chloride was passed in to saturation,

and the reaction mixture was left for two days. The clear colorless solution was vacuum-evaporated, and the residue was dissolved in 25 ml of absolute methanol and again evaporated to dryness. This operation was repeated three times, and the residue was vacuum-dried over phosphorus pentoxide and crystallized from absolute isopropyl alcohol. The yield of the ester dihydrochloride was 52%; m.p. 139-141° (from isopropyl alcohol), undepressed by admixture of a known sample. On treating the ester dihydrochloride with alcoholic alkali we obtained a 60% yield of 4-amino-5-methyl-3-isoxazolidinone, identical with the sample which we prepared previously [11].

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SUMMARY

1. A new simple method for the preparation of 3-(aminooxy)alanine and its homologs is proposed.

2. The chemical properties of 3-(aminooxy)alanine are studied, and some of its derivatives, at the amino and at the aminooxy group, are synthesized.

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