HYDROXYLAMINE DERIVATIVES

COMMUNICATION 10. SYNTHESIS OF ESTERS OF AMINOCARBOHYDROXAMIC ACIDS

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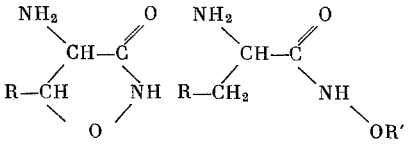
Several years ago we started to study the mechanism of the inhibition of pyridoxal enzymes by cycloserine (4-amino-3-isoxazolidinone). In studying the reaction of cycloserine and its derivatives with pyridoxal and pyridoxal 5-phosphate it was found that the cyclic esters of hydroxamic acids are capable of manifesting acylating properties, and this is a necessary condition for the inhibition of pyridoxal enzymes [1-3]. These tenets were confirmed in a number of enzymatic studies [4-6].

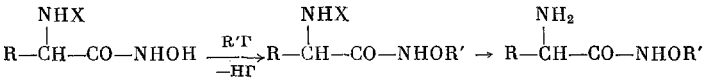
We made a detailed study of the mechanism for the inhibition of L-alanine aminotransferase (L-alanine: 2-oxoglutarate of aminotransferase, KF 2.6.1.2 [7], which possesses a high and specific sensitivity toward cycloserine, by the antibiotic and related compounds. Cycloserine, being a peculiar analog of alanine, is attached to the substrate portion of the active center of the enzyme. The amidooxy fragment of the ring (pK_a 4.5) plays the role of the modified carboxyl group, and the amino group of the inhibitor, similar to the amino group of the substrate, enters into the usual reaction with the coenzyme. Acylation of the catalytically important group of the protein takes place in one of the steps of the normal transamination process, which leads to the irreversible inhibition of the enzyme. Here it is important that the relatively weak acylating properties of the inhibitor be fully manifested due to its being complementary with the substrate portion.

In view of these concepts it seemed of considerable interest to study the properties and reaction of the esters of aminocarbohydroxamic acids, as being peculiar analogs of cycloserine and other active cyclic esters of hydroxamic acids, with enzymes.



In this paper we describe the synthesis of these previously unknown compounds.*

Inasmuch as the aminocarbohydroxamic acids themselves are known and available, then one of the possible paths for the synthesis of their esters could consist in the alkylation of acids with a protected amino group.



However, the alkylation of hydroxamic acids, as is known [10], goes in ill-defined manner. In addition, it could be assumed that a certain similarity exists in the properties of such acyclic esters and the 3-isoxazolidinones, in particular, an adequate lability. For this reason the selection of a protective group and a method for its removal without involving the amidooxy fragment were not clear. In view of this it seemed expedient to use a different route, and specifically, the aminoacylation of various O-substituted hydroxylamines, the preparation of which was described previously [11, 12]. Taking into account the sec-

* The preparation of these compounds and some of their properties were briefly reported by us previously [8]. While this work was in progress there appeared in the press a paper [9] on the synthesis of the amidooxy peptides of aminooxyacetic acid and canaline by similar procedures.

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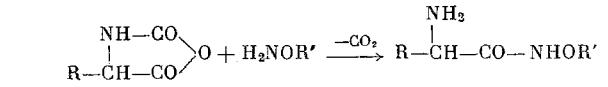
ond consideration expressed above, it seemed necessary, if only in the first steps, to select such methods of activating the carboxyl group as would not stipulate a special protection of the amino group.

The initial attempts to react the esters of amino acids directly with O-substituted hydroxylamines, under the conditions used to prepare aminocarbohydroxamic acids, proved unsuccessful. We were able to synthesize the first members of the aminocarbohydroxamic acid esters by the acylation of O-hydroxylamines with the N-carboxy anhydrides of amino acids.

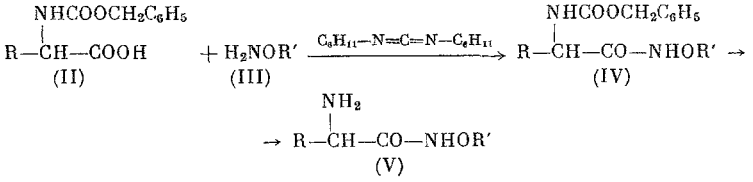


(I)

a	b	c	d	e	f
R=CH_3; R'=CH_3;	CH_3; H;	$(CH_3)_2CH$; CH_3;	$(CH_3)_2CH$; $C_6H_5CH_2$;	$(CH_3)_2CHCH_2$; CH_3;	$(CH_3)_2CHCH_2$ $C_6H_5CH_2$

Although the reaction went quite smoothly and in good yields, an attempt to find synthesis methods suitable for obtaining the corresponding derivatives of various amino acids, caused us to also examine other methods.

As was to be expected, the carbobenzoxyamino acids (II) reacted easily with the O-substituted hydroxylamines (III) when dicyclohexylcarbodiimide was added.



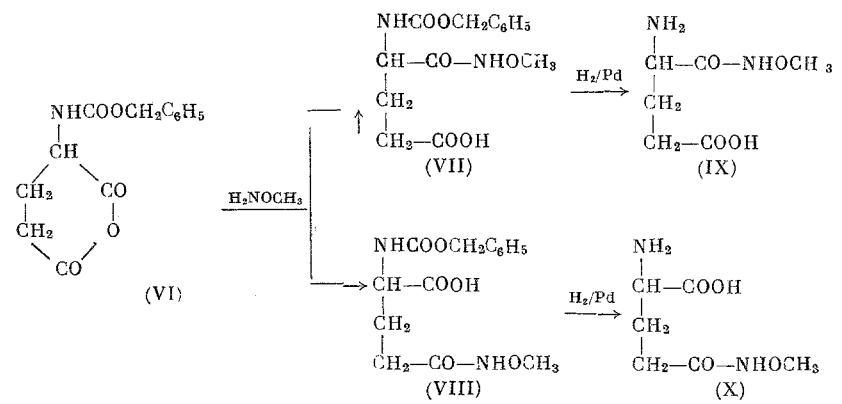
(II), (III), (IV), (V)

a	b	c	d
R=CH_3; R'=CH_3;	CH_3; C_2H_5;	CH_3; CCl_3CH_2;	$(CH_3)_2CH$ $C_6H_5CH_2OOC-CH_2-CH_2$

with the exception of (Vd), where R' = $HOOCCH_2CH_2$

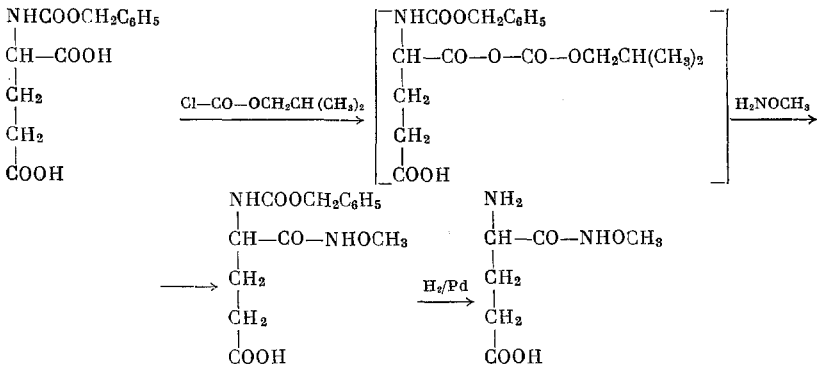
Also in the simplest cases, removal of the protection did not present any difficulties and could be accomplished by the usual techniques (hydrogenolysis in the presence of palladium black or treatment with an acetic acid solution of hydrobromic acid). It should be mentioned that the various aminocarbohydroxamic acid esters differ considerably in their stability, in particular, to reduction. Thus, in the preparation of the β,β,β-trichloroethyl ester of alaninehydroxamic acid (Vc), the synthesis of which was associated with the desire to obtain a compound with a structure that in its acylating properties approximated as close as possible those of the 3-isoxazolidinone, removal of the protection could be accomplished only by treatment with HBr in CH_3COOH, since rupture of the N–O bond also occurred during hydrogenolysis. As a result, to obtain the esters of aminocarbohydroxamic acids it proved possible to use a number of the methods used in the synthesis of peptides.

As a representative of the monoamino dicarboxylic acids we selected glutamic acid, which is the substrate of the highly purified glutamate aspartate aminotransferase (L-asparate: 2-oxoglutarate aminotrans-



ferase, KF 2.5.1.2) – an enzyme that was used by us in the further testing of a number of esters of aminocarbohydroxamic acids. One of the methods for the preparation of the amides and peptides of glutamic acid is the reaction of its N-substituted anhydride with the appropriate amines and amino acids. Here, as a rule, the α- and γ-derivatives are formed, in which connection the ratio of the isomers depends on many factors (nature of the attacking nucleophilic agent, type of N-protection, solvent, etc.) [13-15]. As was ascertained by us, the reaction of O-substituted hydroxylamines with the anhydride of carbobenzoxyglutamic acid (VI) also goes in a similar manner.

The α- and γ-isomers, differing sharply in their properties and easily separable, are formed in apporximately equal amounts, and their ratio does not change for different hydroxylamines. α-L-Glutamyl-[O-methyl]-hydroxylamine (IX) was also obtained by us by another independent route and its structure was proved by reduction to isoglutamine.



In subsequent communications we will publish the results of our studies on the reaction of the synthesized esters of aminocarbohydroxamic acids with pyridoxal and pyridoxal enzymes. Here, it should be mentioned that, contrary to the theory expressed in [9], the simple esters of substrate aminocarbohydroxamic acids, completely free of traces of the O-substituted hydroxylamine derivative, are not inhibitors of pyridoxal enzymes.

EXPERIMENTAL

Synthesis from N-Carboxy Anhydrides of Amino Acids

Methyl Ester of D,L-Alaninehydroxamic Acid (Ia). To a solution of 1.15 g of the N-carboxy anhydride of D,L-alanine in 10 ml of absolute ethanol, with vigorous stirring and cooling to 0°, was added at one time a solution of 1.0 g of the O-methylhydroxylamine free base in 5 ml of ethanol. The stirring was continued for 30 min at 0° and at room temperature for 1 h, after which the precipitate was filtered, washed with ether, and dried in vacuo. We obtained 0.83 g (70%) of the methyl ester (Ia), mp 127 to 128° (aqueous alcohol). Found %: N 23.89. $C_4H_{10}N_2O_2$. Calculated %: N 23.71.

Hydroxamic Acid of D,L-Alanine (Ib). Obtained in the same manner as the preceding. Yield of (Ib) 77%, mp 169-170°. Found %: N 26.82. $C_3H_8N_2O_2$. Calculated %: N 26.90.

Methyl Ester of L-Valenehydroxamic Acid (L-Ic). Under similar conditions, from 0.9 g of the N-carboxy anhydride of L-valine and 0.45 g of O-methylhydroxylamine we obtained 0.42 g of methyl ester (Ic), mp 95-96°. Found %: N 19.11. $C_6H_{14}N_2O_2$. Calculated %: N 19.16.

Benzyl Ester of L-Valinehydroxamic Acid (L-Id). Under similar conditions, from 0.9 g of the N-carboxy anhydride of L-valine and 1.16 g of O-benzylhydroxylamine we obtained 0.51 g (37.8%) of benzyl ester (Id), mp 96°. Found %: N 12.59. $C_{12}H_{18}N_2O_2$. Calculated %: N 12.60.

Methyl Ester of L-Leucinehydroxamic Acid (L-Ie). Under similar conditions, from 0.99 g of the N-carboxy anhydride of L-leucine and 0.45 g of O-methylhydroxylamine we obtained 0.64 g (64%) of methyl ester (Ie), mp 110-111°. Found %: N 17.35. $C_7H_{16}N_2O_2$. Calculated %: N 17.48.

Benzyl Ester of L-Leucinehydroxamic Acid (L-If). Under similar conditions, from 0.9 g of the N-carboxy anhydride of L-leucine and 1.16 g of O-benzylhydroxylamine we obtained 0.8 g (60.7%) of benzyl ester (If), mp 81-82°. Found %: N 12.64. $C_{13}H_{20}N_2O_2$. Calculated %: N 11.85.

Synthesis from Esters of N^{α}-Carbobenzoxyaminocarbohydroxamic Acids

Methyl Ester of N^{α}-Carbobenzoxy-D,L-alaninehydroxamic Acid (IVa). To a solution of 2.1 g of carbobenzoxy-D,L-alanine (IIa) and 0.5 g of O-methylhydroxylamine in 15 ml of absolute tetrahydrofuran (THF), with vigorous stirring and cooling to 0°, was gradually added a solution of 2 g of dicyclohexylcarbodiimide in 6 ml of THF. The mixture was stirred at 20° for 2 h, filtered, and the clear solution was evaporated in vacuo at 20°.

The residue was dissolved in an equimolar amount of 0.2 N aqueous NaOH solution, filtered, and the filtrate was saturated with CO_2. The obtained crystals were separated, washed with cold water, and dried. The yield of the methyl ester (IVa) was 1.86 g (75%), mp 113-114° (from benzene). Found %: N 11.32. $C_{12}H_{16}N_2O_4$. Calculated %: N 11.15.

Methyl Ester of N^{α}-Carbobenzoxy-L-alaninehydroxamic Acid (L-IVa). Under similar conditions, from 2.1 g of carbobenzoxy-L-alanine (L-IIa), 0.5 g of O-methylhydroxylamine and 2 g of dicyclohexylcarbodiimide we obtained 1.65 g (70%) of (L-IVa), mp 112-113° (from benzene). Found %: N 11.24. $C_{12}H_{16}N_2O_4$. Calculated %: N 11.15.

Ethyl Ester of N^{α}-Carbobenzoxy-D,L-alaninehydroxamic Acid (IVb). From 2.23 g of carbobenzoxy-D,L-alanine (IIa), 0.61 g of O-ethylhydroxylamine and 2 g of dicyclohexylcarbodiimide we obtained 2.5 g (94%) of ethyl ester (IVb), mp 114-116° (from dichloroethane). Found %: N 10.37. $C_{13}H_{18}N_2O_4$. Calculated %: N 10.52.

Trichloroethyl Ester of N^{α}-Carbobenzoxy-D,L-alaninehydroxamic Acid (IVa). To the well-stirred reaction mixture, containing 0.04 mole of carbobenzoxy-D,L-alanine (IIa), were added 0.004 mole of the trichloroethylhydroxylamine hydrochloride and 0.004 mole of triethylamine, and 30 moles of dicyclohexylcarbodiimide in 10 ml of THF. After stirring for 2 h, the precipitate was filtered, the filtrate was treated with 15-20 ml of absolute ether, the mixture filtered again, and the filtrate was evaporated in vacuo at 20°. The yield of the trichloroethyl ester (IVc) was 54%, mp 135-140° (from ethyl acetate). Found %: Cl 29.48. $C_{13}H_{15}O_4N_2Cl_3$. Calculated %: Cl 28.78.

β-Carbobenzoxyethyl Ester of N^{α}-Carbobenzoxy-L-valinehydroxamic Acid (L-IVd). In a similar manner, from 1.2 g of the benzyl ester of β-aminooxypropionic acid [12], 1.67 g of carbobenzoxy-L-valine (L-IId) and 1.36 g of dicyclohexylcarbodiimide we obtained 2.13 g (80%) of (L-IVd), mp 104-106° (from benzene–heptane). Found %: C 64.63; H 6.68; N 6.46. $C_{23}H_{28}N_2O_6$. Calculated %: C 64.86; H 6.58; N 6.53.

Anhydride of Carbobenzoxy-D,L-glutamic Acid (VI). To a solution of 2.8 g of carbobenzoxy-D,L-glutamic acid in 15 ml of THF, with stirring, at 0°, was added a solution of 0.62 g of dicyclohexylcarbodiimide in 3 ml of THF, after which the mixture was stirred for 2 h, filtered, the filtrate was evaporated in vacuo, and the residue was recrystallized from a mixture of chloroform and ether. The yield of anhydride (VI) was 50%, mp 107-108°. Literature data [13]: mp 94°.

Carbobenzoxy-α-L-glutamyl[O-methyl]-hydroxylamine (L-VII). To a solution of 4 g of carbobenzoxy-L-glutamic acid (L-IX) and 2.9 g of triethylamine in 25 ml of THF at −40° was added 2 g of the isobutyl ester of chlorocarbonic acid, and the mixture was stirred between −35 and −20° for 10 min. Then a solution of 1.1 g of O-methylhydroxylamine in 5 ml of THF was added in drops, the mixture was stirred for 20 min, followed by the addition of 100 ml of absolute ether, and when the temperature of the mixture reached 20° the ether layer was decanted from the precipitate. Then the precipitate was dissolved in 10 ml of water, the solution was acidified with hydrochloric acid, and the crystals were filtered and washed with ice water. The yield of the methyl ester (L-VII) was 2.4-2.6 g (55-60%), mp 176-177° (from ethyl acetate). Found %: N 9.14. $C_{14}H_{18}N_2O_6$. Calculated %: N 9.03.

Carbobenzoxy-L-glutamyl-[O-methyl]-hydroxylamines (L-VII) and (L-VIII). To a solution of 0.35 g of O-methylhydroxylamine in 3 ml of chloroform at 0° was added a chloroform solution of 1.3 g of the anhydride of carbobenzoxy-L-glutamic acid (VI), after which the mixture was stirred for 30 min and the precipitate of the α-isomer (L-VII) 1 g, 58%, mp 176-177°) was filtered. The filtrate was evaporated in vacuo. We obtained 0.6 g (35%) of carbobenzoxy-γ-L-glutamyl-[O-methyl]-hydroxylamine as an oil.

Carbobenzoxy-D,L-glutamyl-[O-methyl]-hydroxylamines (VII) and (VIII). The opening of the anhydride of carbobenzoxy-D,L-glutamic acid (VI) with O-methylhydroxylamine was done in the same manner as the preceding. The yield of carbobenzoxy-D,L-glutamyl-[O-methyl]-hydroxylamine was 50%, mp 146°. From the filtrates we obtained the γ-isomer as an oil in 40% yield.

Methyl Ester of D,L-Alaninehydroxamic Acid (Ia). A solution of 2.5 g of the methyl ester of N^{α}-carbobenzoxy-D,L-alaninehydroxamic acid in 30 ml of methanol was hydrogenated over palladium black in the presence of several drops of acetic acid until the CO_2 evolution ceased. The catalyst was filtered, and the solvent was removed in vacuo at 20°. The yield of the methyl ester (Ia) was 0.86 g, mp 127-128° (from methanol–ethyl acetate).

Methyl Ester of L-Alaninehydroxamic Acid (L-Ia). Obtained in the same manner as described above from (L-IVa). Yield of (L-Ia) 72%; mp 126-127° (from aqueous alcohol). Found %: N 23.80. $C_4H_{10}N_2O_2$. Calculated %: N 23.71.

Ethyl Ester of D,L-Alaninehydroxamic Acid (Vb). Obtained in the same manner as the preceding from (IVb). Yield of ethyl ester (Vb) 67%, mp 131° (from aqueous alcohol). Found %: N 21.31. $C_5H_{12}O_2N_2$. Calculated %: N 21.19.

Carboxyethyl Ester of L-Valinehydroxamic Acid (L-Vd). A solution of 1 g of the carbobenzoxyethyl ester of L-valinehydroxamic acid (L-IVd) in 75 ml of absolute ethanol was hydrogenated over palladium black in the same manner as described above. The catalyst was filtered, the filtrate was decolorized with activated carbon, and filtered again. The clear filtrate was evaporated in vacuo at 30° to dryness. Yield 0.38 g (80%), mp 154° (from isopropanol). Found %: C 47.32; H 8.10; N 13.42. $C_8H_{16}N_2O_4$. Calculated %: C 47.43; H 7.89; N 13.71. The compound decomposes when stored.

Trichloroethyl Ester of Hydroxamic Acid of D,L-Alanine (Vc). To 0.001 mole of the carbobenzoxy derivative (VIa) was added 1.2 ml of a solution of hydrogen bromide in glacial acetic acid, after which the clear solution was kept at 20° for 15-20 min, then 15 ml of absolute ether, after which it was dissolved in the minimum amount of absolute alcohol and reprecipitated by the addition of excess ether. The hygroscopic substance was separated and dried in vacuo over P_2O_5. The yield of the trichloroethyl ester (Vc) was 35%. Found %: N 9.13. $C_5H_{10}O_2N_2Cl_3Br$. Calculated %: N 9.84.

Glutamyl-[O-methyl]-hydroxylamines (IX) and (X). The carbobenzoxy derivatives of the methyl esters of the glutamylhydroxamic acids were dissolved in aqueous alcohol and then hydrogenated in the presence of several drops of glacial acetic acid over palladium black until the CO_2 evolution ceased. The solvent was removed in vacuo at 20° and the residue was recrystallized from alcohol. The following compounds were obtained in nearly quantitative yields: α-L-glutamyl-[O-methyl]-hydroxylamine (L-IX) with mp 142-143°. Found %: N 15.82. $C_6H_{12}N_2O_4$. Calculated %: N 15.90; γ-L-glutamyl-[O-methyl]-hydroxylamine (L-X) with mp 138-139°. Found %: N 15.85. $C_6H_{12}N_2O_4$. Calculated %: N 15.90; α-D,L-glutamyl-[O-methyl]-hydroxylamine (IX) with mp 142-143°. Found %: N 15.82. $C_6H_{12}N_2O_4$. Calculated %: N 15.90 γ-D,L-glutamyl-[O-methyl]-hydroxylamine (X) with mp 147-148°. Found %: N 15.72. $C_6H_{12}N_2O_4$. Calculated %: N 15.90.

CONCLUSIONS

Employing the methods used for the synthesis of peptides, some esters of aminocarbohydroxamic acids were synthesized.

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