HYDROXYLAMINE DERIVATIVES

COMMUNICATION 7. SYNTHESIS OF 4-SUBSTITUTED 3-ISOXAZOLIDINONES

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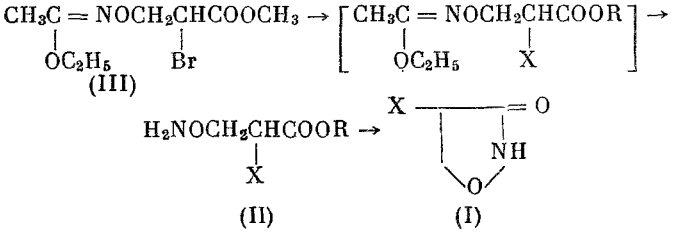
In the course of a study of the molecular mechanism of the biological activity of the antibiotic cycloserine we established a relation between the chemical properties of the antibiotic and its action. It was shown that the antibacterial activity of cycloserine is related to its effect on amino acid metabolism and in particular to the inhibition of enzymes to which pyridoxal phosphate acts as coenzyme [1, 2]. As a result of an investigation of the interaction of cycloserine and some of its derivatives with PP-enzymes and pyridoxal phosphate we were able to establish that an essential feature for the appearance of an inhibiting action is the presence of a free amino group in combination with a hydroxamic cyclic ester grouping. On the basis of these premises we decided to undertake the synthesis of compounds derived from O-substituted hydroxylamine in which these requirements would be realized in some degree. We recently developed a simple and convenient method for the preparation of functional derivatives of O-substituted hydroxylamine [3]. In [4] we applied this method for the preparation of 3-(aminooxy)alanine, a principal intermediary in a new synthesis of cycloserine. We now report the synthesis of some 4-substituted 3-isoxazolidinones in accordance with the scheme:



(III) (II) (I)

(I) a. $X = H_2NO-$; b. $X = C_6H_5O-$; c. $X = C_5H_{11}N$.

(II) a. $X = H_2NO-$; b. $X = C_6H_5CONHO-$; c. $X = C_6H_5O-$; d. $X = C_5H_{11}N$.

$R = C_2H_5$; $R = CH_3$; $R = C_2H_5$; $R = CH_3$

As starting substance we chose the ester (III), in which the α-bromine is highly reactive in substitution reactions. Moreover, the protection on the aminooxy group can be removed under extremely mild conditions, which makes it simple to pass to the 3-isoxazolidinone system. Some simple substituted 3-isoxazolidinones have already been synthesized from 3-(isopropylideneaminooxy)propionic esters, but these compounds were found to be unsuitable for the preparation of more complex analogs of the antibiotic, because the elimination of the isopropylidene group was accompanied by the destruction of the substituent in the α-position. We must point out also that halogen in the α-position of substances of this kind is extremely difficult to replace by other groups, which limits the possibilities of this method substantially. All these circumstances led us to the necessity of using the bromo ester (III) (we have already described the synthesis and some of the properties of this ester [3, 4]) forthe preparation of 4-substituted 3-isoxazolidinones.

Among 4-substituted 3-isoxazolidinones the greatest interest was presented by the compound (Ia), which has a 4-(aminooxy) group which can react readily with aldehydes and ketones with formation of oximes. The synthesis of this particular compound was prompted by the desire to create a structure in which the principal chemical properties

of the antibiotic cycloserine are preserved, but there is a substantial increase in the power to combine with enzymes containing pyridoxal phosphate, for which aldehyde-pyridoxal phosphate serves as coenzyme.

To prepare the bisaminooxypropionic ester (IIa), the key compound for the synthesis of (Ia), we decided to alkylate alkali-metal benzohydroxamates with the bromo ester (III). The reaction of the bromo ester (III) with benzohydroxamic acid goes with difficulty, and the yield of the alkylation product (IIb) does not exceed a few percent. However, when an aqueous solution of a salt of the bromo acid was heated with acetohydroxamic ester the latter was alkylated smoothly. Subsequent esterification led to the ester (IIa) in 60-70% yield. The structure of this ester was proved by its hydrogenation to glyceric ester.

By the treatment of the ester (IIa) with various alkaline agents (Ia) was formed, and this, like all 3-isoxazolidinones, gave a characteristic color with sodium nitroprusside and ferric chloride, formed a silver salt, and decomposed rapidly in aqueous and alcoholic solutions; its hydrogenation gave 4-hydroxy-3-isoxazolidinone, identical with the previously described sample [5]. The reaction of (III) with such compounds as phenol, thiols, Na_2S, etc., went under conditions similar to those found for the alkylation of the hydroxamic ester. By way of example, in Experimental we describe the preparation of 4-phenoxy-3-isoxazolidinone (Ib).

The bromo ester (III) reacts particularly readily with various amines. In the alkylation of piperidine we obtained the corresponding ester in more than 80% yield. This ester was then converted under mild conditions and in high yield into 4-piperidino-3-isoxazolidinone (Ic). It may be supposed that this method for the preparation of 4-amino-3-isoxazolidinones substituted in the amino group, unlike the syntheses described in the literature for individual members of this class of substances [6], is fairly general and that the most varied analogs of the antibiotic can be prepared by the scheme that we have developed.

As would be expected, 4-(aminooxy)-3-isoxazolidinone is biologically active; according to our results it is the most powerful of all known inhibitors of pyridoxal enzymes [7]. Also, preliminary results of tests for antimicrobial activity show that (Ia) has bacteriostatic activity in vitro against Mycobacterium tuberculosis that is comparable to the activity of the antibiotic cycloserine [8].

EXPERIMENTAL

3-(Aminooxy)-N-benzoylalanine Methyl Ester Hydrochloride (IIb). A solution of 8 g of sodium benzohydroxamate and 15 g of the bromo ester (III) in methanol was heated for 8 h at 65-70°. The mixture was vacuum-concentrated and dry ether was added. The solution was filtered, and the filtrate was evaporated to dryness and treated with 5 ml of concentrated hydrochloric acid. The mixture was vacuum-dried and dissolved in dichloroethane; ethyl acetate was added. After one day crystals of the hydrochloride (IIb) were filtered off; yield 8%; m.p. 179° (alcohol–ethyl acetate). Found: Cl 12.41%. $C_{11}H_{15}O_5N_2Cl$. Calculated: Cl 12.19%.

Ethyl 2,3-Bisaminooxypropionate Dihydrochloride (IIa). 27 g of methyl 2-bromo-3-(1-ethoxyethylideneaminooxy)propionate was hydrolyzed at 30-35° with 40% aqueous potassium hydroxide. To the solution of the potassium salt we added 12 g of ethyl acetohydroxamate, 7 g of potassium hydroxide, and 15 ml of water, and the mixture was heated with stirring in a steam bath for 30-40 min. It was cooled to −10° and cautiously acidified with 20% hydrochloric acid to Congo Red. The mixture was extracted twice with methylene chloride, the organic layer was filtered through anhydrous magnesium sulfate, and the filtrate was vacuum-evaporated to dryness. The residue was dissolved in 75 ml of absolute alcohol, and the solution was saturated with heating with dry hydrogen chloride and filtered; the filtrate was boiled for 3 h. After 12 h the dihydrochloride was filtered off, washed with absolute isopropyl alcohol, and vacuum-dried over phosphorus pentoxide; yield 14.2 g (60%); m.p. 151° (ethanol–isopropyl alcohol). Found: C 25.70, 25.52; H 5.70; 5.94; Cl 30.15; 30.13%. $C_5H_{14}O_4N_2Cl_2$. Calculated: C 25.31; H 5.94; Cl 29.93%.

4.74 g of the dihydrochloride (IIa) was dissolved in alcohol and hydrogenated over platinum oxide (0.5 g) until the theoretical amount of hydrogen had been absorbed. Catalyst was then filtered off, and after distillation we obtained 1.5 g (58%) of ethyl glycerate, b.p. 71-73° (0.5 mm); amide, m.p. 100° [5], undepressed by admixture of a known sample.

4-(Aminooxy)-3-isoxazolidinone (Ia). 1.2 g of sodium hydroxide was dissolved in 50 ml of absolute ethanol, 2.37 g of the ester dihydrochloride (IIa) was added, and the mixture was stirred for 1 h. The mixture was cooled to −15°, sodium chloride was separated, and the filtrate was rapidly vacuum-evaporated at 5-10° until crystallization began. The hygroscopic precipitate was filtered off and vacuum-dried. The yield of the sodium salt of (Ia) was 0.7 g (50%); R_f 0.72 (butyl alcohol–acetic acid–water 4 : 1 : 5; development with sodium nitroprusside). Found: N 17.46%. $C_3H_5O_3N_2Na\ H_2O$. Calculated: N 17.72%.

Hydrochloride of (Ia). Hygroscopic substance, rapidly deliquescing in air. Found: Cl 12.38%, 2 · $C_3H_6O_3N_2$ HCl. Calculated: Cl 13.06%.

Picrate of (Ia). The sodium salt of (Ia) was dissolved in alcohol and treated with the calculated amount of an alcoholic solution of hydrogen chloride; sodium chloride was filtered off. From the filtrate we prepared the picrate of (Ia); m.p. 290°; mol. wt. found 346.3, calculated 347.1.* From the mother liquors we isolated the picrate of the dimer of (Ia); m.p. 90°; mol. wt. found 728.9; calculated 694.2.

Hydrogenation of (Ia). (Ia) was hydrogenated in alcoholic solution at 20° over platinum oxide until one molecular proportion of hydrogen had been absorbed. The product was 4-hydroxy-3-isoxazolidinone, identical in paper chromatography with a known sample (butyl alcohol–acetic acid–water 4 : 1 : 5; development with sodium nitroprusside).

Ethyl 3-(Aminooxy)-2-phenoxypropionate Hydrochloride (IIc). The synthesis was similar to that of (IIa). From 13.5 g of the bromo ester (III) and 5 g of phenol we obtained 3.9 g (30 %) of (IIc), m.p. 131-132°. Found: Cl 13.57; N 5.27%. $C_{11}H_{16}O_4Cl$. Calculated: Cl 13.55; N 5.35%.

4-Phenoxy-3-isoxazolidinone. 2.05 g of the ester (IIc) was added to 31 ml of 1 N NaOH at 0°, the mixture was stirred for 1 h, the clear solution was acidified with glacial acetic acid to pH 4.5-5, and the precipitate was filtered off, washed with water, and vacuum-dried over phosphorus pentoxide. The yield of (Ic) was 1 g (71%); m.p. 141-142°. Found: N 7.66%. $C_9H_9O_3N$. Calculated: N 7.81%.

Methyl 3-(1-Ethoxyethylideneaminooxy)-2-piperidinopropionate. 27.1 g of methyl 2-bromo-3-(1-ethoxyethylideneaminooxy)propionate was dissolved in 30 ml of dry benzene, 17 g of piperidine was added dropwise with stirring, and the mixture was then boiled for 2.5 h. The reaction mixture was cooled, 100 ml of ether was added, and the mixture was filtered. The ethereal solution was washed twice with water, dried over magnesium sulfate, and vacuum-distilled. We obtained 23 g (85%) of the ester; b.p. 114-116° (1 mm); n_D^{25} 1.4645. Found: N 8.94%. $C_{13}H_{24}O_4N_2$. Calculated: N 10.29%. It was a colorless mobile liquid which decomposed rapidly at room temperature.

Methyl 3-(Aminooxy)-2-piperidinopropionate Hydrochloride (IId). 0.18 g of water and 0.4 g of hydrogen chloride dissolved in dry ether were added to 2.7 g of the ester, and the reaction mixture was shaken for 30 min. A colorless oil was precipitated, and when this was rubbed out with dry ether it crystallized completely. The yield of the hydrochloride was 2.5 g (91%); m.p. 112-115° (decomp.). Found: C 45.43; H 7.97; Cl 14.92%. $C_9H_{19}O_3N_2Cl$. Calculated: C 45.27; H 8.02; Cl 14.85%.

4-Piperidino-3-isoxazolidinone (Ic). 2.4 g of the ester was added to 30 ml of 1 N NaOH at 0°, and the mixture was stirred for 1 h. The clear solution was passed through the cation exchanger Dowex 50 × 8 (50-100), which was then washed with 100 ml of water. Elution was with 1 N ammonia, and the fraction giving a characteristic blue color with an alkaline solution of sodium nitroprusside was collected. The solution was vacuum-evaporated to dryness at 10-15°. We obtained 0.85 g (57%) of product, m.p. 98-100°. Found: C 56.43; H 8.16%. $C_8H_{14}O_2N_2$. Calculated: C 56.45; H 8.29%.

SUMMARY

1. A new general method was developed for the preparation of 4-substituted 3-isoxazolidinones from acetohydroxamic ester.

2. 4-(Aminooxy)-3-isoxazolidinone was synthesized. This substance is a powerful inhibitor of pyridoxal enzymes and has high bacteriostatic activity in vitro against Mycobacterium tuberculosis.

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* The molecular weight was determined by Cunningham's method [9].

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