SYNTHESIS OF 4-ALKYL-SUBSTITUTED DERIVATIVES OF CYCLOSERINE*

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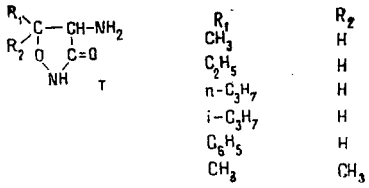
In recent years, various derivatives of 3-isoxazoline have been studied in many aspects, and the simplest representative of them – 4-amino-3-isoxazolidone or cycloserine (I, $R_1 = R_2 = H$) – has proved to be a highly effective broad-spectrum antibiotic [2-6].

Some workers have obtained its derivatives with alkyl (mono- and di-) and aryl radicals in position 5. These compounds (I) can be represented in the following way [7-17]:

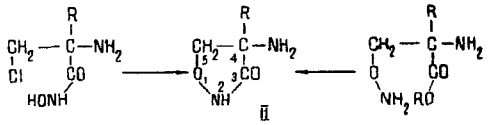


R_1	R_2
CH_3	H
C_2H_5	H
$n\text{-}C_3H_7$	H
$i\text{-}C_3H_7$	H
C_6H_5	H
CH_3	CH_3

The above-mentioned compounds were obtained from β-hydroxy α-amino acids. Antibacterial tests on them have shown that R and Ar in position 5 have a considerable influence on the antibiotic activity.

On the basis of previous investigations [18-20], the synthesis from α-alkylserines of 4-alkyl-substituted cycloserines of the general formula (II) has become possible.

When the α-alkylserines (III) are available, the preparation of 4-alkylcycloserines (II) from them is possible by two routes: intramolecular O-alkylation [7] or intramolecular N-acylation [8], as follows:



Intramolecular O-alkylation, used by Plattner et al. [7] for the synthesis of cycloserine and five of its derivatives, did not lead to the desired result in our case. Intramolecular N-acylation by Stammer's method [8] via the corresponding oxazoline derivatives yielded esters of β-aminooxy α-amino acids, which were then subjected to intramolecular N-acylation. However, this method of synthesis also starts from the corresponding esters of β-hydroxy α-amino acids. Since it had proved impossible to prepare their esters from the α-alkylserines (III), we solved this problem in the following way [1]:

*See the preliminary communication [1].

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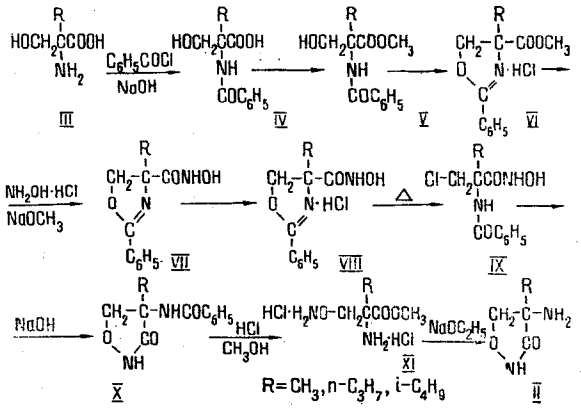
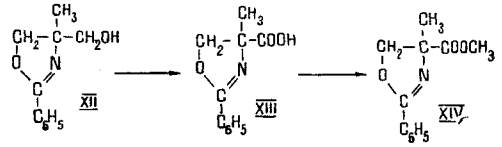


The first stage of the synthesis was the preparation of the N-benzoyl-α-alkylserine (IV) by the Schotten-Baumann method with a yield of 45-50%, and this was then converted into the corresponding methyl esters of the α-alkylserines (V) by means of diazomethane in almost quantitative yield. These esters were treated with thionyl chloride [21-22] and converted into the esters of the corresponding 4-alkyl-2-phenyloxazoline-4-carboxylic acids (VI). From the latter, by using HCl and dioxane, the hydrochlorides of the corresponding hydroxamic acids (VIII) were obtained. In this way, the hydrochlorides of 2-phenyl-4-propyloxazoline-4-hydroxamic acid (VIII, R = N-C_3H_7) and 4-isobutyl-2-phenylisoxazoline-4-hydroxamic acid (VIII, R = i-C_4H_9) were synthesized. The first compound was obtained from α-propylserine and the second from α-isobutylserine.

However, it was impossible to synthesize an oxazolinecarboxylic acid from the simplest representative, α-methylserine (III, R = CH_3), since in this case Schotten-Baumann benzoylation took place in very low yield. The hydroxamic acid was obtained by another method based on the oxidation of 4-hydroxymethyl-4-methyl-2-phenyloxazoline (XII) by potassium permanganate [23]. The oxazolinecarboxylic acid (XIII) was converted into its ester (XIV) with diazomethane in the following way:



The methyl 4-methyl-2-phenyloxazoline-4-carboxylate (XIV) was converted into the hydrochloride of the hydroxamic acid (information on the 4-alkyl-2-phenylisoxazoline-4-hydroxamic acids is given in the experimental part).

The opening of the oxazoline ring is a stage of the synthesis which takes place with a very poor yield. It was possible to open the ring only in the case of the 4-isobutyloxazoline-4-carbohydroxamic acid with a very low yield. This circumstance made necessary a subsequent special study of the transformation of 4-isobutyl-2-phenyloxazoline-4-carbohydroxamic acid (VII, R = i-C_4H_9) into the α-benzamido-β-chloro derivative (IX). The hydrochloride of the hydroxamic acid (VIII) is best isolated from dioxane with only the calculated amount of HCl, in order to avoid side reactions after the opening of the ring.

We could not obtain α-benzamido-β-chloro-α-isobutylpropionohydroxamic acid (IX, R = i-C_4H_9). It was converted directly into N-benzoyl-4-isobutylcycloserine (X, R = i-C_4H_9). The cyclization was performed with NaOH at pH 9.8 in the presence of a Thymol Blue indicator. It is known [24] that at pH 10, because of the enolization of the benzamido group, ring closure to the oxazoline takes place primarily. The benzoyl group was split off with HCl in methanol, the ring simultaneously undergoing cleavage with the formation of the dihydrochloride of the ester of α-amino-β-aminooxy-α-isobutylpropionic acid (XI, R = i-C_4H_9), which it was impossible to isolate in the crystalline state. The reclosure of the 3-isoxazolidone ring was effected with sodium methoxide. 4-Isobutylcycloserine (II, R = i-C_4H_9) was obtained from an aqueous solution of its sodium salt by acidification with acetic acid to pH 6. Since, unlike cycloserine, 4-isobutylcycloserine is very sparingly soluble in water, it crystallizes satisfactorily from water. In the isopropanol—ammonia—water (80 : 2 : 18) system, the substance gave a single spot with R_f 0.69.

Cycloserine and its derivatives give a specific reaction with sodium nitroprusside: in a weakly acid medium an intensely blue complex is formed. The UV spectrum shows that for 4-isobutylcycloserine the absorption maximum is in the 640 nm region, for N-benzoyl-4-isobutylcycloserine it is at 670 nm, and for cycloserine it is at 625 nm. The IR spectrum confirmed the presence of the characteristic groups in the molecule of 4-isobutylcycloserine.

EXPERIMENTAL

N-Benzoyl-α-propylserine (IV, R = n-C_3H_7). With vigorous stirring, 27 ml (32.4 g, 0.23 mole) of benzoyl chloride and 12 ml of 2 N caustic soda were added over 1 h at 0° C to 29.4 g (0.2 mole) of α-propylserine in 120 ml of 2 N caustic soda; the pH of the solution remained alkaline until the end of the reaction (to a Thymol Blue indicator). Then another 20 ml of 2 N caustic soda was added to the solution. The mixture was stirred at 0° C for 1 h and at room temperature for 2 h. With cooling and stirring, the solution was acidified with hydrochloric acid (1 : 1) to pH 1. An oily substance began to precipitate which crystallized on further stirring. For purification from benzoic acid, the substance was extracted three times with hot ether (100 ml). This gave 23.5 g (46%) of a product with mp 143-146° C. This substance was boiled with activated carbon in 40 ml of ethanol, after which the carbon was filtered off and 100 ml of water was added to the hot solution, giving 19.4 g (38.7%) of a substance $C_{13}H_{17}O_4N$ with mp 151° C. The analysis of this compound and those of all the subsequent compounds corresponded to the calculated figures.

N-Benzoyl-α-isobutylserine (IV, R = i-C_4H_9). With vigorous stirring at 0° C, 13.5 ml (16.2 g, 0.115 mole) of benzoyl chloride and 60 ml of 2 N caustic soda were added to 16.1 g (0.1 mole) of α-isobutylserine in 60 ml of 2 N caustic soda over 1 h. Then the reaction mixture was treated in the same way as in the preceding experiment. This gave a substance with the composition $C_{14}H_{19}O_4N$ in a yield of 13.2 g (49.8%); mp 159-161° C (from 33% ethanol).

Methyl Ester of N-Benzoyl-α-propylserine (V, R = n-C_3H_7). A hot solution of 0.14 mole of diazomethane in 150 ml of absolute ether was added to a suspension of 17.57 g (0.07 mole) of N-benzoyl-α-propylserine in 150 ml of absolute ether. After the reaction mixture had begun to boil, the insoluble part was filtered off and the filtrate was evaporated in vacuum to dryness. The residual oil did not crystallize. The weight of the oil was 18.5 g (≈100%); it was fairly pure and was suitable for further synthesis ($C_{14}H_{19}O_4N$).

Methyl Ester of N-Benzoyl-α-isobutylserine (V, R = i-C_4H_9). To a stirred suspension of 15.3 g (0.058 mole) of N-benzoyl-α-isobutylserine in 170 ml of absolute ether was added 0.116 mole of diazomethane in 120 ml of absolute ether. The resulting solution was filtered and evaporated to dryness. On standing, the oil crystallized. The crystals were washed with petroleum ether. Yield 14.8 g (90.8%), mp 92-94°C, composition $C_{15}H_{21}O_4N$.

Hydrochloride of Methyl 2-Phenyl-4-propyloxazoline-4-carboxylate (VI, R = n-C_3H_7). With cooling to 0° C and stirring, 44 ml of thionyl chloride was added by drops over 45 min to 18.5 g (0.07 mole) of the methyl ester of N-benzoyl-α-propylserine in 200 ml of absolute ether. There was a vigorous evolution of HCl and the solution became turbid. Cooling and stirring were continued for another 1 h, until crystallization began. After the mixture had been kept in the refrigerator, the crystals were filtered off, washed with ether, and dried. Weight 13.1 g (66%), mp 105-106° C. The substance was slightly hygroscopic. The filtrate yielded an additonal 2.5 g (12.5%) of substance with mp 102-104° C. The total yield was 15.6 g (78.5%); composition $C_{14}H_{17}O_3NCl$.

Hydrochloride of Methyl 4-Isobutyl-2-phenyloxazoline-4-carboxylate (VI, R = i-C_4H_9). To a suspension of 53.75 g (0.19 mole) of the methyl ester of N-benzoyl-4-isobutylserine in 550 ml of absolute ether was gradually added 115 ml of thionyl chloride. The substance dissolved completely. The crystals that separated from the reaction mixture after it had been kept in the refrigerator were filtered off and washed with absolute ether. This gave 32.9 g of a substance with mp 103-107° C (hygroscopic). The filtrate yielded an additional 12.83 g of product with mp 95-99° C. The total yield was 55.75 g (82.4%); composition $C_{15}H_{19}O_3N \cdot HCl$.

4-Methyl-2-phenyloxazoline-4-carboxylic Acid (XIII). To a suspension of 5.73 g (0.03 mole) of 4-hydroxymethyl-4-methyl-2-phenyloxazoline (XII) [23] in 90 ml of water were added 0.8 g (0.02 mole) of caustic soda and 6.3 g (0.019 mole) of potassium permanganate at such a rate that the temperature of the mixture did not exceed 40° C. The manganese dioxide was filtered off and washed with water, and the

combined filtrates were acidified with HCl to pH 3 and were then extracted six times with chloroform. The chloroform solution was dried over calcined magnesium sulfate and evaporated to dryness. The resulting oil crystallized, and the crystals were washed with petroleum ether. This gave 3.8 g (62%) of a substance $C_{11}H_{11}O_2N$ with mp 130-132° C.

Methyl-4-Methyl-2-phenyloxazoline-4-carboxylate (XIV). With stirring, 0.04 mole of diazomethane in 50 ml of ether was added to a suspension of 4.1 g (0.02 mole) of compound (XIII) in 50 ml of absolute ether. Stirring was continued at room temperature for 2 h. This gave 4.3 g (≈ 100%) of an oily substance which crystallized on standing ($C_{13}H_{17}O_4N$). When it was treated with HCl, white crystals deposited which, on filtration, changed into an oil. Consequently, to obtain the hydroxamic acid the free ester was used.

4-Methyl-2-phenyloxazoline-4-carbohydroxamic Acid (VII, R = CH_3). A solution of sodium methoxide (1.15 g of Na in 20 ml of absolute methanol) was added with stirring at 0° C to a solution of 4.3 g (0.02 mole) of compound (XIV) and 2.1 g (0.03 mole) of hydroxylamine hydrochloride in 30 ml of absolute methanol. A precipitate of sodium chloride immediately deposited. The mixture was left at room temperature for a day. The precipitate that deposited was dissolved in 25 ml of water and the solution was filtered. Then a current of CO_2 was passed through it for 2 h. The crystals that deposited were filtered off, washed with water, and dried. This gave 3.05 g (68.0%) of a substance with mp 160-163° C. After recrystallization from aqueous ethanol, the substance had mp 161-163° C; composition $C_{11}H_{12}O_3N_2$.

The Hydrochloride (VIII) (R = CH_3). At room temperature, 6.8 ml (0.015 mole) of a solution of HCl in dioxane was added to a suspension of 3.3 g (0.015 mole) of the hydroxamic acid (VII, R = CH_3) in 50 ml of absolute dioxane. First the substance was converted into a dough-like mass, and then it dissolved. In 15-20 min, the solution deposited crystals, and after some time in the refrigerator they were filtered off, washed with dioxane and ether, and dried. This gave 3.75 g (97.5%) of a substance with mp 201-205° C (decomp.). After recrystallization from a mixture of methanol and ether, the melting point had not changed; composition $C_{11}H_{12}O_3N_2 \cdot HCl$.

2-Phenyl-4-propyloxazoline-4-carbohydroxamic Acid (VII, R = nC_3H_7). A solution of sodium methoxide prepared from 50 ml of absolute methanol and 3.02 g of sodium was added to a solution of 10.0 g (0.0355 mole) of compound (VI, R = nC_3H_9 and 3.68 g (0.0533 mole) of hydroxylamine hydrochloride in 100 ml of absolute methanol at 0°C. After standing at room temperature for a day, the reaction mixture was evaporated and the residue was dissolved in 150 ml of water. The solution was filtered and acidified with acetic acid to pH 5. After some hours, crystals deposited, which were filtered off, washed with water, and dried. This gave 7.65 g (87.0%) of a substance with mp 156-159° C (from aqueous ethanol); composition $C_{13}H_{16}O_3N_2$.

Hydrochloride of VIII (R = nC_3H_9). To a suspension of 16.1 g (0.065 mole) of the hydroxamic acid (VII, R = nC_3H_7) in 100 ml of dioxane was added 29.5 ml (0.065 mole) of a 2.2 N solution of HCl in dioxane. The substance dissolved completely, and then crystals deposited which were filtered off and washed successively with absolute dioxane and ether. This gave 16.99 g (92%) of a product with mp 157-159° C (decomp.). After recrystallization from a mixture of methanol and ether, the melting point had not changed; composition $C_{13}H_{16}O_3N_2 \cdot HCl$.

4-Isobutyl-2-phenyloxazoline-4-carbohydroxamic Acid (VII, R = i-C_4H_9). With stirring at 0° C, a solution of sodium methoxide (50 ml of absolute methanol and 3.02 g of sodium) was added to a solution of 10.6 g (0.0355 mole) of compound (VI, R = i-C_4H_9) and 3.68 g (0.53 mole) of hydroxylamine hydrochloride in 120 ml of absolute methanol. This gave 8.25 g (87.7%) of a substance with mp 147-149° C (from ethanol), $C_{14}H_{18}O_3N_2$.

The Hydrochloride (VIII, R = i-C_4H_9). To a suspension of 11.8 g (0.044 mole) of the hydroxamic acid (VII, R = i-C_4H_9) in 60 ml of absolute dioxane was added 16.7 ml (0.044 mole) of a solution of HCl in dioxane. The reaction mixture was treated in the same way as in the preceding experiments, giving 10.8 g (80%) of a substance with mp 135-139° C; composition $C_{14}H_{18}O_3N_2 \cdot HCl$.

N-Benzoyl-4-isobutylcycloserine (X, R = i-C_4H_9). A suspension of 10.8 g (0.036 mole) of the hydrochloride (VIII, R = i-C_4H_9) in 60 ml of absolute dioxane was heated in the water bath at 100° C for 45 min and was then left at room temperature for several hours. The crystals that deposited proved to be hydroxylamine hydrochloride. The filtrate was evaporated to dryness in vacuum and the oil residue was dissolved in 30 ml of ethanol. The solution was filtered and titrated with 1 N NaOH in the presence of Thymol Blue

indicator until its color changed to blue. Then another 30 ml of 1 N caustic soda was added and the solution was kept at room temperature for 3 h. After this it was acidified with acetic acid to pH 7, a small amount of activated carbon and 150 ml of water were added, and it was filtered. When the filtrate was acidified with HCl to pH 1, a yellow oily substance deposited. The mixture was kept for several hours in the cold and decanted. The oily residue was treated several times with water and dried in a desiccator until it was completely solid. This gave 2.8 g, and the filtrate gave another 0.85 g, making a total of 3.65 g (40%) of a substance which contained 42% of N-benzoyl-4-isobutylcycloserine. This was boiled with 45 ml of ethyl acetate with the addition of a small amount of activated carbon. After the carbon had been removed, the mixture was left in the cold, and the resulting crystals of N-benzoyl-4-isobenzylcycloserine were filtered off, washed first with ethyl acetate and then with petroleum ether, and dried. The yield of white crystals with mp 163-163.5° C, composition $C_{14}H_{18}O_3N_2$, was 1.37 g (15%).

4-Isobutylcycloserine (II, R = i-C_4H_9). A current of dry hydrogen chloride was passed through a suspension of 6.25 g (0.024 mole) of compound X (R = i-C_4H_9) in 150 ml of absolute ethanol to saturation, whereupon the substance dissolved completely. Then the solution was evaporated to dryness and the oily residue was dissolved in 120 ml of absolute ethanol and the solution was likewise saturated with HCl, after which it was left overnight at room temperature and was then heated in the water bath at 80°C for 3 h and was then repeatedly evaporated with ethanol. The oily residue was treated several times with ether and, to eliminate the ether, with benzoic acid. The residual oil was dried in a desiccator to constant weight. A foam-like, very hygroscopic substance was obtained. It was dissolved in 50 ml of absolute ethanol and to the stirred solution was added a solution of sodium ethoxide (1.91 g of sodium and 50 ml of ethanol); the temperature of the reaction mixture rose to 40° C. The mixture was left at room temperature for 3 h and was then evaporated to dryness in vacuum. The residue was dissolved in 20 ml of water, and the solution was treated with a small amount of activated carbon and filtered. The filtrate was acidified with acetic acid to pH 6. Crystals deposited, and after standing in the refrigerator for several hours they were filtered off, washed with water and with ethanol, and were dried. This gave 1.1 g (29.5%) of a substance with mp 175° C; after recrystallization from water, mp 180-185° C, composition $C_7H_{14}O_2N_2$.

SUMMARY

1. A method for the synthesis of 4-isobutylcycloserine (4-amino-4-isobutyl-3-oxazolidone) has been developed.

2. 4-Methyl-, 4-propyl-, and 4-isobutyloxazoline-4-carbohydroxamic acids have been obtained from the corresponding α-alkylserines.

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