HYDROXYLAMINE DERIVATIVES

COMMUNICATION 4*. SYNTHESIS OF CYCLOCANALINE (HOMOCYCLOSERINE) AND RELATED COMPOUNDS**

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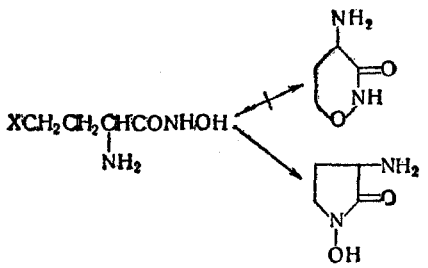
Amongst the most interesting natural hydroxylamine derivatives is the antibiotic cycloserine (CS). Study of the mechanism of action of CS has shown that it is a powerful inhibitor of nitrogen-exchange enzymes, the coferment of which is pyridoxal phosphate [3]. We have shown that the specific biological activity of CS is probably due to its ability to react with pyridoxal enzymes as an acylating agent; the acylating properties of the antibiotic are determined by the conjunction of a hydroxamic ester grouping and an amino group in the α-position in its molecule [4]. On the basis of the relationships that have been established it may be supposed that biological activity will also be possessed by the compound which, like CS, contains a catenation of an α-amino group and a hydroxamic ester grouping, but, unlike CS, contains these not in a five-, but in a six-membered ring. Interest in the synthesis of just this compound arises not only from its similarity to CS, but also from the fact that its structure is based on the amino acid canaline [2-amino-4-(aminooxy)-butyric acid], which shows a multiformity of biological activity. This compound, therefore, can be regarded, on the one hand, as a peculiar six-membered homolog of CS (homocycloserine) or, on the other, as a canaline derivative (cyclocanaline).

After the discovery of the antibiotic CS and the establishment of its structure, several authors made attempts to synthesize cyclocanaline (CC) [4-aminodihydro-2H-1,2-oxazin-3(4H)-one] and its derivatives by the cyclization of the corresponding halo hydroxamic acids. It was found that the reaction resulted in the formation of another heterocyclic system: 1-hydroxy-2-pyrrolidinone [5].



The lack of success was probably associated with the fact that at that time there was no information about the six-membered heterocyclic system dihydro-2H-1,2-oxazin-3(4H)-one, from which cyclocanaline is derived. We therefore investigated possible ways of synthesizing this heterocycle and showed that dihydro-2H-1,2-oxazin-3(4H)-one is formed only by the action of basic agents on 4-(aminooxy)butyric esters. We gave rigorous proof of the structure of the product [2]. On the basis of these results we made an attempt to synthesize CC.

This paper is concerned with the synthesis of CC and some of its derivatives. We first attempted the synthesis of CC as follows:

*For Communications 1, 2, and 3 see [1, 2, 3]. The main themes in this paper were expounded at the Fifth International Biochemistry Congress.

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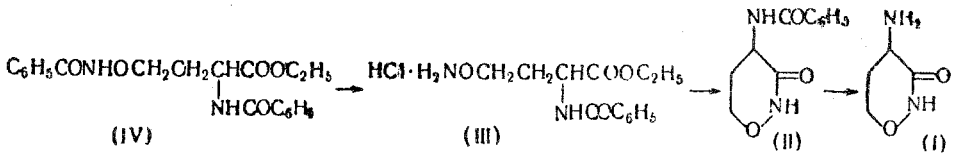
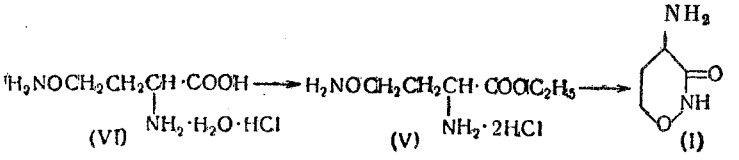


As our main starting substance we took the dibenzoyl derivative of canaline ester [6]. The dibenzoyl ester (IV) was hydrolyzed with alcoholic hydrogen chloride, as a result of which only one benzoyl group was eliminated. The hydrochloride (III) was then treated with various alkaline agents. By cyclization under the action of alkali-metal alkoxides or in the presence of alcoholic alkali we isolated N-benzoylcyclocanaline (II) in high yield. N-Benzoylcyclocanaline is the second dihydro-2H-1,2-oxazin-3(4H)-one discovered and is a stable crystalline substance, soluble in alkali and in most organic solvents; it gives a specific color with sodium nitroprusside; it readily decomposes in an acid medium. However, all our attempts to convert (II) into cyclocanaline were uncussessful, for cleavage of the amide linkage in the ring first occurred and we obtained, depending on the hydrolysis conditions, either (III), or 2-amino-4-(aminooxy)butyric acid. Comparison of the properties of N-benzoylcyclocanaline with those of dihydro-2H-1,2-oxazin-3(4H)-one prepared by us earlier indicated that this new heterocyclic system is very labile. It must be supposed, therefore, that those methods of synthesizing (I) which include the removal of the protective groups at the last stage will probably be unsuitable. Consequently, for the preparation of 4-aminodihydro-2H-1,2-oxazin-3(4H)-one (I) we selected the following scheme;



In this synthesis the main starting compound is canaline. The most convenient syntheses of canaline, which start from γ-butyrolactone, are fairly complicated, multistage, difficultly reproducible processes [6, 7]. This fact, and also the absence of a good method of purifying canaline, made the preparation of any considerable amounts of pure canaline difficult. In view of this we worked out a simple method of preparing canaline from acrolein and showed that the pure amino acid can be obtained in the form of the monohydrochloride [8]. Success in the synthesis of (I) by the above scheme is largely determined by the purity of the canaline used. We showed that only from samples

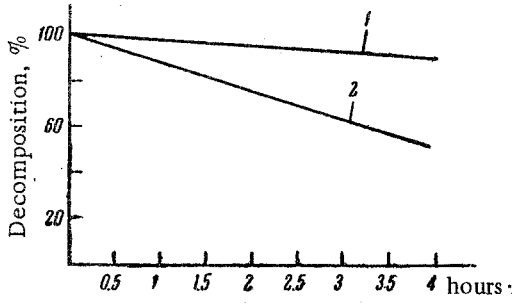


Fig. 1. Rates of decomposition of cycloserine and of cyclocanaline in 0.1 N aqueous solution at pH 6.5: 1) cycloserine; 2) cyclocanaline.

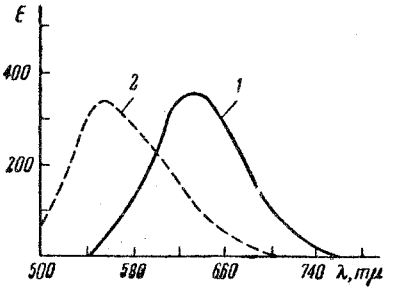


Fig. 2. Absorption spectra of cycloserine (1) and cyclocanaline (2) in presence of aqueous sodium nitroprusside solution.

of analytically pure canaline monohydrochloride can a high yield of crystalline canaline ethyl ester dihydrochloride (V) was observed (chromatography on paper), but we were unable to isolate it in the crystalline state.

We then studied the conditions for the conversion of (V) into (I) under the action of various alkaline agents. It was found that reaction goes in more than one way and depends on the conditions (concentrations of reactants, temperature, solvent) and the nature of the alkaline agent; as well as (I), by-products, probably various canaline peptides, were always formed*. We isolated (I) from the reaction mixture, as the potassium salt or as the oxalate,

*When the present work had been completed, there appeared a communication with no experimental section devoted to attempts to synthesize cyclocanaline derivatives by an analogous scheme [9]. However, the authors were able to isolate only linear canaline peptides.

after the dihydrochloride (V) had been treated with alcoholic alkali for a short time. The appreciable difficulties met in carrying out this stage of the synthesis are probably to be explained by the presence of definite strain in the six-membered ring. As a result, the formation of (I) does not occur so readily with absence of side reactions as in the case of CS. Moreover, comparison of the rates of decomposition of CS and of (I) in aqueous solution at pH 6.5 shows that CC is less stable and decomposes much more rapidly (Fig. 1). These facts probably explain the formation of linear peptides in attempts to prepare CC made by us and other authors.

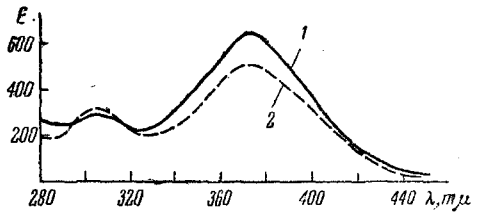
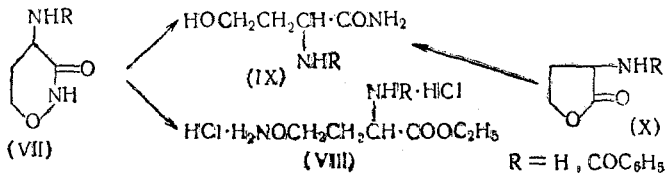


Fig. 3. Ultraviolet spectra of pyridoxal (P) in presence of cycloserine and of cyclocanaline in alcoholic alkali ($3.3 \cdot 10^{-3}$ M NaOH in absolute ethanol): 1) $1 \cdot 10^{-4}$ M P + $1 \cdot 10^{-4}$ M CS; 2) $1 \cdot 10^{-4}$ M P + $1 \cdot 10^{-4}$ M CC.

Cyclocanaline and its derivatives give a specific color reaction with sodium nitroprusside with an absorption maximum at 540 mμ and thus lying at a shorter wavelength than the absorption maximum for CS (Fig. 2). We proved the structures of (I) and of its benzoyl derivative (VII) unequivocally by hydrolysis, hydrogenolysis, molecular weight determinations, and their absorption spectra.



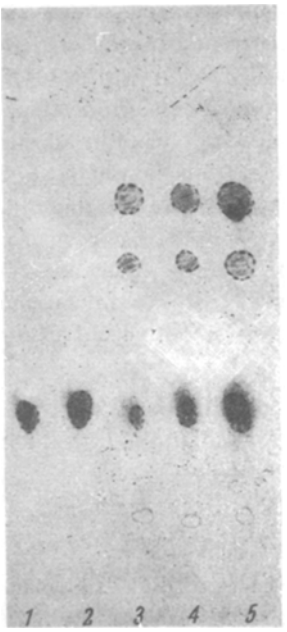


Fig. 4. Reaction of cyclocanaline with pyridoxal: 1, 2) known pyridoxamine; 3, 4, 5) products of the reaction of pyridoxal with cyclocanaline (chromatogram in 4 : 1 : 5 butyl alcohol-acetic acid-water; development with a 0.5% solution of ninhydrin in acetone).

By the acid alcoholic hydrolysis of (VII) we isolated the corresponding ester dihydrochlorides (VIII), and on hydrogenation over platinum oxide we obtained the amides (IX). Confirmatory syntheses of previously undescribed amides of homoserine were carried out by the decyclization of the lactones (X) with liquid ammonia. With a view to checking our theoretical surmise of the presence in (I) of biological activity correlated with the inhibition of pyridoxal enzymes, we studied the interaction of (I) in a model system with pyridoxal, its action on glutamate- aspartate-transaminase, and its influence on the growth of Mycobacterium tuberculosis. It was found that (I), like CS, reacts in an alcoholic medium with pyridoxal with formation of an azomethine derivative, which then, as in the case of CS, can undergo further transformations (Fig. 3). In particular, pyridoxamine was found in the reaction mixture (Fig. 4). When (I) was tested on a highly purified (above 95% pure) preparation of glutamate-aspartate-transaminase, it was shown that it is a powerful inhibitor of the enzyme and is several times as effective as CS [10]. This result is in good accord with the fact that (I) has more strongly marked acylating properties than CS.

Preliminary tests on antituberculosis activity in vitro showed that (I) has a certain bacteriostatic activity toward Mycobacterium tuberculosis (about 50 γ/ml). The low activity of (I), as compared with CS, is probably associated with its more labile character [11].

EXPERIMENTAL

Ethyl 4-(Aminooxy)-2-benzamidobutyrate Hydrochloride (III). A solution of 1.6 g of ethyl 2-benzamido-4-(benzamidooxy)butyrate (IV) in 50 ml of absolute ethanol was saturated with dry hydrogen chloride for one hour and boiled for four hours. The clear solution was vacuum-evaporated, and the residue was ground with dry ether. The yield of the hydrochloride (III) was 1.2 g (92%); m.p. 166-168° (from isopropyl alcohol). Found: C 51.80; H 6.48; N 9.04; Cl 11.90. $C_{13}H_{19}O_4N_2Cl$%. Calculated: C 51.60; H 6.33; N 9.3; Cl 11.75%.

4-Benzamidodihydro-2H-1,2-oxazin-3(4H)-one (N-Benzoylcyclocanaline) (II). 2 g of the ester hydrochloride (III) was dissolved with heating in absolute alcohol, and 13.5 ml of 1.016 N methanolic sodium hydroxide was added. The mixture was stirred for two hours and evaporated to dryness at 20-25°. The residue was suspended in 15 ml of absolute ethanol, alcoholic hydrogen chloride was added to pH 6.5, and the precipitate was filtered off. The precipitate was boiled for 15-20 minutes with dry chloroform, and the solution was filtered and left to crystallize at 0°. We obtained 0.8 g of (II), m.p. 187-189° (from chloroform). From the filtrates we isolated a further 0.3 g of (II). The total yield was 1.1 g (75%). Found: C 59.50; H 5.59; N 12.51%. $C_{11}H_{13}O_3N_2$. Calculated: C 59.96; H 5.49; N 12.72%.

Fig. 5. Hydrogenation of cyclocanaline: 1) cyclocanaline; 2) known amide of homoserine; 3) products of the hydrogenation of cyclocanaline (chromatogram in 4:1:5 butyl alcohol-acetic acid-water; development with a 0.5% solution of ninhydrin in acetone).

Alcoholic Hydrolysis of (II). 0.44 g of (II) was suspended in 2 ml of absolute alcohol, and the suspension was saturated with dry hydrogen chloride for 30-40 minutes. After 20 hours the precipitate was filtered off, washed with ether, and dried. We obtained 0.4 g (85%) of (III), m.p. 166-168° (from isopropyl alcohol). A mixture with a known sample of (III) melted without depression.

N-Benzoylhomoserine Amide (IX; R = COC_6H_5). a) 4 g of α-benzamidobutyrolactone in 25 ml of liquid ammonia was heated in an autoclave for five hours at 60°. Ammonia was removed, and the residue was ground with absolute ethanol. We obtained 3.3 g (80%) of the N-benzoyl amide (IX), m.p. 176-177° (from ethanol). Found: N 12.54%. $C_{11}H_{14}O_3N_2$. Calculated: N 12.62%.

b) 0.22 g of N-benzoylcyclocanaline in absolute ethanol was hydrogenated over platinum oxide. After the absorption of one molecular proportion of hydrogen the hydrogenation ceased. Catalyst was filtered off, and the clear solution was vacuum-evaporated to dryness. From the residue, after grinding it with absolute ethanol and chloroform, we isolated 0.19 g (85%) of crystalline amide (IX; R = COC_6H_5), m.p. 175-176°. A mixture with a known sample of (IX; R = COC_6H_5) melted without depression.

Ethyl 2-Amino-4-(aminooxy)butyrate Dihydrochloride (V). A suspension of 1 g of canaline monohydrochloride (VI) in 10 ml of absolute ethanol was boiled for three hours with passage of a rapid stream of dry hydrogen chloride. After 24 hours the precipitate formed was washed with cold ethanol and with ether and was vacuum-dried over P_2O_5 and over caustic alkali. We obtained 1 g (80%) of (V), m.p. 144-146°. Found: C 30.05; H 7.10; N 11.50; Cl 30.34%. $C_6H_{16}O_3N_2Cl_2$. Calculated: C 30.64; H 6.85; N 11.91; Cl 30.16%.

4-Aminodihydro-2H-1,2-oxazin-3(4H)-one (Cyclocanaline) (I). A mixture of 0.47 g of the ester dihydrochloride (V) and 5 ml of 1.6 N ethanolic KOH was stirred for ten minutes at room temperature and filtered; the precipitate was washed with 2 ml of absolute ethanol, and the clear solution was boiled for ten minutes. Alcoholic hydrogen chloride was added dropwise to the cooled solution of the potassium salt of (I) to bring the pH to 6.2, the precipitate was filtered off, and to the clear solution we added a solution of 0.18 g of oxalic acid in absolute ethanol. The resulting precipitate of the oxalate of (I) was filtered off and washed with ether. We obtained 0.145 g (35%) of the oxalate of (I), m.p. 174-176°; R_f 0.32. Found: C 34.53; H 4.91%. $C_6H_{10}O_6N_2$. Calculated: C 34.96; H 4.85%. The picrate of (I) was prepared from the potassium salt of (I) and picric acid in absolute ethanol; m.p. 152-155°. The molecular weight of the picrate of (I) (determined by the spectrophotometric method in accordance with [12]) was 363.1 (theory requires 345.1).

Hydrogenolysis of (I). 0.012 g of (I) in 40 ml of absolute ethanol was hydrogenated over platinum oxide. To a chromatogram we applied (see Fig. 5) 0.1 ml of the solution before hydrogenation (Point 1) and 0.2 ml of the solution after hydrogenation (Point 3). Point 2 is given by authentic homoserine amide.

Homoserine Amide (IX; R = H). 4.8 g of aminobutyrolacetone hydrobromide was suspended in 50 ml of dry chloroform, and 50 ml of chloroform saturated with ammonia was added with stirring, which was continued further for 20 minutes. The precipitate of ammonium bromide was filtered off, and the filtrate was evaporated quickly in a vacuum at 20-25°. We obtained 2.3 g (87%) of oily α-aminobutyrolacetone. 2.3 g of α-aminobutyrolacetone was added to 20 ml of liquid ammonia and heated in an autoclave for six hours at 65°. Ammonia was removed, and the residue was ground with dry ether and crystallized from absolute alcohol. We obtained 1.2 g (52%) of homoserine amide, m.p. 58-60°; R_f = 0.26. Found: C 40.59; H 8.50; N 22.93%. $C_4H_{10}N_2O_2$. Calculated: C 40.66; H 8.53; N 23.71%.

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SUMMARY

1. 4-Aminodihydro-2H-1,2-oxazin-3(4H)-one (cyclocanaline or homocycloserine) and some of its derivatives were synthesized.

2. Some properties and the biological activity of the compounds obtained were studied.

LITERATURE CITED

1. R. M. Khomutov, Zh. obshch. khimii 31, 1992 (1961).
2. R. M. Khomutov, M. Ya. Karpeiskii, and E. S. Severin, Izv. AN SSSR. Otd. khim. n. 1074 (1962).
3. A. E. Braunshtein, R. M. Azarkhi, and Hsu T'ing-sen, Biokhimiya 882 (1961); R. M. Khomutov, M. Ya. Karpeiskii, E. S. Seberin, and N. V. Gnuchev, Dokl. AN SSSR 140, 492 (1961); O. L. Polyanovskii and Yu. M. Torchinskii, Dokl. AN SSSR 141, 488 (1961).
4. R. M. Khomutov, M. Ya. Karpeiskii, and E. S. Severin, Biokhimiya 26, 772 (1961).
5. J. Smrt, J. Beranek, and M. Horak, Collect. Czechosl. chem. commun., 24, 1672 (1959); N. K. Kochetkov, R. M. Khomutov, E. S. Severin, M. Ya. Karpeiskii, É. I. Budovskii, and V. I. Erashko, Zh. obshch. khimii 29, 3417 (1959).
6. J. Knobler and M. Frankel, J. Chem. Soc. 632 (1958).
7. D. D. Nyberg and B. A. Christensen, J. Amer. Chem. Soc. 79, 1222 (1957).
8. M. Ya. Karpeiskii, R. M. Khomutov, and E. S. Severin, Zh. obshch. khimii 32, 1357 (1962).
9. M. Frankel, I. Knobler, and G. Zvilichowsky, Tetrahedron Letters, No. 18 (1960).
10. M. Ya. Karpeiskii, R. M. Khomutov, O. L. Polyanovskii, E. S. Severin, and Yu. N. Brusov, Biokhimiya, (in the press) (1963).
11. R. M. Khomutov, M. Ya. Karpeiskii, M. A. Breger, and E. S. Severin, Voprosy meditsinskoi khimii 8, 389 (1962).
12. K. G. Cunningham, W. Dawson, and F. S. Spring, J. Chem. Soc. 2305 (1951).
