SYNTHESIS OF SOME HOMOSERINE DERIVATIVES

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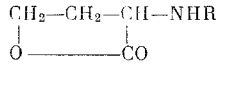
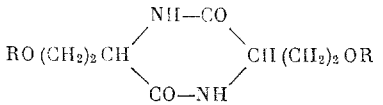
Previously we had described the synthesis of the N-bis(β-chloroethylaminophosphoryl) derivatives of some amino acids (serine, glycine) [1]. We postulated that these compounds, in contrast to the known and effective compound endoxan [2], will be activated in the tumor by phosphamidase, an enzyme that easily cleaves the P–N bond of phosphorylated amino acids [3] and does not activate endoxan [4]. The purpose of the present study was to synthesize the N-bis(β-chloroethylaminophosphoryl) derivatives of homoserine. The intermediate compounds needed for this were prepared first. Thus, the formylation of homoserine with the mixed anhydride of formic and acetic acids gave us, together with the expected α-(formylamino)-γ-butyrolactone (I), also N,O-diformylhomoserine (II) and 3,6-bis(β-formyloxyethyl)-2,5-diketopiperazine (III) (R = CHO). The latter compound is formed from the diketopiperazine of homoserine, present as impurity in the starting homoserine, and can be obtained by the formylation of (III) (R = H).

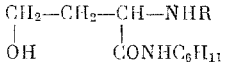


(I) R = CHO, $COOCH_2Ph$



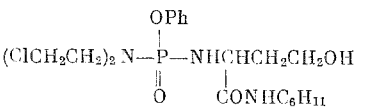
(III) R = H, CHO

When treated with cyclohexylamine, the α-formylamino- and α-carbobenzoxyamino-γ-butyrolactones are cleaved at the O-acyl linkage with the formation of the corresponding cyclohexylamides of homoserine (IV).



(IV) R = H, CHO, $COOCH_2Ph$

When (IV) (R = $COOCH_2Ph$) is treated with thionyl chloride it is converted to the corresponding γ-chlorobutyric acid derivative (V). The catalytic hydrogenation of (IV) (R = $COOCH_2Ph$) or the deformylation of (IV) (R = CHO) gave the cyclohexylamide of homoserine (IV) (R = H). Instead of the cyclic analog of endoxan, reaction of (IV) with bis-(β-chloroethyl)-phosphoramidic dichloride gives a complex mixture of noncrystallizing compounds. However, the reaction of O-phenyl-bis-(β-chloroethyl)phosphoramidic chloride [5] with homoserine cyclohexylamide gave the O-phenyl-N-[(α-cyclohexylamido-γ-hydroxy)propyl]amide of N'-bis(β-chloroethyl)phosphoramidic acid (VI).



(VI)

EXPERIMENTAL

3,6-bis(β-Formyloxyethyl)-2,5-diketopiperazine (III) (R = CHO). 4 g of homoserine was dissolved in 54 ml of mixture A, representing a mixture of 100% formic acid and acetic anhydride in a 3:1 ratio. The solution was heated for 45 min at 45-50°, evaporated in vacuo to dryness, and the residue was treated with a small amount of an ethanol–methanol mixture (1:1) and then allowed to stand in the refrigerator overnight. Yield of (III) (R = CHO) 1 g; m.p. 165-168°. After recrystallization from an ethanol–methanol mixture, and then from methanol, the m.p. was 168-170°. Found %: C 46.41; H 5.58; N 10.85. $C_{10}H_{14}O_6N_2$. Calculated %: C 46.51; H 5.42; N 10.85. The main mother liquor from the separation of (III) (R = CHO) was evaporated in vacuo, and the residue, an oil, was shown to contain α-(formylamino)-γ-butyrolactone (see below).

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A solution of 0.3 g of (III) (R = H) in 4 ml of mixture A was treated in the same manner as described above. We obtained 0.38 g (97.4%) of (III) (R = CHO), m.p. 170° (methanol). The mixed melting point with a sample obtained by the formylation of homoserine was not depressed. (III) (R = CHO) is easily deformylated by heating with a solution of cyclohexylamine in absolute alcohol.

N,O-Diformylhomoserine (II). 5 g of homoserine, from which the diketopiperazine impurity had been removed, was dissolved in 67.5 ml of mixture A. The solution was heated on the water bath for 45 min at 45-50°, and then it was evaporated in vacuo to dryness. The residue, an oil, was treated with a small amount of absolute methanol and placed in the refrigerator. The obtained precipitate was filtered. Yield of (II) 2 g (27.4%), m.p. 130-133.5°. Found %: C 41.38; H 5.24; N 8.04. $C_6H_9NO_5$. Calculated %: C 41.14; H 5.14; N 8.00. The filtrate was evaporated. The residue, an oil, contains α-(formylamino)-γ-butyrolactone (see below). The treatment of (II) with cyclohexylamine in alcohol solution gives the cyclohexylammonium salt; m.p. 101-102° (ethyl acetate). Found %: C 52.54; H 7.99; N 10.20. $C_{12}H_{22}N_2O_5$. Calculated %: C 52.55; H 8.02; N 10.22.

N-Formylhomoserine Cyclohexylamide (V) (R = CHO). The oily residue (3 g) (see above), obtained in the formylation of homoserine, was dissolved in 20 ml of absolute ethanol. To the solution was added 3 g of cyclohexylamine. The reaction mixture was heated up to the boil, after which it was cooled, and then ethyl acetate and petroleum ether were added until a cloudiness appeared. On standing in the refrigerator a crystalline (IV) (R = CHO) was obtained, yield 1.3 g (24.5%), m.p. 158-160°. After recrystallization from absolute ethanol, m.p. 159-160°. Found %: C 57.88; H 8.72; N 12.28. $C_{11}H_{20}N_2O_3$. Calculated %: C 57.89; H 8.77; N 12.29.

N-Carbobenzoxyhomoserine Cyclohexylamide (IV) (R = $OCOCH_2Ph$). To a solution of 2.3 g of (III) (R = $OCOCH_2PH$) in absolute ethanol was added 3 g of cyclohexylamine. The reaction mixture was refluxed for 5 min, and the solution was evaporated in vacuo. Crystalline (IV) (R = $OCOCH_2Ph$) was obtained, m.p. 155-157°, yield 3.35 g (100%). After recrystallization from methanol and ethanol, m.p. 166-167°. Found %: C 64.53; H 7.96; N 8.35. $C_{18}H_{26}N_2O_4$. Calculated %: C 64.67; H 7.78; N 8.08. The compound fails to give a color with ninhydrin, and is insoluble both in HCl (1N and concentrated) and in NaOH. After treatement with NaOH solution, m.p. 164-165°. The mixed melting point with an authentic specimen was not depressed. With cooling (ice bath), 1.67 g of (IV) (R = $OCOCH_2Ph$) was dissolved in 5 ml of thionyl chloride. With stirring, the solution was gradually brought up to room temperature, and then it was heated up to the boil. After cooling, the solution was treated with petroleum ether until cloudiness appeared and then it was placed in the refrigerator for an hour. We obtained 1.7 g (96.6%) of the cyclohexylamide of α-carbobenzoxyamino-γ-chlorobutyric acid with m.p. 152-153°. Found %: C 61.22; H 7.19; N 8.08; Cl 10.29. $C_{18}H_{25}N_2O_3Cl$. Calculated %: C 61.28; H 7.09; N 7.91; Cl 10.07.

Homoserine Cyclohexylamide (IV) (R = H). 17 g of (IV) (R = $OCOCH_2Ph$) was stirred up in 200 ml of methanol. To the suspension was added 1 g of Pt black. The hydrogenation was run until the precipitate dissolved (0.5 h), and then for another 2 h. We obtained 10 g (98%) of (IV) (R = H) with m.p. 148-149°. The compound fails to give a color with ninhydrin. After recrystallization from benzene and ethanol, m.p. 150°. Found %: C 59.80; H 9.99; H 14.22. $C_{10}H_{20}N_2O_2$. Calculated %: C 60.00; H 10.00; N 14.00.

1 g of (IV) (R = CHO) was dissolved in 20 ml of absolute methanol, saturated with HCl. The solution was allowed to stand overnight, after which it was refluxed for 5 min, and then evaporated in vacuo. The oily residue was dissolved in 2 ml of chloroform, and the solution was treated with chloroform, saturated with ammonia at 0°. The ammonium chloride precipitate was filtered. The filtrate was evaporated in vacuo to dryness. We obtained 0.7 g (80%) of (IV) (R = H). After recrystallization from benzene and ethanol, m.p. 147°. The mixed melting point with the compound obtained above was 147°.

The cyclohexylamide of 2-[bis(β-chloroethyl)amino]-2-oxo-4-carboxyl-1,3,2-oxazaphosphorine could not be obtained by the reaction of (IV) (R = H) with N-bis(β-chloroethyl)phosphoramidic dichloride. Instead of the expected cyclic product we obtained a substance with a mol. wt. of approximately 1500. The material was not identified further.

O-Phenyl-N-(α-cyclohexylamido-γ-hydroxypropyl)amide of N'-bis(β-chloroethyl)phosphoramidic Acid (VI). With stirring, a mixture of 1 g of (IV) (R = H) and 0.5 g of triethylamine in 20 ml of chloroform was added dropwise in 1 h to a solution of 1.58 g of O-phenyl-bis-(β-chloroethyl)phosphoramidic chloride (the temperature of the reaction mixture did not exceed 20°). The solution was refluxed for 5 min, and then it was evaporated in vacuo to dryness. The oily residue crystallized

on standing. The crystalline deposit was washed with ether, then with water, and dried. Yield of (VI) 1.4 g (77%). After recrystallization from ethyl acetate and alcohol, m.p. 152-152.5°. Found %: 50.38; H 6.70; N 8.73; Cl 14.60. $C_{20}H_{33}N_3O_4Cl_2P$. Calculated %: C 50.00; H 6.71; N 8.74; Cl 14.77.

CONCLUSIONS

1. Some new homoserine derivatives, substituted on the hydroxy, amino and carboxyl groups, were obtained.

2. The N-phosphorylation of homoserine cyclohexylamide was accomplished using O-phenyl-N-bis(β-chloroethyl)phosphoramidic chloride.

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