SYNTHESIS AND HYDROLYSIS OF p-[DI-(2-CHLOROPROPYL)AMINO]-DL-PHENYLALANINE

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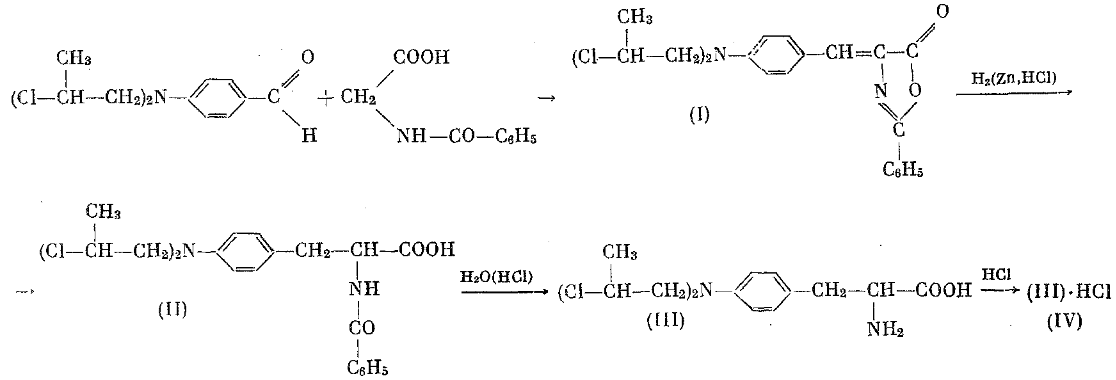
In a search for antitumor compounds capable of being used for perfusion, we undertook the synthesis of p-[di-(2-chloropropyl)amino]-DL-phenylalanine and a study of the hydrolysis rate of the chlorine atom. In order to obtain this compound we resorted to one of the methods used to synthesize sarcolysin [1]. The condensation of p-[di-(2-chloropropyl)amino]benzaldehyde [2] with hippuric acid gave p-[di-(2-chloropropyl)amino]benzylidenephenyloxazolone (I). The reduction of oxazolone (I) with zinc dust in a mixture of hydrochloric and acetic acids, with the simultaneous hydrolysis of the intermediate saturated oxazolone, gave N-benzoyl-p-[di-(2-chloropropyl)amino]-DL-phenylanaline (II)



The hydrolysis of compound (II) with hydrochloric acid leads to the formation of p-[di-(2-chloropropyl)amino]-DL-phenylalanine (III), which was isolated as the free base from the reaction mixture by treatment with saturated sodium acetate solution. Compound (III) was converted to the water-soluble hydrochloride (IV) by the addition of the calculated amount of HCl in ethanol.

A comparative study of the hydrolysis rate of the chlorine atom in the synthesized compounds (I), (II), and (IV), and also in sarcolysin, by the conductometric method in aqueous dioxane at 60° disclosed that the hydrolysis rate of the hydrochloride (IV) is one order of magnitude higher than in the case of sarcolysin; at the same time, oxazolone (I) in general does not undergo hydrolysis under the given conditions. The overall hydrolysis constant $K \cdot 10^3$, min^{-1}, is 45 for (II), 11.6 for (IV), and 1.15 for sarcolysin.

EXPERIMENTAL METHOD

The melting points were determined on a Kofler microheating block. The hydrolysis of the C–Cl bond in compounds (I), (II), and (IV), and in sarcolysin, was studied by the conductometric method in a thermostatted hermetically sealed glass cell, equipped with Pt electrodes, in aqueous dioxane medium (5 : 6, by volume), at 60 ± 0.1°, and a concentration of 0.8 mM for the investigated solutions. The kinetic curves were recorded automatically. The reproducibility of the curves was ±1.5%. The hydrolysis curve plotted in semilogarithmic coordinates was a straight line. The analysis of the reaction mixtures and of the

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obtained hydrolysis products, and the hydrolysis mechanism in the case of the aromatic di-(2-chloroalkyl)-amines, are described in [3]. The overall hydrolysis constants of the C–Cl bond were calculated from the kinetic curves by the modified Guggenheim method [4] for first-order reactions. In the hydrolysis of compounds (I), (II), (IV), and sarcolysin the reaction was run up to 80% conversion of the starting compounds. The arithmetical mean error in calculating the overall hydrolysis rate constants was ±7%.

p-[Di-(2-chloropropyl)amino]benzylidenephenyloxazolone (I). A stirred mixture of 2.74 g of p-[di-(2-chloropropyl)amino]benzaldehyde, 1.79 g of hippuric acid, 0.82 g of well-ground anhydrous $NaOOCCH_3$, and 3 ml of $(CH_3CO)_2O$ was heated up to 120° and, after solution was achieved, for 25 min on the steam bath. Then the solution was cooled and 10 ml of water was added. The obtained oil was extracted with benzene, dried over $MgSO_4$, and poured into petroleum ether. The obtained orange precipitate was filtered and recrystallized from ethanol. We obtained 2.16 g (52%) of (I) with mp 153-155°. Found: N 6.55; Cl 16.79%. $C_{22}H_{22}N_2O_2Cl_2$. Calculated: N 6.71; Cl 17.00%.

N-Benzoyl-p-[di-(2-chloropropyl)amino]-DL-phenylalanine (II). With stirring and gentle heating, 2 g of (I) was dissolved in 10 ml of glacial CH_3COOH and 15 ml of conc. HCl. With cooling, 4 g of Zn dust was added to the solution in small portions, and the mixture was stirred until the color disappeared. The residual Zn dust was filtered, while the filtrate was gradually poured, with stirring, into 200 ml of distilled water. The obtained precipitate was filtered, washed with water, and purified by reprecipitation from ethyl acetate solution with petroleum ether. We obtained 1.32 g (66%) of (II) with mp 147-149°. Found: N 6.20; Cl 16.03%. $C_{22}H_{26}N_2O_3Cl$. Calculated: N 6.40; Cl 16.21%.

p-[Di-(2-chloropropyl)amino]-DL-phenylalanine (III). A mixture of 1 g of (II) and 20 ml of conc. HCl was refluxed for 2 h. The solution was cooled and the precipitated benzoic acid was filtered. The filtrate was diluted in half with water, heated with active carbon, filtered, and to the cooled solution, with stirring, anhydrous $NaOOCCH_3$ was gradually added to pH 5. The obtained white precipitate was filtered and recrystallized from ethanol. We obtained 0.38 g (50%) of (III) with mp 165-167° (decomp.). Found: N 8.67; Cl 21.10%. $C_{15}H_{22}N_2O_2Cl_2$. Calculated: N 8.40; Cl 21.27%.

p-[Di-(2-chloropropyl)amino]-DL-phenylalanine Hydrochloride (IV). Compound (III) (1.4 g) was dissolved in 15 ml of absolute ethanol that contained 0.14 g of HCl. The solution was kept at room temperature for 30 min, after which 30 ml of absolute ether was added, and the mixture was cooled. The obtained white precipitate was filtered and recrystallized from a mixture of acetone and absolute ether. We obtained 1 g (65%) of (IV) with mp 110° (decomp.). Found: N 7.39; Cl 28.69%. $C_{15}H_{23}N_2O_2Cl_3$. Calculated: N 7.57; Cl 28.76%.

CONCLUSIONS

We synthesized p-[di-(2-chloropropyl)amino]-DL-phenylalanine and studied the hydrolysis rate of the chlorine atom in the cytotoxic group.

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