SYNTHESIS OF A SUGGESTED AGGRESSION PEPTIDE

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Four different schemes for the synthesis by the classical method of pyroglutamylasparaginylglycine – a suggested aggression peptide have been described. The best is a scheme using C- and N-terminal protective groups of the benzyl type at the stage of obtaining the protected tripeptide. Literature information according to which the compound possesses biological activity has been refuted.

Reichelt et al. [1] have reported the isolation from biological fluids, the purification, and the determination of the structure of the tripeptide pyroglutamylasparaginylglycine (here and below, the amino acids, apart from glycine, are of the L-series), which is capable on subcutaneous injection of causing aggressive behavior in mice. In the same paper, it was reported that a synthetic tripeptide of the same structure possessed an identical biological action, but no information on its synthesis is given either in Reichelt's book [1] or in subsequent publications.

Within the framework of a search for factors of peptide nature causing resistance and predisposition to stress reactions, we have synthesized this tripeptide by the methods of classical peptide synthesis in solution and have performed trials of its biological activity. We used several schemes to synthesize the desired compound: 1) combining the sodium salt of asparaginylglycine with the trichloro-, pentachloro-, or pentafluorophenyl esters of N-benzyloxycarbonylpyroglutamic acid; 2) condensing the sodium salt of asparaginylglycine with pentafluorophenyl pyroglutamate; 3) coupling the methyl ester of asparaginylglycine with trichlorophenyl N-benzyloxycarbonylpyroglutamate followed by the simultaneous elimination of the protective groups by the action of hydrogen bromide in acetic acid with the addition of water; and 4) condensing the benzyl ester of asparaginylglycine with pentafluorophenyl N-benzyloxycarbonylpyroglutamate.

The first three variants did not give the desired results: in the first two cases the high solubility of the product in water greatly complicated the isolation and purification of the desired compound; in the third case, the simultaneous deblockage of the protected tripeptide led to the formation of a complex mixture of compounds, apparently through the acidolytic cleavage of the pyroglutamic acid ring [2] and the formation of aminosuccinyl derivatives from the asparagine residue in the AsnGly section [3].

The most successful proved to be the fourth scheme, using benzyl protection at the C-terminal group of the peptide. The asparagine residue was introduced in the form of the p-nitrophenyl ester of N-tert-butoxycarbonylasparagine. The benzyloxycarbonyl group of the protected pyroglutamic acid was eliminated simultaneously with the C-terminal benzyl group by catalytic hydrogenolysis.

Biological activity was tested on random-bred mice and rats in the V. P. Serbskii All-Union Scientific Research Institute of General and Forensic Psychiatry. On subcutaneous injection in doses of 0.5-1 mg/kg, on injection into the medial and lateral ventricles of the brain in doses of 0.1 mg in 25 μl and 0.25 mg in 50 ml of Jenck's solution, respectively and on injection into the heart in a dose of 0.2 mg in a volume of 200 μl it was observed that the synthetic peptide did not produce aggressive behavior in mice and rats and did not cause the appearance of the phenomenon of muricide in rats. A structural analog of this tripeptide – prolylasparaginylglycine, which we synthesized by coupling the sodium salt of asparaginylglycine with the p-nitrophenyl ester of N-benzyloxycarbonylproline, followed by catalytic hydrogenolysis, did not cause the appearance of aggressiveness in rats, either.

It must be mentioned that a report [4] on the isolation and purification of the anorexogenic peptide pyroglutamylhistidylglycine was not confirmed in the aspect of biological activity [5].

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EXPERIMENTAL

The individuality of the compounds obtained was checked with the aid of TLC on Silufol 25 × 50 mm plates in the following systems: 1) benzene–acetone (1:1); 2) benzene–acetone (1:2); 3) butan-1-ol–acetic acid–water (3:1:1); 4) acetone–water (1:1); 5) butan-1-ol–acetic acid–water (4:1:5), lower phase. The individuality of the deblocked compound was confirmed by electrophoresis on Whatman FN 12 paper [sic] in 2% acetic acid at a working voltage of 1200 V. Specific rotations were measured in a 1 dm tube on a single Winkel-Zeiss polarimeter. The results of C,H,N-analysis of all the compounds corresponded to the calculated figures.

Benzyl Ester of N-tert-Butoxycarbonylasparaginylglycine. Yield 88%, R_f 0.75 (system 1); $[\alpha]_D^{20}$ − 18° (c 1; dimethylformamide); after deblocking, E_{Gly} 1.30.

Benzyl Ester of N-Benzyloxycarbonylpyroglutamylasparaginylglycine. Yield 55%, R_f 0.20 (system 1); 0.50 (system 2); 0.95 (system 3); $[\alpha]_D^{20}$ − 29° (c 1; dimethylformamide).

Pyroglutamylasparaginylglycine. Yield 94%; R_f 0.10 (system 3); 0.90 (system 4); 0.90 (system 5); $[\alpha]_D^{20}$ − 85° (c 1; water), E_{Gly} 0.50.

SUMMARY

Pyroglutamylasparaginylglycine has been synthesized by the methods of classical peptide synthesis in solution. Contrary to statements in the literature, the compound did not cause aggressive behavior in mice and rats.

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