SYNTHESIS AND UTEROTONIC ACTIVITY OF [4-METHIONINE]OXYTOCIN

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Among the analogs of the hypophyseal hormone oxytocin modified in position 4 there are many compounds possessing a high physiological activity [1]. We have synthesized an analog in which the glutamine residue in position 4 has been replaced by the more hydrophobic methionine residue. The uterotonic activity of our preparation measured on the isolated rat uterus amounted to 17.5 ± 4 units/mg (mean of seven separate experiments), which is almost 30 times less than the activity of oxytocin and practically coincides with the activity of the isosteric analog [4-norleucine]oxytocin (20.5 ± 0.5 units/ mg).

The protected nonapeptide was synthesized by the condensation of benzyloxycarbonyl-S-benzylcysteinyltyrosylisoleucine with the amide of methionylasparaginyl-S-benzylcysteinylprolylleucylglycine by the carbodiimide method in the presence of N-hydroxysuccinimide at −10°C. After elimination of the protective groups with anhydrous hydrogen fluoride in the presence of anisole, closure of the disulfide bond was performed by oxidation with atmospheric oxygen. For purification, the freeze-dried oxidation product was subjected to two gel filtrations on a column of Sephadex G-15 (90 × 1.5 cm) using 25% acetic acid as the eluent in the first separation and the upper phase of the butan-1-ol−n-propanol−3.5% acetic acid system in the second separation, the column being saturated with the lower phase of the same system. The peaks in gel filtration were identified from the absorption of the eluate at 280 nm. Individual [4-methionine]-oxytocin was obtained by freeze-drying the second peak. According to elementary analysis, the freeze-dried product also contained acetic acid and mineral salts. The amount of the main substance was 71%.

The sample for amino-acid analysis was hydrolyzed with 6 N hydrochloric acid in the presence of thioglycolic acid [2] to prevent the oxidation of methionine and tyrosine. Under these conditions a considerably higher amount of proline was found [2], and cysteine was not determined (an intense peak of the mixed disulfide of cysteine and thioglycolic acid, issuing before the aspartic acid, appeared). The result of the amino acid analysis was Asp 1.01 (0.95), Pro 1.62 (1.04), Gly 1.00 (1.00), Met 0.85 (0.73), He 0.93 (0.76), Leu 0.99 (0.95), Tyr 0.93 (0.46) (performed by A. O. Smirnov on an AAA 881 automatic analyzer; the figures in parentheses are those obtained on hydrolysis in the absence of thioglycolic acid).

LITERATURE CITED

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