SYNTHESIS OF NEW ANALOGS OF OXYTOCIN MODIFIED SIMULTANEOUSLY IN POSITIONS 2 AND 4

O. A. Kaurov, V. F. Martynov, and E. I. Grigor'ev

UDC 547.466.1+547.583.4+541.69

Earlier, on the basis of a study of the structure-activity interrelationship in a number of analogs of the neurohypophysary hormone oxytocin the hypothesis was put forward that in the stimulation of uterotonic activity the side chain of tyrosine leaves its original localization in the receptor [1]. Developing this idea further, it may be suggested that as a result a conformation corresponding to Walter's "biologically active" conformation is formed [2]. According to Walter, in such a conformation the side chain of the tyrosine is turned back into the cyclic part of the molecule. The conformation may be stabilized through the interaction of the phenolic hydroxyl of tyrosine with the amide grouping of glutamine. In favor of the hypothesis put forward are the results of biological tests of the [2-Phe(NH_2), 4-Glu]oxytocin (T) that we have synthesized, in which the "active" conformation can be stabilized through an electrostatic interaction between the p-aminophenylalanine (Phe(NH_2)) and the glutamic acid.

In contrast to other analogs modified in positions 2 and 4, in which the formation of an "active" conformation is less likely, compound (I) possesses a greater uterotonic activity than that predicted by the rule of additivity [3] on the basis of biological tests of the corresponding analogs modified in only one of the positions under consideration. Thus, the uterotonic activity of compound (I) is 1.1 U/mg as compared with a predicted 0.1 U/mg, while for [2-Ile, 4-Ile]oxytocin the figures are 0.53 U/mg [4] and 1.6, and for [2-Phe-4-Abu]oxytocin they are 1.6 [5] and 5.33, respectively. The fact that the uterotonic activity of the other analog that we have synthesized, [2-Phe(NH_2), 4-Glu]deaminooxytocin (IV) is lower than that of compound (I) indicates that the other conformation suggested for oxytocin with the tyrosine turned back in the direction of the amino group of the cysteine [6] can hardly be "biologically active". In compound (I), unlike (IV), the appearance of such a conformation is difficult because of the possible electrostatic repulsion between the aromatic p-amino group of the aminophenylalanine and the α-amino group of the cysteine (compound (IV) possesses a uterotonic activity equivalent to 0.025 U/mg).

The analogs (I) and (IV) were synthesized by the successive addition of the p-nitrophenyl esters of benzyloxycarbonylamino acids, beginning from the C end. The C end of the pentapeptide was synthesized by literature methods [7]. In the process of synthesis, the following new compounds were obtained: Z-Glu(OBu^t)-Asn-Cys-(Bzl)-Pro-Leu-GlyNH_2, yield 76%, mp 198-199°C, $[\alpha]_D^{20}$ −76.0° (c 1.0; DMF); Z-Ile-Gly-Asn-Cys(Bzl)-Pro-Leu-GlyNH_2, yield 87%, mp 194-196°C, $[\alpha]_D^{20}$ −55.0° (c 1.0; DMF); Z-Phe(NO_2)-Ile-Glu-Asn-Cys(Bzl)-Pro-Leu-GlyNH_2, yield 87%, mp 206-208°C, $[\alpha]_D^{20}$ −40.0° (c 1.0; DMF); Z-Cys(Bzl)-Phe(NO_2)-Ile-Glu-Asn-Cys(Bzl)-Pro-Leu-GlyNH_2 (V), yield 71%, mp 210-211°C $[\alpha]_D^{20}$ −46.0° (c 1.0; DMF); and β-Mpr(Bzl)-Phe(NO_2)-Ile-Glu-Asn-Cys(Bzl)-Pro-Leu-GlyNH_2 (VI), yield 93%, mp 220-221°, $[\alpha]_D^{20}$ −31.0° (c 1.0; DMF).

The elementary analyses of all the compounds corresponded to the calculated figures. The nonapeptides (V) and (VI) were treated with sodium in liquid ammonia. The products obtained were oxidized by atmospheric air and purified by gel chromatography on Sephadex G-15. In the subsequent stages of purification we used the following eluents: 50% acetic acid; butan-1-ol−n-propanol−3.5% acetic acid (2:1:3); and 2 N acetic acid. Products were collected which showed a positive reaction for an aromatic amino group [8]. The purified preparations were characterized by amino-acid analyses and UV and IR spectra.

A. A. Zhdanov Leningrad State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 433-434, May-June, 1977. Original article submitted February 1, 1977.

LITERATURE CITED

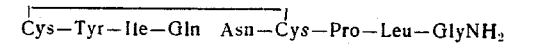
1. O. Kaurov, V. F. Martynov, Ju. D. Michaylov, O. A. Popernaczky, and M. P. Smirnova, Peptides 1972, Elsevier, Amsterdam–New York (1973), p. 450.
2. R. Walter, Structure-Activity Relationship of Protein and Polypeptide Hormones, New York (1971), p. 181.
3. J. Rudinger, in: Drug Design (ed. F. J. Ariens), Academic Press, New York, Vol. 2 (1971), p. 319.
4. V. G. Hruby, V. du Vigneaud, and W. J. Chan, J. Med. Chem., 13, 185 (1970).
5. V. du Vigneaud, J. Biol. Chem., 241, 4051 (1966).
6. J. Fric, M. Kodicek, K. Yost, and K. Blaha, Peptides 1972, Elsevier, Amsterdam-New York (1973), p. 318.
7. O. A. Kaurov, V. F. Martynov, and V. B. Morozov, Zh. Obshch. Khim., 40, 908 (1970).
8. S. Veibel, Identification of Organic Compounds, G. E. C. Gad, Copenhagen (1954).

SYNTHESIS OF A CYCLIC TRIPEPTIDE MODELLING THE "ACTIVE" CONFORMATION OF OXYTOCIN

E. I. Grigor'ev, E. A. Krol', and O. A. Kaurov

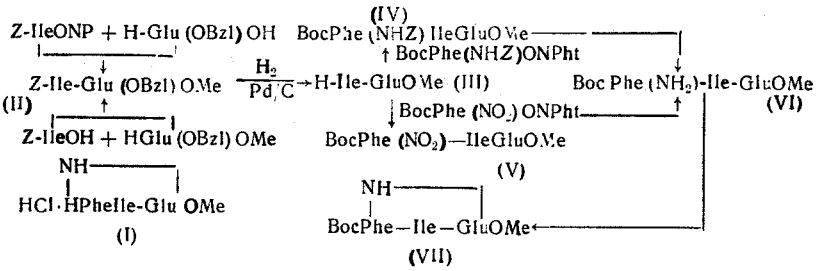
UDC 547.466.1+547.583.4

The opinion exists that in the stimulation of biological activity, the tyrosine residue leaves its original localization in the receptor, forming a "pseudobicyclic" structure through the formation of hydrogen bonds between its phenolic hydroxyl and the amide grouping of asperagine [1]:



Oxytocin

It appeared of interest to synthesize a cyclic tripeptide modelling the "pseudo-ring" of the active conformation of the hormone molecule and to study its biological activity. With this aim, we have synthesized the cyclic tripeptide (I), which proved to be inactive on testing on the rat uterus in vitro at concentrations of up to 1.0 mg/ml. The peptide (I) was synthesized in the following way:



The intermediate dipeptide (II) was obtained by the p-nitrophenyl ester method followed by treatment with diazomethane (yield 54%) and by the carbodiimide method starting from the diester of glutamic acid (yield 65%). The melting point of compound (II) was 123–124°C, $[\alpha]_D^{20}$ −16.0° (c 1.0; methanol). The initial tripeptide for cyclization (VI) was also obtained by two methods: by the hydrogenation of compounds (V) and (IV). Compounds (IV) and (V) were obtained from compound (III) and the hydroxyphthalimide esters of the corresponding substituted amino acids [tripeptide (IV) – yield 55%, mp 151–152°C, $[\alpha]_D^{20}$ −21.0° (c 1.0; methanol); tripeptide (V) – yield 73%, mp 145–147°C, $[\alpha]_D^{20}$ −26.0° (c 1.0; methanol)]. Both active esters were synthesized by the carbodiimide method: Boc-Phe(NHZ)-ONPht, mp 160–161°C, $[\alpha]_D^{20}$ −44.0° (c 1.0; ethyl acetate). Compound (VI) was subjected to cyclization immediately after its preparation.

A. A. Zhdanov Leningrad State University.

Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 434–435, May-June, 1977. Original article submitted February 16, 1977.