AMINO ACIDS AND PEPTIDES. CI.*

THE PREPARATION OF N^{α}-ACETYL-2-O-METHYLTYROSINE-OXYTOCIN, A POWERFUL INHIBITOR OF THE UTEROTONIC ACTIVITY OF OXYTOCIN

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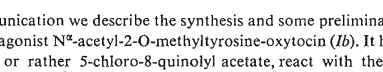
Received February 20th, 1970

Substances with an inhibitory action on the typical biological activities of the neurohypophysial hormones oxytocin and vasopressin belong to a variety of structural types[1]. Of particular interest are those antagonists which are derived from the maternal hormones structurally. Of a large number of synthetic analogues of these hormones, only a very few antagonise the action of oxytocin *in vivo*. The structural modifications of these antagonists involve changes at the amino end of the molecule: hemicysteine** in sequence position 1 is replaced by a penicillamine residue (L or D) or by a β-mercaptoisovaleric acid residue[3,4]; the second case is N-carbamyl-2-O-methyltyrosine-oxytocin[5].

In the present communication we describe the synthesis and some preliminary pharmacological data on an oxytocin antagonist N^{α}-acetyl-2-O-methyltyrosine-oxytocin (*Ib*). It has been determined that acetic anhydride, or rather 5-chloro-8-quinolyl acetate, react with the free amino group of 2-O-methyltyrosine-oxytocin[6] (*Ia*) under conditions described under Experimental, with no significant degree of transsulphidation reaction. A small part of the unreacted starting compound *Ia* was removed by chromatography on CM-Sephadex in pyridine–acetate buffer and a very small amount of dimerised product was removed by filtration on Biogel P-4.



Ia, X = H
Ib, X = CH_3CO

According to preliminary pharmacological tests carried out by V. Pliška of this Institute and I. Krejčí of the Research Institute for Pharmacy and Biochemistry, Prague, compound *Ib* has no intrinsic uterotonic activity in standard *in vitro* tests. At concentrations 15—30× greater than those of oxytocin it acted as an antagonist, the probability being for a competitive type of inhibition. In *in vivo* tests (oestrus or pro-oestrus) *Ib* inhibited the contractile activity of the uterus; there was a 50% decrease in oxytocin activity with an antagonist: oxytocin ratio of 7—12, complete inhibition was seen with ratios of 15—20. On the isolated rat mammary gland analogue *Ib* had a weak, oxytocin-like activity with no signs of inhibition, and a very weak inhibitory effect on the antidiuretic action of lysine-vasopressin. A detailed analysis of the pharmacological properties of this analogue will be published separately by these investigators.

* Part C: This Journal *36*, 234 (1971).

** All amino acids in this work were L-configuration. Terminology and symbols for hormone analogues are according to published suggestions[2].

EXPERIMENTAL

N^{α}-Acetyl-2-O-methyltyrosine-oxytocin

a) *Using 5-chloro-8-quinolyl acetate*: To a solution of 158·5 mg of 2-O-methyltyrosine-oxytocin[6] in 1 ml dimethylformamide we added 104 mg 5-chloro-8-quinolyl acetate[7]. After 60 h at 0°C the reaction mixture was diluted with 20 ml ether and the product which separated out was filtered and washed on the filtre with ether. The yield was 133·3 mg. After dissolving in 2 ml of the following buffer: water–pyridine–acetic acid (90 : 10 : 0·295, pH 6·5) the solution was placed on a column (40 × 1·4 cm; 100—270 mesh; 4·5 m Equiv/g) of CM-Sephadex. The peak corresponding to acylated peptide (localised by optical density at 280 nm) was freeze-dried and the dry powder was dissolved in 1M acetic acid and filtered through a column (100 × 1 cm in 1M acetic acid) of Biogel P-4. The peptide material corresponding to the monomer was again isolated by freeze-drying. The yield was 100 mg (63%); $[\alpha]_D$ −104·7° (*c* 0·23, 1M acetic acid). R_F 0·55 (1-butanol–acetic acid–water 4 : 1 : 1), 0·75 (1-butanol–pyridine–acetic acid–water 15 : 10 : 3 : 6), 0·38 (2-butanol–90% formic acid–water, 75 : 13·5 : 11·5). 2-O-methyloxytocin under the same conditions showed R_F 0·15, 0·75, 0·05. The amino acid analysis (20 h hydrolysis in 6M-HCl): Asp 1·00, Cys 1·1, Glu 1·00, Gly 0·96, Ile 0·92, Leu 1·00, Pro 0·92, Tyr 0·68, Tyr(Me) 0·28; Tyr + Tyr(Me) 0·96.

The sample for elemental analysis was dried 24 h at room temperature *in vacuo* of 1 mm Hg over P_2O_5; the loss with drying was 3·6%. For $C_{46}H_{70}N_{12}O_{13}S_2.2\,H_2O$ (1099·2) calculated: 50·27% C, 6·42% H, 15·29% N; found: 50·38% C, 6·87% H, 14·75% N (calculated on 1% ash content).

b) *Using acetic anhydride*. To a solution of 20 mg of 2-O-methyltyrosine-oxytocin in 0·1 ml dimethylformamide we added 6 mg acetic anhydride. After 60 h at 0°C the reaction mixture was diluted with 10 ml ether, the separated product filtered and washed on the filtre with ether, and then purified as in *a*) above. The yield was 10 mg (50%) of a product with identical properties as those of product *a*) above.

Our thanks are due to Professor J. Rudinger for stimulating discussion. Mr P. Moritz and Miss J. Koutníková rendered valuable technical assistance. Amino-acid analyses were carried out by Mr J. Zbrožek, elemental analyses by the staff of the Analytical Laboratory and optical rotations were measured by Miss J. Šafaříková.

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Translated by J. Cort.