EFFECT OF METHYLATION OF THE HYDROXYL GROUP OF TYROSINE IN [1-β-MERCAPTOPROPIONIC ACID, 8-D-ARGININE]-VASOPRESSIN ON ITS BIOLOGICAL EFFECTS*

Yiktor KRCHŇÁK and Milan ZAORAL

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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Using the method of solid-phase synthesis, S-benzyl-β-mercaptopropionyl-O-methyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^G-p-toluenesulfonyl-D-arginyl-glycine amide (*I*) was prepared which after removal of the protecting groups, oxidation, and purification afforded [1-β-mercaptopropionic acid, 2-O-methyltyrosine, 8-D-arginine]-vasopressin (*II*). *II* shows a low antidiuretic effect, c. 10 I.U./mg. It is without effect on rat uterus *in vitro* and on the blood pressure of rat *in vitro*.

The replacement of glutamine in position 4 of lysine-vasopressin by asparagine increases the specificity of the antidiuretic effect[1]. If this replacement is carried out in the molecule of [Mpr[1], D-Arg[8]]vasopressin (DDAVP)** a compound showing a high and practically pure antidiuretic effect is obtained[2]. The methylation of the hydroxyl group of the tyrosine residue of lysine-vasopressin also increases the specificity of the antidiuretic effect[3-6] as judged by the test on rat. We considered interesting to investigate whether the methylation of the tyrosine hydroxyl in DDAVP will have an effect similar to that observed with lysine-vasopressin or resulting from the introduction of asparagine into position 4 of DDAVP, *i.e.* whether the specificity of the antidiuretic effect will increase without a drastic decrease of its magnitude. To provide an answer to this question we synthetized [Mpr[1], Tyr(Me)[2], D-Arg[8]]-vasopressin. The synthesis of *II* was carried out in solid phase following a scheme developed earlier[7]. We employed the usual combination of protecting groups: the benzyl residue to protect sulfur, the *p*-toluenesulfonyl residue to protect the guanidine group, and the tert-butyloxycarbonyl residue to protect the α-amino groups. The peptide was split off from the resin by ammonolysis. The crude reaction product was purified by crystallization from aqueous acetic acid. The free peptide was obtained by re-

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** The abbreviations and symbols common in peptide chemistry are used. Mpr = β-mercaptopropionic acid. Unless stated otherwise the optically active amino acids are of L-configuration.

moving the protecting groups by sodium in liquid ammonia, oxidative closure of the disulfide ring by potassium ferricyanide, desalting of the product on a column of a carboxyl cation exchanger, and purification by continuous free-flow electrophoresis. The antidiuretic, pressor, and uterotonic effect were determined by conventional methods[8-12]. When tested for its uterotonic effect the analog was inactive up to a dose of $5 . 10^{-2}$ mg, when tested for its pressor effect up to a dose of $5 . 10^{-2}$ mg. The antidiuretic effect of *II* was determined with respect to a standard batch of DDAVP. We found that the antidiuretic effect of *II* corresponds to 0·021% of the antidiuretic effect of the standard, *i.e.* to c. 10 I.U./mg. Hence *II* has a pure yet low antidiuretic effect. Whereas the O-methylation of the tyrosine residue in lysine vasopressin decreases its antidiuretic effect to about 1/3 (see[3]), the same structural alteration of DDAVP results in a decrease of its antidiuretic effect by three orders. This result is in accordance with our previous experience from which the amino-, deamino-, L-, D-series *etc.* should be regarded as representing individual groups of analogs in which a structural change of the same type can exert an entirely different influence on biological effects.

EXPERIMENTAL

All the general experimental details including information on the instruments used for the measurement and purification have been reported earlier[13].

t-Butyloxycarbonyl-O-methyltyrosine

The tert-butyloxycarbonylation of O-methyltyrosine[14] was carried out according to Grzonka and Lammek[15] the reaction time being 24 h. The oily product was converted into the dicyclohexylammonium salt. The yield was 83%; after recrystallization of the sample for analysis from ethyl acetate the m.p. was 153—155°C, $[\alpha]_D^{20}$ +34·4 (*c* 0·5, methanol). For $C_{27}H_{44}N_2O_5$ (476·7) calculated: 68·04% C, 9·30% H, 5·88% N; found: 67·99% C, 8·71% H, 5·61% N.

S-Benzyl-β-mercaptopropionyl-O-methyltyrosyl-phenylalanyl-glutaminyl-asparaginyl--S-benzylcysteinyl-prolyl-N^G-*p*-toluenesulfonyl-D-arginyl-glycine Amide (*I*)

The synthesis was effected on the chloromethylated polystyrene resin cross-linked by 2% of divinylbenzene (Calbiochem, Los Angeles, U.S.A., chlorine content 5·9%). The resin was esterified by Boc-Gly and contained 0·86 mmol of Gly/g of resin. The synthesis was carried out in a manually operated synthetizer according to the program described in the preceding communication[7] (program No 6). The resin (1·3 g) afforded after ammonolysis[7] 1·26 g (80%) of the crude reaction product, m.p. 201—214°C. The yield of compound *I* after recrystallization from a mixture of acetic acid and water was 0·85 g (54%), m.p. 215—218°C; $[\alpha]_D^{20}$ −18·4° (*c* 0·5, dimethylformamide). Amino acid analysis: Tyr 0·9, Phe 0·91, Glu 0·99, Asp 1·05, Cys (SBzl) 1·0, Pro 1·09, Arg 1·09, Gly 1·05. For $C_{68}H_{86}N_{14}O_{14}S_3$ (1 419·7): calculated: 57·53% C, 6·10% H, 13·81% N; found: 57·76% C, 5·89% H, 13·52% N.

[Mpr^1, Tyr(Me)^2, D-Arg^8]-vasopressin (*II*)

Compound *II* was prepared from protected precursor *I* in the usual manner[13]: 250 mg of protected octapeptide amide *I* afforded 140 mg of the 1st lyophilisate. Purification by continuous free-flow electrophoresis afforded 75 mg of 2nd lyophilisate containing 82% of compound *II* (mean value from polarographic determination of the content and calculated from the nitrogen content of the sample); $[\alpha]_D^{20}$ −64·0° (*c* 0·1, water). Amino acid analysis: Tyr 1·0, Phe 1·01, Glu 0·98, Asp 1·02, Pro 1·05, Arg. 0·92, Gly 1·01. The purity of the sample was checked by thin-layer chromatography in the system n-butanol–tert-butanol–acetic acid–water (2 : 2 : 1 : 1) and by paper electrophoresis. The sample for elemental analysis was dried 10 h at 100°C and a pressure of 10 Pa over phosphorus pentoxide. For $C_{47}H_{66}N_{14}O_{12}S_2.CH_3COOH.2\ H_2O$ (1179) calculated: 49·90% C, 6·32% H, 16·62% N; found: 50·10% C, 6·11% H, 16·54% N.

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