[1-β-MERCAPTOPROPIONIC ACID, 8-α,β-DIAMINOPROPIONIC ACID] VASOPRESSIN AND [1-β-MERCAPTOPROPIONIC ACID, 8-D-α,β-DIAMINOPROPIONIC ACID] VASOPRESSIN. TWO LYSINE-VASOPRESSIN ANALOGS WITH CONSIDERABLE ANTIDIURETIC EFFECT*

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Two lysine-vasopressin analogs, [1-β-mercaptopropionic acid, 8-α,β-diaminopropionic acid]-vasopressin (*IVa*) and [1-β-mercaptopropionic acid, 8-D-α,β-diaminopropionic acid]vasopressin (*IVb*) were prepared by solid phase synthesis. *IVa* and *IVb* show a considerable antidiuretic effect (1 079 and 1 000 I.U./mg, resp.) and a low pressor effect (14·0 and 22·8 I.U./mg, resp.). The uterotonic effect of *IVa* is 0·9 I.U./mg and of *IVb* 1·72 I.U./mg.

The biological effects of the vasopressins depend, *inter alia*, on the length of the side chain of the amino acid occupying position 8. The profile of this dependence in the deamino-L-lysine-vasopressin series shows a minimum with the analog containing three carbon atoms in its side chain, *i.e.* with [Mpr[1], Orn[8]]VP[1], and a maximum with a lower homolog, [Mpr[1], Dab[8]]VP (ref.[2]). This analog is so far the most active compound of the lysine-vasopressin series. The situation with respect to the deamino-D-series is different: we observe a minimum with [Mpr[1], D-Dab[8]]VP (ref.[3]) and a maximum with [Mpr[1], D-Orn[8]]VP (ref.[2]). The first members of both series, *i.e.* [Mpr[1], Dap[8]]VP (*IVa*) and [Mpr[1], D-Dap[8]]VP (*IVb*) have been missing as yet. The present paper fills up this gap.

Both analogs were prepared by solid phase synthesis following a scheme developed earlier[4]; the usual combination of protecting groups (SH-Bzl, β-NH_2-Tos, α-NH_2-Boc) was used. The condensations were effected by dicyclohexylcarbodiimide;

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Abbreviations and symbols common in peptide chemistry were used. Other abbreviations: Dap α,β-diaminopropionic acid, Dab α,β-diaminobutyric acid, Mpr β-mercaptopropionic acid, VP vasopressin, LVP lysine-vasopressin, AVP arginine vasopressin. Unless stated otherwise the optically active amino acids are of L-configuration.

N-hydroxybenzotriazole was added to the reaction mixture in steps involving Boc-Asn and in the subsequent steps. The Boc residue was removed by 80% trifluoroacetic acid in dichloromethane. After completion of the synthesis the peptide was split off from the resin by ammonolysis. The protected linear peptides obtained were converted into *IVa* and *IVb* by the usual sequence of reactions. The biological effects of *IVa* and *IVb* were determined by conventional methods[5–9]. Both analogs show nearly the same, relatively high antidiuretic effect. They are approximately five times more efficient that LVP and twice than AVP. A surprising finding is the low pressor effect of [Mpr^1, Dap^8]VP (*IVa*) which is considerably lower even than the pressor effect of the corresponding D-analog. Both analogs represent therefore compounds showing a considerably high and a considerably specific antidiuretic effect.

EXPERIMENTAL

The melting points (uncorrected) were measured on a Kofler block; the samples for analysis were dried 24 h over phosphorus pentoxide at a pressure of 10 Pa and a temperature by 50°C lower than the m.p. The analyses and measurements were made in Model 6020 Amino Acid Analyzer (Instrument Development Workshops, Czechoslovak Academy of Sciences, Prague), in Type 141 Perkin–Elmer Spectrophotometer, Specord UV-VIS Spectrophotometer, and LP-7 Polarograph (Laboratorní přístroje, Prague). The purification of the analogs was carried out by continuous free-flow electrophoresis according to Hannig[10] (Workshops of the Czechoslovak Academy of Sciences, Prague). The purity of the compounds was checked by thin-layer chromatography on silica gel layer sheets (Silufol, Kavalier, Votice, Czechoslovakia) in the systems n-butanol–acetic acid–water (4 : 1 : 1 and 4 : 1 : 5, system A, B, resp.), chloroform–methanol (9 : 1, system C), tert-butanol–n-butanol–acetic acid–water (2 : 2 : 1 : 1, system D), and by electrophoresis on paper Whatman No 3 MM at 700 V in dilute acetic acid (pH 2·5).

N$^\alpha$-Benzyloxycarbonyl-N$^\beta$-*p*-toluenesulfonyl-α,β-diaminopropionic Acid (*Ia*)

N$^\alpha$-Benzyloxycarbonyl-α,β-diaminopropionic acid[11] (13·6 g, 5·7 mmol) was dissolved in 57 ml of 1M-NaOH and a solution of 22·6 g (11·5 mmol) of *p*-toluenesulfonyl chloride in 50 ml of acetone was added; the pH of the stirred reaction mixture was kept in the alkaline range (pH 10) 1 h by the addition of 1M-NaOH. The mixture was extracted three times with ether, acidified by hydrochloric acid to pH 2 and the product extracted with ethyl acetate. The ethylacetate solution was dried over Na_2SO_4 and evaporated under reduced pressure. The dry residue was crystallized from ethyl acetate–light petroleum, yield 15·7 g (70%), m.p. 143—145°C; $[\alpha]_D^{20}$ —11·7° (*c* 1, methanol). For $C_{18}H_{20}N_2O_6S$ (392·4) calculated: 55·10% C, 5·10% H, 7·14% N; found: 54·88% C, 5·33% H, 7·18% N.

N$^\alpha$-Benzyloxycarbonyl-N$^\beta$-*p*-toluenesulfonyl-D-α,β-diaminopropionic Acid (*Ib*)

In analogy to the preparation of compound *Ia*, 11·6 g (4·86 mmol) of N$^\alpha$-benzyloxycarbonyl-D-α,β-diaminopropionic acid[11] afforded 13·5 g (71%) of product *Ib*, m.p. 142—144°C; $[\alpha]_D^{20}$ 9·6° (*c* 1, methanol). For $C_{18}H_{20}N_2O_6S$ (392·4) calculated: 55·10% C, 5·10% H, 7·14% N; found: 55·27% C, 5·26% H, 7·01% N.

N$^{\alpha}$-Tert-butyloxycarbonyl-N$^{\beta}$-*p*-toluenesulfonyl-α,β-diaminopropionic Acid (*IIa*)

Compound *Ia* (12·1 g, 3·1 mmol) was allowed to stand 10 min in a solution of 50 ml of acetic acid at 60°C with 50 ml of 35% hydrogen bromide in acetic acid. The hydrobromide was precipitated with dry ether and ether was decanted off. The greasy product was dried 2 h over KOH *in vacuo*, dissolved in a small volume of water, and the pH of the solution adjusted to *c.* 7 by ammonium hydroxide. The precipitate was filtered off one hour later, washed with water, and dried. The N$^{\alpha}$-*p*-toluenesulfonyl-α,β-diaminopropionic acid obtained was tert-butyloxycarbonylated for 24 h according to Grzonek[3]. The yield after crystallization from ethyl acetate–light petroleum was 7·1 (64%), m.p. 138—140°C, after recrystallization 142—143°C. $[\alpha]_D^{20}$ —3·0° (*c* 1, methanol), $[\alpha]_D^{20}$ 16·1° (*c* 0·5, 1M-NaOH). The compound was chromatographically homogeneous in systems A, B, and C. For $C_{15}H_{22}N_2O_6S$ (358·4) calculated: 50·41% C, 6·19% H, 7·82% N; found: 50·36% C, 6·16% H, 8·22% N.

N$^{\alpha}$-Tert-butyloxycarbonyl-N$^{\beta}$-*p*-toluenesulfonyl-D-α,β-diaminopropionic Acid (*IIb*)

In analogy to the preparation of compound *IIa*, 10·5 g (26·8 mmol) yielded 6·5 g (68%) of product *IIb*, m.p. 143—145°C, $[\alpha]_D^{20}$ 3·13° (*c* 1, methanol), $[\alpha]_D^{20}$ —15·6° (*c* 0·5, 1M-NaOH). The product was chromatographically homogeneous in systems A, B, and C. For $C_{16}H_{22}N_2O_6S$ (358·4) calculated: 50·41% C, 6·19% H, 7·82% N; found: 50·62% C, 6·02% H, 7·89% N.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N$^{\beta}$-*p*-toluenesulfonyl-α,β-diaminopropionyl-glycine Amide (*IIIa*)

The synthesis was effected on chloromethylated polystyrene resin cross-linked by 2% of divinylbenzene (Fluka, Buchs, Switzerland, chlorine content 2·75%). The resin was esterified by Boc-Gly[4] and contained 0·15 mmol of Gly/g of resin. The synthesis was carried out in a manually operated synthetizer accorrding to the program developed earlier[4] (program No 3). The resin (10·6 g) yielded after the synthesis and ammonolysis 1·59 g (71·5%) of the crude product, m.p. 200—215°C. The yield after recrystallization from a mixture of acetic acid and water was 1·12 g (50·5%), m.p. 216—219°C; $[\alpha]_D^{20}$ —26·1° (*c* 0·5, dimethylformamide), amino acid analysis Tyr 0·91, Phe 1·01, Glu 1·01, Asp 1·01, Cys (Bzl) 1·01, Pro 1·01, Dap 1·06, Gly 1·00. For $C_{64}H_{78}N_{12}O_{14}S_3$ (1 335) calculated: 57·56% C, 5·89% H, 12·58% N; found: 57·91% C, 6·07% H, 12·49% N.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N$^{\beta}$-*p*-toluenesulfonyl-D-α,β-diaminopropionyl-glycine Amide (*IIIb*)

The synthesis was carried out with the chloromethylated resin cross-linked by 2% of divinylbenzene (Calbiochem, Los Angeles, U.S.A., chlorine content 5·9%), esterified with Boc-Gly (content of 0·80 mmol of Gly/g of resin). The synthesis was carried out in the same manner as the synthesis of compound *IIIa*. The resin (1·45 g) afforded 1·20 g (77·5%) of crude product *IIIb*, m.p. 193—211°C. The yield after crystallization from a mixture of acetic acid and water was 0·91 g (59%), m.p. 214—218°C; $[\alpha]_D^{20}$ —30·6° (*c* 0·5, dimethylformamide), amino acid analysis Tyr 0·98, Phe 1·02, Glu 0·93, Asp 1·00, Cys (Bzl) 1·11, Pro 0·96, Dap 1·04, Gly 0·95. For $C_{64}H_{78}N_{12}O_{14}S_3$ (1 335) calculated: 57·56% C, 5·89% H, 12·58% N; found: 57·74% C, 6·09% H, 12·41% N.

[Mpr^1, Dap^8]vasopressin (*IVa*)

The protected octapeptide amide *IIIa* (400 mg) afforded after reduction by sodium in liquid ammonia, oxidation by ferricyanide, desalting on Amberlite IRC 50, and lyophilization[12] 134 mg of a crude product which after purification by continuous free-flow electrophoresis gave 47 mg of a lyophilisate containing 83% of product *IVa* (average value obtained by polarographic determination of the product content and by the determination of the nitrogen content of the lyophilisate), $[\alpha]_D^{20}$ —8·8° (*c* 0·1, water); amino acid analysis Tyr 0·97, Phe 1·04, Glu 1·02, Asp 1·02, Pro 0·97, Dap 1·04, Gly 0·93. The purity of the product was checked by thin-layer chromatography (system D) and by paper electrophoresis. The sample for elemental analysis was dried 10 h over phosphorus pentoxide at a pressure of 10 Pa and 100°C. For $C_{43}H_{58}N_{12}O_{12}S_2 . CH_3COOH . H_2O$ (1077) calculated: 50·18% C, 5·99% H, 15·60% N; found: 49·97% C, 6·11% H, 15·42% N.

[Mpr^1, D-Dap^8]vasopressin (*IVb*)

By an analogous procedure 80 mg of the crude product was obtained from 250 mg of precursor *IIIb*. The yield of peptide *IVb* after purification was 32 mg (peptide content 86%). $[\alpha]_D^{20}$ —7·6° (*c* 0·1, water), amino acid analysis Tyr 0·94, Phe 1·02, Glu 1·00, Asp 1·03, Pro 1·05, Dap 1·03, Gly 0·94. For $C_{43}H_{58}N_{12}O_{12}S_2 . CH_3COOH . H_2O$ (1077) calculated: 50·18% C, 5·99% H, 15·60% N; found: 50·22% C, 5·67% H, 15·73% N.

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