PREPARATION OF [1-β-MERCAPTOPROPIONIC ACID, 8-HOMOARGININE]-, AND [1-β-MERCAPTOPROPIONIC ACID, 8-D-HOMOARGININE]-VASOPRESSIN*

M.ZAORAL and F.BRTNÍK

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

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Condensation of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzyl-cysteine with prolyl-N^{ε}-benzyloxycarbonyllysyl-glycine amide (*VIIa*) and with prolyl-N^{ε}-benzyl-oxycarbonyl-D-lysyl-glycine amide (*VIIb*) by the azide method afforded β-benzylthiopropionyl--tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^{ε}-benzyloxycarbonyl-lysyl-glycine amide (*VIIIa*) and its analog *VIIIb*, respectively. [1-β-Mercaptopropionic acid, 8-homoarginine]-vasopressin (*Ia*) and its 8-D-homoarginine analog (*Ib*) were obtained from *VIIIa* and *VIIIb* by decarbobenzoxylation, guanidation, oxidation, and purification. *Ia* showed approximately 40% of the activity of [1-β-mercaptopropionic acid, 8-D-arginine]-vasopressin, 1/4 to 1/3 of the pressor effect of lysine vasopressin, and 1/50 of the uterotonic effect of oxytocin. *Ib* showed approximately 1/20 of the antidiuretic effect, 1/3000 of the pressor effect, and 1/6 of the uterotonic effect of *Ia*.

In our studies on the importance of the length of the side chain of the basic amino acid for the biological effects of the vasopressins[1] we prepared [1-β-mercapto-propionic acid, 8-homoarginine]-vasopressin (*Ia*) and [1-β-mercaptopropionic acid, 8-D-homoarginine]-vasopressin (*Ib*). Both products were intended to contribute to the elucidation of the problem of the optimal length of this chain in the L- and D-series of analogs of deaminoarginine-vasopressin.

A recent report by Bodanszky and coworkers[2] on the synthesis and certain pharmacological properties of [8-homoarginine]-vasopressin and [1-β-mercaptopropionic acid, 8-homoarginine]-vasopressin led us to publish some of our results.

The synthesis of *Ia* and *Ib* was effected by the "guanidation" procedure[3] which involved the preparation of the corresponding octapeptide amide derivatives containing in position 8 lysine with ε-amino group protected by the benzyloxycarbonyl residue, the removal of this protecting group by a solution of hydrogen bromide in glacial acetic acid, and guanidation of ε-amino groups by 1-guanido-3,5-dimethyl-pyrazole nitrate[4]. The "guanidation" procedure will be discussed in greater detail in a forthcoming communication. We wish merely to mention here that the guanida-

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tion proceeded in our hands incompletely (to 60–80%) and that it was complicated by side reactions.

The lysine octapeptide amide derivatives were prepared according to the usual scheme[5] namely by condensation of fragment 1–6 with tripeptide amides 7–9 by the azide method[6]. β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine hydrazide was obtained by the procedure described earlier[7]. Tripeptide amides *VIIa* and *VIIb* were prepared by stepwise synthesis from the carboxyl terminus. The α-amino groups and the ε-amino groups of lysine were protected by tert-butyloxycarbonyl and by benzyloxycarbonyl group, respectively. The benzyloxycarbonyl residues were removed by treating the peptides with a mixture of trifluoroacetic acid and dichloromethane (4 : 1), 20 min at room temperature[8]. Partial cleavage of the ε-benzyloxycarbonyl residue of lysine was virtually eliminated under these conditions. Additional details of the preparation of protected octapeptide amide derivatives follow from Scheme 1.

H-Gly-NH_2.HBr
Boc-Lys(Z)-OH
↓
Boc-Lys(Z)-Gly-NH_2 (*II*)
↓
TFA.H-Lys(Z)-Gly-NH_2 (*III*)
↓
H-Lys(Z)-Gly-NH_2 (*IV*)
Boc-Pro-OH
↓
Boc-Pro-Lys(Z)-Gly-NH_2 (*V*)
↓
TFA-H-Pro-Lys(Z)-Gly-NH_2 (*VI*)
↓
H-Pro-Lys(Z)-Gly-NH_2 (*VII*)
Mpr(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-$NHNH_2$
↓
Mpr(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Z)-Gly-NH_2 (*VIII*)
↓
Mpr(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys-Gly-NH_2
↓
Mpr(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Har-Gly-NH_2* (*IX*)
↓
Mpr-Tyr-Phe-Gln-Asn-Cys-Pro-Har-Gly-NH_2 (*I*)

SCHEME 1

Vertical bars at the left-hand side of the formulas indicate coupling steps.

* Har = homoarginine. The remaining symbols and abbreviations are identical with those used in the preceding study[11].

Peptides *IXa* and *IXb* were freed of the protecting groups by reduction with sodium in liquid ammonia, the disulfide ring was closed by oxidation with potassium ferricyanide[9], the reaction mixture was desalted on Amberlite CG and purified by continuous free-flow electrophoresis[10,11]. The peptides obtained were characterized and identified by elemental analyses, amino-acid analysis, polarography[12], UV-spectra measurement, paper electrophoresis, and thin-layer chromatography.

The amino-acid analysis of *Ia* and *Ib* indicated the presence of a certain quantity of the corresponding lysine peptides which we have not been able to remove either by an extensive purification of protected octapeptide amide derivatives *IXa* and *IXb* or by additional purification of the products obtained by continuous free-flow electrophoresis. Lysine found in the hydrolysates of *Ia* and *Ib* is derived, however, only partly from the lysine peptides present. It also originates from partial deguanidation of homoarginine during hydrolysis. A comparison of *Ia* with the product prepared by a different procedure (from homoarginine) showed that the presence of lysine peptides (5–10% according to our estimate made with regard to analogous cases, course of purification by continuous free-flow electrophoresis, paper electrophoresis, and results of amino-acid analyses) does not affect the results of biological titrations.

The antidiuretic, pressor, and uterotonic effect of both analogs, *Ia* and *Ib*, was examined. The antidiuretic activity was determined according to Burn[13]. When using a modification of Sawyer's method[14], our experience was similar to a number of preceding cases: the antidiuretic effect of analogs of the D-series can be determined by this method with great difficulties only. The values often vary over a wide range and frequently erroneously high results are obtained. We meet with similar difficulties even with some analogs of the L-series, especially with those which show a high antidiuretic effect. This may perhaps account, *inter alia*, for discrepancies in the values of antidiuretic activity recorded for [1-β-mercaptopropionic acid,8-homolysine]-vasopressin[2,15].

The pressor effect was measured essentially according to Landgrebe and coworkers[16] and the uterotonic effect according to Munsick[17].

The antidiuretic activity was determined using a standard batch of Adiuretin-SD (DDAVP) as the comparison sample. The pressor and uterotonic effect was compared with that of synthetic standards of lysine-vasopressin (pressor effect 200 I.U./mg) and oxytocin (uterotonic effect 400 I.U./mg); their activities were established by titration against 3rd International Standard.

The antidiuretic activity of *Ia* was approximately 40% of that of DDAVP, the pressor effect was 1/2–3/4 and the uterotonic effect approximately 1/30 of the effect of the corresponding standards. The biological activities of *Ib* were considerably lower than those of *Ia*. The antidiuretic effect of *Ib* was approximately 1/20, the pressor effect approximately 1/3000, and the uterotonic effect approximately 1/6 of the effect of *Ia*.

The elongation of the side chain of the basic amino acid in deaminoarginine-vaso-

pressin leads to a product with a considerable antidiuretic and pressor effect. The results obtained as yet do not permit us, however, to make any other better defined conclusions on the optimal length of this chain. The change of configuration in position 8 brings about a marked decrease of antidiuretic effect and a drastic decrease of the pressor effect[7]. [1-β-Mercaptopropionic acid, 8-D-homoarginine]-vasopressin shows dissociation of the antidiuretic and pressor effect which is typical of vasopressin analogs of the D-series. The results obtained indicate that the series of arginine-vasopressin analogs is governed by similar regularities as the series of lysine-vasopressin analogs. These regularities are in accordance with our conclusions on the relation between the length of the side chain of the basic amino acid of vasopressin and their typical biological activities, on the predominant importance of configuration, length, and size of side chain, and on the secondary importance of its basicity.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. The optical activity was measured on Zeiss-Winkel 0·01 polarimeter and Perkin Elmer type 141 polarimeter. The UV spectrophotometry was carried out on VSU-2P Zeiss (Jena) spectrophotometer. The polarographic measurements were performed on LP 7 polarograph (Laboratorní přístroje, Prague). Electrophoresis on a preparative scale was carried out in an apparatus for continuous free-flow electrophoresis (a modification of the apparatus of Hannig, constructed in the workshops of this Institute). The purity of intermediary and of final products was checked by thin-layer chromatography on silica gel coated aluminum sheets (Silufol, Kavalier) in the systems n-butanol–acetic acid–water (4 : 1 : 1 and 4 : 1 : 5) and ethyl acetate–ethanol (9 : 1) and by paper electrophoresis (5% acetic acid and pyridine acetate buffer at pH 5·7, on Whatman No 3 paper). Samples whose melting points are lower than 90°C were dried 3 days at room temperature and 0·1 Torr over phosphorus pentoxide before being analyzed. The remaining samples except the lyophilisates were dried 8 h at 80°C under identical conditions.

N^{α}-tert-Butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-glycine Amide (*IIa*)

A solution of 7·2 g (19 mmol) of N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysine in 20 ml of dimethylformamide was treated with a solution of 3·0 g (19 mmol) of glycine amide hydrobromide, 2·56 ml of N-ethylpiperidine and 3·1 g (38 mmol) of N-hydroxybenztriazole in 15 ml of dimethylformamide. The mixture was cooled to 0°C and a solution of 4·3 g (21 mmol) of dicyclohexylcarbodiimide in 5 ml of dimethylformamide was added. The mixture was set aside for 1 h at 0°C and for 20 h at room temperature. Dicyclohexyl urea which had separated was filtered off (approximately quantitative yield), the filtrate was taken to dryness, and the dry residue dissolved in 200 ml of chloroform. This solution was washed 4-times with 100 ml of 5% $NaHCO_3$, 4-times with 100 ml of 10^{-3}M-HCl, once with 100 ml of water, and dried by sodium sulfate. Chloroform was distilled off and the dry residue was crystallized from 2-propanol–diisopropyl ether. The yield after three-fold crystallization from the same system was 7·0 g of *IIa* (85%), m.p. 76–76·5°C, $[\alpha]_D^{25}$ −3·4° (*c* 1, ethanol). For $C_{21}H_{32}N_4O_6$ (436·4) calculated: 57·79% C, 7·38% H, 12·84% N; found: 57·88% C, 7·53% H, 12·82% N. The values recorded[18] for the same product prepared by ammonolysis of Boc-Lys(Z)-Gly-OEt are m.p. 76°C, $[\alpha]_D^{22}$ −3°, (*c* 1, methanol).

N$^{\alpha}$-tert-Butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-D-lysyl-glycine Amide (*IIb*)

N$^{\alpha}$-tert-Butyloxycarbonyl-N$^{\varepsilon}$-benzyloxycarbonyl-D-lysine (7·6 g), glycine amide hydrobromide (3·1 g), N-ethylpiperidine (2·7 ml), N-hydroxybenztriazole (5·4 g), and dicyclohexylcarbodiimide (4·5 g) afforded 7·0 g (80%) of *IIb*, m.p. 76–78°C, $[\alpha]_D^{25}$ +2·9° (*c* 1, methanol). For $C_{21}H_{32}N_4O_6$ (436·4) calculated: 57·79% C, 7·38% H, 12·84% N; found: 57·55% C, 7·36% H, 12·87% N.

N$^{\varepsilon}$-Benzyloxycarbonyllysyl-glycine Amide Trifluoroacetate (*IIIa*)

Compound *IIa* (4·3 g, 10 mmol) was treated with 8 ml of concentrated trifluoroacetic acid and 2 ml of dichloromethane. The mixture was allowed to stand 20 min at room temperature. The solvents were distilled off *in vacuo*. The dry residue afforded after repeated trituration with ether a precipitate which was crystallized from a mixture of ethyl acetate and ether. The sample for analysis was recrystallized from methanol–diisopropyl ether. The yield was 4·0 g (89%) of *IIIa*, m.p. 133–134°C, $[\alpha]_D^{25}$ +12·2° (*c* 1, methanol). For $C_{16}H_{24}N_4O_4.CF_3CO_2H$ (450·4) calculated: 48·00% C, 5·60% H, 12·44% N; found: 48·23% C, 5·37% H, 12·71% N.

N$^{\varepsilon}$-Benzyloxycarbonyl-D-lysyl-glycine Amide Trifluoroacetate (*IIIb*)

Compound *IIb* (5·0 g) afforded after two crystallizations from ethyl acetate–ether 4·5 g (90%) of *IIIb*, m.p. 93–95°C, $[\alpha]_D^{25}$ −12·8° (*c* 1, methanol). For $C_{16}H_{24}N_4O_4.CF_3CO_2H$ (450·4) calculated: 48·00% C, 5·60% H, 12·44% N; found: 48·20% C, 5·54% H, 12·67% N.

N$^{\varepsilon}$-Benzyloxycarbonyllysyl-glycine Amide (*IVa*)

A solution of 4·3 g (9·5 mmol) of *IIIa* in methanol was filtered through a column of Ostion AT (200 ml, OH-form) (a strongly basic ion-exchange resin, produced by Spolek pro chemickou a hutní výrobu, Ústí nad Labem). The effluents were taken to dryness *in vacuo* and the dry residue was recrystallized three-times from methanol–ether. The yield was 3·0 g (94%), m.p. 110–111°C, $[\alpha]_D^{25}$ +13·8° (*c* 0·44, methanol). For $C_{16}H_{24}N_4O_4$ (336·4) calculated: 57·13% C, 7·19% H, 16·66% N; found: 57·15% C, 7·19% H, 16·77% N.

N$^{\varepsilon}$-Benzyloxycarbonyl-D-lysyl-glycine Amide (*IVb*)

Compound *IIIb* (4·5 g, 10 mmol) gave after three crystallizations from methanol–ether 3·0 g (89%) of *IVb*, m.p. 116–117°C, $[\alpha]_D^{25}$ −11·4° (*c* 1, methanol). For $C_{16}H_{24}N_4O_4$ (336·4) calculated: 57·13% C, 7·19% H, 16·66% N; found: 57·14% C, 7·20% H, 16·53% N.

Tert-butyloxycarbonylprolyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-glycine Amide (*Va*)

A solution of 3·2 g (10 mmol) of *IVa* and 2·2 g of tert-butyloxycarbonylproline (10 mmol) in 10 ml of dimethylformamide was treated with a solution of 2·7 g (20 mmol) of N-hydroxybenztriazole in 5 ml of dimethylformamide. The mixture was cooled to 0°C and a solution of 2·3 g of dicyclohexylcarbodiimide in 5 ml of dimethylformamide was added. The subsequent treatment of the reaction mixture was identical with that described for *IIa*. After two crystallizations from a mixture 2-propanol–diisopropyl ether–ether the yield was 4·1 g of *Va*, m.p. 86–90°C, $[\alpha]_D^{25}$ −35·6° (*c* 0·5, methanol). For $C_{26}H_{39}N_5O_7$ (533·6) calculated: 58·52% C, 7·37% H, 13·12% N; found: 58·39% C, 7·39% H, 13·36% N.

Tert-butyloxycarbonylprolyl-N$^{\varepsilon}$-benzyloxycarbonyl-D-lysyl-glycine Amide (*Vb*)

The same quantity of starting components as in the preceding case yielded 4·0 g (84%) of *Vb*, m.p. 174–175°C, $[\alpha]_D^{25}$ −14·4° (*c* 1, methanol). For $C_{26}H_{39}N_5O_7$ (533·6) calculated: 58·52% C, 7·37% H, 13·12% N; found: 58·70% C, 7·30% H, 13·01% N.

Prolyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-glycine Amide Trifluoroacetate (*VIa*)

The splitting of the tert-butyloxycarbonyl group was carried out as described for *IIIa*. Compound *Va* (3·5 g) gave after three crystallizations from methanol–ether 3·4 g (92%) of *VIa*, m.p. 69·5 to 70°C, $[\alpha]_D^{25}$ −20·8° (*c* 0·8, methanol). For $C_{21}H_{31}N_5O_5.CF_3CO_2H$ (547·5) calculated: 50·45% C, 5·89% H, 12·79% N; found: 50·46% C, 5·96% H, 12·90% N.

Prolyl-N$^{\varepsilon}$-benzyloxycarbonyl-D-lysyl-glycine Amide Trifluoroacetate (*VIb*)

Compound *Vb* (3·0 g) afforded after three crystallizations from methanol–ether 2·7 g of *VIb* (88%), m.p. 141–143°C, $[\alpha]_D^{25}$ −11·4° (*c* 1, methanol). For $C_{21}H_{31}N_5O_5.CF_3CO_2H$ (547·5) calculated: 50·45% C, 5·89% H, 12·79% N; found: 50·38% C, 5·96% H, 12·90% N.

Prolyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-glycine Amide (*VIIa*)

The deionization of *VIa* was carried out as described for *IVa*. After two crystallizations from ethyl acetate–diisopropyl ether, 3·0 g of *VIa* yielded 2·2 g (84%) of *VIIa*, m.p. 72–73°C, $[\alpha]_D^{25}$ −33·0° (*c* 0·85, methanol). For $C_{21}H_{31}N_5O_5.1/2\,H_2O$ (442·5) calculated: 57·00% C, 7·29% H, 15·83% N; found: 56·91% C, 7·26% H, 15·80% N. Amino-acid composition: Pro 1·02, Lys 1·04, Gly 1·00.

Prolyl-N$^{\varepsilon}$-benzyloxycarbonyl-D-lysyl-glycine Amide (*VIIb*)

The same quantity of trifluoroacetate *VIb* as in the preceding case afforded after two crystallizations from ethyl acetate–diisopropyl ether and after additional crystallization from methanol–diisopropyl ether 2·2 g (84%) of *VIIb*, m.p. 72–73°C, $[\alpha]_D^{25}$ −11·9° (*c* 1, methanol). For $C_{21}H_{31}N_5O_5.1/2\,H_2O$ (442·5) calculated: 57·00% C, 7·29% H, 15·83% N; found: 57·00% C, 7·24% H, 15·64% N. Amino-acid composition: Pro 1·03, Lys 1·00, Gly 0·98.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N$^{\varepsilon}$-benzyloxycarbonyllysyl-glycine Amide (*VIIIa*)

A solution of 1·434 g (1·5 mmol) of β-benzylthiopropionyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine hydrazide[1] in 15 ml of dimethylformamide was treated with 0·8 ml of 4N solution of hydrogen chloride in dioxane free of peroxides. The solution was cooled to −20°C. Amyl nitrite (0·175 g, 1·5 mmol) in 1 ml of dimethylformamide was added with stirring and the course of the reaction was followed by the Griess reagent at 5 min intervals. After a period of 20 min the mixture was cooled to −40°C, neutralized with N-ethylpiperidine and treated with a solution of 0·916 g (40% excess) of *VIIa* in 5 ml of dimethylformamide. The mixture was allowed to stand 12 h at 0°C and 3 h at room temperature. The solvent was distilled off *in vacuo*, the pasty residue was cooled to room temperature, and 200 ml of approximately 1% hydrochloric acid was added. The precipitate was washed on the filter with 200 ml of saturated solution of $NaHCO_3$, and with 100 ml of water. After two crystallizations from dimethylformamide–water the yield was 2·0 g of *VIIIa*, m.p. 215–218°C, $[\alpha]_D^{22}$ −35·9° (*c* 1, dimethylformamide).

For $C_{68}H_{84}N_{12}O_{14}S_2$ (1357·5) calculated: 60·17% C, 6·24% H, 12·38% N, 4·72% S; found: 59·95% C, 6·13% H, 12·18% N, 4·70% S. Amino-acid composition: Tyr 0·90, Phe 1·05, Glu 1·09, Asp 1·12, Pro 0·96, Lys 0·92, Gly 1·00.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--N^{ε}-benzyloxycarbonyl-D-lysyl-glycine Amide (*VIIIb*)

The same quantities of starting compounds as in the preceding case afforded after two crystallizations from dimethylformamide–water 2·0 g of *VIIIb*, m.p. 211–213°C, $[\alpha]_D^{22}$ −19·2° (*c* 1, dimethylformamide). For $C_{68}H_{84}N_{12}O_{14}S_2$ (1357·5) calculated: 60·17% C, 6·24% H, 12·38% N, 4·72% S; found: 60·03% C, 6·18% H, 12·24% N, 4·68% S. Amino acid composition: Tyr 0·89, Phe 1·11, Glu 1·09, Asp 1·09, Pro 0·94, Lys 0·99, Gly 1·00.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--homoarginyl-glycine Amide (*IXa*)

Compound *VIIIa* (1·7 g, 1·25 mmol) was dissolved in 4 ml of hot acetic acid and 4 ml of approximately 35% solution of hydrogen bromide in acetic acid was added. The mixture was heated 15 min at 65°C, taken to dryness *in vacuo*, the dry residue cooled to room temperature, and triturated with saturated solution of $NaHCO_3$. The resulting precipitate of β-benzylthiopropionyl--tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-lysyl-glycine amide was washed on the filter with water and ether and dried 24 h over phosphorus pentoxide *in vacuo*. 1-Guanyl-3,5-dimethylpyrazole nitrate[4] (1·09 g, 5·4 mmol) was added to a solution of 1·036 g (0·77 mmol) of the lysine-octapeptide amide derivative in 7 ml of dimethylformamide. The pH was adjusted to 9·5 by triethylamine and the mixture was set aside for 4 days at room temperature. It was subsequently neutralized by acetic acid, taken to dryness *in vacuo* and the residue triturated with 75 ml of water. The resulting precipitate was filtered off, washed with water, and recrystallized twice from dimethylformamide–water. Yield 0·7 g (72%) of *IXa*, m.p. 187–189°C, $[\alpha]_D^{22}$ −36·7° (*c* 1, dimethylformamide). For $C_{61}H_{80}N_{14}O_{12}S_2$ (1265·5) calculated: 57·89% C, 6·37% H, 15·50% N, 5·07% S; found: 58·02% C, 6·37% H, 14·74% N, 4·91% S.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--D-homoarginyl-glycine Amide (*IXb*)

Compound *VIIIb* (1·35 g, 1·1 mmol) yielded 0·6 g of *IXb*, m.p. 209–211°C, $[\alpha]_D^{22}$ −20·0° (*c* 1, dimethylformamide). For $C_{61}H_{80}N_{14}O_{12}S_2$ (1265·5) calculated: 57·89% C, 6·37% H, 15·50% N, 5·07% S; found: 57·94% C, 6·42% H, 14·93% N, 4·91% S.

[1-β-Mercaptopropionic Acid, 8-homoarginine]-vasopressin (*Ia*)

Compound *IXa* (300 mg) was reduced with sodium in 400 ml of liquid ammonia (until a blue color permanent for 30 s was obtained). The solution was evaporated under reduced pressure and: he dry residue was dissolved in 600 ml of 2·5% acetic acid. A small quantity of undissolved material was filtered off. The filtrates were extracted 5-times with 100 ml of ether and their pH was adjusted to 6·75 by ammonia. Subsequently the filtrates were oxidized by 0·01M solution of $K_3[Fe(CN)_6]$. The pH of the oxidation mixture was adjusted to 4·4 by acetic acid and the solution was desalted by filtration through a column of Amberlite CG 50 (25 ml). The column was washed with 250 ml of 0·25% acetic acid. Crude *Ia* was eluted from the column by 50% acetic acid. A total of 30 ml of effluent was collected, made up to 50 ml with water and lyophilized.

The yield of first lyophilisate was 145·6 mg. The product was purified by continuous free-flow electrophoresis as described in the preceding report[11]. The voltage used was 3 500 V (180 mA) and the temperature of cooling air was −2°C. The yield of 2nd lyophilisate was 44 mg. $[\alpha]_D^{25}$ −79·8° (*c* 0·5, 1M-CH_3COOH). Bodanszky and coworkers[2] report $[\alpha]_D^{25}$ −87·9° (*c* 0·5, 1M-CH_3COOH). The sample for analysis was dried 8 h at 100°C and 0·1 Torr over phosphorus pentoxide. The analysis corresponded to monoacetate. For $C_{49}H_{70}N_{14}O_{14}S_2$ (1143·3) calculated: 51·48% C, 6·17% H, 17·15% N, 5·61% S; found: 51·30% C, 6·07% H, 16·90% N, 5·57% S. Amino-acid analysis: Tyr 0·94, Phe 1·01, Glu 0·95, Asp 0·93, Pro 1·02, Har 0·91, Gly 0·91. UV (water acidified by hydrochloric acid to pH 3·2) λ_{max} 228, λ_{min} 250, λ_{max} 275.

[1-β-Mercaptopropionic Acid, 8-D-homoarginine]-vasopressin (*Ib*)

The same quantity of protected octapeptide amide derivative afforded 80·4 mg of 2nd lyophilisate. $[\alpha]_D^{25}$ −59·4° (*c* 0·4, 1M-CH_3COOH). The sample for analysis was dried as described for the preceding product. The analytical data also corresponded to monoacetate. Found: 51·60% C, 6·20% H, 16·95% N, 5·63% S. Amino-acid analysis: Tyr 0·94, Phe 1·05, Glu 1·07, Asp 1·05, Pro 1·03, Har 0·77, Gly 0·90. UV (water acidified by hydrochloric acid to pH 3·2) λ_{max} 227, λ_{min} 250, λ_{max} 275.

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