4-PHENYLALANINE ANALOGUES OF VASOPRESSIN: SYNTHESIS, PHARMACOLOGICAL AND CHIROPTICAL PROPERTIES*

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Synthesis, some pharmacological properties and CD spectra of [4-phenylalanine, 8-arginine]vasopressin and [4-phenylalanine, 8-lysine]vasopressin are described.

Oxytocin and vasopressin analogues**, modified in position 4, usually retain a high degree of biological activities[3]. [4-Phenylalanine]oxytocin[4] represents an exception having only a very low uterotonic activity and being inactive in the pressor and avian vasodepressor tests. However, it weakly inhibits the avian vasodepressor effect of oxytocin and, in relatively high doses, shows natriuretic activity. Its reported[4-6] inhibitory activity in the antidiuretic test has not been confirmed[7]. Some of the pertinent pharmacological data have been published in journals not easily accessible to us[8,9].

It appeared interesting to prepare similar analogues in the vasopressin series. Our present paper concerns the synthesis, pharmacological activity and chiroptical properties of [4-phenylalanine, 8-arginine]vasopressin (*Ia*) and [4-phenylalanine, 8-lysine]vasopressin (*Ib*). Both were prepared according to the "6 + 3" fragment condensation scheme, using benzyloxycarbonyl group for protection of the lysine ε-amino group, *p*-toluenesulfonyl group for blocking the arginine guanyl group and 2,4,6-trimethylbenzyl group for protecting the sulfhydryl group in the cysteine residues[10]. All the protecting groups were removed in the last step by treatment with trifluoromethanesulfonic acid[10,11].

The amino-terminal hexapeptide *IIa* was prepared by azide condensation of benzyloxycarbonyl-S-(2,4,6-trimethylbenzyl)cysteinyl-tyrosyl-phenylalanine hydrazi-

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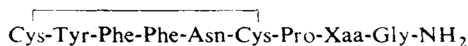
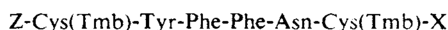
** The nomenclature and symbols of the amino acids, peptides and protecting groups obey the published recommendations[1,2]; Cys(Tmb) denotes S-(2,4,6-trimethylbenzyl)cysteine. The chiral amino acids used in this work are of the L-configuration.

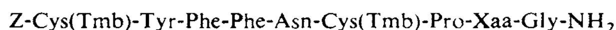
de[12] with phenylalanyl-asparaginyl-S-(2,4,6-trimethylbenzyl)cysteine methyl ester, obtained by stepwise synthesis *via* active esters. The hexapeptide-ester *IIa* was converted into the hydrazide *IIb* by the usual procedure. The azide, prepared from *IIb*, was condensed either with prolyl-N^G-*p*-toluenesulfonylarginyl-glycine amide[13] to give compound *IIIa* or with prolyl-N^ε-benzyloxycarbonyllysyl-glycine amide (obtained by stepwise synthesis *via* activated esters) to afford the nonapeptide *IIIb*. Both the protected nonapeptides were completely deblocked by treatment with trifluoromethanesulfonic acid in trifluoroacetic acid in the presence of thioanisole and the obtained disulfhydryl compounds were oxidized with air oxygen. The analogues *Ia* and *Ib* were purified by continuous free-flow electrophoresis.



Ia, Xaa = Arg, *Ib*, Xaa = Lys



IIa, X = OMe *IIb*, X = N_2H_3



IIIa, Xaa = Arg *IIIb*, Xaa = Lys

Their biological activities are given in Table I. As seen, similarly to [4-phenylalanine]oxytocin, also in the case of the analogues *Ia* and *Ib* replacement of the glutamine moiety with phenylalanine reduces strongly all biological activities. The analogue *Ib* has no inhibitory effect on the oxytocin-induced antidiuretic activities.

Vasopressin exhibits strong analgesic activity[14]. All the hitherto tested vasopressin analogues have been less potent than the natural hormone (for a review see ref.[3]). The analogues *Ia* and *Ib*, tested in the tail-flick test[15], exhibited only low activities. The effect occured 30–60 min after intracerebroventricular (30 μg/rat) and 90 min

TABLE I

Biological activities (*I.U.*/mg) of the synthesized analogues and parent hormones (rat)

Compound	Uterotonic (*in vivo*)	Galactogogic (*in vivo*)	Pressor	Antidiuretic
[8-Arginine]vasopressin[29]	17	69[a]	412	465
[8-Lysine]vasopressin[29]	5	42[a]	285	260
Ia	0·04	0·28	~0·5	17·2
Ib	<0·05	3·6	~0·5	0·3

[a] Rabbit.

after intraperitoneal (40 μg/rat) administration. Applied intracerebroventricularly, the analogues exhibited a 30% increase of response latency (compared with the base-line). The low activity of both analogues in the tail-flick test shows that the vasopressin receptor, mediating the analgesic effect, is closely similar to the known receptors of the V_1 or V_2 type.

The general shape of CD spectra of both the [Phe^4] analogues is approximately parallel to the curve of [8-arginine]vasopressin when recorded under the same experimental conditions (Table II, Fig. 1). However, a detailed inspection reveals differences which cannot be ascribed to the sole manifestation of the additional aromatic chromophore introduced by the Phe^4 substitution. In fact, there is almost no change of the long wavelength aromatic B_{2u} band at about 265 nm. Minor direct contribution of the additional aromatic chromophore is indicated in the region of the short wavelength E_{1u} transition (at about 190 nm). These observations do not support the possibility of stacking of the three neighbouring aromatic side chains within the cyclic moiety. Significant changes are detected in two spectral regions: Both analogues exhibit a distinct decrease of overall intensity of dichroic absorption within the short-wavelength region (185–250 nm) and marked changes of disulfide bands above 290 nm. The changes are not identical for both analogues but they are consistent with respect to the curve of [8-arginine]vasopressin. The overall decrease of CD intensity which concerns mainly the amide n-π* and π-π* transitions indicates a conformational change within the peptide backbone. We cannot distinguish whether this change occurs in the cyclic moiety or in the C-terminal part since there are arguments for both possibilities. It is to be noted that Spasov and Popov[16] in their computational study of [8-arginine]vasopressin conformation in aqueous solution detected important stabilizing interactions between the Phe^3 side chain and the C-terminal part. It cannot be excluded that introduction of Phe^4 residue affects these interactions. On the other hand, remarkable changes of disulfide bands indicate a conformational change within the cyclic moiety. The long-wavelength negative disulfide band is seriously bathochromically shifted (the maximum is located at 330–340 nm). According to Maxfield and Scheraga[17] (see also Frič and coworkers[18]) this observation can be interpreted by increased population of conformers possessing disulfide group seriously deviated from the rectangular conformation that is lowest in energy in the absence of other interactions. Apparently, these changes are reflected in low values of biological activities found for both [Phe^4] analogues.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over phosphorus pentoxide at room temperature and 150 Pa. Thin-layer chromatography was performed on silica gel plates (Silufol, Kavalier) in the systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S2),

1-butanol–acetic acid–water (4 : 1 : 1) (S3), 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4), n-heptane–tert-butyl alcohol–pyridine (5 : 1 : 1) (S5), 1-butanol–acetic acid–ethyl acetate–water (1 : 1 : 1 : 1) (S7), 1-butanol–water–acetic acid (50 : 40 : 15) (S13), toluene–acetone (20 : 1) (S19), ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3) (S23). Electrophoresis was carried

TABLE II

Short-wavelength circular dichroism data of the prepared analogues as compared to [8-arginine]-vasopressin

Compound	$\lambda_{max}([\Theta]_{max} \cdot 10^{-3})$[a]					
[Phe^4, Arg^8] VP	228·5 (+8·7)	204·5 (−23)	197 (−21)[b]	–	–	185 (−46)[c]
[Phe^4, Lys^8] VP	229·0 (+12·8)	204·0 (−34)	198 (−34)[b]	193 (−40)	189 (−32)[b]	185 (−62)[c]
[Arg^8] VP	227·0 (+16·5)	203·0 (−45)	195 (−68)	–	188 (−42)[b]	185 (−128)[c]

[a] λ_{max} wavelength of the maximum in nm, $[\Theta]_{max}$ molar ellipticity of the maximum in deg cm^2 . dmol^{-1}; [b] negative minimum; [c] end value.

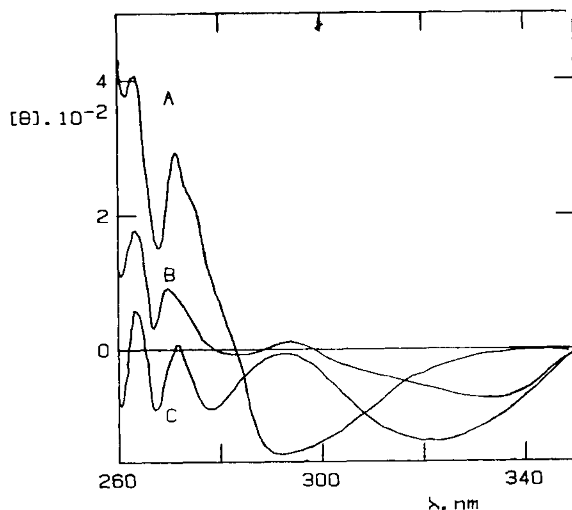


FIG. 1

Long-wavelength region of CD spectra of [8-arginine]vasopressin (A), analogue *Ia* (B), and *Ib* (C) (in 0·02 mol l^{-1} neutral phosphate buffer)

out on a Whatman 3MM paper in a moist chamber at 20 V/cm for 1 h in 1M-acetic acid (pH 2·4) or in a pyridine–acetate buffer, pH 5·7. Compounds, containing *o*-nitrobenzenesulfenyl protecting group, were deblocked with hydrogen chloride prior to electrophoresis. The compounds were detected with ninhydrin or by the chlorination method. Solvents were evaporated on a rotatory evaporator at bath temperature 30°C, dimethylformamide at the same temperature and 150 Pa. Samples for amino acid analyses were hydrolyzed with 6M-HCl at 150°C for 20 h. The amino acid analyses were performed on a two-column instrument (type 6020, Developmental Workshops of Czechoslovak Academy of Sciences). Preparative free-flow electrophoresis was done on a previously described instrument[19,20].

S-(2,4,6-Trimethylbenzyl)cysteine Methyl Ester Hydrochloride

Thionyl chloride (8 ml) was added dropwise to methanol (50 ml) at a temperature below −5°C. To this solution, S-(2,4,6-trimethylbenzyl)cysteine[10] (7·5 g) was added in portions, the temperature being kept below −5°C. After stirring at −5°C for 30 min and at 43°C for 2 h, the solvent was distilled off and the residue codistilled several times with methanol. The crystalline part was suspended in water (15 ml) and mixed with a solution of sodium hydrogen carbonate (7·5 g) in water (75 ml). The suspension was filtered, the filtrate extracted with ethyl acetate, the extract washed with saturated solution of sodium hydrogen carbonate and water, and dried over sodium sulfate. After evaporation of the solvent, the residue was mixed with 2M-HCl in ether (3 ml). Crystallization from methanol and ether gave 7·0 g (78%) of the product, m.p. 166–168°C; $[\alpha]_D$ +23·9° (*c* 0·5, dimethylformamide); R_F 0·58 (S1), 0·57 (S2), 0·54 (S3), 0·65 (S4), 0·78 (S7); $E^{His}_{2.4}$ 0·73, $E^{His}_{5.7}$ 0·80. For $C_{14}H_{21}NO_2S$. HCl (303·9) calculated: 55·34% C, 7·30% H, 4·61% N, 10·55% S; found: 55·61% C, 7·41% H, 4·67% N, 10·30% S.

Benzyloxycarbonylasparaginyl-S-(2,4,6-trimethylbenzyl)cysteine Methyl Ester

Benzyloxycarbonylasparagine *p*-nitrophenyl ester (1·16 g) was added to a solution of S-(2,4,6-trimethylbenzyl)cysteine methyl ester hydrochloride (0·91 g) and triethylamine (0·42 ml) in dimethylformamide (6 ml). The mixture was stirred for 12 h, the formed gel was mixed with water, the solid material filtered and washed successively with saturated solution of sodium hydrogen carbonate, water, 10% hydrochloric acid and acetone. Crystallization from aqueous acetic acid afforded 1·3 g (84%) of the dipeptide, m.p. 178–180°C, $[\alpha]_D$ −10·7° (*c* 0·5, dimethylformamide); R_F 0·72 (S1), 0·60 (S2), 0·72 (S3), 0·71 (S4), 0·31 (S5). For $C_{26}H_{33}N_3O_6S$ (515·6) calculated: 60·56% C, 6·45% H, 8·15% N, 6·35% S; found: 60·73% C, 6·42% H, 8·11% N, 6·22% S.

Benzyloxycarbonylphenylalanyl-asparaginyl-S-(2,4,6-trimethylbenzyl)cysteine Methyl Ester

A mixture of methyl ester of the protected dipeptide (3·09 g) in acetic acid (12 ml) and 4M-HBr in acetic acid (6 ml) was set aside for 30 min at room temperature. The peptide hydrobromide was precipitated by addition of ether ($E^{Gl}_{2.4}$ 0·88, $E^{His}_{5.7}$ 0·53). The hydrobromide was dissolved in dimethylformamide (15 ml) and the solution adjusted to pH 10 with N-ethylpiperidine. Benzyloxycarbonylphenylalanine 2,4,5-trichlorophenyl ester (2·87 g) was added and the mixture was stirred for 48 h. After evaporation of the dimethylformamide, the residue was triturated successively with water, saturated sodium hydrogen carbonate solution, water and ether. Crystallization from methanol gave 3·4 g (86%) of the tripeptide methyl ester, m.p. 214·5–215°C, $[\alpha]_D$ −14·7° (*c* 0·5, dimethylformamide); R_F 0·87 (S1), 0·50 (S2), 0·83 (S3), 0·83 (S4). For $C_{35}H_{42}N_4O_7S$ (662·6) calculated: 63·43% C, 6·39% H, 8·45% N, 4·84% S; found: 63·24% C, 6·30% H, 8·63% N, 4·94% S.

Benzyloxycarbonyl-S-(2,4,6-trimethylbenzyl)cysteinyl-
-tyrosyl-phenylalanyl-phenylalanyl-asparaginyl-S-(2,4,6-
-trimethylbenzyl)cysteine Methyl Ester (*IIa*)

A solution of the protected tripeptide methyl ester (1·99 g) in acetic acid (10 ml) was mixed with 4M-HBr in acetic acid (5 ml). After standing for 30 min at room temperature, the tripeptide hydrobromide was precipitated with ether and dried ($E_{5.7}^{His}$ 0·53, $E_{2.4}^{Gly}$ 0·88). A 4·6M-HCl solution in dioxane (1·3 ml) was added to a suspension of hydrazide of the protected tripeptide[12] (2·14 g) in dimethylformamide (15 ml) and the mixture was stirred at room temperature to homogeneity. The solution was cooled to −20°C, butyl nitrite (0·31 g) in dimethylformamide (1 ml) was added and the mixture was stirred at this temperature for 20 min. After neutralization with N-ethylpiperidine at −40°C, a solution of the above-described tripeptide methyl ester hydrobromide in dimethylformamide (15 ml), adjusted with N-ethylpiperidine to pH 10, was added and the mixture was allowed to stand for 60 h at 0°C. Dimethylformamide was evaporated, and the residue was washed with water, saturated sodium hydrogen carbonate solution, water, 0·5M-HCl, water, methanol, and ether. Yield 3·2 g (88%) of *IIa*, m.p. 237−238°C (dimethylformamide–water); $E_{2.4}^{Gly}$ 0·80, $E_{5.7}^{His}$ 0·27 (after removal of the benzyloxycarbonyl group); $[\alpha]_D$ −32·8° (*c* 0·5, dimethylformamide). For $C_{66}H_{77}N_7O_{11}S_2$ (1 208) calculated: 65·60% C, 6·42% H, 8·11% N, 5·31% S; found: 65·84% C, 6·13% H, 8·02% N, 5·11% S.

Benzyloxycarbonyl-S-(2,4,6-trimethylbenzyl)cysteinyl-
-tyrosyl-phenylalanyl-phenylalanyl-asparaginyl-S-(2,4,6-
-trimethylbenzyl)cysteine Hydrazide (*IIb*)

Hydrazine hydrate (80%; 2 ml) was added to a solution of the methyl ester *IIa* (2·41 g) in dimethylformamide (20 ml). After standing for 48 h at room temperature, the crude product was precipitated with water, collected on filter and washed with water. Crystallization from dimethylformamide and water gave 2·0 g (83%) of *IIb*, m.p. 251−253°C; $[\alpha]_D$ −40·6° (*c* 0·5, dimethylformamide). For $C_{65}H_{77}N_9O_{10}S_2 . H_2O$ (1 226) calculated: 63·65% C, 6·49% H, 10·28% N, 5·23% S; found: 63·59% C, 6·57% H, 10·11% N, 5·23% S.

N^{α}-*o*-Nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysine *o*-Nitrophenyl Ester

Dicyclohexylammonium salt of N^{α}-*o*-nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysine (6·15 g) was suspended in ethyl acetate (300 ml) and converted into the free acid by treatment with an aqueous solution of potassium hydrogen sulfate and potassium sulfate (pH 2). The obtained solution in ethyl acetate was washed with water, dried and taken down. The residue was dissolved in dimethylformamide (20 ml) and *o*-nitrophenol (3·78 g) was added. After cooling to −10°C and addition of dicyclohexylcarbodiimide (2·06 g), the mixture was stirred for 1 h at −10°C and 12 h at room temperature. The precipitated dicyclohexylurea was filtered off, dimethylformamide evaporated and the residue dissolved in ethyl acetate. The organic solution was extracted successively with 0·1M-NaOH, saturated solution of sodium sulfate, solution of $KHSO_4$ and K_2SO_4 (pH 2), and again with saturated solution of Na_2SO_4. After evaporation of the solvent, the residue was crystallized from toluene and diisopropyl ether to give 4·2 g (79%) of the active ester, m.p. 66−68°C, $[\alpha]_D$ −49·3° (*c* 0·5, dimethylformamide), R_F 0·81 (S1), 0·80 (S3), 0·77 (S4), 0·16 (S5), 0·17 (S19). For $C_{26}H_{26}N_4O_8S$ (554·6) calculated: 56·31% C, 4·73% H, 10·10% N, 5·78% S; found: 56·19% C, 4·86% H, 10·33% N, 5·96% S.

N^{α}-*o*-Nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysine Pentachlorophenyl Ester

N^{α}-*o*-Nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysine was obtained from its dicyclohexylammonium salt (6·15 g) by treatment with a solution of $KHSO_4$ and K_2SO_4 (pH 2). Pentachlorophenol (2·66 g) in ethyl acetate (20 ml) was added to a solution of the protected lysine in ethyl acetate (30 mg). Dicyclohexylcarbodiimide (2·06 g) was added at −10°C and the mixture was stirred at −10°C for 1 h and then at room temperature for 12 h. Dicyclohexylurea was filtered off, washed with ethyl acetate and the filtrate was washed successively with saturated solutions of sodium sulfate, sodium hydrogen carbonate and sodium sulfate, with a $KHSO_4/K_2SO_4$ solution and again with saturated sodium sulfate solution. After drying over sodium sulfate, the ethyl acetate was evaporated, the residue crystallized from ethyl acetate–light petroleum and recrystallized from ethanol, affording 5·6 g (85%) of the active ester, m.p. 98–99°C, $[\alpha]_D$ −34·3° (*c* 1, ethyl acetate); R_F 0·84 (S3), 0·88 (S4), 0·42 (S5), 0·23 (S19). For $C_{26}H_{22}Cl_5N_3O_6S$ (681·8) calculated: 45·80% C, 3·25% H, 6·16% N, 26·00% Cl, 4·70% S; found: 45·62% C, 3·15% H, 6·08% N, 25·97% Cl, 4·79% S.

N^{α}-*o*-Nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysyl-glycine Amide

N-Ethylpiperidine (0·7 ml) and N^{α}-*o*-nitrobenzenesulfenyl-N^{ε}-benzyloxycarbonyllysine (3·2 g) were added with cooling to a solution of glycinamide hydrobromide (0·78 g) in dimethylformamide (15 ml). The mixture was stirred at room temperature, the dimethylformamide evaporated, the residue dissolved in ethyl acetate and the solution purified as described in the preparation of the pentachlorophenyl ester. Crystallization from ethyl acetate and light petroleum gave 2·0 g (84%) of the dipeptide amide, m.p. 142–143°C, $[\alpha]_D$ −12° (*c* 1, acetone), 0° (*c* 2, dimethylformamide); R_F 0·78 (S1), 0·60 (S2), 0·72 (S3), 0·75 (S4); $E_{2.4}^{His}$ 0·67, $E_{5.7}^{His}$ 0·70. For $C_{22}H_{27}N_5O_6S$ (489·6) calculated: 53·98% C, 5·56% H, 14·31% N, 6·55% S; found: 53·72% C, 5·47% H, 14·08% N, 6·48% S.

o-Nitrobenzenesulfenylprolyl-N^{ε}-benzyloxycarbonyllysyl-glycine Amide

The above-described protected dipeptide amide (2·4 g) was dissolved in dimethylformamide (15 ml) and the *o*-nitrobenzenesulfenyl group was removed by addition of 3·45M-HCl in ether (6 ml). The product was precipitated with ether and dried over phosphorus pentoxide and potassium hydroxide. A solution of the obtained hydrochloride in dimethylformamide (15 ml) was adjusted to pH 10 with N-ethylpiperidine and, after addition of *o*-nitrobenzenesulfenylproline 2,4,5-trichlorophenyl ester (2·24 g), the mixture was stirred at room temperature for 20 h. Dimethylformamide was evaporated, the residue dissolved in ethyl acetate and the product purified as described for the preparation of the pentachlorophenyl ester. Crystallization from ethyl acetate and light petroleum afforded 2·3 g (78%) of the protected tripeptide, m.p. 128–130°C; $[\alpha]_D$ −30·7° (*c* 0·6, dimethylformamide); R_F 0·61 (S1), 0·50 (S2), 0·65 (S3), 0·67 (S4); $E_{5.7}^{His}$ 0·51, $E_{2.4}^{Gly}$ 0·84. For $C_{27}H_{34}N_6O_7S$ (586·7) calculated: 55·28% C, 5·84% H, 14·32% N, 5·47% S; found: 55·14% C, 5·98% H, 14·08% N, 5·62% S.

Benzyloxycarbonyl-S-(2,4,6-trimethylbenzyl)cysteinyl-
-tyrosyl-phenylalanyl-phenylalanyl-asparaginyl-S-(2,4,6-
-trimethylbenzyl)cysteinyl-prolyl-N^{ε}-benzyloxycarbonyllysyl-glycine Amide (*IIIb*)

Ethereal 3M-HCl was added to a solution of *o*-nitrobenzenesulfenylprolyl-N^{ε}-benzyloxycarbonyllysyl-glycine amide (352 mg) in dimethylformamide (1 ml). After standing for 4 min at room

temperature, the product was triturated with ether and dried. A solution of the hydrazide *IIb* (483 mg) in dimethylformamide (4 ml) was mixed with 3M-HCl in dioxane (0·26 ml), cooled to −20°C, and butyl nitrite (42 mg) in dimethylformamide (0·5 ml) was added. The mixture was stirred for 25 min at −20°C, cooled to −40°C and neutralized with N-ethylpiperidine. A solution of the above-prepared tripeptide amide hydrochloride in dimethylformamide (2 ml), adjusted to pH 10 with N-ethylpiperidine, was added and the mixture was set aside at 0°C for 70 h. After evaporation of dimethylformamide, the residue was triturated successively with 0·5M-HCl, water, saturated sodium hydrogen carbonate solution and water. Crystallization from dimethylformamide and water afforded 600 mg (93%) of *IIIb*, m.p. 204–206°C; $[\alpha]_D$ −36·3° (*c* 0·5, dimethylformamide). For $C_{86}H_{104}N_{12}O_{15}S_3$ (1 610) calculated: 64·16% C, 6·51% H, 10·44% N, 3·98% S; found: 63·98% C, 6·32% H, 10·28% N, 3·87% S.

Benzyloxycarbonyl-S-(2,4,6-trimethylbenzyl)cysteinyl-
-tyrosyl-phenylalanyl-phenylalanyl-asparaginyl-S-(2,4,6-
-trimethylbenzyl)cysteinyl-prolyl-N^G-*p*-toluenesulfonylarginyl-glycine Amide (*IIIa*)

A solution of benzyloxycarbonylprolyl-N^G-*p*-toluenesulfonylarginyl-glycine amide (418 mg) in acetic acid (1 ml) was mixed with 4M-HBr in acetic acid (1 ml). After standing at room temperature for 30 min, the tripeptide amide hydrochloride was precipitated with ether and dried.

A solution of hexapeptide *IIb* (483 mg) in dimethylformamide (4 ml) was mixed with 3M-HCl in dioxane (0·26 ml), cooled to −20°C and butyl nitrite (0·42 ml) in dimethylformamide (0·5 ml) was added. After stirring at −20°C for 25 min, the mixture was neutralized at −40°C with N-ethylpiperidine and the above-described tripeptide hydrobromide in dimethylformamide (2 ml), adjusted to pH 10 with N-ethylpiperidine, was added. The mixture was allowed to stand at 0°C for 70 h, the dimethylformamide was evaporated and the residue triturated successively with 1M-HCl, water, saturated solution of sodium hydrogen carbonate, and water. Three crystallizations from dimethylformamide–water yielded 632 mg (95%) of *IIIa*, m.p. 201–203°C, $[\alpha]_D$ −31·4° (*c* 0·5, dimethylformamide). For $C_{85}H_{104}N_{14}O_{15}S_3$ (1 658) calculated: 61·58% C, 6·32% H, 11·83% N, 5·80% S; found: 61·42% C, 6·04% H, 11·78% N, 5·69% S.

[4-Phenylalanine, 8-lysine]vasopressin (*Ib*)

Trifluoromethanesulfonic acid (1·6 ml), precooled to 0°C, was added at 0°C to a solution of the protected nonapeptide *IIIb* (200 mg) and thioanisole (160 µl) in trifluoroacetic acid (2·4 ml). After standing for 30 min at 0°C, ether was added, the precipitate collected, washed on filter with ether, and dissolved in water (600 ml). The solution was adjusted to pH 6·8 with 0·1M-NaOH, oxidized with air for 1 h, adjusted to pH 4·4 with concentrated acetic acid and filtered through a column of Amberlite CG-50 (25 ml). The column was washed with 0·2% acetic acid and the crude analogue was eluted with 50% acetic acid. The eluate was freeze-dried and the obtained material (65 mg) was purified by free-flow electrophoresis (U = 2 500 V, I = 148 mA). Fractions, containing the pure analogue, were combined and freeze-dried to give 22·5 mg (17%) of pure *Ib*, $[\alpha]_D$ −15·8° (*c* 0·3, 0·2M-acetic acid); R_F 0·24 (S7), 0·62 (S4), 0·51 (S13), 0·90 (S23); $E^{His}_{5.7}$ 0·71, $E^{His}_{2.4}$ 0·65. Amino acid analysis: Tyr 0·94, Phe 2·02, Asp 1·02, Pro 0·98, Gly 0·98, Lys 1·07, Cys(O_3H) 1·92 (after oxidation). For $C_{50}H_{66}N_{12}O_{11}S_2 . 2 C_2H_4O_2 . 2 H_2O$ (1 171) calculated: 53·32% C, 6·37% H, 14·35% N; found: 53·21% C, 6·18% H, 14·11% N.

[4-Phenylalanine, 8-arginine]vasopressin (*Ia*)

The protected nonapeptide *IIIa* (200 mg) was deblocked and the product oxidized in the same manner as described for the analogue *Ib*. Analogously were performed also the desalting and the

purification by free-flow electrophoresis. Yield 27·1 mg (20%) of pure *Ia*; $[\alpha]_D$ −25·1° (*c* 0·4, 0·2M-acetic acid); $E^{His}_{5.7}$ 0·63, $E^{His}_{2.4}$ 0·62, R_F 0·61 (S4), 0·28 (S7), 0·57 (S13), 0·93 (S23). Amino acid analysis: Phe 2·02, Tyr 0·96, Asp 1·00, Pro 1·02, Arg 0·99, Gly 1·03, $Cys(O_3H)$ 1·98 (after oxidation). For $C_{50}H_{66}N_{14}O_{11}S_2 . 3 C_2H_4O_2 . H_2O$ (1319) calculated: 50·98% C, 6·24% H, 14·86% N; found: 50·77% C, 5·98% H, 15·03% N.

Pharmacological Methods

Uterotonic activity was determined on an isolated strip of rat uterus[21,22]. Galactogogic activity was assayed on ethanol anesthetized rats[23,24], pressor activity on despinalized rats[25] and antidiuretic potency on anesthetized rats[26,27]. Analgesic activity was determined by the tail-flick test[15] on rats.

Spectral Measurements

CD measurements were carried out using an Auto-Dichrographe Mark V (Jobin Yvon, France) driven by the on-line Silex microcomputer. The process of the measurements was controlled by the Dichrosoft program designed in our laboratory[28]. The spectra were recorded in quartz cells with the optical path length of 0·02—1 cm for about $3 . 10^{-4}$ mol l^{-1} solutions in 0·02 mol l^{-1} phosphate buffer (pH 7·5) at ambient temperature. The experimental curves were obtained as averages of four to sixteen single scans (higher number of scans refer to short wavelength region of the spectrum). The data were plotted after manual smoothing.

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