COMPARISON OF THE POTENCY OF 8-L-ARGININE, 8-D-ARGININE AND 8-D-HOMOARGININE VASOPRESSIN ANALOGS WITH SUBSTITUTED PHENYLALANINE IN POSITION 2*

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An analysis of the uterotonic potencies of all analogs having substituted L- or D-tyrosine or -phenylalanine in position 2 and L-arginine, D-arginine or D-homoarginine in position 8 was made. The series of analogs already published was completed by the solid phase synthesis of ten new analogs having L- or D-Phe, L- or D-Phe(2-Et), L- or D-Phe(2,4,6-triMe) or D-Tyr(Me) in position 2 and either L- or D-arginine in position 8. All newly synthesized analogs were found to be uterotonic inhibitors. Deamination increases both the agonistic and antagonistic potency. In the case of phenylalanine analogs the change of configuration from L to D in position 2 enhances the uterotonic inhibition for more than 1 order of magnitude. The L to D change in position 8 enhances the inhibitory potency negligibly. Prolongation of the side chain of the D-basic amino acid in position 8 seems to decrease slightly the inhibitory potency if there is L-substituted amino acid in position 2. On the other hand there is a tendency to the increase of the inhibitory potency if there is D-substituted amino acid in position 2.

Recently we have described the inhibitory qualities of vasopressin analogs** having D-homoarginine in position 8 and substituted phenylalanine in position 2 (refs[2–6]). The considerably high antagonistic potency of these analogs prompted us to analyze which of the carried out modifications (deamination, change of configuration, Har introduction) has the greatest role. At the present time we cannot namely unambiguously say which of these modifications are necessary for inhibitory activity and which are only enhancing the inhibitory potency.

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** All the chiral amino acids unless otherwise stated are of the L-series. The nomenclature and symbols of the amino acids and peptides obey the published recommendations[1]: Phe(2-Et) denotes the 2-ethylphenylalanine, Phe(2,4,6-triMe) the 2,4,6- trimethylphenylalanine and Tyr(Me) the *O*-methyltyrosine.

The analogs described in literature can be divided according to modification in position 2 into two groups. The first one, i.e. tyrosine analogs, having alkoxy substituent and the second one, i.e. phenylalanine analogs, having alkyl substituent. For the above mentioned analysis, there are available in the group of tyrosine analogs six amino- and nine deaminoanalogs (see Table I, refs[3, 4,11–14,17–20]) having mostly D-arginine or D-homoarginine in position 8 and in the group of phenylalanine analogs two analogs with unsubstituted phenylalanine ([Phe^2]AVP in amino and deamino series, refs[10,11]) and 14 analogs with substituted phenylalanine in position 2 and D-homoarginine in position 8 (see refs[2,3,5,6]). All described amino or deamino analogs with unsubstituted L-tyrosine, both with L- and D-basic amino acid in position 8, have intrinsic activity (they are agonists). The comparison of these analogs with *O*-substituted deamino analogs from both AVP and LVP series (see ref.[4]) shows that *O*-substituted tyrosine in position 2 is a sufficient modification for inhibitory activity and that the configuration of tyrosine is not crucial for the inhibitory activity. One analog, [Mpa^1,Tyr(Me)^2,D-Arg^8]VP, having zero agonistic activity (ref.[11]), was not tested for inhibitory activity. On the other hand combination of unsubstituted D-tyrosine in position 2 and of a basic amino acid of D-configuration in position 8 is sufficient for the uterotonic inhibitory activity.

It is evident that there is no difference between amino and deamino analogs as far as the quality of activity in uterus in vitro test is concerned (agonistic or antagonistic activity), but the potency is enhanced by deamination (see the values for amino and deamino analogs, respectively: [Tyr(Me)^2,D-Har^8]VP, pA_2 7.7 and 8.1; [D-Tyr(Me)^2,D-Har^8]VP, pA_2 7.9 and 9.0; [D-Tyr(Et)^2,D-Har^8]VP, pA_2 7.3 and 8.8).

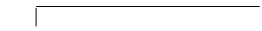
We cannot, however, say anything about the significance of the basic amino acid in position 8 (both configuration and size) and about the significance of phenylalanine substitution in position 2 (in contrast to the tyrosine series, unsubstituted analog with basic D-amino acid in position 8 has not been prepared yet).

To estimate the role of D-homoarginine, i.e. the effect of the prolongation of the side chain in position 8, on the selectivity and potency of vasopressin analogs in uterus in vitro test, we have synthesized eight new analogs with D-arginine in position 8. We have chosen analogs having in D-homoarginine series the strongest inhibitory activity in vitro ([Mpr^1,D-Tyr(Me)^2,D-Har^8]VP, [D-Phe(2-Et)^2,D-Har^8]VP) and in vivo ([D-Phe(2,4,6-triMe)^2,D-Har^8]VP). The inhibitory activity of the tyrosine analog is indubitable, however the question is the magnitude of the pA_2. In the phenylalanine series it was necessary to synthesize for completeness two analogs having unsubstituted phenylalanine and L- or D-arginine in position 8.

Ten analogs of vasopressin ($I - X$) described in this paper having L- or D-arginine in position 8 and unsubstituted or diversely substituted phenylalanine in position 2 were synthesized in the same manner as the 8-D-homoarginine vasopressin analogs[2–6].

The syntheses of analogs with L-arginine were performed by usual solid phase technique on *p*-methylbenzhydrylamine resin with 4-[(α-(9-fluorenylmethoxycarbonyl-

amino)-2′,4′-dimethoxybenzyl]phenoxyacetic acid linker[21]. As the α-amino group protection we have used fluorenylmethoxycarbonyl (Fmoc) group. For the side chain protection we have used a 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Arg) or -trityl (Cys) group.



I,	X = Cys;	Y = L-Phe(2-Et);	Z = L-Arg
II,	X = Cys;	Y = D-Phe(2-Et);	Z = L-Arg
III,	X = Cys;	Y = L-Phe(2-Et);	Z = D-Arg
IV,	X = Cys;	Y = D-Phe(2-Et);	Z = D-Arg
V,	X = Cys;	Y = L-Phe(2,4,6-triMe);	Z = D-Arg
VI,	X = Cys;	Y = D-Phe(2,4,6-triMe);	Z = D-Arg
VII,	X = Mpa;	Y = D-Tyr(Me);	Z = D-Arg
VIII,	X = Cys;	Y = L-Phe;	Z = D-Arg
IX,	X = Cys;	Y = D-Phe;	Z = D-Arg
X,	X = Cys;	Y = D-Phe;	Z = L-Arg

Fmoc-DL-2-ethylphenylalanine was prepared from DL-2-ethylphenylalanine[5]. Protected amino acids were coupled by *N,N′*-diisopropylcarbodiimide (DIC) in the presence of *N*-hydroxybenzotriazole (HOBt) in dimethylformamide. Acid labile side chain protecting groups (Pmc, Trt) were cleaved simultaneously with the cleavage of the peptide from the resin by trifluoroacetic acid.

The syntheses of analogs with D-arginine were performed by the solid phase technique on *p*-methylbenzhydrylamine resin. A *tert*-butoxycarbonyl group or fluorenylmethoxycarbonyl group (in the case of analogs *VIII* and *IX*) were used for the α-amino group protection. For the side chain protection either tosyl group (D-Arg) or 4-methylbenzyl group (Cys, Mpa) was used. N^{α}-*tert*-Butoxycarbonyl-N^{G}-tosyl-D-arginine was prepared by modification (protection of amino group by di-*tert*-butyl dicarbonate) of the described method[22]. N^{α}-*tert*-Butoxycarbonyl-*O*-methyl-D-tyrosine was prepared using dimethyl sulfate for alkylation of Boc-D-Tyr-OH. The protected amino acids were then coupled by *N,N′*-dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) in dimethylformamide. Side chain protecting group removal was performed simultaneously with the cleavage of the peptide from the resin by liquid hydrogen fluoride.

Sulfhydryl group oxidation was performed by potassium ferricyanide. Because of easy separability of diastereoisomeric peptides by means of RP HPLC (refs[2–6,23–25]), the syntheses of analogs *I – VI* were performed using racemic amino acids D,L-2-ethylphenylalanine or D,L-2,4,6-trimethylphenylalanine. Peptides containing the appropriate diastereoisomers were separated at the end of the preparation. Formation of both diastereoisomers was accomplished using only 1.1 equivalents of protected racemic amino

acid (*tert*-butoxycarbonyl-D,L-2-ethylphenylalanine or *tert*-butoxycarbonyl-D,L-2,4,6-trimethylphenylalanine) (see ref.[2]). Chirality of the amino acid in the pure peptide was determined in hydrolysates either by digestion, using L-amino acid oxidase[26,27] (digestion time 100 h), or on chiral TLC plates[28]. In HPLC on reverse phase determined *k* value was in both cases lower for the L-diastereoisomer, which is consistent with previous findings[2–6,23,24].

Analogs containing L-amino acid in position 2 are significantly more basic (electrophoresis in pyridine–acetate buffer, pH 5.7) than those containing D-amino acid (see[4–6] and compare[27,28]).

Biological activities of the analogs are given in Table I. All the analogs were found to be antagonists of oxytocin-evoked uterine contractions. Earlier publications have reported that the combination of D-amino acids in both positions, i.e. position 2 and 8, results generally in more potent inhibitors than analogs having D-amino acid in position 8 only. The analogs prepared in this study confirm that finding. Comparison of analogs with D-arginine and D-homoarginine in position 8 shows, that in analogs with L-substituted amino acid in position 2 prolongation of the side chain by CH_2 group (D-Arg→ D-Har) slightly reduced the magnitude of inhibitory activity (see *III* and *V* and corresponding D-Har analogs), but in analogs with D- substituted amino acid in position 2 the same change tends to increase the inhibitory potency (see *IV* and *VI* and corresponding D-Har analogs).

From previously published data it is also evident, that all amino and deamino analogs with *O*-substituted D-tyrosine in position 2 are pressor inhibitors. On the other hand analogs with *O*-substituted L-tyrosine have very low agonistic activity or no activity at all with the exception of [Tyr(Et)^2,D-Har^8]VP. The analog *VII* prepared in this paper has low pressor inhibitory activity. The analogs with substituted D-phenylalanine in position 2 which have inhibitory activity are always stronger inhibitors than analogs with substituted L-phenylalanine in position 2. The prolongation of the side chain of the D-basic amino acid in position 8 usually decreases the inhibitory potency thus making the D-homoarginine analogs more selective for the uterotonic inhibition.

The prepared analogs were tested also for galactogogic activity. The ones having D-amino acid in position 2 were found to be inhibitors (*IV*, pA_2 6.6; *VI*, pA_2 6.7; *VII*, pA_2 7.3), the ones having L-amino acid (*III* and *V*) had no activity.

Comparison of uterotonic activities of analogs with D-basic amino acid in position 8 and substituted tyrosine in position 2 summarized in Table I clearly shows that *O*-alkylation of hydroxyl group of L-tyrosine leads to the change of agonist into antagonist and the change of D-arginine for D-homoarginine may lead to the enhancement of the inhibitory potency.

In the case of the D-tyrosine analogs having basic amino acid in position 8 (the analogs that have already inhibitory qualities), the *O*-alkylation seems to lead to the increase of inhibitory potency, as well as the change from D-Arg to D-Har.

TABLE I
Biological activities (rat) of arginin vasopressin analogs (I.U./mg or pA_2) with modifications in position 2

Compound	Activity		
	uterotonic (in vitro)	pressoric	ref.
AVP	17	412	7
[D-Arg^8]VP	0.4	4.1	8
[D-Har^8]VP	–	0.83	9
	0.9	–	2
[Phe^2]AVP	~0.2	122	10
[D-Phe^2]AVP, (*X*)	$pA_2 = 7.3$	12.8	*b*
[Phe^2,D-Arg^8]VP, (*VIII*)	$pA_2 = 6.0$	0^a	*b*
[D-Phe^2,D-Arg^8]VP, (*IX*)	$pA_2 = 7.4$	$pA_2 = 6.6$	*b*
[L-Phe(2-Et)^2]AVP, (*I*)	$pA_2 = 6.7$	0^a	*b*
[L-Phe(2-Me)^2,D-Har^8]VP	$pA_2 = 6.4$	$pA_2 < 6.0$	5
[D-Phe(2-Me)^2,D-Har^8]VP	$pA_2 = 7.8$	0^a	5
[L-Phe(2-Et)^2,D-Arg^8]VP, (*III*)	$pA_2 = 7.0$	0^a	*b*
[L-Phe(2-Et)^2,D-Har^8]VP	$pA_2 = 6.8$	0^a	5
[D-Phe(2-Et)^2]AVP, (*II*)	$pA_2 = 7.8$	$pA_2 = 7.2$	*b*
[D-Phe(2-Et)^2,D-Arg^8]VP, (*IV*)	$pA_2 = 8.1$	$pA_2 = 6.57$	*b*
[D-Phe(2-Et)^2,D-Har^8]VP	$pA_2 = 8.4$	$pA_2 = 5.6$	5
[L-Phe(2,4,6-triMe)^2,D-Arg^8]VP, (*V*)	$pA_2 = 6.7$	$pA_2 = 6.7$	*b*
[LPhe(2,4,6-triMe)^2,DHar^8]VP	$pA_2 = 6.1$	$pA_2 = 5.8$	6
[D-Phe(2,4,6-triMe)^2,D-Arg^8]VP, (*VI*)	$pA_2 = 7.9$	$pA_2 = 7.1$	*b*
[D-Phe(2,4,6-triMe)^2,D-Har^8]VP	$pA_2 = 8.1$	$pA_2 = 6.1$	6
[L-Phe(4-Me)^2,D-Har^8]VP	$pA_2 = 6.85$	0.04	2
[D-Phe(4-Me)^2,D-Har^8]VP	$pA_2 = 7.78$	0.04	2
[L-Phe(4-Et)^2,D-Har^8]VP	$pA_2 = 7.4$	$pA_2 = 6.5$	2
[D-Phe(4-Et)^2,D-Har^8]VP	$pA_2 = 8.15$	$pA_2 = 6.5$	2
[Mpa^1,Phe^2]AVP	0.3	29	11
[Mpa^1,L-Phe(4-Me)^2,D-Har^8]VP	$pA_2 = 7.50$	$pA_2 = 6.2$	3
[Mpa^1,D-Phe(4-Me)^2,D-Har^8]VP	$pA_2 = 8.20$	0^a	3
[Mpa^1,L-Phe(4-Et)^2,D-Har^8]VP	$pA_2 = 8.00$	$pA_2 = 6.2$	3
[Mpa^1,D-Phe(4-Et)^2,D-Har^8]VP	$pA_2 = 8.30$	$pA_2 = 6.35$	3

TABLE I
(*Continued*)

Compound	Activity		
	uterotonic (in vitro)	pressoric	ref.
[D-Tyr^2]AVP	1.53	194	12
	0.01	39.5	13
[Tyr(Me)^2]AVP	pA_2 = 7.4	9.7	14
[Tyr(Me)^2,D-Har^8]VP	pA_2 = 7.7	0.04	4
[Tyr(Et)^2,D-Har^8]VP	pA_2 = 6.9	pA_2 = 6.8	4
[D-Tyr(Me)^2,D-Har^8]VP	pA_2 = 7.9	pA_2 = 6.9	4
[D-Tyr(Et)^2,D-Har^8]VP	pA_2 = 7.3	pA_2 = 7.2	4
[Mpa^1]AVP	27	370	15
	63	346	16
[Mpa^1,D-Arg^8]VP	5.1	11	17
[Mpa^1,D-Har^8]VP	–	0.06	9
	2	0.5	18
	2	0.05	19
	0.8	0.28	3
[Mpa^1,Tyr(Me)^2,D-Arg^8]VP	0	0	11
[Mpa^1,Tyr(Et)^2,D-Arg^8]VP	pA_2 = 6.6	–	20
[Mpa^1,Tyr(Me)^2,D-Har^8]VP	pA_2 = 8.1	0[a]	4
[Mpa^1,D-Tyr^2]AVP	0.045	483	13
[Mpa^1,D-Tyr^2,D-Arg^8]VP	pA_2 = 7.9	0.1	13
[Mpa^1,D-Tyr(Me)^2,D-Arg^8]VP, (*VII*)	pA_2 = 8.23	pA_2 = 6.7	[b]
[Mpa^1,D-Tyr(Me)^2,D-Har^8]VP	pA_2 = 9.0	pA_2 = 6.9	4
[Mpa^1,D-Tyr(Et)^2,D-Har^8]VP	pA_2 = 8.8	pA_2 = 7.0	4

[a] 0 means inactive up to the dose $2 . 10^{-2}$ mg/rat; [b] this paper.

In the case of phenylalanine analogs, already the analog having L-basic amino acid in position 8 is an antagonist if D-phenylalanine is in position 2 (both unsubstituted or substituted). The L to D change in position 2 as well as in position 8 enhances the inhibitory effect.

EXPERIMENTAL

General methods: Thin-layer chromatography (TLC) was carried out on silica gel coated plates (Silufol, Kavalier, The Czech Republic) in the following systems: 1-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 6, S4), petroleum ether–ethyl acetate (1 : 1, S22). Paper electrophoresis was performed in a moist chamber in 1 M acetic acid (pH 2.4) and in pyridine–acetate buffer (pH 5.7) on Whatmann 3MM paper at 20 V/cm for 60 min. Spots in the TLC and electrophoresis were developed with ninhydrin or by the chlorination method. Samples for amino acid analysis were hydrolyzed with 6 M HCl at 105 °C for 20 h using a mixture of propionic acid–hydrochloric acid (1 : 1) at 160 °C for 15 min, and analyzed on Amino Acid Analyzer T 339 (Mikrotechna Praha,) or D-500 analyzer (Durrum, U.S.A.). Optical rotations were determined on Perkin–Elmer instrument type 141 MCA (Norwalk, U.S.A.) at room temperature. Fast atom bombardment mass spectra were obtained on a ZAB-EQ spectrometer (VG Analytical, Manchester, U.K.) at 8 kV with xenon as the bombarding gas. High performance liquid chromatography (HPLC) was carried out on SP-8800 instrument equipped with SP-8450 detector and SP-4290 integrator (all from Spectra Physics, Santa Clara, U.S.A.). Preparative HPLC was carried out using column Vydac 218TP-510 (5 μm, 250 × 10 mm). Purity of the products was determined on the column of Vydac 218TP54. Before use, all amino acid derivatives were subjected to the ninhydrin test[31].

Solid Phase Peptide Synthesis

Incorporation of each amino acid residue into the growing peptide chain consisted of the cycles described below. The synthesis was monitored using the bromophenol blue method[32].

Fmoc method cycle. 1) Cleaving the Fmoc group by 20% piperidine in dimethylformamide; *2*) washing with dimethylformamide; *3*) washing with dichloromethane; *4*) washing with dimethylformamide; *5*) adding the Fmoc-protected amino acid derivative in dimethylformamide followed by HOBt and by DIC; *6*) washing with dimethylformamide.

Boc-method cycle. 1) Cleaving the Boc group by 50% trifluoroacetic acid in dichloromethane containing 5% anisole, 5 min and 30 min; *2*) washing with dichloromethane, 2-propanol and dichloromethane; *3*) neutralizing by 5% diisopropylethylamine in dichloromethane, 2 and 5 min; *4*) washing with dichloromethane and dimethylformamide; *5*) adding the Boc-protected amino acid derivative in dimethylformamide followed by HOBt, followed by DCC and stirring for 1 – 2.5 h; *6*) washing with dimethylformamide, dichloromethane, 2-propanol and dichloromethane.

N^{α}-Fluorenyloxycarbonyl-D,L-2-ethylphenylalanine

The D,L-2-ethylphenylalanine[5] (0.6 g; 3 mmol) was suspended in the mixture of water (50 ml) and 10% Na_2CO_3 (pH 8.5 – 9) and Fmoc-ONSu (1.1 g; 3.3 mmol) in acetone (50 ml) was added. After stirring for 2 h at room temperature acetone was evaporated, the residue was extracted with ether, acidified to pH 2.0 with 1 M HCl (20 ml) and the product was extracted into ethyl acetate. Extract was washed successively with saturated solution of $KHSO_4$, saturated solution of Na_2SO_4, water and dried with sodium sulfate, filtered and evaporated. The residue was crystallized. The crystals were filtered and washed with ethyl acetate and petroleum ether. Yield 1.08 g (87%), m.p. 159 – 161 °C; R_F 0.38 (S22); HPLC: *k* 4.38; methanol–0.05% trifluoroacetic acid 7 : 3 (v/v). For $C_{26}H_{25}NO_4$ (415.5) calculated: 75.16% C, 6.06% H, 3.37% N; found: 75.21% C, 6.12% H, 3.30% N. FAB MS (*m*/*z*): 416.3 ($M + H^+$).

Fmoc-Nonapeptide Resin.

p-Methylbenzhydrylamine resin (Peptides International, 0.92 mol/g, 0.75 g) was suspended in dichloromethane and after washing with 5% diisopropylethylamine in dichloromethane and with dimethylformamide was coupled with 4-[α-9-(fluorenylmethoxycarbonylamino)-2′,4′-dimethoxybenzyl] phenoxyacetic acid linker (2 molar excess) in the presence of *N*-hydroxybenzotriazole and diisopropylcarbodiimide in dimethylformamide. Coupling was finished after 2 h and the resin substitution (0.57 mmol/g) was determined by UV spectroscopy[33]. Fmoc-Amino acids were coupled to the resin by the DIC/HOBt procedure as described above. All reagents used were in 3 molar excess. Protected derivatives were used in the following order: Fmoc-Gly-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asn-OH, Fmoc-Gln-OH, Fmoc-Phe-OH, Fmoc-D,L-Phe(2-Et)-OH (two equivalents) or Fmoc-D-Phe-OH and Fmoc-Cys(Trt)-OH.

Boc-Nonapeptide Resin A

p-Methylbenzhydrylamine resin (Peptides International, 0.92 mol/g, 0.5 g) was suspended in dichloromethane and after washing with 5% diisopropylethylamine in dichloromethane and with dimethylformamide Fmoc-Gly-OH was coupled (3 molar excess) in the presence of *N*-hydroxybenzotriazole and diisopropylcarbodiimide in dimethylformamide. Coupling was finished after 2 h and the resin substitution (0.78 mmol/g) was determined by UV spectroscopy[33]. The polymer was then acetylated (5 ml acetanhydride, 2 ml triethylamine in 50 ml dichloromethane). Protected amino acids were coupled using cycles described above in the following order: Boc-D-Arg(Tos)-OH (TFA was used for deprotection), Fmoc-Pro-OH, Fmoc-Cys(4-MeBzl)-OH, Fmoc-Asn-OH, Fmoc-Gln-OH and Fmoc-Phe-OH. Following this step the resin was divided into two equal portions. The portions were then coupled according to the general scheme with either Fmoc-L-Phe-OH or Fmoc-D-Phe-OH and with Boc-Cys(4-MeBzl)-OH (ref.[34]).

Boc-Nonapeptide Resin B

p-Methylbenzhydrylamine resin (Peptides International, 0.79 mol/g, 1.5 g) was suspended in dichloromethane. After washing with 5% diisopropylethylamine in dichloromethane and with dimethylformamide, it was coupled with of Boc-Gly-OH (3 molar excess) in the presence of *N*-hydroxybenzotriazole and dicyclohexylcarbodiimide in dimethylformamide. The coupling reaction was interrupted after 2 h and the resin was washed consequently by dimethylformamide (3 times) and dichloromethane (3 times). The resin substitution was determined by amino acid analysis (0.33 mmol/g). The polymer was then acetylated (5 ml acetanhydride, 2 ml triethylamine in 50 ml dichloromethane). Boc-Amino acids were coupled to the resin by the DCC/HOBt procedure as described above (Boc-method cycle). All reagents were used in 3 molar excess. The protected derivatives were used in the following order: Boc-D-Arg(Tos)-OH, Boc-Pro-OH, Boc-Cys(4-MeBzl)-OH (ref.[34]), Boc-Asn-OH, Boc-Gln-OH and Boc-Phe-OH. Amino acid analysis on the resin: Asp 1.03, Glu 1.08, Pro 1.20, Gly 1.06, Cys 0.50, Phe 0.90, Arg 0.93. Following this step the resin was divided into three equal portions. The portions were then coupled according to the general scheme with either Boc-D,L-Phe(2-Et)-OH (ref.[5]) or Boc-D,L-Phe(2,4,6-triMe)-OH (ref.[6]) or Boc-D-Tyr(Me)-OH and with Boc-Cys(4-MeBzl)-OH (ref.[34]).

Cleavage of the Peptides from the Resins

A. After deprotection of Fmoc group, the nonapeptide resin was treated 4 h (2 × 5 ml) with the mixture of trifluoroacetic acid, thioanisol, thiophenol and water (93.5 : 2.5 : 1.5 : 2.5). The resin was

TABLE II
Physico-chemical and analytical data for analogs *I* – *X*

Compound	Yield %/mg	*k*	R_F S4	$E^{Gly}_{2.4}/E^{His}_{5.7}$	$[\alpha]_D$[a]	FAB MS[b]
I	25/56.1	0.76[c]	0.46	0.94/0.55	–22.0	1 096.0
II	21/48.2	1.65[c]	0.48	0.94/0.50	–55/8	1 096.0
III	27/25.0	0.90[c]	0.45	0.98/0.53	–4.6	1 096.1
IV	23/21.5	1.98[c]	0.48	0.95/0.45	–37.2	1 096.1
V	35/33.0	0.71[d]	0.47	0.92/0.60	–4.4	1 110.2
VI	28/26.4	1.60[d]	0.48	0.88/0.45	–99.1	1 110.2
VII	54/98.8	1.14[c]	0.51	0.81/0.31	–50.4	1 083.0
VIII	24/50.0	0.96[e]	0.42	1.00/0.56	–6.6	1 068.7
IX	25/53.0	2.39[e]	0.43	0.95/0.55	–31.8	1 068.7
X	18/36.8	1.80[e]	0.44	0.90/0.47	–60.7	1 068.7

[a] (*c* 0.1; 1 M AcOH); [b] (*m*/*z*) for $M + H^+$; [c] methanol–0.05% trifluoroacetic acid (5 : 5), Vydac; [d] methanol–0.05% trifluoroacetic acid (5.5 : 4.5), Vydac; [e] methanol–0.05% trifluoroacetic acid (4 : 6), Vydac.

TABLE III
Amino acid analysis for analogs *I* – *X*

Amino acid	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*
Asp	1.00	0.95	1.00	0.99	1.00	0.97	0.94	0.99	1.01	0.99
Glu	1.03	0.92	1.05	1.09	1.05	0.86	1.04	1.03	1.03	0.88
Pro	1.00	0.95	1.12	1.20	1.12	1.06	0.85	0.96	1.00	1.02
Gly	1.00	1.05	0.9	1.06	0.96	1.02	1.05	1.03	1.01	0.99
Cys	1.32	1.25	1.12	1.76	1.22	1.21	0.48	1.33	1.15	1.15
Phe	0.99	1.01	0.94	1.06	0.94	0.99	1.05	1.94	2.01	1.93
Phe(OEt)	1.00	1.10	0.82	0.87	–	–	–	–	–	–
Phe(triMe)	–	–	–	–	1.00	1.14	–	–	–	–
Tyr	–	–	–	–	–	–	0.93	–	–	–
Arg	1.00	0.95	1.07	0.91	0.93	0.95	1.07	1.00	0.96	1.01

filtered off and filtrate was evaporated. The residue was triturated with ether, decanted and the precipitate was resolved in 3 M acetic acid and then lyophilized.

B. After removal of the Boc-protecting group, the nonapeptide resin was treated with liquid hydrogen fluoride (15 ml, 60 min, 0 °C) in the presence of anisole (1.5 ml). After the evaporation of hydrogen fluoride, the nonapeptide together with the resin was triturated with ether, filtered, and washed with ethyl acetate. The free peptide was successively extracted with acetic acid, 50% acetic acid and water, and then lyophilized.

Oxidation and Purification of Analogs *I* – *X*

The linear peptide was dissolved in water (200 ml) and the pH of the solution was adjusted with 0.1 M NaOH to 7.0. Potassium ferricyanide (0.01 M solution) was added to this solution until a stable yel-

TABLE IV
Elemental analyses of analogs *I* – *X*

Compound	Formula	M.w.	Calculated/Found		
			% C	% H	% N
I	$C_{48}H_{69}N_{15}O_{11}S_2$. 3 TFA . 2 H_2O	1 474.4	43.99	5.19	14.25
			43.84	5.00	14.21
II	$C_{48}H_{69}N_{15}O_{11}S_2$. 3 TFA . 3.5 H_2O	1 501.4	43.20	5.30	13.98
			42.79	4.78	13.64
III	$C_{48}H_{69}N_{15}O_{11}S_2$. 3 TFA . 2 H_2O	1 474.4	43.99	5.19	14.25
			43.91	5.01	14.41
IV	$C_{48}H_{69}N_{15}O_{11}S_2$. 3 TFA . 3 H_2O	1 492.4	43.45	5.26	14.07
			43.30	4.95	14.09
V	$C_{49}H_{71}N_{15}O_{11}S_2$. 3 TFA . 3.5 H_2O	1 515.5	43.59	5.38	13.85
			43.25	5.01	13.85
VI	$C_{49}H_{71}N_{15}O_{11}S_2$. 3 TFA . 4.5 H_2O	1 533.5	43.08	5.46	13.69
			42.80	4.93	13.66
VII	$C_{47}H_{66}N_{14}O_{12}S_2$. 2 TFA . 4 H_2O	1 383.4	44.27	5.53	14.18
			44.18	5.07	13.89
VIII	$C_{46}H_{65}N_{15}O_{11}S_2$. 3 TFA . 4 H_2O	1 482.4	42.13	5.17	14.17
			42.37	4.71	13.84
IX	$C_{46}H_{65}N_{15}O_{11}S_2$. 3 TFA . 4 H_2O	1 482.4	42.13	5.17	14.17
			42.09	4.78	13.82
X	$C_{46}H_{65}N_{15}O_{11}S_2$. 3 TFA . 3 H_2O	1 464.4	42.65	5.09	14.35
			42.90	4.90	13.94

low color persisted. During the oxidation (20 min), the pH was maintained at 7.2 by adding 0.1 M NaOH and then adjusted with acetic acid to pH 4.5. The solution was then put on a column of Amberlite CG-50I (15 ml), which was washed with 0.25% acetic acid and the product eluted with 50% acetic acid (50 ml). After freeze-drying, the crude product was purified by HPLC on a Vydac 218TP510 column in the gradient running from 30% to 50% methanol in 0.05% trifluoroacetic acid for 60 min (analogs *I*, *II*, *V* and *VI*) or from 25% to 50% methanol in 0.05% trifluoroacetic acid for 50 min (analogs *III*, *IV* and *VII*) or from 20% to 50% methanol in 0.05% trifluoroacetic acid for 60 min (analogs *VIII*, *IX* and *X*) and lyophilized.

Yields, *k*, R_F, electrophoretic mobility, optical rotation and FAB MS are given in Table II, results of amino acid analyses in Table III and that of elemental analysis in Table IV.

Pharmacological Methods

All pharmacological tests were performed using Wistar rats weighing 200 – 300 g. The uterotonic potency in vitro was evaluated using the Holton procedure[35] in Munsick[36] solution. Inhibitory activity was characterized by the pA_2 value[37]. Pressor activity was tested on pithed rat according to refs[38,39].

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REFERENCES

1. *IUPAC-IUB Joint Commission on Biochemical Nomenclature*. Eur. J. Biochem. *138*, 9 (1984).
2. Zertova M., Prochazka Z., Blaha I., Barth T., Slaninova J., Maletinska L., Lebl M.: Collect. Czech. Chem. Commun. *55*, 3000 (1990).
3. Zertova M., Prochazka Z., Barth T., Slaninova J., Skopkova J., Blaha I., Lebl M.: Collect. Czech. Chem. Commun. *56*, 1761 (1991).
4. Zertova M., Prochazka Z., Slaninova J., Skopkova J., Barth T., Lebl M.: Collect. Czech. Chem. Commun. *57*, 604 (1992).
5. Zertova M., Prochazka Z., Slaninova J., Barth T., Majer P., Lebl M.: Collect. Czech. Chem. Commun. *57*, 1103 (1992).
6. Zertova M., Prochazka Z., Slaninova J., Barth T., Majer P., Lebl M.: Collect. Czech. Chem. Commun. *58*, 2751 (1993).
7. Jost K., Lebl M., Brtnik F.: *CRC Handbook of Neurohypophyseal Hormone Analogs*, Vol. II, Part 2. CRC Press, Boca Raton 1987.
8. Zaoral M., Kolc J., Sorm F.: Collect. Czech. Chem. Commun. *31*, 382 (1966).
9. Lindeberg E. G. G., Melin P., Larsson L. E.: Int. J. Pept. Protein Res. *8*, 193 (1976).
10. Huguenin R. L., Boissonnas R. A.: Helv. Chim. Acta *45*, 1629 (1962).
11. Krchnak V., Zaoral J.: Collect. Czech. Chem. Commun. *44*, 1642 (1979).
12. Hruby V. J., Upson D. A., Yamamoto D. M., Smith C. W., Walter R.: J. Am. Chem. Soc. *101*, 2717 (1979).
13. Zaoral J.: Int. J. Pept. Protein. Res. *26*, 561 (1985).

14. Bankowski K., Manning M., Haldar J., Sawyer W. H.: J. Med. Chem. *21*, 850 (1978).
15. Zaoral M., Kolc J., Sorm F.: Collect. Czech. Chem. Commun. *32*, 1250 (1967).
16. Huguenin R. L., Boissonnas R. A.: Helv. Chim. Acta *49*, 695 (1966).
17. Manning M., Balaspiri L., Moehring J., Haldar J., Sawyer W. H.: J. Med. Chem. *19*, 842 (1976).
18. Brtnik F.: *Ph. D. Thesis*. Institute of Organic Chemistry and Biochemistry, Prague 1976.
19. Zaoral M., Brtnik F.: Collect. Czech. Chem. Commun. *40*, 905 (1975).
20. Pliska V.: *8th Am. Pept. Symp.*, Tucson 1983. Abstr. 5-21.
21. Atherton E., Sheppard R. C.: *Solid Phase Peptide Synthesis, A Practical Approach*, p. 63. IRL Oxford University Press, Oxford 1989.
22. Holton H. H., Branda L. A., Ferrier B. M.: Can. J. Chem. *51*, 1910 (1973).
23. Larsen B., Fox B. L., Burke M. F., Hruby V. J.: Int. J. Pept. Protein Res. *13*, 12 (1979).
24. Lebl M.: J. Chromatogr. *264*, 459 (1983).
25. Lebl M., Barth T., Servitova L., Slaninova J., Jost K.: Collect. Czech. Chem. Commun. *50*, 132 (1985).
26. Riniker B., Schwyzer R.: Helv. Chim. Acta *44*, 658 (1961).
27. Riniker B., Schwyzer R.: Helv. Chim. Acta *47*, 2357 (1964).
28. Toth G., Lebl M., Hruby V. J.: J. Chromatogr. *504*, 450 (1990).
29. Blumenstein M., Hruby V. J., Yamamoto D. M., Yang Y. C. S.: FEBS Lett. *81*, 347 (1977).
30. Hruby V. J., Deb K. K., Spatola A. F., Upson D. A., Yamamoto D. M.: J. Am. Chem. Soc. *101*, 202 (1979).
31. Kaiser E., Colescott R. L., Bossinger C. D., Cook P. I.: Anal. Biochem. *34*, 595 (1970).
32. Krchnak V., Vagner J., Safar P., Lebl M.: Collect. Czech. Chem. Commun. *53*, 2542 (1988).
33. Chang C. D., Felix A. M., Jimenez M. H., Meienhofer J.: Int. J. Pept. Protein Res. *15*, 485 (1980).
34. Cosand W. L., Merrifield R. B.: Proc. Natl. Acad. Sci. U.S.A. *74*, 2771 (1977).
35. Holton P.: Br. J. Pharmacol. *3*, 328 (1984).
36. Munsick R. A.: Endocrinology *66*, 451 (1960).
37. Schild H. O.: Br. J. Pharmacol. *2*, 189 (1947).
38. Shipley R. E., Tilden J. H.: Proc. Soc. Exp. Biol. N.Y. *64*, 453 (1947).
39. Krejci I., Kupkova B., Vavra I.: Br. J. Pharmacol. Chemother. *30*, 497 (1967).

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