AMINO AND DEAMINO ANALOGS OF 8-D-HOMOARGININ--VASOPRESSIN WITH MODIFIED TYROSINE IN POSITION 2: SYNTHESIS AND SOME BIOLOGICAL PROPERTIES*

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Solid phase methodology on benzhydrylamine or *p*-methylbenzhydrylamine resin was used for the synthesis of seven analogs of amino or deamino vasopressin with non-coded amino acid, D-homoarginine, in position 8 and D- or L- O-methyl or O-ethyl tyrosine in position 2. [L-Tyr(Me)^2, D-Har^8]vasopressin (*I*), [D-Tyr(Me)^2, D-Har^8]vasopressin (*II*), [L-Tyr(Et)^2, D-Har^8]-vasopressin (*III*), [D-Tyr(Et)^2, D-Har^8]vasopressin (*IV*), [Mpr^1, L-Tyr(Me)^2, D-Har^8]-vasopressin (*V*), [Mpr^1, D-Tyr(Me)^2, D-Har^8]vasopressin (*VI*) and [Mpr^1, D-Tyr(Et)^2, D-Har^8]vasopressin (*VII*) were synthesized. All analogs have very low antidiuretic activity. Analogs containing O-methyltyrosine of D-configuration or O-ethyltyrosine of both D- and L-configuration are low pressor inhibitors. All analogs were found to be uterotonic inhibitors, the most potent one in vitro being [Mpr^1, D-Tyr(Me)^2, D-Har^8]vasopressin (*VI*) with $pA_2 = 9{\cdot}0$ and [Mpr^1, D-Tyr(Et)^2, D-Har^8]vasopressin (*VII*) with $pA_2 = 8{\cdot}8$.

Basicity in the side chain of the amino acid in position 8 is very important for vasopressin-like activities (for the recent reviews on structure–activity relationship in neurohypophysial hormone analogs see e.g. refs[1-3]). Surprisingly, the basic amino acid is also advantageous in the design of uterotonic inhibitors. Up to now synthesized potent inhibitors of oxytocin in the in vitro and in vivo uterotonic test derived from oxytocin molecule had bulky substituents on the β-carbon in position 1 or the D-configuration of a substituted aromatic amino acids in position 2 or both modifications together. The inhibitory potency of these analogs is enhanced if the basic amino acid is present in position 8.

Literature search suggests that for uterotonic inhibitors derived from vasopressin molecule (i.e. Phe in position 3) the presence of bulky substituents on the β-carbon in position 1, has been accepted not only as a sufficient condition (see [Cpp^1,Arg^8]-vasopressin or [Pen^1,Lys^8]vasopressin** with $pA_2 = 8{\cdot}15$, and 6·60 in vitro resp.),

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** All the chiral amino acids, mentioned in this work, are of the L-series. The nomenclature and symbols of the amino acids and peptides obey the published recommendations[4]: Har denotes the homoarginine moiety.

but also as a necessary condition[5]. However, several vasopressin analogs with uterotonic inhibitory activity not containing bulky substituent on the β-carbon of cysteine in position 1 were synthesized (see Table I). It is very probable that a number of recently prepared analogs exhibiting inhibitory activity in pressor and antidiuretic tests (see e.g. ref.[6]) would be found to be potent uterotonic inhibitors; however, they were not evaluated in this test. It was shown that the sufficient condition for inhibiting

TABLE I

Uterotonic activities (rat in vitro) of amino and deamino vasopressin analogs with the modification in the position 2

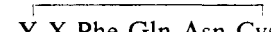
Compound	Position 1	Position 2	Position 8	Uterotonic activity[a]
AVP	Cys	Tyr	Arg	17
[D-Arg^8]VP	Cys	Tyr	D-Arg	0·4
[D-Har^8]VP	Cys	Tyr	D-Har	0·9
[D-Lys^8]VP	Cys	Tyr	Lys	1
[Mpr^1]AVP	Mpr	Tyr	Arg	63
[Mpr^1, D-Arg^8]VP	Mpr	Tyr	D-Arg	5·1
[Mpr^1, D-Har^8]VP	Mpr	Tyr	D-Har	2
[Mpr^1, D-Lys^8]VP	Mpr	Tyr	D-Lys	0·07
[D-Tyr^2]AVP	Cys	D-Tyr	Arg	1·53
[Mpr^1, D-Tyr^2]AVP	Mpr	D-Tyr	Arg	0·045
[Tyr(Me)^2]LVP	Cys	Tyr(Me)	Lys	inhib.
[Tyr(Et)^2]LVP	Cys	Tyr(Et)	Lys	inhib.
[Tyr(Me)^2]AVP	Cys	Tyr(Me)	Arg	$pA_2 = 7{\cdot}44$
I	Cys	Tyr(Me)	D-Har	$pA_2 = 7{\cdot}70$
III	Cys	Tyr(Et)	D-Har	$pA_2 = 6{\cdot}9$
[Mpr^1, Tyr(3,5Br$_2$)^2]LVP	Mpr	Tyr(3,5Br$_2$)	Lys	$pA_2 = 6{\cdot}29$
[Mpr^1, Tyr(Bu)^2]LVP	Mpr	Tyr(Bu)	Lys	$pA_2 = 6{\cdot}22$
[Mpr^1, Tyr(Me)^2,D-Arg^8]VP	Mpr	Tyr(Me)	D-Arg	0
[Mpr^1, Tyr(Et)^2,D-Arg^8]VP	Mpr	Tyr(Et)	D-Arg	$pA_2 = 6{\cdot}25$
V	Mpr	Tyr(Me)	D-Har	$pA_2 = 8{\cdot}10$
II	Cys	D-Tyr(Me)	D-Har	$pA_2 = 7{\cdot}9$
IV	Cys	D-Tyr(Et)	D-Har	$pA_2 = 7{\cdot}3$
[Mpr^1, D-Tyr^2, D-Arg^8]VP	Mpr	D-Tyr	D-Arg	$pA_2 = 7{\cdot}9$
[Mpr^1, D-Tyr^2, Abu^4,D-Arg^8]VP	Mpr	D-Tyr	D-Arg	$pA_2 = 8{\cdot}2$
VI	Mpr	D-Tyr(Me)	D-Har	$pA_2 = 9{\cdot}0$
VII	Mpr	D-Tyr(Et)	D-Har	$pA_2 = 8{\cdot}8$

[a] According to ref.[1].

activity, in the absence of the bulky substituents on the β-carbon of amino acid in position 1, is a substituted L-tyrosine in position 2. It may be speculated that the configuration of tyrosine is not crucial for the inhibitory activity, but no one analog with substituted D-tyrosine in position 2 as the only modification was tested. The presence or absence of an amino group in position 1 and the configuration of basic amino acid in position 8 is not crucial either. However, in analogs with a basic amino acid of D-configuration in position 8, the substitution of position 2 by D-tyrosine (unsubstituted) is sufficient for the uterotonic inhibitory activity.

The aim of our project is to continue studies of the influence of unnatural amino acids in position 8 in connection with various modifications in position 2, on inhibition of the uterotonic activity. Several new amino (ref.[7]) and deamino (ref.[8]) analogs were prepared where in position 2 tyrosin was replaced by L- or D- *p*-methyl- or *p*-ethylphenylalanine. They exhibited high uterotonic inhibitory activity. This fact prompted us to synthesize more analogs both with and without amino group in position 1. Preliminary results of their biological evaluation were already published[9].

This work describes the synthesis of seven analogs of vasopressin (*I*–*VII*) with D-homoarginine in position 8 and modified hydroxyl of tyrosine in position 2.



Y-X-Phe-Gln-Asn-Cys-Pro-D-Har-Gly-NH_2

I	X = L-Tyr(Me)	Y = Cys
II	X = D-Tyr(Me)	Y = Cys
III	X = L-Tyr(Et)	Y = Cys
IV	X = D-Tyr(Et)	Y = Cys
V	X = L-Tyr(Me)	Y = Mpr
VI	X = D-Tyr(Me)	Y = Mpr
VII	X = D-Tyr(Et)	Y = Mpr

Synthesis of the analogs followed the approach used in the previous communications[7,8]. N^{α}-Tert-butoxycarbonyl-N^{G}-nitrohomoarginine[7] was used in the solid phase synthesis. O-Methyltyrosine residue was introduced in the form of racemic amino acid, prepared from tert-butoxycarbonyl-O-methyl-L-tyrosine by racemization in dimethylformamide solution with the use of dimethylaminopyridine. For comparison and identification of particular diastereoisomers, synthesis of analog *I* was performed with the use of Boc-L-Tyr(Me)-OH.

Syntheses of all seven analogs were performed by solid phase technique on the benzhydrylamine or *p*-methylbenzhydrylamine resin. As the α-amino group protection we have used tert-butoxycarbonyl group. For the side chain protection we have used: nitrogroup (D-Har), 4-methoxybenzyl (Cys) and 4-methylbenzyl (Cys, Mpr). Protected amino acids were coupled by N,N'-dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt) in dimethylformamide. Side chain protecting

groups were cleaved simultaneously with the cleavage of the peptide from the resin by liquid hydrogen fluoride. Sulfhydryl groups oxidation was performed by potassium ferricyanide and analogs were purified by HPLC. In syntheses of analogs with O-methyltyrosine we made use of the fact that diastereoisomeric peptides can be easily separated by reversed phase chromatography[7,8,10-12]. Syntheses of these analogs were performed with the racemic amino acid and appropriate diastereoisomers were separated at the end of synthesis. Identification of L- or D-amino acid was performed by digestion with L-amino acid oxidase[13,14]. Besides that, the k value in HPLC on reversed phase was always lower for L-diastereoisomer, which is consistent with previous findings[7,8,11]. For the formation of both diastereoisomers only 1·1 equivalents of protected racemic amino acid have to be used due to the preferential formation of one diastereoisomer (tert-butoxycarbonyl-D,L-O-methyltyrosine, see for example[7]).

Electrophoresis in pyridine–acetate buffer (pH 5·7) can be used for separation of the diastereoisomeric mixtures too. However, electrophoretic mobility in 1M acetic acid is identical for both diatereoisomers. Analogs containing L-amino acid in position 2 are significantly more basic than analogs with D-amino acid in the same position. This difference is observable only in analogs with cysteine in position 1.

TABLE II

Biological activities (rat) of vasopressin analogs (I.U./mg) modified in the position 2

Compound	Uterotonic		Pressor	Antidiuretic	Ref.
	in vitro	in vivo			
AVP	17[a]		412[a]	465[a]	—
[D-Har[8]]VP	—		0·83	84	17
	0·9		—	1% dDAVP	7
[Mpr[1], D-Har[8]]VP	—		0·06	196	17
	2		0·5	1 540	18
	2		0·05	2% dDAVP	19
	0·8		0·28	5% dDAVP	8
I	$pA_2 = 7·7$	—	0·04	$<10^{-4}$% DDAVP	[b]
II	$pA_2 = 7·9$	$pA_2 = 6·9$	$pA_2 = 6·9$	—	[b]
III	$pA_2 = 6·9$	$pA_2 = 7·0$	$pA_2 = 6·8$	$<10^{-4}$% DDAVP	[b]
IV	$pA_2 = 7·3$	$pA_2 = 6·3$	$pA_2 = 7·2$	$<10^{-4}$% DDAVP	[b]
V	$pA_2 = 8·1$	—	0	—	[b]
VI	$pA_2 = 9·0$	$pA_2 = 7·1$	$^*pA_2 = 6·9$	—	[b]
VII	$pA_2 = 8·8$	$pA_2 = 6·6$	$pA_2 = 7·0$	$<10^{-4}$% DDAVP	[b]

[a] According to ref.[1]; [b] this paper.

This means that the configuration of amino acid in position 2 controls p*K* of N--terminal amino group. Influence of configuration of an amino acid onto p*K* of its amino group was described[15,16] for oxytocin analogs with L- or D-cysteine in position 1, but the transfer of this effect over one amino acid residue was quite surprising.

Biological activities of the analogs are given in the Table II. Substitution in position 2 led to a substantial decrease of the antidiuretic activity. The analogs with alkylated tyrosine hydroxyl group in position 2 have either no or negligible intrinsic pressor activity (*I*, *V*) or low inhibitory activity (*II*, *III*, *IV*, *VI* and *VII*). As well as in the case of α-amino[7] and desamino[8] analogs with substituted phenylalanine in position 2, weak uterotonic agonists [D-Har[8]]VP and [Mpr[1], D-Har[8]]VP were transformed by modification of tyrosine hydroxyl group to potent uterotonic inhibitors in vitro. As can be seen in Table I, earlier described analogs show that more potent inhibitors result from the combination of D-amino acids both in position 2 and 8. This holds true also for analogs prepared in this study (compare *I* and *II*, *III* and *IV*, *V* and *VI* in Table II). In the in vitro test deamino analogs are more potent than analogs containing α-amino group (compare *I* and *V*, *II* and *VI*, *IV* and *VII*). Analog *VI* is one of the most potent in vitro uterotonic inhibitors described up to now (see ref.[5]). However in the uterotonic test in vivo the inhibitory activity is much lower. The comparison of the uteronic and pressor inhibitory activities of analogs *I* – *VII* is worth mentioning. If we compare the activities in the most commonly used uterotonic in vitro test to the activities in the pressor test, we will find high selectivity in favour of the antiuterotonic potency. However if we compare the values from the uterotonic in vivo test and that from pressor test (which should be considered to be more proper), we shall see that the values are in the same order of magnitude. It means that the distribution in the organism (the so called bioavailability) and/or ion composition plays a big role in the biological potency of these analogs.

EXPERIMENTAL

General Methods

Thin-layer chromatography (TLC) was carried out on silica gel coated plates (Silufol, Kavalier, Czechoslovakia) in the following systems: 2-butanol–98% formic acid–water (10 : 3 : 8) (S1), 1-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 6) (S4). Paper electrophoresis was performed in a moist chamber in 1M acetic acid (pH 2·4) and in pyridine-acetate buffer (pH 5·7) on Whatman 3MM paper at 20 V/cm for 60 min. Spots in TLC and electrophoresis were detected with ninhydrin or by chlorination method[20]. Samples for amino acid analysis were hydrolyzed with 6M HCl at 105°C for 20 h or with mixture propionic acid–hydrochloric acid (1 : 1) at 160°C for 15 min and analyzed on an Amino acid analyzer T 339 (Mikrotechna Praha, Czechoslovakia) or D-500 analyzer (Durrum Corp., U.S.A.). Optical rotations were determined on a Perkin--Elmer instrument type 141 MCA (Norwalk, U.S.A.). Fast atom bombardment mass spectra were obtained on a ZAB-EQ spectrometer (VG Analytical Ltd., Manchester) with xenon at

8 kV as the bombarding gas. High performance liquid chromatography (HPLC) was carried out on an SP-8800 instrument equipped with an SP-8450 detector and SP-4290 integrator (all from Spectra Physics, Santa Clara, U.S.A.). HPLC purities of products were determined on a Vydac 218TP54 column. Preparative liquid chromatography was carried out on above described equipment using a Vydac 218TP510 (5 μm, 250 × 10 mm) column. Before use, all amino-acid derivatives were subjected to ninhydrin test[21].

Solid-Phase Peptides Synthesis

A cycle for incorporation each amino acid residue into the growing peptide chain consisted of the following: *1*) cleaving the Boc group by 50% trifluoracetic acid in dichloromethane containing 5% anisole; *2*) washing with dichloromethane, isopropanol and dichloromethane; *3*) neutralizing by 5% diisopropylethylamine in dichloromethane; *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, isopropanol and dichloromethane. The synthesis was monitored by bromophenol blue method[22].

Heptapeptide-Resin (Peptide Resin A or B)

Benzhydrylamine resin (UCB, 0·56 mmol NH_2/g, 4·46 g, 2·5 mmol) or *p*-methylbenzhydrylamine resin (Peptides International, 0·79 mmol NH_2/g, 8·0 g) was suspended in dichloromethane and after washing with 5% diisopropylethylamine in dichloromethane and with dimethylformamide it was coupled with 3 molar excess of Boc-Gly-OH in the presence of N-hydroxybenzotriazole and dicyclohexylcarbodiimide in dimethylformamide. Coupling was interrupted after 2 h, the resin was washed consequently by dimethylformamide (3 × 40 ml) and dichloromethane (3 × 40 ml) and resin substitution was determined by amino acid analysis (0·46 resp. 0·55 mmol/g). Polymer was acetylated (5 ml acetanhydride, 2 ml triethylamine in 50 ml dichloromethane). The free amino groups disappeared during 2 h (according to the ninhydrin test). The following procedure was performed according to general scheme given in the beginning of experimental part (starting from the point *1*). Boc-amino acids were coupled to the resin by the DCCI/HOBt procedure. All reagents were used in 3 molar excess and coupling was monitored by bromophenol blue method[22]. Protected derivatives were used in the following order: Boc-D-Har(NO_2)--OH (ref.[7]), Boc-Pro-OH, Boc-Cys(4-Me-Bzl)-OH (ref.[23]), Boc-Asn-OH, Boc-Gln-OH and Boc-Phe-OH. Yield for benzhydrylamine resin A: 6·9 g. Amino-acid analysis on resin: Asp 0·95, Glu 1·13, Pro 1·01, Gly 0·67, Cys 0·50, Phe 1·10, Har 0·82. Yield for *p*-methylbenzhydrylamine resin B: 14·5 g. Amino acid analysis on resin: Asp 0·90, Glu 1·03, Pro 1·06, Gly 1·02, Cys 0·70, Phe 1·01, Har 0·98.

[2-O-Methyl-L-tyrosine,8-D-Homoarginine]vasopressin (*I*)

Peptide-resin A (1·7 g, 0·48 mmol) was coupled according to general scheme with Boc-Tyr(Me)--OH (released from dicyclohexylammonium salt[24]) and Boc-Cys(4-Me-Bzl)-OH (ref.[23]). Boc--protecting group was cleaved and nonapeptide-resin (1·8 g) was treated with liquid hydrogen fluoride (15 ml, 60 min, 0°C) in the presence of anisole (1 ml). Unprotected nonapeptide, together with the resin, was triturated with ether after evaporation of hydrogen fluoride, filtered off, washed with ethylacetate and the free peptide was extracted successively by acetic acid, 50% acetic acid, water and lyophilized (0·69 g). The lyophilizate was dissolved in water (700 ml) and the pH of the solution was adjusted with 0·1M NaOH to 7·0. Potassium ferricyanide (0·01M solu-

tion) was added to this solution until stable yellow colour persisted. During the oxidation (20 min) pH was maintained at 7·2 by addition of 0·1M NaOH and then adjusted with acetic acid to 4·5. The solution was put on a column of Amberlite CG-50I (25 ml), the column was washed with 0·25% acetic acid and the product eluted with 50% acetic acid (75 ml). After freeze-drying, the crude product (353 mg) was purified by HPLC on a Vydac 218TP510 column in slow gradient running from 20% to 40% methanol in 0·05% trifluoroacetic acid in 60 min. Lyophilization of the corresponding fractions afforded 160 mg of the product pure according to HPLC (k 1·13, methanol–0·05% trifluoroacetic acid 35 : 65). R_F 0·02 (S1), 0·41 (S4). $E_{2,4}^{Gly}$ 1·08, $E_{5,7}^{His}$ 0·54. $[\alpha]_D$ 0·0° (c 0·1, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·00, Pro 0·97, Gly 0·99, Cys 1·8, Tyr(Me) 0·27, Tyr 0·75, Phe 1·00, Har 0·86. For $C_{48}H_{69}N_{15}O_{12}S_2.3$ TFA.3 H_2O (1 508·4) calculated: 43·00% C, 5·21% H, 13·93% N; found: 42·73% C, 4·83% H, 13·93% N. FAB MS (m/z): 1 113 ($M + H^+$).

Preparation of Racemic Tert-butoxycarbonyl-O-methyl-D,L-tyrosine

Dicyclohexylamonnium salt of tert-butoxycarbonyl-O-methyl-L-tyrosine (2·4 g, 5 mmol) was suspended in ethyl acetate and the solution was extracted with 0·5M H_2SO_4, water and dried by Na_2SO_4. Ethyl acetate solution was evaporated, the residue was dissolved in dimethylformamide (30 ml) and dimethylaminopyridine (0·61 g, 5 mmol) was added. Racemisation was followed by optical rotation measurement. Starting value of $[\alpha]_D$ −10·6° (c 1·0, dimethylformamide) decreased during 140 h to $[\alpha]_D$ −3·6°. In the last 100 h the value changed very slowly. 1-Hydroxybenzotriazol (0·7 g) and dicyclohexylcarbodiimide (5 ml of 1M solution) were added to the solution and optical rotation was measured again $[\alpha]_D$ −5·3° (c 1·0; dimethylformamid). After 24 h its value stabilized on $[\alpha]_D$ −2·1°. Dicyclohexylurea was filtered off and the active ester solution was used directly for the coupling reaction.

[2-O-Methyl-L-tyrosine, 8-D-Homoarginine]vasopressin (*I*)
and [2-O-Methyl-D-tyrosine, 8-D-Homoarginine]vasopressin (*II*)

Peptide-resin B (2·0 g, 0·6 mmol) was coupled according to general scheme with 1·1 equivalents Boc-L,D-Tyr(Me)-OH for 24 h. Coupling was repeated twice with another 0·5 equivalents for 24 and 3 h and with Boc-Cys(4-MeO-Bzl)-OH (ref.[25]). Treatment of protected nonapeptide was analogous to that of compound *I*. Freeze-drying afforded 582 mg of crude product, which was purified by HPLC on a Vydac 218TP510 column in a slow gradient running from 30% to 50% MeOH in 0·05% trifluoracetic acid in 60 min. Lyophilization of the corresponding fractions afforded 192 mg and 25 mg of the products pure according to HPLC.

The first product (192 mg; k 1·13, methanol–0·05% trifluoroacetic acid 35 : 65) was identical to the analog *I* prepared above.

The second product (25 mg; k 2·45, methanol–0·05% trifluoroacetic acid 35 : 65) corresponds to analog (*II*) with O-methyl-D-tyrosine in position 2. R_F 0·02 (S1), 0·43 (S4). $E_{2,4}^{Gly}$ 1·05; $E_{5,7}^{His}$ 0·43. $[\alpha]_D$ −33·7° (c 0·1, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·00, Pro 0·96, Gly 1·00, Cys 1·80, Tyr(Me) 0·21, Tyr 0·71 Phe 1·02, Har 0·65. For $C_{48}H_{69}N_{15}O_{12}S_2.3·5$ TFA. .3·5 H_2O (1 574·4) calculated: 41·95% C, 5·09% H, 13·34% N; found: 41·72% C, 4·70% H, 13·78% N. FAB MS (m/z): 1 113 ($M + H^+$).

[2-O-Ethyl-L-tyrosine, 8-D-Homoarginine]vasopressin (*III*)

Peptide-resin A (1·7 g, 0·48 mmol) was coupled according to general scheme with Boc-L-Tyr(Et)-OH and with Boc-Cys(4-MeBzl)-OH (ref.[23]). Treatment of protected nonapeptide was ana-

logous to that of compound *I*. Freeze-drying afforded 401 mg of crude product, which was purified by HPLC on a Vydac 218TP510 column in a slow gradient running from 20% to 40% MeOH in 0·05% trifluoroacetic acid in 60 min. Lyophilization of the corresponding fractions afforded 145 mg of the product pure according to HPLC (k 1·07, methanol–0·05% trifluoroacetic acid 4 : 6). R_F 0·02 (S1), 0·45 (S4). $E_{2.4}^{Gly}$ 1·03, $E_{5.7}^{His}$ 0·54. $[\alpha]_D$ +6·0° (c 0·1, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·00, Pro 1·04, Gly 1·00, Cys 1·40, Tyr 0·65, Phe 1·08, Har 1·09. For $C_{49}H_{71}N_{15}O_{12}S_2.3$ TFA.4·5 H_2O (1 549·5) calculated: 42·63% C, 5·40% H, 13·56% N; found: 42·19% C, 4·98% H, 13·96% N. FAB MS (m/z): 1 126·4 ($M + H^+$).

[2-O-Ethyl-D-tyrosine, 8-D-Homoarginine]vasopressin (*IV*)

Peptide-resin B (0·5 g, 0·15 mmol) was coupled according to general scheme with Boc-D-Tyr(Et)--OH and with Boc-Cys(4-MeO-Bzl)-OH (ref.[25]). Treatment of protected nonapeptide was analogous to that of compound *I*. Freeze-drying afforded 104 mg of crude product, which was purified by HPLC on a Vydac 218TP510 column in a slow gradient running from 20% to 40% of MeOH in 0·05% trifluoroacetic acid in 60 min. Lyophilization of the corresponding fractions afforded 34 mg of the product pure according to HPLC (k 2·48, methanol–0·05% trifluoroacetic acid 4 : 6). R_F 0·02 (S1), 0·46 (S4). $E_{2.4}^{Gly}$ 1·01, $E_{5.7}^{His}$ 0·44. $[\alpha]_D$ −27·9° (c 0·1, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·00, Pro 1·00, Gly 1·00, Cys 1·70, Tyr 0·85, Phe 1·00, Har 0·87. For $C_{49}H_{71}N_{15}O_{12}S_2.2$ TFA.AcOH (1 414·4) calculated: 46·70% C, 5·48% H, 14·85% N; found: 46·34% C, 5·27% H, 15·13% N. FAB MS (m/z): 1 126·7 ($M + H^+$).

[1-Mercaptopropionic Acid, 2-O-Methyl-L-tyrosin, 8-D-Homoarginine]vasopressin (*V*) and [1-Mercaptopropionic Acid, 2-O-Methyl-D-tyrosin, 8-D-Homoarginine]vasopressin (*VI*)

Peptide-resin B (2·0 g, 0·6 mmol) was coupled according to general scheme with 1·1 equivalents of Boc-D,L-Tyr(Me)-OH for 5 h and with another 0·7 equivalents for 24 h and with Mpr(4-Me--Bzl)-OH. Treatment of protected nonapeptide was analogous to that of compound *I*. Freeze--drying afforded 405 mg of crude product, which was purified by HPLC on a Vydac 218TP510 column in a slow gradient running from 25% to 45% MeOH in 0·05% trifluoroacetic acid in 60 min. Lyophilization of the corresponding fractions afforded 108 mg and 19·3 mg of the products pure according to HPLC.

The first product (108 mg; k 2·82 methanol–0·05% trifluoroacetic acid 4 : 6) corresponds to analogue (*V*) with O-methyl-L-tyrosine in position 2. R_F 0·06 (S1), 0·51 (S4). $E_{2.4}^{Gly}$ 0·65; $E_{5.7}^{His}$ 0·30 (detection by chlorination method). $[\alpha]_D$ −54·3° (c 0·1, 1M acetic acid). Amino acid analysis: Asp 1·01, Glu 1·01, Pro 0·95, Gly 1·00, Cys 0·41, Tyr(Me) 0·26, Tyr 0·78, Phe 1·04, Har 0·90. For $C_{48}H_{53}N_{14}O_{12}S_2.2$ TFA.2·5 H_2O (1 370·4) calculated: 45·58% C, 5·51% H, 14·31% N; found: 45·43% C, 5·22% H, 14·67% N. FAB MS (m/z): 1 097 (M^+).

The second product (19·3 mg; k 3·95, methanol–0·05% trifluoroacetic acid 4 : 6) corresponds to analogue (*VI*) with O-methyl-D-tyrosine in position 2. R_F 0·05 (S1), 0·51 (S4). $E_{2.4}^{Gly}$ 0·64; $E_{5.7}^{His}$ 0·30 (detection by chlorination method). $[\alpha]_D$ −63·7° (c 0·1, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·01, Pro 0·95, Gly 1·00, Cys 0·40, Tyr(Me) 0·25, Tyr 0·79, Phe 1·03, Har 0·87. For $C_{48}H_{58}N_{14}O_{12}S_2.2$ TFA.3 H_2O (1 379·4) calculated: 45·27% C, 5·55% H, 14·22% N; found: 44·92% C, 5·15% H, 14·49% N. FAB MS (m/z): 1 097 (M^+).

[1-Mercaptopropionic Acid, 2-O-Ethyl-D-tyrosine, 8-D-Homoarginine]vasopressin (*VII*)

Peptide-resin B (0·5 g, 0·15 mmol) was coupled according to general scheme with Boc-D-Tyr(Et)--OH and with Mpr(4-Me-Bzl)-OH. Treatment of protected nonapeptide was analogous to that

of compound *I*. Freeze-drying afforded 95 mg of crude product, which was purified by HPLC on a Vydac 218TP510 column in a slow gradient running from 25% to 45% of MeOH in 0·05% trifluoracetic acid in 60 min. Lyophilization of the corresponding fractions afforded 21·3 mg of the product pure according to HPLC (k 3·22, methanol–0·05% trifluoroacetic acid 4 : 6). R_P 0·06 (S1), 0·53 (S4). $E_{2,4}^{Gly}$ 0·64, $E_{5,7}^{His}$ 0·30 (detection by chlorination method). $[\alpha]_D$ −37·0° (c 0·1, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·02, Pro 0·97, Gly 0·97, Cys 0·26, Tyr(Et) 0·05, Tyr 0·89, Phe 1·03, Har 0·92. For $C_{49}H_{70}N_{14}O_{12}S_2.2\,TFA.3\,H_2O$ (1 393·4) calculated: 45·68% C, 5·63% H, 14·06% N; found: 45·76% C, 5·55% H, 14·15% N. FAB MS (m/z): 1 111·7 ($M + H^+$).

Pharmacological Methods

All pharmacological test were performed using Wistar rats weighing 200–300 g. The uterotonic potency in vitro was evaluated using the Holton procedure[26] in Munsick[27] solution. For the determination of the in vivo activity[28] oestrogenized rats in ethanol anaesthesia were used. Inhibitory activity is characterized by pA_2 value (refs[29–31]). Pressor activity was tested on pithed rat preparation according to refs[32,33]. Antidiuretic potency on nonanaesthetized rat was followed according to the method in ref.[34]. As the standard in the antidiuretic test [D-arginin[8]] deaminovasopressin (dDAVP) was used.

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