THE ANALOGS OF 8-D-HOMOARGININE-VASOPRESSIN WITH *p*-SUBSTITUTED PHENYLALANINE IN POSITION 2; SYNTHESIS AND SOME BIOLOGICAL PROPERTIES*

Miroslava ŽERTOVÁ, Zdenko PROCHÁZKA, Ivo BLÁHA, Tomislav BARTH, Jiřina SLANINOVÁ, Lenka MALETÍNSKÁ and Michal LEBL

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

Received May 17, 1990
Accepted June 2, 1990

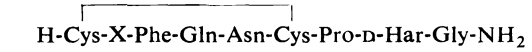
Solid phase methodology on benzhydrylamine resin was used for the synthesis of five analogs of vasopressin with non-coded amino acid, D-homoarginine, in position 8 and *p*-substituted D or L phenylalanine in position 2. Besides the mother analog, [D-Har^8]vasopressin (*I*), [L-Phe(Me)^2, D-Har^8]vasopressin (*II*), [D-Phe(Me)^2, D-Har^8]vasopressin (*III*), [L-Phe(Et)^2, D-Har^8]vasopressin (*IV*), and [D-Phe(Et)^2, D-Har^8]vasopressin (*V*) were synthesized. All analogs have very low antidiuretic and pressor activities. Analogs containing *p*-ethylphenylalanine of both D and L configuration are pressor inhibitors. All analogs substituted in position 2 were found to be uterotonic inhibitors, the most potent being [D-Phe(Et)^2, D-Har^8]vasopressin (*V*) with pA_2 8·15.

It is known that some analogs of neurohypophyseal hormones can significantly increase the level of blood coagulation factors in the human serum[1,2]. This is just one of many important biological activities of this class of biologically active peptides. One of analogs used in a clinical practice is dDAVP ([8-D-arginine]desamino-vasopressin), whose factor-VIII increasing activity is practically utilized[3,4]. From the practical reasons, it is obviously advantageous if the analog potentially used for the exploatation of this activity has all other biological activities decreased or, if possible, eliminated. Based on our previous knowledge, it seemed necessary to keep the basic side chain functionality in the molecule to preserve high factor-VIII increasing activity and modify other positions to achieve decrease of the other peripheral activities (antidiuretic, pressor, uterotonic). Homologization of amino acid of D-configuration in position 8 by methylene group is known to result in decreased antidiuretic activity, as well as modification of position 2 by substituted phenylalanine both of D- or L-configuration (for the structure activity relationships of neurohypophyseal hormone analogs see ref.[5]). We were interested to find the influence of D-homoarginine substitution in position 8 and its combination with the

* Part CCXVIII in the series Amino Acids and Peptides; Part CCXVII: Int. J. Pept. Protein Res. *36*, 321 (1990).

introduction of *p*-methyl of *p*-ethylphenylalanine of both D and L configuration into position 2.

Five analogs of vasopressin* (*I*–*V*) with D-homoarginine in position 8 and modified phenylalanine in position 2 were synthesized.



I, X = Tyr
II, X = L-Phe(*p*Me)
III, X = D-Phe(*p*Me)
IV, X = L-Phe(*p*Et)
V, X = D-Phe(*p*Et)

N^{α}-Tert-butoxycarbonyl-N^{G}-nitrohomoarginine was found to be suitable derivative of D-homoarginine for solid phase synthesis. N^{G}-Nitrohomoarginine was prepared by nitroguanylation of D-lysine using the described method[7]. Direct protection of amino group by tert-butoxycarbonylanhydride provided desired derivative. D,L-*p*-Methylphenylalanine was prepared by modified acetamidomalonane method[8,9] starting from *p*-methylbenzyl chloride, D,L-*p*-ethylphenylalanine was prepared by the same method starting from the mixture of *o*- and *p*-ethylbenzyl chloride. Amino group of racemic amino acids were protected by Boc-group.

The syntheses of all five analogs were performed by solid phase technique on the benzhydrylamine resin. As the α-amino group protection we have used tert-butoxycarbonyl group. Side chain functional groups were protected by: nitrogroup (D-Har), 4-methylbenzyl (Cys) and 2,4-dichlorobenzyl or benzyloxycarbonyl (Tyr). 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 the liquid hydrogen fluoride. Sulfhydryl groups oxidation was performed by potassium ferricyanide and analogs were purified by HPLC and gel filtration. In syntheses of analogs with *p*-ethylphenylalanine and *p*-methylphenylalanine we made use of fact that diastereoisomeric peptides can be easily separated by reversed phase chromatography[10–12]. Syntheses of these analogs were performed with the racemic amino acids 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]. In both cases the value of *k* in HPLC on reversed phase was always lower for L-diastereoisomer, what is consistent with previous findings[11]. Using

* 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[6]: Har. denotes the homoarginine moiety, Phe(Et) the *p*-ethylphenylalanine and Phe(Me) the *p*-methylphenylalanine moiety.

three molar excess of D,L-*p*-methylphenylalanine in solid phase synthesis, only one analog was obtained. It was therefore necessary to use only 1·1 equivalent of protected racemic amino acid (tert-butoxycarbonyl-D,L-*p*-methylphenylalanine or tert-butoxycarbonyl-D,L-*p*-ethylphenylalanine) for the formation of both diastereoisomers. During the characterization of the diastereoisomeric analogs we have observed different behaviour of L and D-amino acid containing compounds in FAB MS. Spectra of analogs *III* and *V* contain significantly higher amount of $(M + 2H + H)^+$ ions (in the case of compound *V* this ion is the prevailing in the spectrum) than spectra of analogs *II* and *IV*. This finding may suggest easier reducibility of D-amino acid containing compounds, what may be caused by their higher conformational tension.

Biological activities of the analogs are given in the Table I. [8-D-Homoarginine]-vasopressin (*I*) was found to have approximately 1% of the antidiuretic activity of dDAVP. This result is comparable to the finding of Swedish authors[16], who found the activity of this analog in the test on anaesthetized rat to be 84 I.U./mg. This analog was also found to be a weak agonist in the uterotonic test in vitro. Substitution in position 2 led to a substantial decrease of antidiuretic activity. Pressor activity of analogs with substituted position 2 was either very low (*II*, *III*) or very low inhibitory activity appeared (*IV*, *V*). Most interesting were results of uterotonic activity evaluation. A weak uterotonic agonist *I* was transformed by the modification of position 2 to rather potent uterotonic inhibitor. And again, as described earlier[5,12], *p*-ethylphenylalanine of D-configuration was found superior in producing an inhibitor.

Because the evaluation of factor-VIII increasing activities of the prepared analogs will take much longer time and because the uterotonic inhibitory activity was so high (analog *V* is one of the twenty most potent uterotonic inhibitors described up

TABLE I

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

Compound	Uterotonic[a]	Pressor	Antidiuretic
AVP[b]	17	412	465
[D-Har[8]]VP[c]	—	0·83	83
I	0·9	—	1% dDAVP
II	pA_2 6·85	0·04	<0·1% dDAVP
III	pA_2 7·78	0·04	<0·1% dDAVP
IV	pA_2 7·4	pA_2 6·5	<0·1% dDAVP
V	pA_2 8·15	pA_2 6·5	<0·1% dDAVP

[a] In vitro; [b] according to ref.[15]; [c] ref.[16].

to now – see ref.[5]), we decided to publish the synthesis and activities of these analogs already now.

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 (75 : 13·5 : 11·5, S1); 2-butanol–25% ammonia–water (85 : 7·5 : 7·5, S2); 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. Samples for amino acid analysis were hydrolyzed with 6M-HCl at 105°C for 20 h or with propionic acid–hydrochloric acid (1 : 1) mixture 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-8700 instrument equipped with an SP-8400 detector and SP-4100 integrator (all from Spectra Physics, Santa Clara, U.S.A.). HPLC purities of products were determined on the column of Separon SIX C-18 (S), Spherisorb ODS II 3 μm (Sph) or Vydac 218TP5 (V). Preparative liquid chromatography was carried out on above described equipment using column Vydac 218TP5 (5 μm, 25 × 1·0 cm) or on modular instrument Knauer (Knauer HPLC Programmer 50, Knauer HPLC Pump 364 and Knauer Variable Wavelength Detector) on column filled with Separon SGX-C18 (10 μm, 250 × 16 mm). As the final purification step, the Biogel P-2 column chromatography in 1M acetic acid was used (1 × 100 cm). Chelaton III was added to the sample in order to remove the residues of the heavy metals if present. Before use, all amino-acid derivatives were subjected to ninhydrin test[17].

Solid-phase peptide synthesis: Synthesis was performed on home-made semiautomatic solid phase synthesizer. A cycle for incorporation each amino acid residue into the growing peptide chain consisted of the following: 1. cleavage of the Boc group by adding 15 ml of 45% trifluoroacetic acid in dichloromethane containing 5% anisole, one treatment for 5 min, and second for 30 min; 2. washing with dichloromethane (3 × 15 ml, 1 min/wash); 3. washing with 2-propanol (3 × 15 ml, 1 min/wash); 4. washing with dichloromethane (3 × 15 ml, 1 min/wash); 5. neutralization by addition 15 ml of 5% diisopropylethylamine in dichloromethane, one treatment for 2 min, a second for 5 min; 6. washing with dichloromethane (3 × 15 ml, 1 min/wash); 7. washing with dimethylformamide (3 × 15 ml, 1 min/wash); 8. addition of the Boc-protected amino acid derivative in 10 ml of dimethylformamide followed by HOBt, followed by DCCI and stirring for 2–5 h; 9. washing with dimethylformamide (3 × 15 ml, 1 min/wash); 10. washing with dichloromethane (3 × 15 ml, 1 min/wash); 11. washing with 2-propanol (3 × 15 ml, 1 min/wash); 12. washing with dichloromethane (3 × 15 ml, 1 min/wash). The synthesis was monitored by bromophenol blue method[18].

N^{α}-Tert-butoxycarbonyl-N^{G}-nitro-D-homoarginine

Hydrochloride of D-lysine (5·4 g; 30 mmol) was dissolved in water (30 ml), basic copper carbonate (6·6 g) was added, suspension was heated to the boiling point, insoluble part was filtered off, filtrate was cooled to 0°C, and 1M-NaOH (30 ml) was added. 2-Methyl-1-nitroisourea (3·72 g) was then added during 30 min. Reaction mixture was stirred 2 h at 0°C and then at room tempera-

ture and crystals of cupric complex were filtered off and washed by water, ethanol and ether. Intermediate (6·84 g) was suspended in water (120 ml), heated, and hydrogen sulfide was introduced until blue color disappeared. After addition of 1M-HCl (30 ml) and short boiling the mixture was filtered. Combined filtrates were evaporated and the oily residue (R_F 0·11 (S1), 0·02 (S2); $E_{2,4}^{Gly}$ 0·49, $E_{5,7}^{His}$ 0·00) was dissolved in dioxane (50 ml), water (30 ml) and 1M-NaOH (30 ml). Boc-anyhdride (7·2 g) was added to this mixture and stirring continued 3 h at room temperature. pH was kept by addition of 1M-NaOH (about 70 ml) in the range 9—10. After washing with ether the aqueous layer was cooled to 0°C and acidified by 20% citric acid. Product was extracted by ethyl acetate. Washing by water, 0·5M-H_2SO_4, water, drying (Na_2SO_4) and evaporation afforded 6·16 g (62%) of pure product as amorphous foam. R_F 0·74 (S1), 0·18 (S2); HPLC (*k* 2·69, methanol–0·05% trifluoroacetic acid 4 : 6 — Separon).

Part of the product (0·67 g; 2 mmol) was transformed to dicyclohexylammonium salt. Crystallization from ether and petroleum ether gave 0·8 g (78%); m.p. 93—96°C. $[\alpha]_D$ +14·3° (*c* 0·2; pyridine). For $C_{24}H_{46}N_6O_6$ (514·7) calculated: 56·01% C, 9·00% H, 16·32% N; found: 56·14% C, 9·00% H, 16·31% N.

D,L-*p*-Methylphenylalanine

Diethyl 4-methylbenzylacetamidomalonate was prepared from 4-chloromethyltoluene similarly as described[7] for diethyl benzylacetamidomalonate. Yield: 79%, m.p. 109—111°C. HPLC (*k* 3·38, methanol–0·05% trifluoroacetic acid 7 : 3 — Separon). Lit.[8] m.p. 108—110°C (after recrystalization from ethanol–petrolether 111—113°C).

Starting from diethyl 4-methylbenzylacetamidomalonate D,L-*p*-methylphenylalanine was prepared by the procedure described for synthesis of 3,5-dimethylphenylalanine[8]. Yield: 95%, m.p. 200—207°C; HPLC (*k* 1·51, methanol–0·05% trifluoroacetic acid 1 : 1 — Separon). Lit.: m.p. 276—279°C (refs[8,19]), 215—216°C (ref.[20]), 226—229°C (Kofler) and 275°C (capillary) (ref.[21]).

Tert-butoxycarbonyl-D,L-*p*-methylphenylalanine

Solution of D,L-*p*-methylphenylalanine (1·8 g; 10 mmol) in the mixture of dioxan (20 ml), water (10 ml), and 1M-NaOH (10 ml) was treated with Boc-anhydride (2·4 g; 11 mmol) under stirring at pH 8—9 (adjusted by addition of 1M-NaOH) for 4 h. Reaction mixture was extracted by ether, aqueous layer was cooled to 0°C, acidified by 20% citric acid and product was extracted by ethyl acetate. Extracts were washed by water, 0·5M-H_2SO_4, water and dried by sodium sulfate. Filtration and evaporation afforded 2·51 g (90%) of pure product, m.p. 112—117°C; HPLC (*k* 2·29, methanol–0·05% trifluoroacetic acid 7 : 3; *k* 9·23, methanol–0·05% trifluoroacetic acid 6 : 4 — Separon). For $C_{15}H_{21}NO_4$ (279·3) calculated: 64·50% C, 7·58% H, 5·01% N; found: 64·85% C, 7·58% H, 5·01% N.

Tert-butoxycarbonyl-D,L-*p*-ethylphenylalanine

Acylation of D,L-*p*-ethylphenylalanine[9] (1·93 g; 10 mmol) was performed as above. Yield 2·72 g (93%), m.p. 135—138°C. HPLC (*k* 3·09, methanol–0·05% trifluoroacetic acid 7 : 3; *k* 11·09, methanol–0·05% trifluoroacetic acid 6 : 4 — Separon). For $C_{16}H_{23}NO_4$ (293·4) calculated: 65·51% C, 7·90% H, 4·77% N; found: 65·70% C, 7·89% H, 4·83% N.

Heptapeptide-resin (Peptide-resin A)

Benzhydrylamine resin (UCB, 0·6 mmol/g, 2·0 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 by dimethylformamide (3 × 20 ml) and dichloromethane (3 × 20 ml) and resin substitution was determined by amino acid analysis — 0·48 mmol/g. Polymer was acetylated (5 ml acetanhydride, 2 ml triethylamine in 50 ml dichloromethane). The free amino groups disappeared during 3 h (the ninhydrin test). The following procedure was performed according to general scheme given at the beginning of experimental part. 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[18]. Protected derivatives were used in the following order: Boc-D-Har(NO_2)-OH, Boc-Pro-OH, Boc-Cys(4-Me-Bzl)-OH (ref.[22]), Boc-Asn-OH, Boc-Gln-OH and Boc-Phe-OH. In the case of homoarginine and proline coupling the reaction was not complete even after 15 h and therefore repeated coupling in the presence of 1 equivalent of 4-dimethylaminopyridine was used. Starting from asparagine, couplings were completed in 1 to 2 h. Yield: 3·00 g. In the second batch heptapeptide with substitution 0·56 mmol/g (9·0 g) was prepared.

[8-D-Homoarginine]vasopressin (*I*)

Peptide-resin A (0·9 g; 0·33 mmol) was coupled with Boc-Tyr(2,6-diCl-Bzl)-OH (ref.[23]) and Boc-Cys(4-Me-Bzl)-OH (ref.[22]). After cleavage of Boc-protecting group the nonapeptide-resin (1 g) was treated with liquid hydrogen fluoride (10 ml, 60 min, 0°C) in the presence of anisole (1·5 ml). Unprotected nonapeptide, together with the resin, was triturated with ether after evaporation of hydrogen fluoride, filtered off, washed with ethyl acetate and then free peptide was extracted successively by acetic acid, 50% acetic acid, water and combined extracts were lyophilized. The lyophilizate was dissolved in water (300 ml) and the pH of the solution was adjusted with 0·1M-NaOH to 7·0. Potassium ferricyanide (0·01M solution) 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 it was adjusted with acetic acid to 4·5. The solution was put on a column of Amberlite CG-50I (15 ml), the column was washed with 0·25% acetic acid and the product eluted with 50% acetic acid. After freeze-drying, the product (148 mg) was purified by HPLC in a slow gradient of methanol in 0·1% trifluoroacetic acid on a column of Separon SGX-C18 and lyophilization of the corresponding fractions afforded 52 mg of the product pure according to HPLC (k 2·23, methanol–0·05% trifluoroacetic acid 4 : 6; k 4·68, methanol–0·05% trifluoroacetic acid 3·5 : 6·5 — Separon). R_F 0·00 (S1), 0·30 (S4); $E_{2.4}^{Gly}$ 1·24, $E_{5.7}^{His}$ 0·61; $[\alpha]_D$ −2·8° (c 0·2; 1M acetic acid). Amino acid analysis: Asp 1·02, Glu 1·19, Pro 1·02, Gly 0·92, Cys 2·05, Tyr 0·75, Phe 1·04, Har 0·94. For $C_{47}H_{67}N_{15}O_{12}S_2.3\,TFA.3\,H_2O$ (1 494·4) calculated: 42·59% C, 5·12% H, 14·05% N; found: 42·37% C, 5·00% H, 13·79% N. FAB MS (m/z): 1 099 ($M + H^+$). Lit.[2]: $[\alpha]_D$ +3·0° (c 0·5; 1M acetic acid).

[2-*p*-Methyl-L-phenylalanine, 8-D-homoarginine]vasopressin (*II*)
and [2-*p*-methyl-D-phenylalanine, 8-D-homoarginine]vasopressin (*III*)

Peptide-resin A (3·4 g, 0·96 mmol) was coupled according to general scheme with 1·1 equivalents of Boc-L,D-Phe(Me)-OH for 18 h and subsequently with another 0·5 equivalents for 4 h and with Boc-Cys(4-Me-Bzl)-OH (ref.[22]). Treatment of protected nonapeptide was analogous to that of compound *I*. Freeze-drying afforded 614 mg of crude product, which was purified by HPLC on Vydac column 218TP5 in slow gradient running from 25% to 50% methanol in 0·05% trifluoroacetic acid in 50 min. Lyophilization of the corresponding fractions afforded 40 mg and 16·2 mg of the products pure according to HPLC.

The first product (40 mg; k 3·18, methanol–0·05% trifluoroacetic acid 55 : 45 (S)) corresponds to analog with p-methyl-L-phenylalanine in position 2. R_F 0·01 (S1), 0·45 (S4); $E_{2.4}^{Gly}$ 1·24; $E_{5.7}^{His}$ 0·61; $[\alpha]_D$ −4·0° (c 0·2; 1M-acetic acid). Amino acid analysis: Asp 1·08, Glu 1·01, Pro 0·87,Gly 1·08, Cys (as cysteic acid) 2·04, 4-Me-Phe 0·5, Phe 0·92, Har 1·01. For $C_{48}H_{67}N_{13}O_{12}S_2.3TFA.2\ H_2O$ (1 474·4) calculated: 43·99% C, 5·20% H, 14·25% N; found: 44·19% C, 4·96% H, 14·41% N. FAB MS (m/z): 1 097 $(M + H)^+$.

The second product (16·2 mg; k 5·18, methanol–0·05% trifluoroacetic acid 55 : 45 (S)); corresponds to analog containing p-methyl-D-phenylalanine in position 2. R_F 0·01 (S1), 0·45 (S4); $E_{2.4}^{Gly}$ 1·24, $E_{5.7}^{His}$ 0·52; $[\alpha]_D$ −44·3° (c 0·2; 1M acetic acid). Amino acid analysis: Asp 0·89, Glu 1·02, Pro 1·00, Gly 1·05, Cys (as cysteic acid) 2·03, 4-Me-Phe 0·88, Phe 1·00, Har 1·08. For $C_{48}H_{67}N_{13}O_{12}S_2.3·5\ TFA.1·5\ H_2O$ (1 522·4) calculated: 43·39% C, 5·00% H, 13·80% N; found: 43·24% C, 4·78% H, 14·13% N. FAB MS (m/z): 1 097 $(M + H)^+$ and 1 099 $(M + 2\ H + H)^+$.

[2-p-Ethyl-L-phenylalanine, 8-D-homoarginine]vasopressin (*IV*)
and [2-p-ethyl-D-phenylalanine, 8-D-hmoarginine]vasopressin (*V*)

Peptide-resin A (1·35 g; 0·5 mmol) was coupled according to general scheme with 1·1 equivalents of Boc-L,D-Phe(Et)-OH for 24 h and coupling was repeated with another equivalent in the presence of dimethylaminopyridine for 30 min. After coupling of Boc-Cys(4-Me-Bzl)-OH (ref.[22]) the treatment of the resin was analogous to that described above. After freeze-drying, the product (185 mg) was purified by HPLC (gradient of methanol in 0·1% trifluoroacetic acid on Separon SGX-C18 column) and lyophilization of the corresponding fractions afforded 18 mg and 11·5 mg of the products pure according to HPLC.

The first product (18 mg; k 2·2, methanol–0·05% trifluoroacetic acid 3 : 2 (S); k 7·44, methanol –0·05% trifluoroacetic acid 1 : 1 (S); k 1·4, methanol–0·05% trifluoroacetic acid 1 : 1 (V)) corresponds to analog with p-ethyl-L-phenylalanine in position 2. R_F 0·00 (S1), 0·45 (S4); $E_{2.4}^{Gly}$ 1·17, $E_{5.7}^{His}$ 0·58; $[\alpha]_D$ −3·1° (0·2; 1M-acetic acid). Amino acid analysis: Asp 0·99, Glu 1·00, Pro 1·12, Gly 1·01, Cys (as cysteic acid) 2·12, 4-Et-Phe 0·70, Phe 1·02, Har 0·80. For $C_{49}H_{71}N_{15}.O_{11}S_2.4\ TFA.2\ H_2O$ (1 602·5) calculated: 42·79% C, 4·97% H, 13·11% N; found: 42·85% C, 5·05% H, 13·37% N. FAB MS (m/z): 1 111 $(M + H)^+$.

The second product (11·5 mg; k 3·60, methanol–0·05% trifluoroacetic acid 3 : 2 (S); k 19·57, methanol–0·05% trifluoroacetic acid 1 : 1 (S); k 2·77, methanol–0·05% trifluoroacetic acid 1 : 1 (V)) corresponds to analog containing p-ethyl-D-phenylalanine in position 2. R_F 0·05 (S1), 0·45 (S4); $E_{2.4}^{Gly}$ 1·19, $E_{5.7}^{His}$ 0·51; $[\alpha]_D$ −28·5° (c 0·2; 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 0·98, Pro 1·05, Gly 1·12, Cys (as cysteic acid) 2·02, 4-Et-Phe 0·68, Phe 1·05, Har 0·85. For $C_{49}H_{71}N_{15}O_{11}S_2.4$ TFA (1 566·4) calculated: 43·71% C, 4·83% H, 13·41% N; found: 43·74% C, 5·16% H, 13·52% N. FAB MS (m/z): 1 113 $(M + 2\ H + H)^+$.

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[24] in Munsick[25] solution. Inhibitory activity is characterized by pA_2 value (ref.[26]). Pressor activity was tested on pithed rat preparation according to refs[27,28]. Antidiuretic potency on nonanaesthetized rat was followed according to the method in ref.[29]. As the standard in the antidiuretic test [8-D-arginine]deamino-vasopressin was used.

The authors are indebted to Mrs V. Holoubková, Mrs I. Hošková and Mrs J. Kellerová for skillful technical assistance in the case of peptide synthesis and pharmacological evaluation, resp. to Mr. J. Zbrožek for the amino acid analyses, Mrs Z. Ledvinová for the optical rotation measurements

and to Mr M. Ryba for the preparative liquid chromatography of analogs I, IV and V. The elemental analyses were carried out in the Analytical Laboratory of our Institute (Dr V. Pechanec, Head) and mass spectra in the Laboratory of the Mass Spectroscopy (Dr K. Ubik, Head).

REFERENCES

1. Ludlam C. A., Peake I. R., Allen N., Davies B. L., Furlong R. A., Bloom A. L.: Br. J. Haematol. *45*, 499 (1980).
2. Garcia V. V., Silva I. A., Borrasca A. L.: Thromb. Haemost. *48*, 91 (1982).
3. Mikaelsson M., Nilsson I. M., Vilhardt H., Wiechel B.: Transfusion *22*, 229 (1982).
4. Nilsson I. M., Mikaelsson M., Vilhardt H., Walter H.: Thromb. Res. *15*, 263 (1979).
5. *Handbook of Neurohypophyseal Hormone Analogs* (K. Jošt, M. Lebl and F. Brtník, Eds). CRC Press, Boca Raton 1987.
6. *IUPAC-IUB Joint Commission on Biochemical Nomenclature:* Eur. J. Biochem. *138*, 9 (1984).
7. Heyboer N., Heymens Visser G., Kerling K. E. T.: Rec. Trav. Chim. *81*, 69 (1962).
8. Snyder H. R., Shekleton J. F., Lewis C. D.: J. Am. Chem. Soc. *67*, 310 (1945).
9. Herr R. R., Enkoji T., Dailey J. P.: J. Am. Chem. Soc. *79*, 4229 (1957).
10. Larsen B., Fox B. L., Burke M. F., Hruby V. J.: Int. J. Peptide Protein Res. *13*, 12 (1979).
11. Lebl M.: J. Chromatogr. *264*, 459 (1983).
12. Lebl M., Barth T., Servitová L., Slaninová J., Jošt K.: Collect. Czech. Chem. Commun. *50*, 132 (1985).
13. Riniker B., Schwyzer R.: Helv. Chim. Acta *44*, 658 (1961).
14. Riniker B., Schwyzer R.: Helv. Chim. Acta *47*, 2357 (1964).
15. Ref.[5], Vol. II, Part 2, p. 134.
16. Lindeberg E. G. G., Melin P., Larsson L. E.: Int. J. Pept. Protein Res. *8*, 193 (1976).
17. Kaiser E., Colescott R. L., Bossinger C. D., Cook P. I.: Anal. Biochem. *34*, 595 (1970).
18. Krchňák V., Vágner J., Šafář P., Lebl M.: Collect. Czech. Chem. Commun. *53*, 2542 (1988).
19. Dakin H. D.: J. Biol. Chem. *9*, 151 (1911).
20. Cleland G. H.: J. Org. Chem. *34*, 744 (1969).
21. Zhuze A. L., Jošt K., Kasafírek E., Rudinger J.: Collect. Czech. Chem. Commun. *29*, 2648 (1964).
22. Cosand W. L., Merrifield R. B.: Proc. Natl. Acad. Sci. U.S.A. *74*, 2771 (1977).
23. Yamashiro D., Li C. H.: J. Am. Chem. Soc. *95*, 1310 (1973).
24. Holton P.: Br. J. Pharmacol. *3*, 328 (1984).
25. Munsick R. A.: Endocrinology *66*, 451 (1960).
26. Eggena P., Schwartz I. L., Walter R.: J. Gen. Physiol. *56*, 250 (1970).
27. Shipley R. E., Tilden J. H.: Proc. Soc. Exp. Biol. NY *64*, 453 (1947).
28. Krejčí I., Kupková B., Vávra I.: Br. J. Pharmacol. Chemother. *30*, 497 (1967).
29. Burn J. H., Finney D. J., Goodwin L. G.: *Biological Standardization*, 2nd ed., p. 187. Oxford University Press, London 1950.

Translated by the author (M.L.).