[1-β-MERCAPTOPROPIONIC ACID, 8-NORARGININE]VASOPRESSIN AND [1-β-MERCAPTOPROPIONIC ACID, 8-D-NORARGININE]VASOPRESSIN. TWO ANALOGS WITH STRONG BIOLOGICAL EFFECTS*,**

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[1-β-Mercaptopropionic acid, 8-norarginine]vasopressin (L^8, D^8; *I*, *II*) was prepared by condensation of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine with prolyl-N$^\gamma$-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine amide (L^2, D^2) by the azide or carbodiimide method, respectively, removal of the benzyloxycarbonyl residue, guanidination of γ-amino groups, removal of protecting groups, closing of the disulfide bridge, and electrophoretic purification. *I* has an almost 2 times higher antidiuretic effect than DDAVP and a 3 times higher pressor effect than AVP. *II* has 20—25% of the antidiuretic effect of DDAVP and 16 IU/mg of the pressor effect.

Systematic homologization experiments[2] permit us to determine the "activity profile" of a biologically active peptide with respect to the given position (dependence of biological effect on the length of the side chain of the amino acid). So far the two norarginine analogs have been missing in the homologous series of deaminoarginine-vasopressin (L, D). The results of this study fill up the gap.

We based the synthesis of the protected peptides used as starting material for the preparation of both analogs on the guanidination procedure[3]. The guanidine group was introduced into synthetized, partially protected octapeptide derivatives, Mpr(Bzl)--Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Dab-Gly-NH_2 (L^8, D^8; *III*, *IV*). The fully protected

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** The symbols and abbreviations usual in peptide chemistry were used. Other abbreviations: Mpr β-mercaptopropionic acid, Nar norarginine. Unless stated otherwise the optically active amino acids are of L-configuration. LVP lysine-vasopressin, AVP arginine-vasopressin, AD antidiuretic effect, BP pressor effect, DDAVP [1-β-mercaptopropionic acid, 8-D-arginine]-vasopressin, DCI N,N′-dicyclohexylcarbodiimide. As a homologous series we regard groups of vasopressin analogs of the same type (amino-, deamino-,AVP, LVP, *etc.*) homologized at one position (position 8 in this case). Some results of this study have been presented in a preliminary communication[1].

precursors of *III* and *IV* with γ-amino groups blocked by the benzyloxycarbonyl residue (peptides *V* and *VI*) were prepared by two different procedures. The first one involved the condensation of Mpr(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-N_3 with amides H-Pro-Dab(Z)-Gly-NH_2 (L[2], D[2]) (the synthesis of H-Pro-D-Dab(Z)-Gly-NH_2 was carried out in the same manner as the synthesis of the all-L peptide[2]). The azide syntheses required a large excess (40%) of tripeptide amides[4]. To avoid this complication we prepared peptides *V* and *VI* also by a modified carbodiimide method[5]. The requisite Mpr(Bzl)Tyr-Phe-Gln-Asn-Cys(Bzl)-OH (ref.[1]) was obtained by condensation of Boc-Asn-OH (ref.[6]) with H-Cys(Bzl)-OBzl (ref.[7]), removal of the Boc group in trifluoroacetic acid, release of dipeptide ester from the trifluoroacetate by sodium bicarbonate, condensation of the free dipeptide ester with Nps-Gln-OH(DCI) (ref.[8]), removal of the Nps group in methanolic solution of hydrogen chloride, and release of the tripeptide ester from the hydrochloride by ammonium hydroxide. The condensation of Mpr(Bzl)-Tyr-Phe-OH with H-Gln-Asn-Cys(Bzl)-OBzl by the modified carbodiimide method afforded Mpr(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-OBzl. The latter was treated with hydrogen bromide in acetic acid[9] to yield Mpr(Bzl)-Tyr--Phe-Gln-Asn-Cys(Bzl)-OH. The fully protected octapeptide amide derivatives *V* and *VI*, prepared by azide synthesis and the modified carbodiimide method showed the same melting points and the same optical activities. The benzyloxycarbonyl residues were removed from the γ-position of peptides *V* and *VI* by treatment with hydrogen bromide in acetic acid and the hydrobromides of the partially protected peptides *III* and *IV* were converted into the free bases by a solution of sodium bicarbonate. Peptides *III* and *IV* were guanidinated by 1-guanyl-3,5-dimethylpyrazole-nitrate[10]. Like in many preceding experiments the guanidination was not complete. Efforts to remove the unguanidinated product at the stage of protected peptides (ion-exchangers, chromatography) were unsuccessful. Better results gave the electrophoretic purification of the analogs synthetized. The D-analog was obtained in pure form. We were not able, however, to remove traces of the unguanidinated product from the all-L peptide. The synthesis of *I* and *II* from *III* and *IV* and the assay of their biological effects were carried out by standard methods[4,11,12].

Biological activities of *I* and *II* are very high. The antidiuretic effect of *I* is at the same dose level practically twice higher than that of DDAVP and the pressor effect approximately three times higher than the same effect of AVP. *II* has also a high antidiuretic effect yet markedly lower than that of *I* (approximately 10% of *I* and 20% of DDAVP). The pressor effect of *II* is low, like with all analogs of the D-series. The AD/BP ratio of *I* is markedly shifted in favor of AD (Table I) similarly to the corresponding analog of the deamino-LVP series, [Mpr^1, Dab^8]VP.[2]

The high biological activity of [Mpr^1, Nar^8]VP (*I*) and the lower AD of [Mpr^1, D-Nar^8] (*II*) was expected. There is a marked activity maximum in the deamino-LVP series represented by [Mpr^1, Dab^8]VP[2], in the corresponding D-series the AD maximum is shifted toward the ornithine and arginine derivative respectively; the

activity profile of the configurationally related series is similar. The homologization experiments in the vasopressin series will be discussed in detail later. It is obvious, though, that the biological activity in homologous vasopressin series varies with the size of the side chain of the basic amino acid. The character of this dependence is not simple yet shows a certain trend. The knowledge of the latter permits us to predict – to a certain degree of probability – the properties of new members of the series.

EXPERIMENTAL

The instruments and analytical methods used here are the same as those described before[3]. Silica-gel sheets (Kavalier, Votice) and the following solvent systems were used for thin-layer chromatography: A, 1-butanol–acetic acid–water (4 : 1 : 1), B, 1-butanol–acetic acid–water (4 : 1 : 5), C, acetonitrile–water (3 : 1), D, ethanol–chloroform (4 : 1).

N^{α}-Tert-butyloxycarbonyl-N^{γ}-benzyloxycarbonyl-D-α,γ-diaminobutyryl-glycine Amide

To the solution of 7 g (20 mmol) of N^{α}-tert-butyloxycarbonyl-N^{γ}-benzyloxycarbonyl-D-α,γ-diaminobutyric acid (prepared from N^{α}-benzyloxycarbonyl-D-α,γ-diaminobutyric acid[15] according to[16]; dicyclohexylammonium salt, m.p. 120–122°C, $[\alpha]_D^{20}$ +17·6° (*c* 2, methanol), +7·0° (*c* 2, dimethylformamide). For $C_{29}H_{47}N_3O_6$ (533·7) calculated: 65·26% C, 8·88% H, 7·87% N; found: 65·03% C, 8·76% H, 7·93% N) was added a solution of 3 g (22 mmol) of N-hydroxybenzotriazole in 10 ml of dimethylformamide, a solution of 3·1 g (20 mmol) of glycine amide hydrobromide and 2·7 ml (20 mmol) of N-ethylpiperidine in 10 ml of dimethylformamide, and 4·5 g (22 mmol) of dicyclohexylcarbodiimide dissolved in 10 ml of dimethylformamide, at 0°C. The mixture was set aside for 1 h at 0°C and for 20 h at room temperature. Dicyclohexylurea which had separated was filtered off, the filtrate was taken to dryness, and the residue dissolved in 200 ml of chloroform. The solution was washed 4 times with 100 ml of 5% $NaHCO_3$, 4 times with 100 ml of 1 mM-HCl, once with 100 ml of water, and then dried over magnesium sulfate. Chloroform was distilled off and the residue was crystallized from methanol–ether. The yield

TABLE I
Biological Activity of Vasopressin Analogs

Compound	AD[a]	BP[b]	UT[b]
[Mpr[1]]AVP (ref.[13])	2·6	370 ± 20	27 ± 4
I[Mpr[1], Nar[8]]VP	~ 200	~ 1 500	7·4
II[Mpr[1], D-Nar[8]]VP	~ 20	16·3	16·2

[a] Expressed in % of a standard DDAVP batch. The actual content of peptides in lyophilisates was taken as the basis for the calculation of biological activities[14] of *I* and *II*. [b] IU/mg.

was 5·8 g (72%) of dipeptide amide chromatographically pure in systems A and B. M.p. 127—128°C. $[\alpha]_D^{24}$ +6·8° (*c* 1, dimethylformamide), $[\alpha]_D^{24}$ +9·5° (*c* 1, methanol). For $C_{19}H_{18}N_4O_6$ (408·5) calculated: 55·87% C, 6·91% H, 13·72% N; found: 55·81% C, 6·88% H, 13·58% N.

Nγ-Benzyloxycarbonyl-D-α,γ-diaminobutyryl-glycine Amide

The preceding product (4·7 g, 11·5 mmol) afforded after removal of the tert-butyloxycarbonyl group in a mixture of 8 ml of trifluoroacetic acid and 2 ml of dichloromethane (after 20 min) a trifluoroacetate. The latter was dissolved in 11 ml of methanol and filtered through a 100-ml column of Ostion AT. The effluents were taken to dryness and the residue was crystallized from a mixture of methanol and ether. The yield of the free dipeptide was 3·2 g (96%) and was chromatographically homogeneous in systems A and C. M.p. 133—134°C and $[\alpha]_D^{24}$ −12·3° (*c* 1, methanol). For $C_{14}H_{20}N_4O_4$ (308·3) calculated: 54·4% C, 6·54% H, 18·17% N; found: 54·30% C, 6·47% H, 18·15% N.

Tert-butyloxycarbonylprolyl-Nγ-benzyloxycarbonyl-D-α,γ-diaminobutyryl-glycine Amide

Tert-butyloxycarbonylproline[17] (2·2 g, 10 mmol) and N-hydroxybenzotriazole (1·5 g, 11 mmol) were dissolved in 7 ml of dimethylformamide. The mixture was cooled to 0°C, 2·3 g (11 mmol) of dicyclohexylcarbodiimide in 5 ml of dimethylformamide was added, the mixture was set aside for 1 h at 0°C and 15 h at room temperature, and then treated as described for the protected dipeptide. Three-fold crystallization from ethanol afforded 4·7 g (83%) of protected tripeptide chromatographically pure in systems A and D. M.p. 193—194°C, $[\alpha]_D^{24}$ −17·8° (*c* 1, 95% acetic acid). For $C_{24}H_{35}N_5O_7$ (505·5) calculated: 57·02% C, 6·97% H, 13·85% N; found: 57·32% C, 7·16% H, 13·86% N.

Prolyl-Nγ-benzyloxycarbonyl-D-α,γ-diaminobutyryl-glycine Amide Trifluoroacetate

The Boc-group was removed from 4 g (8 mmol) of the protected tripeptide in the same manner as described for the preparation of the free dipeptide. The trifluoroacetate, homogeneous in systems A and B, was obtained after three-fold crystallization from methanol–ether and the yield was 4 g (96%). M.p. 149—150°C and $[\alpha]_D^{20}$ −5·7° (*c* 1, methanol). For $C_{19}H_{27}N_5O_5.CF_3COOH$ (519·5) calculated: 48·56% C, 5·24% H, 13·48% N; found: 48·67% C, 5·22% H, 13·57% N.

Prolyl-Nγ-benzyloxycarbonyl-D-α,γ-diaminobutyryl-glycine Amide

The deionization of 3·5 g (6·7 mol) of the preceding trifluoroacetate on Ostion AT (50 ml) gave after crystallization from methanol–ether 2·6 g (96%) of free tripeptide amide chromatographically homogeneous in systems A, C, D. M.p. 157—158°C and $[\alpha]_D^{20}$ −13·6° (*c* 0·9, methanol). For $C_{19}H_{27}N_5O_5.1/2\,H_2O$ (414·5) calculated: 55·06% C, 6·81% H, 16·95% N; found: 54·81% C, 6·55% H, 16·95% N. Amino acid composition: Pro 1·06, Dab 0·91, Gly 1·03.

Tert-butyloxycarbonylasparaginyl-S-benzylcysteine Benzyl Ester

Tert-butyloxycarbonylasparagine[6] (6·05 g, 28 mmol) was dissolved in 50 ml of chloroform by the addition of N-ethylpiperidine (3·9 ml, 28 mmol) and pyridine (2·25 ml, 28 mmol). The solution was cooled to −10°C treated with pivalic chloride (3·5 ml, 28 mmol) and after 5 min at 0°C with S-benzylcysteine benzyl ester (9·6 g, 28 mmol) in 20 ml of chloroform. After 1 h at room temperature the mixture (solid gel) was partly freed of the solvent *in vacuo* (water pump, 1 h),

the residue was triturated three times with 2% HCl and a saturated solution of sodium bicarbonate, filtered off, and dried. Yield 13·7 g (95%), m.p. 137–144°C. Recrystallization from 80% methanol afforded 12·0 g (83%) of a product of m.p. 143–146°C. The sample for analysis was recrystallized from 80% methanol. M.p. 145–147°C. $[\alpha]_D^{21}$ −48·6° (*c* 0·5, methanol). According to literature[18] the m.p. of the product prepared by nitrophenyl ester synthesis is 145–147°C and the yield 66%.

o-Nitrophenylsulphenylglutaminyl-asparaginyl-S-benzylcysteine Benzyl Ester

Tert-butyloxycarbonylasparaginyl-S-benzylcysteine benzyl ester (6·7 g, 13 mmol) was allowed to stand with 60 ml of trifluoroacetic acid 1 h at room temperature. The solution was taken to dryness under reduced pressure (water pump) and the residue was recrystallized from ethyl acetate–light petroleum. Yield 6·8 g (97%), m.p. 62–66°C. This product was immediately used for additional synthesis. The sample for analysis was recrystallized from the same solvents. M.p. 64–67°C, $[\alpha]_D^{20}$ −26·7° (*c* 1·3, H_2O). For $C_{21}H_{25}N_3O_4S.CF_3COOH.0{\cdot}5\,H_2O$ (538·5) calculated: 51·31% C, 5·05% H, 7·80% N; found: 51·04% C, 5·20% H, 7·67% N. Ethyl chloroformate (1·14 ml, 12 mmol) was added to a solution of *o*-nitrophenylsulphenylglutamine[19] (3·45 g, 12 mmol) and N-ethylpiperidine (1·65 ml, 12 mmol) in dimethylformamide (30 ml) cooled to − 10°C. After 10 min at 0°C the mixture was treated with a solution of asparaginyl-S-benzylcysteine benzyl ester trifluoroacetate (6·5 g, 12 mmol) and N-ethylpiperidine (1·65 ml, 12 mmol) in 45 ml of dimethylformamide. The mixture was set aside for 60 min at room temperature. The product was isolated as described in the preceding experiment except that it was not triturated with dilute HCl. The yield was 6·0 g of the crude product, m.p. 201–203°C, which was immediately used for further work. After twofold crystallization from dimethylformamide–water the m.p. of the product was 208–209°C, $[\alpha]_D^{25}$ −31·9° (*c* 0·5, dimethylformamide). For $C_{32}H_{36}.N_6O_8S_2$ (696·9) calculated: 55·15% C, 5·22% H, 12·06% N, 9·20% S; found: 55·30% C, 5·42% H, 11·79% N, 9·33% S.

Glutaminyl-asparaginyl-S-benzylcysteine Benzyl Ester

o-Nitrophenylsulfenylglutaminyl-asparaginyl-S-benzylcysteine benzyl ester (5·0 g, 7·2 mmol) was dissolved in 50 ml of 1M solution of hydrogen chloride in methanol. The solution was allowed to stand 5 min at room temperature and then evaporated under reduced pressure. The residue was triturated with ether, dried, suspended in water, and the pH of the suspension adjusted to 8–9 by ammonium hydroxide. The mixture was set aside for 1 h at room temperature, the product was then filtered off, washed with water, dried, and recrystallized from ethanol–ether. Yield 3·2 g (82%), m.p. 189–192°C, $[\alpha]_D^{25}$ −26·3° (*c* 0·3, 95% CH_3COOH). For $C_{26}H_{33}N_5O_6S.1/2\,H_2O$ (552·6) calculated: 56·51% C, 6·20% H, 12·67% N; found: 56·81% C, 6·08% H, 12·69% N.

β-Benzylthiopropionyl-tyrosyl-phenylalanine

β-Benzylthiopropionyl-tyrosyl-phenylalanine methyl ester[19,20] (10·0 g, 19 mmol) was shaken 45 min with 50 ml of 1M-NaOH. The mixture was acidified by hydrochloric acid to pH 1–2, the product (oil) which separated was extracted into ethyl acetate, the extract was dried over sodium sulfate and taken to dryness under reduced pressure. The residue yielded after twofold crystallization from ethyl acetate–light petroleum 7·0 g (70%) of a product of m.p. 125°C, $[\alpha]_D^{25}$ +10·5° (*c* 0·6, 95% CH_3COOH). For $C_{28}H_{30}N_2O_5S.H_2O$ (524·6) calculated: 64·10% C, 6·13% H, 5·33% N, 6·12% S; found: 64·32% C, 6·36% H, 5·03% N, 6·04% S.

β-Benzylthio propionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Benzyl Ester

To the solution of β-benzylthiopropionyl-tyrosyl-phenylalanine (2·9 g, 5·5 mmol), glutaminyl--asparaginyl-S-benzylcysteine benzyl ester (3·0 g, 5·5 mmol), and N-hydroxybenzotriazole (2·3 g, 17 mmol) in 20 ml of dimethylformamide was added at 0°C a solution of dicyclohexylcarbodiimide (1·25 g, 6 mmol), in 5 ml of dimethylformamide. The mixture was allowed to stand 2 h at 0°C and at room temperature overnight. The N,N'-dicyclohexylurea which separated was filtered off, the filtrates were taken to dryness under reduced pressure, and the residue was triturated three times with 3% hydrochloric acid and a saturated solution of sodium bicarbonate. The product was filtered off, washed on the filter with water, and dried. The yield was 5·4 g (99%) of crude product which was reprecipitated from dimethylformamide–water. Yield 4·9 g (89%), m.p. 237—239°C, $[\alpha]_D^{25}$ —31·5° (*c* 0·15, dimethylformamide). The data recorded in literature[18] are m.p. 235—238°C, $[\alpha]_D^{23}$ —27·7° (*c* 1, dimethylformamide) for a product prepared. by stepwise synthesis from the carboxyl terminus by the method of activated *p*-nitrophenyl estersr

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine benzyl este (4·7 g, 4·5 mmol) was dissolved in 20 ml of acetic acid, the solution was treated with 20 ml of c. 35% solution of hydrogen bromide in acetic acid, and the mixture was heated 15 min at 60°C. The solution was taken to dryness under reduced pressure and water was layered over the residue. After 1 h of standing at 0°C the product which separated was filtered off and dried (3·8 g, 90%). The yield of the product obtained after reprecipitation from dimethylformamide–water was 2·8 g (66%), m.p. 218—219°C, $[\alpha]_D^{20}$ —30·9° (*c* 0·5, dimethylformamide). For $C_{47}H_{55}N_{10}O_{10}S_2$ (942·10) calculated: 59·90% C, 5·88% H, 10·41% N, 6·81% S; found: 59·71% C, 5·88% H, 10·18% N, 6·85% S.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--N$^{\gamma}$-benzyloxycarbonyl-D-α,γ-diaminobutyryl-glycine Amide (*VI*)

The azide synthesis was effected as described for *V* (ref.[2]). From 1·43 g (1·5 mmol) of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine hydrazide, 0·85 g (40% excess) of prolyl-N$^{\gamma}$-benzyloxycarbonyl-D-α,γ-diaminobutyryl-glycine amide, 1·9 g of the product (95% yield) was obtained; m.p. 215—217°C, $[\alpha]_D^{20}$ —19·9° (*c* 1·0, dimethylformamide). For $C_{66}H_{80}N_{12}O_{14}S_2$ (1 329·4) calculated: 59·62% C, 6·05% H, 12·65% N, 4·83% S; found: 59·49% C, 6·14% H, 12·69% N, 4·77% S.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--norarginyl-glycine Amide

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N$^{\gamma}$--benzyloxycarbonyl-α,γ-diaminobutyryl-glycine amide[2] (3·2 g, 2·4 mmol) was dissolved in acetic acid (4 ml), a solution of hydrogen bromide in acetic acid (35%, 4 ml) was added and the mixture was heated 15 min at 65°C. Subsequently the mixture was taken to dryness under reduced pressure and the residue was triturated with saturated solution of sodium bicarbonate. The product was filtered off, washed on the filter with water, and dried *in vacuo* over phosphorus pentoxide. The yield was 1·8 g (63%). The dry product (1·2 g, 1·0 mmol) was dissolved in 7 ml of dimethylformamide and the solution was treated with 1-guanyl-3,5-dimethylpyrazole nitrate (1·4 g,

7 mmol). The pH of the solution was adjusted to 9·5 by triethylamine and the mixture was set aside for 4 days at room temperature. Subsequently it was neutralized with acetic acid and taken to dryness under reduced pressure. The solid residue was triturated with water (75 ml). The product was filtered off, washed on the filter with water and twice recrystallized from dimethylformamide–water. Yield 0·8 g (72%), m.p. 187—189°C, $[\alpha]_D^{25}$ —37·0° (*c* 1·0, dimethylformamide). For $C_{59}H_{76}N_{14}O_{12}S_2$ (1 237·5) calculated: 57·12% C, 6·19% H, 15·85% N, 5·18% S; found: 57·00% C, 6·11% H, 15·03% N, 5·12% S. The low nitrogen value indicates that guanidination was incomplete.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--D-norarginyl-glycine Amide

The decarbobenzoxylation and guanidination were carried out as described for the preceding experiment; the same quantity of reaction components was used. M.p. 189—191°C, $[\alpha]_D^{22}$ —28·5° (*c* 1·0, dimethylformamide). For $C_{59}H_{76}N_{14}O_{12}S_2$ (1 237·5) calculated: 57·12% C, 6·19% H, 15·85% N, 5·18% S; found: 57·08% C, 6·00% H, 15·12% N, 5·12% S.

[1-β-Mercaptopropionic Acid, 8-norarginine]vasopressin (*I*)

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-α--amino-γ-guanidinobutyryl-glycine amide (0·5 g, 0·405 mmol) was dissolved in 500 ml of distilled ammonia and the solution was treated with sodium with stirring until a blue color stable for 30 s developed. The solution was decolorized by the addition of acetic acid, taken to dryness under reduced pressure, the residue freed of ammonia by 1 h evacuation (water pump) at room temperature and dissolved in 600 ml of 2·5% acetic acid. The solution was extracted 5-times with ether free of peroxides, and its pH was adjusted to 6·75 by ammonium hydroxide; subsequently it was oxidized by 0·01M solution of $K_3Fe(CN)_6$. The pH of the reaction mixture was adjusted to 4·4 by acetic acid and the solution was desalted by passage through an Amberlite CG 50 column (25 ml) and washing of the column with 250 ml of 0·25% acetic acid. The crude product was eluted from the column by 50% acetic acid. Effluents containing the product (30 ml) were diluted to 50 ml with water and lyophilized. The lyophilisate was subjected to purification by free-flow electrophoresis. The absorbance of the fractions was measured at 275 nm. The fractions which contained the pure product were pooled, filtered, and lyophilized. The yield of the analog lyophilisate was 109·0 mg, $[\alpha]_D^{25}$ —72·1° (*c* 0·37, 1M-CH_3COOH). The elemental analysis of the lyophilisate dried 20 h at 100°C and 0·1 Torr over phosphorus pentoxide corresponded to the monoacetate. For $C_{45}H_{62}N_{14}O_{12}S_2.CH_3CO_2H$ (1 115·3) calculated: 50·62% C, 5·97% H, 17·58% N, 5·75% S; found: 50·48% C, 5·83% H, 17·41% N, 5·69% S. Amino acid analysis: Tyr 1·03, Phe 1·05, Glu 1·00, Asp 0·96, Pro 0·98, Nar 0·91, Gly 0·99. UV (water acidified to pH 3·3 with hydrochloric acid) λ_{min} 250 nm, λ_{max} 275 nm.

[1-β-Mercaptopropionic Acid, 8-D-norarginine]vasopressin (*II*)

The analog was prepared by the procedures described for the preceding experiment. β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-D-norarginyl-glycine amide (0·3 g) afforded 54·8 mg of the analog lyophilisate. $[\alpha]_D^{20}$ —58·6° (*c* 0·2, 1M-CH_3. .COOH). The analysis of the sample dried 20 h at 100°C and 0·1 Torr over phosphorus pentoxide corresponded to the monoacetate. For $C_{45}H_{62}N_{14}O_{12}S_2.CH_3CO_2H$ (1 115·3) calculated: 50·62% C, 5·97% H, 17·58% N, 5·75% S; found: 50·51% C, 5·88% H, 17·33% N, 5·65% S. Amino acid analysis: Tyr 0·94, Phe 1·06, Glu 1·04, Asp 0·98, Pro 0·96, Nar 0·91, Gly 1·03.

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