[1-β-MERCAPTOPROPIONIC ACID, 8-α,γ-DIAMINOBUTYRIC ACID]-VASOPRESSIN AND [1-β-MERCAPTOPROPIONIC ACID, 8-D-ORNITHINE]VASOPRESSIN. SYNTHESIS AND BIOLOGICAL EFFECTS*

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[1-β-Mercaptopropionic acid, 8-α,γ-diaminobutyric acid]vasopressin (*III*) and [1-β-mercaptopropionic acid, 8-D-ornithine]vasopressin (*IV*), prepared by condensation of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine with prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine amide (*V*) and prolyl-N^{δ}-benzyloxycarbonyl-D-ornithinyl-glycine amide (*VI*) respectively, by removal of the protecting groups, oxidative closure of the disulfide ring, and purification by continuous free-flow electrophoresis, show high biological activities. The antidiuretic effect of *III* (1373 IU/mg) is several times higher than that of desaminolysine-vasopressin and the pressor effect (450 IU/mg) corresponds to the effect of arginine-vasopressin. The antidiuretic effect of *IV* is high (590 IU/mg) and its pressor effect low (4·8 IU/mg), similarly to all the analogs of the D-series.

Homologization experiments in the lysine-vasopressin series[1] have cast light on relations between the size of the side chain of the amino acid in position 8 and the antidiuretic and pressor effect, relations which are useful both from the theoretical and practical viewpoint. We have not performed as yet a similar study with the corresponding desamino compounds which are more interesting from the viewpoint of investigation of antidiuretics derived from vasopressin (they show a higher value and a higher specificity of antidiuretic effect in most cases). In the process of synthetizing arginine–vasopressin analogs by the "guanidation procedure"[2-4] we obtained as intermediary products β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine amide (*I*) and β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^{δ}-benzyloxycarbonyl-D-ornithinyl-glycine amide (*II*), *i.e.* peptides containing the sequence of [Mpr^1, Dab^8]VP** (*III*) and [Mpr^1, D-Orn^8]VP

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** The abbreviations used in this study mostly follow the suggestions of the IUPAC-IUB

(*IV*). In the present study we have made use of *I* and *II* for the preparation of *III* and *IV* in order to complement the existing group of desamino compounds (analogs containing Orn, Lys, and Hly in position 8 are known in the L-series, analogs with D-Dab, D-Lys, and D-Hly in the D-series, see Table I) and to obtain knowledge about the extent to which relations derived from the amino series are valid also in the desamino series.

Both protected octapeptide amide derivatives *I* and *II* were prepared according to the usual scheme[8], *i.e.* by condensation of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine azide with prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine amide (*V*) and prolyl-N^{δ}-benzyloxycarbonyl-D-ornithyl-glycine amide (*VI*) respectively. Amides *V* and *VI* were obtained by coupling N^{α}-tert-butyloxycarbonyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyric acid[9] or N^{α}-tert-butyloxycarbonyl-N^{δ}-benzyloxycarbonyl-D-ornithine[10] respectively with glycine amide, by removal of the tert-butyloxycarbonyl residues from the protected dipeptide amides in a mixture of trifluoroacetic acid and dichloromethane[11], liberation of the partly protected dipeptide amides from the trifluoroacetates by the action of a strongly basic anion exchanger (Ostion AT*), and acylation of the dipeptide amides by tert-butyloxycarbonylproline in the presence of dicyclohexylcarbodiimide. The deblocking of the α-amino groups and the liberation of the partially protected tripeptide amides from the trifluoroacetates was effected in the same manner as with the dipeptide amide derivatives. Analogs *III* and *IV* were prepared from compounds *I* and *II* by the usual procedure involving reduction by sodium in liquid ammonia[12,13], desalting[8] on Amberlite CG, and purification by continuous free-flow electrophoresis[14,15].

The antidiuretic effect of the analogs synthetized was determined by the method of Jeffers and coworkers[16] as modified by Pliška and Rychlík[17], moreover the antidiuretic effect of the analog *IV* was determined by the method of Burn and coworkers[18]. The pressor effect was determined on despinalized rats according to Krejčí and coworkers[19], the uterotonic effect on isolated rat uterus according to Holton[20] using the modification of Munsick[21], the galactagogic effect on the mammary gland of a lactating rat according to Bisset and coworkers[22]. The

Comission on Biochemical Nomenclature[5–7]. Other abbreviations: Dab α,γ-diaminobutyric acid, Dap α,β-diaminopropionic acid, Mpr β-mercaptopropionic acid, VP vasopressin, AD antidiuretic effect, BP pressor effect, UT uterotonic effect, G galactagogic effect. Compounds with cysteine in position 1 and with all optically active amino acids of L-configuration are referred to as analogs of amino-L-series. Compounds containing β-mercaptopropionic acid in position 1 and an amino acid of D-configuration in position 8 we regard as belonging to the desamino-D-series. Some older names of analogs are retained.

* A strongly basic anion exchanger on polystyrene basis, a product of Spolek pro chemickou výrobu, Ústí nad Labem.

TABLE I

Biologic Activity (IU/mg) of Lysine-vasopressin and Desamino-lysine-vasopressin Analogs Containing Homologous Series of Basic Amino Acids in Position 8

	[X^8] vasopressin	AD	BP	UT	G	AD/BP	Ref.
			Amino-L-series				
1	Dap^8	40—60	109	0·45	—	0·5	1
2	Dab^8	120 ± 30	149	3·5	—	0·8	23
3	Orn^8	92 ± 18	375 ± 27	10 ± 2	50	0·2	24
4	Lys^8	209 ± 10	243 ± 3	4·8 ± 0·3	63 ± 10	0·9	25, 26
5	Hly^8	159 ± 18	267 ± 13	...	...	0·6	27
		(300)	(250)			(1·2)	28
			Desamino-L-series				
III	Dab^8	1 373 ± 128	450 ± 30	24·7 ± 7	134 ± 15	3	[a]
6	Orn^8	208 ± 24	365 ± 31	16 ± 2	44 ± 7	0·6	24
7	Lys^8	313 ± 11	131 ± 2	12 ± 0·5	33 ± 2	2	25
8	Hly^8	634 ± 54	153 ± 4	—	—	4	27
		(10 200)[b]	(990)[b]	—	—	(11)	30
			Amino-D-series				
9	Dap^8	150—170	21·2	0·53	—	7	1
10	Dab^8	120 ± 30	3·6	0·1	—	33	14
11	Orn^8	50—70	0·24	0·2	—	250	1
12	Lys^8	6—10	0·75	1·0	—	11	29
13	Hly^8	207 (168—254)	1·83 (1·48—2·28)	—	—	113	31
			Desamino-D-series				
14	Dab^8	360	2·05	0·38	—	176	26
IV	Orn^8	29 (590[c])	4·8 ± 0·9	1·9 ± 0·25	3·1 ± 0·3	123	[a]
15	Lys^8	3·8	1·05	0·07	—	4	26
16	Hly^8	170 (130—217)	0·5	—	—	340	31

[a] This paper, [b] unrealistically high value, [c] according to[18].

antidiuretic and pressor effect of compound *III* is probably the highest of all the compounds of the desamino-L-lysine-vasopressin series known so far. The removal of the amino group from position 1 resulted in an increase of AD by one order of magnitude and in a three-fold increase of BP compared to the corresponding amino compound (Table I, compound *2*). The antidiuretic effect of *IV*, estimated by Burn's assay[18], is relatively high and corresponds to 590 IU/mg. The pressor effect of *IV* is low as with all the analogs of the D-series prepared so far.

A certain similarity will emerge if we compare the amino series with the even incomplete desamino series. The antidiuretic effect shows an ascending trend in the amino series. It attains one maximum with [Dab^8]VP (*2*) and another with LVP (*4*) or perhaps even with [Hly^8]VP (*5*). In the desamino-L-series, namely with [Mpr^1, Dab^8]VP (*III*), a maximum, much more pronounced, is observed too. The antidiuretic effect then increases from [Mpr^1, Orn^8]VP (*6*) to [Mpr^1, Hly^8]VP (*8*), similarly to the amino-L-series. A slightly different situation is encountered with the pressor effect. The latter increases in the amino-L-series from [Dap^8]VP (*1*) to [Orn^8]VP (*3*) (maximum) and then decreases to LVP (*4*). A small yet distinct increase of BP can be observed with [Hly^8]VP (*5*). The desamino-L-series on the other hand probably reaches maximum of BP with [Mpr^1, Dab^8]VP (*III*). BP then decreases to [Mpr^1, Lys^8]VP (*7*). Similarly to the amino-L-series, BP of [Mpr^1, Hly^8]VP (*8*) is slightly yet distinctly higher than BP of desamino-lysine-vasopressin (*7*).

We do not compare compounds of the amino-D-series with compounds of the desamino-D-series in this study. AD was determined by different methods in the two series and this makes a comparison very difficult. Moreover, we are lacking data on the first member of the desamino-D-series, [Mpr^1, D-Dap^8]VP, which can be of essential importance.

Compounds *III* and *IV* show relatively high activities of the vasopressin type. We determined therefore their effect also on the uterus and the mammary gland. On this occassion we compared available data on UT and G in the series discussed. UT can be compared in all four series, G in the desamino-L-series only. Compounds *III* and *IV* are the most active ones in their series, not only as regards their vasopressin effects but also their oxytocin effects. UT parallels the trend of BP in the amino-L-series and desamino-L-series (Table I). The removal of NH_2^1 has the expected effect: it leads to the potentiation of UT. The change of configuration in position 8 decreases UT practically in all cases by one to two orders of magnitude. G decreases in the desamino-L-series from [Mpr^1, Dab^8]VP (*III*) to [Mpr^1, Lys^8]VP (*7*).

EXPERIMENTAL

The instruments and analytical methods used in this study are the same as in the preceding papers[4].

N^{α}-tert-Butyloxycarbonyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine Amide

N^{α}-tert-butyloxycarbonyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyric acid[9] (9·5 g, 27 mmol), glycine amide hydrobromide (4·2 g, 27 mmol), N-ethylpiperidine (3·65 ml, 27 mmol), and N-hydroxybenztriazole (3·8 g, 27 mmol) were dissolved in 20 ml of dimethylformamide. The solution was treated at 0°C with 5·7 g (28 mmol) of dicyclohexylcarbodiimide in 8 ml of dimethylformamide. Dicyclohexylurea which separated after the mixture had been allowed to stand 1 h at 0°C and 15 h at room temperature, was filtered off, the filtrates were evaporated at reduced pressure, and the dry residue was dissolved in 150 ml of chloroform. The chloroform solution was washed with saturated solution of $NaHCO_3$ (3 times), water, dilute hydrochloric acid (10^{-3}M, 3 times), water, dried by sodium sulfate, and taken to dryness. After the dry residue had been crystallized from a mixture of 2-propanol and diisopropyl ether, 9·0 g (82%) of the protected dipeptide amide was obtained. M.p. 125—126°C, $[\alpha]_D^{20}$ —7° (*c* 1·0, dimethylformamide), $[\alpha]_D^{24}$ —9·6° (*c* 1·0, methanol). For $C_{19}H_{28}N_4O_6$ (408·5) calculated: 55·87% C, 6·91% H, 13·72% N; found: 56·04% C, 6·89% H, 13·72% N.

N^{α}-tert-Butyloxycarbonyl-N^{δ}-benzyloxycarbonyl-D-ornithine Amide

Using the procedure described in the preceding experiment and 4·2 g (11·5 mmol) of N^{α}-tert-butyloxycarbonyl-N^{δ}-benzyloxycarbonyl-D-ornithine[10], 1·8 g (11·5 mmol) of glycine amide hydrobromide, 3·1 g (23 mmol) of N-hydroxybenztriazole, and 2·6 g (11·5 mmol) of dicyclohexylcarbodiimide, we obtained 4·0 g (82%) of the protected dipeptide amide, m.p. 127—129°C and $[\alpha]_D^{22}$ 1·8° (*c* 1·0, methanol). For $C_{20}H_{30}N_4O_6$ (422·5) calculated: 56·86% C, 7·16% H, 13·26% N; found: 56·88% C, 6·98% H, 12·95% N.

N^{γ}-Benzyloxycarbonyl-α,γ-diaminobutyryl-glycine Amide

A solution of N^{α}-tert-butyloxycarbonyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine amide (4·0 g, 10 mmol) in 8 ml of trifluoroacetic acid and 2 ml of dichloromethane were allowed to stand 20 min at room temperature. The mixture was taken to dryness under reduced pressure, the dry residue was dissolved in 10 ml of methanol and the solution was filtered through a column of Ostion AT. The effluents were evaporated under reduced pressure and the dry residue was recrystallized twice from a mixture of methanol and ether. The yield was 2·9 g (94%) of the partly protected amide, m.p. 133—134°C, δ $[\alpha]_D^{22}$ 12·3° (*c* 1, methanol). For $C_{14}H_{20}N_4O_4$ (308·3) calculated: 54·54% C, 6·54% H, 18·17% N; found: 54·44% C, 6·50% H, 18·13% N.

N^{δ}-Benzyloxycarbonyl-D-ornithyl-glycine Amide

The same procedure as in the preceding experiment was used. From 3·5 g (8 mmol) of the protected dipeptide amide 2·4 g (93%) of the partly protected dipeptide amide was obtained. M.p. 119—121°C and $[\alpha]_D^{20}$ —11·1° (*c* 1·0, methanol). For $C_{15}H_{22}N_4O_4$ (322·4) calculated: 55·89%C, 6·88% H, 17·38% N; found: 55·66% C, 6·86% H, 17·39% N.

Prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine Amide (*V*)

Tert-butyloxycarbonylproline (2·2 g, 10 mmol) and N-hydroxybenztriazole (1·5 g, 11 mmol) were dissolved in 7 ml of dimethylformamide and a solution of 3·1 g (10 mmol) of N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine amide in 7 ml of dimethylformamide was added. The mixture was cooled to 0°C and 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 for 15 h at room temperature

and was treated afterwards as described for the corresponding dipeptide amide. Yield 3·8 g (73%) (amorphous foam). The removal of the tert-butyloxycarbonyl residue was carried out as in the preceding case. After double crystallization from ethyl acetate–diisopropyl ether 2·0 g (81%) of amide *V*, m.p. 95—100°C and $[\alpha]_D^{20}$ —25·5° (*c* 0·95, methanol) was obtained from 3·0 g of the protected tripeptide amide. For $C_{19}H_{27}N_5O_5.1/2\ H_2O$ (414·5) calculated: 55·06% C, 6·81% H, 16·91% N; found: 54·87% C, 6·90% H, 17·01% N. Amino acid composition: Pro 1·00, Dab 0·97, Gly 1·07.

Prolyl-N^{δ}-benzyloxycarbonyl-D-ornithyl-glycine Amide (*VI*)

Tert-butyloxycarbonylproline (3·3 g, 15 mmol), N-hydroxybenztriazole (2·2 g, 16 mmol), and N^{δ}-benzyloxycarbonyl-D-ornithyl-glycine amide (4·8 g, 15 mmol) were dissolved in 20 ml of dimethylformamide. The solution was treated at 0°C with 3·4 g (16·5 mmol) of dicyclohexylcarbodiimide in 7 ml of dimethylformamide and the mixture was allowed to stand 1 h at 0°C and 20 h at room temperature. The same procedure as that used for the preparation of the corresponding dipeptide amide was employed for the isolation of the reaction product. Double crystallization from a mixture of 2-propanol and diisopropyl ether afforded 6·0 g (78%) of amide *VI*, m.p. 110—111°C and $[\alpha]_D^{20}$ —13·9° (*c* 1·0, methanol). For $C_{25}H_{37}N_5O_7$ (519·6) calculated: 57·79% C, 7·18% H, 13·48% N; found: 57·99% C, 7·28% H, 13·47% N. The partially protected tripeptide amide was prepared in the same manner as *V*. The protected tripeptide amide (5·2 g) afforded, after three-fold crystallization from a mixture of methanol and ether, 4·8 g (90%) of *VI* trifluoroacetate, m.p. 154—155°C and $[\alpha]_D^{20}$ —12·6° (*c* 1·0, methanol). For $C_{20}H_{29}N_5O_5.CF_3CO_2H$ (533·5) calculated: 49·53% C, 5·67% H, 13·13% N; found: 49·41% C, 5·63% H, 12·89% N.

After deionization of *VI* trifluoroacetate (3·0 g, 5·6 mmol) on Ostion AT and crystallization from ethyl acetate–diisopropyl ether and methanol–ether, 2·0 g (83%) of *VI*, m.p. 59—60°C and $[\alpha]_D^{20}$ —12·3° (*c* 1·0, methanol) were obtained. For $C_{20}H_{29}N_5O_5.1/2\ H_2O$ (428·5) calculated: 56·06% C, 7·06% H, 16·34% N; found: 56·13% C, 7·19% H, 16·46% N. Amino acid composition: Pro 0·95, Orn 1·02, Gly 1·03.

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

A solution of β-benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine hydrazide (1·43 g, 1·5 mmol) in 15 ml of dimethylformamide and 0·8 ml of 4M-HCl in dioxane were cooled to —20°C and treated with 0·175 g of amyl nitrite in 1 ml of dimethylformamide. The mixture was cooled to —40°C after 20 min, neutralized with triethylamine, treated with 0·85 g of *V* (a 40% excess) in 5 ml of dimethylformamide, and set aside for 20 h at 0°C. Dimethylformamide was distilled off under reduced pressure and 200 ml of 1% hydrochloric acid was added to the residue. The precipitate which had separated was filtered off, repeatedly (3 times) triturated with a saturated solution of $NaHCO_3$, filtered off, washed with water on the filter, and dried. The yield after crystallization from dimethylformamide–water was 1·9 g (95%) of *I*, m.p. 222—224°C which increased after crystallization from acetic acid–water to 225—228°C, $[\alpha]_D^{20}$ —38·1° (*c* 1·0, dimethylformamide). Recorded data[32]: m.p. 225—228°C, $[\alpha]_D^{20}$ —40·7° (*c* 0·2, 95% acetic acid) for a product prepared by condensation of the same fragments by the carbodiimide method with the addition of N-hydroxybenztriazole. For $C_{66}H_{80}N_{12}O_{14}S_2$ (1 329·5) calculated: 59·62% C, 6·03% H, 12·64% N, 4·83% S; found: 59·45% C, 6·16% H, 12·64% N, 4·77% S.

β-Benzylthiopropionyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl--N^{δ}-benzyloxycarbonyl-D-ornithyl-glycine Amide (*II*)

Compound *II* was prepared by the same procedure as compound *I*. From 0·886 g (a 40% excess) of *VI* and the same quantity of the remaining reaction components we obtained 2·0 g (a quantitative yield) of *II*, m.p. 210–212°C and $[\alpha]_D^{24}$ −22·8° (*c* 1·0, dimethylformamide). For $C_{67}H_{82}N_{12}O_{14}S_2$ (1343·6) calculated: 59·89% C, 6·15% H, 12·51% N, 4·77% S; found: 59·93% C, 6·13% H, 12·70% N, 4·73% S.

[1-β-Mercaptopropionic acid, 8-α,γ-diaminobutyric acid]vasopressin (*III*)

Protected octapeptide amide *I* (300 mg) was reduced by sodium in 500 ml of liquid ammonia[12]. Ammonia was distilled off under reduced pressure and the dry residue was dissolved in 400 ml of 2·5% acetic acid. The solution was extracted with ether (5 portions of 100 ml), its pH was adjusted to 6·75 with ammonia, and it was oxidized by 0·01M-$K_3[Fe(CN)_6]$. The pH of the reaction mixture was adjusted to 4 by the addition of acetic acid and the solution was desalted by filtration through a column of Amberlite CG (25 × 1 cm). The column was washed with 400 ml of 0·25% acetic acid and the peptides were eluted by 50% acetic acid and lyophilized. The 1st lyophilisate (163 mg) was dissolved in acetic acid (5% solution in 20% acetic acid) and purified[14] by free-flow electrophoresis[15] at 3500 V (160 mA). The yield was 68 mg of the 2nd lyophilisate, $[\alpha]_D^{22}$ −89°C (*c* 0·35, 1M-CH_3COOH). The sample for the analysis was dried 8 h at 100°C under reduced pressure (13 Pa) over phosphorus pentoxide. The analysis corresponded to the monoacetate. For $C_{44}H_{60}N_{12}O_{12}S_2.CH_3COOH$ (1073·2) calculated: 51·48% C, 6·01% H, 15·66% N, 5·98% S; found: 51·32% C, 5·95% H, 15·48% N, 5·96% S. Amino acid composition: Tyr 0·98, Phe 1·02, Glu 1·00, Asp 0·99, Pro 1·01, Dab 0·99, Gly 0·99. UV-spectrum (water acidified by hydrochlorid acid to pH 3·2) λ_{min} 251, λ_{max} 275.

[1-β-Mercaptopropionic acid, 8-D-ornithine]vasopressin (*IV*)

Compound *II* (300 mg) afforded by the same procedure as in the preceding case 152 mg of 1st lyophilisate and 58 mg of 2nd lyophilisate, $[\alpha]_D^{22}$ −62° (*c* 0·16, 1M-CH_3COOH). The results of elemental analysis corresponded to the monoacetate. For $C_{45}H_{62}N_{12}O_{12}S_2.CH_3COOH$ (1087·3) calculated: 51·92% C, 6·12% H, 15·46% N, 5·89% S; found: 51·77% C, 6·09% H, 15·36% N, 5·89% S. Amino acid composition: Tyr 0·93, Phe 1·02, Glu 0·98, Asp 0·99, Pro 1·06, Orn 1·04, Gly 0·98. UV-spectrum (water acidified by hydrochloric acid to pH 3·4) λ_{min} 250, λ_{max} 275.

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