AMINO ACIDS AND PEPTIDES. CVIII.*

DESAMINOVASOPRESSIN-(1–6)-, AND DESAMINOOXYTOCIN-(1–6)-HEXAPEPTIDE AMIDE

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The condensation of β-benzylthiopropionic acid *p*-nitrophenyl ester with O-benzyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteine amide and the stepwise elongation of glutaminyl-asparaginyl-S-benzylcysteine amide by phenylalanine, tyrosine, and β-benzylthiopropionic acid, afforded two protected linear pentapeptide amides, *III*, and *VI*, which after deprotection, oxidation, and purification, yielded desaminooxytocin-(1-6)-hexapeptide amide (*I*), and desamino-vasopressin-(1-6)-hexapeptide amide (*II*). The uterotonic activity of *I* and *II* is considerably higher as compared with the corresponding amino compounds.

The cyclic moiety of the vasopressins and of oxytocin retains some of the characteristic properties of both hormones[1–3]. Vasopressin-(1–6)-hexapeptide amide** has a distinct antidiuretic and residual uterotonic and milk-ejecting activity, oxytocin-(1–6)-hexapeptide amide shows, besides the two oxytocin effects (uterotonic and galactogogue), a relatively high diuresis-inhibiting potency. However, the ratios of the individual activities are shifted, none of the fragments shows – in the dose range used – any influence on the blood pressure of the despinalized rat, and biological effects are rather decreased than increased in the presence of Mg^{+2}-ions. The cyclic fragment of the oxytocin and vasopressin molecule behaves thus as an independent entity differing from the complete molecules more profoundly than merely in the degree of biological activities. This result led us to extend our previous study[3] to include two additional compounds, the desamino analogs of both cyclopeptides.

The synthesis of the required linear protected peptides did not represent any problem and was accomplished by acylation of O-benzyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteine amide by β-benzylthiopropionic acid *p*-nitrophenyl ester on the one hand and by stepwise elongation of glutaminyl-asparaginyl-S-benzyl-cysteine amide by phenylalanine, tyrosine, and β-benzylthiopropionic acid on the

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** The nomenclature and abbreviations are those used in the preceding paper[3].

other. The combination of tert-butyloxycarbonyl and benzyl residues was used for the blocking of the functional groups throughout the whole synthesis.

For the preparation of Boc-amino acids, 2,4,5-trichlorophenyl-tert-butylcarbonate[4] was employed. The procedure deserves a brief comment. The acylation reactions proceeded in all instances smoothly but the products were, without any exception, contaminated with trichlorophenol. Their purification by crystallization was both tedious and wasteful, and uncomplete. We found that the products can easily be freed of the impurity as follows: a chloroform or benzene solution of the crude acylation mixture is filtered through a silica gel column (the Boc-amino acids are adsorbed), trichlorophenol is washed out by the same solvent, and the product is eluted by methanol.

The final stage of the syntheses followed the standard sequence of reactions, comprising reduction with sodium in liquid ammonia[5], oxidative closure of the disulfide bridge[6], desalting on a column of Amberlite IRC-50, XE-64, and purification by a continuous-flow electrophoresis[7,8]. Admittedly, the latter procedure is less customary with this type of compounds but our experiments with Sephadex columns yielded unsatisfactory results. The obtained products were identified and characterized as usual (elemental analyses, amino-acid analyses, TLC).

Desaminooxytocin-(1 – 6)-hexapeptide amide (*I*) and desaminovasopressin-(1 – 6)-hexapeptide amide (*II*) were assayed* for antidiuretic and uterotonic activity with synthetic arginine-vasopressin, oxytocin, and the National Standard for Oxytocin and Vasopressin Substances as reference compounds. The bioassays were complicated by the low solubility of the peptides in water and this fact influenced also the accuracy of the determinations. With the vasopressin derivative both the antidiuretic and uterotonic activity were substantially increased as compared with the corresponding amino compound, the first from c. 0·7 I.U./mg (amino compound) to 1·2 I.U./mg, the latter from 0·26 I.U./mg to 1·03 I.U./mg (medium free of Mg^{+2}) respectively. The renal activity of the desamino oxytocin cycle was low, (0·21 I.U./mg), but for the uterotonic effect a relatively high value**, 16·47 I.U./mg, was obtained. The biological effects of the desaminoanalogs complemented by the data on the amino compounds, are summarized in Table I. The data obtained for desaminovasopressin-(1 – 6)-hexapeptide amide show even a deeper elimination of the differencies between the effects which constitute the oxytocin and vasopressin character as compared with the corresponding amino compound. The potentiation of the antidiuretic and uterotonic activity corresponds roughly to the expected effect of the structural change in position 1. However, the relatively high uterotonic potency of *I* is conspicuous. The present results further emphasize qualitative differences existing between the cyclic moiety of the vasopressins and oxytocin and the parent hormones.

* The biological assays were carried out by Dr I. Krejčí and his coworkers, Research Institute for Pharmacy and Biochemistry, Prague, by Doc. MUDr V. Holeček, IIIrd Clinic of Internal Medicine, Charles University, Prague, and by Dr T. Barth, Department of Biochemistry of this Institute.

** See note added in proof.

TABLE I

Biological Effects of Cyclic Fragments of the Oxytocin and Vasopressin Molecule

Cycle	Activity (on rats)		
	anti-diuretic[a]	utero-tonic[b]	pressor[c]
Desaminooxytocin	0·21	16·47	0
Oxytocin	3	2·7	0
Desaminovasopressin	1·2	1·03	—
Vasopressin	0·7	0·26	—

The following assay methods were used: [a]ref.[9,10]; [b]ref.[11]; [c]ref.[12].

EXPERIMENTAL

The determination of the melting points and of the optical activity was carried out as in the preceding communication[3]. The same procedures were employed for checking the purity of the intermediates and preparation of the substances for the elemental analysis.

Purification of tert-Butyloxycarbonyl Amino Acids: tert-Butyloxycarbonyl-S-benzylcysteine

S-Benzylcysteine (4·2 g, 20 mmol) was acylated with tert-butyl-2,4,5-trichlorophenyl carbonate (6·84 g, 23 mmol) as described[4]. Water (40 ml) was added to the residue (after evaporation of the solvent), the mixture was acidified with hydrochloric acid to pH 3—4 and extracted with ethyl acetate. The acetate solution was washed with water, dried, and the solvent was evaporated *i.v.* The residue was dissolved in chloroform (8 ml) and the solution was applied to a column of silica gel (60—120 mesh, 15-fold weight excess). The column was washed with chloroform (250 ml), the Boc-amino acid was eluted with methanol (taken 100 ml of the effluents), the solvent was distilled off *i.v.*, and the residue was recrystallized from ether–light petroleum (or better from cyclohexane). Yield 5·4 g (87%), m.p. 62—64°C, in accordance with the reported data[4]. Essentially the same procedure was successfully used for the preparation of Boc-Tyr-OH, Boc-Arg(Tos)-OH, and Boc-Gln-OH.

S-Benzylthiopropionyl-O-benzyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteine Amide *III*

O-Benzyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteine amide trifluoroacetate[3] (1·5 g, 1·6 mmol) was dissolved in 22 ml of dimethylformamide. Triethylamine (0·22 ml, 1·6 mmol) and β-benzylthiopropionic acid *p*-nitrophenyl ester (0·49 g, 1·6 mmol) were added to the solution. After 16 h at room temperature the reaction mixture was worked up. The yield of the neutral product was 1·2 g (75%). After recrystallization from acetic acid–water 1·0 g (64%) of the pentapeptide amide derivative *III*, m.p. 272—274°C, $[\alpha]_D^{25}$ −35·2° (*c* 0·15, dimethylformamide) was obtained. The product was dried for analysis 8 h/120°C/0·1 Torr over phosphorus pentoxide. The analytical data corresponded to the hemihydrate. For $C_{51}H_{64}N_8O_9S_2.0{\cdot}5\ H_2O$ (1006) calculated: 60·83% C, 6·26% H, 10·91% N; found: 60·87% C, 6·52% H, 11·14% N.

tert-Butyloxycarbonylphenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Amide *IV*

To the solution of tert-butyloxycarbonylphenylalanine (2·4 g, 9·2 mmol) in 20 ml of dimethylformamide were added N-ethylpiperidine (1·29 ml, 9·2 mmol), ethylchloroformate (0·88 ml, 9·2 mmol) (at 0°C), and after 10 min glutaminyl-asparaginyl-S-benzylcysteine amide[3] (4·2 g, 9·2 mmol) in 40 ml of dimethylformamide. After 1 h at room temperature the solvent was evaporated at reduced pressure and the neutral product was isolated. Yield 5·8 g (90%). After recrystallization from acetic acid–water 5·4 g (83%) of *IV*, m.p. 232—233°C, $[\alpha]_D^{25}$ —40·5° (*c* 0·3, dimethylformamide) was obtained. For $C_{33}H_{45}N_7O_8S$ (699·8) calculated: 56·63% C, 6·49% H, 14·01% N, 4·58% S; found: 56·34% C, 6·49% H, 13·84% N, 4·59% S.

tert-Butyloxycarbonyl-O-benzyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Amide *V*

To the solution of *IV* (5·8 g) in 30 ml of acetic acid 25 ml of 35% solution of hydrogen bromide in acetic acid was added. After 10 min at room temperature the hydrobromide was precipitated with excess of ether, filtered off, and dried. It was then dissolved in 60 ml of water, the pH of the solution was adjusted to 8·5—9 by ammonium hydroxide, and the mixture was set aside at 0°C for 2 h. The separated peptide amide was filtered off, washed with water and dried. A part of the tetrapeptide amide (4·0 g, 6·7 mmol) was dissolved in 45 ml of dimethylformamide, tert-butyloxycarbonyl-O-benzyltyrosine 2,4,5-trichlorophenyl ester[4] (3·7 g, 6·7 mmol) was added, and the mixture was allowed to stand at room temperature overnight. The solvent was distilled off at reduced pressure and from the residue the neutral product was isolated in the usual manner. Yield 5·9 g (93%), m.p. 232—234°C. This product was used without purification for further synthesis. Recrystallization from dimethylformamide–water raised the melting point to 234—235°C, $[\alpha]_D^{25}$ —26·1° (*c* 0·45, dimethylformamide). For $C_{49}H_{60}N_8O_{10}S$ (953·1) calculated: 61·75% C, 6·34% H, 11·76% N, 3·36% S; found: 62·01% C, 6·49% H, 11·71% N, 3·66% S.

β-Benzylthiopropionyl-O-benzyltyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Amide *VI*

The Boc group of *V* (5·6 g) was split off by trifluoroacetic acid as usual. The acid was distilled off *i.v.*, the residue was triturated with ether, filtered off, dried, suspended in 25 ml of water and the pH of the mixture was adjusted to 8·5—9. After 2 h at 0°C the separated pentapeptide amide was filtered off, washed with water, recrystallized from aqueous ethanol, and dried. Yield 3·2 g (64%). A part of the product (3·0 g, 3·5 mmol) was coupled with β-benzylthiopropionic acid *p*-nitrophenyl ester (1·1 g, 3·5 mmol) in 30 ml of dimethylformamide. The yield of the neutral product was 2·7 g (75%), m.p. 251—256°C. Repeated crystallization from acetic acid–water afforded *VI*, m.p. 254—256°C, $[\alpha]_D^{25}$ —33·9° (*c* 0·2, dimethylformamide). The product was dried for analysis 10 h/120°C/0·1 Torr over phosphorus pentoxide. The analytical data indicated the presence of a half molecule of water. For $C_{54}H_{62}N_8O_9S_2.0{\cdot}5\ H_2O$ (1040) calculated: 62·35% C, 6·11% H, 10·77% N, 6·17% S; found: 62·31% C, 6·18% H, 10·71% N, 6·09% S.

Desaminooxytocin-(1—6)-hexapeptide Amide *I*

Reduction, oxidation, desalting and purification followed the procedure described earlier[8,13]. Pentapeptide amide derivative *III* (0·53 g, 0·52 mmol) yielded 138 mg of the crude and 32 mg of the purified lyophilisate. For $C_{30}H_{44}N_8O_9S_2$ (724·94) calculated: 15·46% N; found: 14·34% N, *i.e.* the lyophilisate contains 93% of the peptide. $[\alpha]_D^{25}$ —46·2° (*c* 0·19, 20% acetic acid). After drying for 8 h/120°C/0·1 Torr over phosphorus pentoxide the analytical data corresponded

to the sesquihydrate. For $C_{30}H_{44}N_8O_9S_2.1^1/_2\ H_2O$ (751·9) calculated: 47·92% C, 6·31% H, 14·95% N; found: 47·83% C, 6·22% H, 14·82% N. Amino-acid analysis[14]: Cys 1·08, Tyr 0·96, Ile 0·95, Glu 1·02, Asp 1·0.

Desaminovasopressin-(1 — 6)-hexapeptide Amide *II*

The same procedure as in the preceding case afforded (from 0·54 g, 0·51 mmol) of *VI* 69 mg of the crude and 33 mg of the purified lyophilisate. For $C_{33}H_{42}N_8O_9S_2$ (759·0) calculated: 14·76% N; found: 12·42% N, corresponding to 84% of the peptide in the lyophilisate. $[\alpha]_D^{25}$ −51·8° (*c* 0·22, 20% acetic acid). For $C_{33}H_{42}N_8O_9S_2.^1/_2\ CH_3CO_2H$ (789·0) calculated: 51·63% C, 5·63% H, 14·20% N; found: 51·67% C, 5·40% H, 13·99% N. Amino-acid analysis: Cys 1·07, Tyr 0·93, Phe 0·98, Glu 1·02, Asp. 1·0.

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Note added in proof: After this paper had been accepted for publication a communication by Hruby and coworkers and by Ferger and coworkers appeared (J. Am. Chem. Soc. *93*, 5539 (1971), *94*, 982 (1972)) describing the synthesis of *I* and *II* in a different manner. Whereas most of the comparable figures are in a good agreement, an activity of 34·2 ± 3 I.U./mg is given by Hruby for the uterotonic activity of the desaminooxytocin cycle.