AMINO ACIDS AND PEPTIDES. CXIII.*

CARBA[1]-OXYTOCIN: SYNTHESIS AND SOME OF ITS BIOLOGICAL PROPERTIES

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Carba[1]-oxytocin ([6,1-cystathionine]-oxytocin) was synthetized by the stepwise method, mostly with active esters. Its biological activities are similar to the activities of the parent hormone, *i.e.* oxytocin. Similarly to oxytocin, carba[1]-oxytocin is cleaved by human serum oxytocinase.

The preparation and the biological activities of oxytocin analogs in which the disulfide bond has been replaced by other atoms or groups of atoms have been investigated in a number of studies from this Laboratory[1-4]. The majority of these analogs (designated by the generic name of carba analogs) showed high oxytocin-like activities. These activities together with other experimental findings, such as the very similar time profile of their effects and the same slope of the dose-effect curves[5], indicate that the participation of the disulfide bond in the reaction with the receptor is extremely unlikely[6,7], at least as far as the typical oxytocin-like activities are concerned. All the carba analogs described so far have been derived from deamino-oxytocin (*Ib*). It should be assumed on the basis of the knowledge of the relation of the biological activities of oxytocin (*Ia*) and deamino-oxytocin[8] that the deletion of the amino group of cysteine at position 1 would increase the biological activities also of these analogs. Moreover, the absence of this amino group makes the synthetic work considerably easier. The presence of the α-amino group, however, is of essential importance for certain other studies.

We have therefore prepared the basic analog of this series, carba[1]-oxytocin ([6,1-cystathionine]-oxytocin; *Ic*)**. Its synthesis has been effected essentially by the procedure described for the synthesis of deamino-carba[1]-oxytocin[11]. The key intermediary product was the appropriately substituted cystathionine derivative *IIa* or, alternatively *IIb*. The condensation of these products with prolyl-leucyl-glycine amide afforded tetrapeptides *IIIa* or *IIIb*. In both cases dicyclohexylcarbodiimide served as the condensing agent; in the case of product *IIIb* it was used in the presence of 1-hydroxybenzotriazole[12]. The procedure using the *o*-nitrobenzenesulfenyl protecting group

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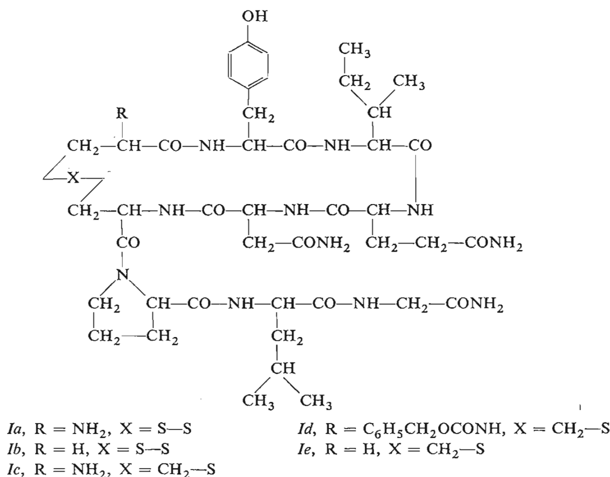
** All amino acids used in this study are of the L-series. The nomenclature and symbols follow the suggestions reported elsewhere[9]. See paper[10] for the nomenclature and system of symbols for cystathionine peptides.

gave slightly lower yields (especially in the synthesis of product *IIIa*) than the alternative method using the *p*-methoxybenzyloxycarbonyl group. The NPS protecting group was removed from product *IIIa* by hydrogen chloride in ether[13], the *p*-methoxybenzyloxycarbonyl group by trifluoroacetic acid in the presence of anisole[14]. The free tetrapeptide esters were acylated by *o*-nitrobenzenesulfenyl-asparagine 2,4,5-trichlorophenyl ester and the identical protected pentapeptide *IV* was obtained. The peptide chain was elongated in a similar manner (removal of the NPS protecting group by hydrogen chloride in ether and acylation by the active ester) by a glutamine and an isoleucine residue (products *V* and *VIa* were obtained). After removal of the



Ia, R = NH_2, X = S—S
Ib, R = H, X = S—S
Ic, R = NH_2, X = CH_2—S
Id, R = $C_6H_5CH_2OCONH$, X = CH_2—S
Ie, R = H, X = CH_2—S

NPS-group and alkaline hydrolysis, peptide *VIb* was isolated by using an ion-exchange resin and acylated by tert-butyloxycarbonyl-O-tert-butyltyrosine N-hydroxysuccinimide ester. Linear octapeptide *VII* was converted into the active ester by treatment with bis-*p*-nitrophenyl sulfite[15] and cyclized in pyridine after the removal of the protecting groups of the tert-butyl type by trifluoroacetic acid. N^{α}-Benzyloxycarbonyl-cyclopeptide *Id* was isolated by countercurrent distribution. The benzyloxycarbonyl group was removed by hydrogen bromide in acetic acid. Analog *Ic* was then released from the hydrobromide by an ion-exchange resin, isolated, and purified by countercurrent distribution and gel filtration.

The biological activities were examined by the conventional pharmacologic tests characterizing the main regions of effect of the neurohypophyseal hormones. The results are given in Table I. The activities of carba[1]-oxytocin are close to the effects

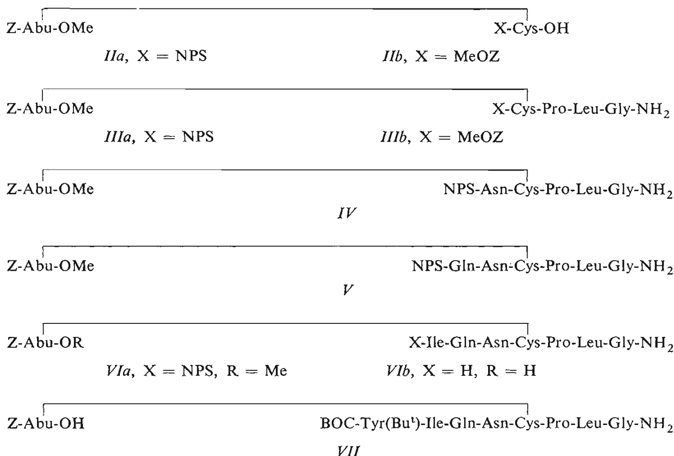


TABLE I

Biological Activities (in international units per mg)

Compound	Rat uterus isolated *in situ*	Vasodepressor mammary gland[a]	Antidiuretic pressor
Oxytocin (*Ia*)[8,26,31]	486	507	2·7
	450[b]	533	3·1
Deamino-oxytocin (*Ib*)[32,33]	803	975	19
	900	—	1·44
Deamino-carba[1]-oxytocin (*Ie*)[2]	1 898	1 127	21
	1 251	562	17·5
Carba[1]-oxytocin (*Ic*)	734	175	9·3
	120	177	2·95

[a] *in vitro*, [b] cat.

of oxytocin. Higher activities were found when the oxytocin activity was assayed *in vitro*. The antidiuretic activity was three-times higher than the activity of oxytocin. The remaining activities of analog *Ic* are lower than those of oxytocin. There is a striking difference between analog *Ic* and oxytocin especially in the effect on uterus *in vitro* and *in vivo*. We observed a lower *in vivo* activity also with deamino-carba^1-oxytocin. From what is known about the enzymatic cleavage of the neurohypophyseal hormones, it is hardly probable that the lower activities observed in the *in vivo* test could be caused by the difference in the rate of enzymatic inactivation during the transport or directly in the receptor compartment. Analog *Ic* – with respect to its chemical structure should be cleaved in the organism at a lower rate or at least at the same rate as oxytocin. Oxidation may represent an exception since this process is faster with thio ethers than with disulfides[16].

The results of the digestion of oxytocin and carba^1-oxytocin by serum oxytocinase, isolated from the blood of pregnant women, indicate that the affinity of carba^1-oxytocin for the enzyme (K_m 2 . 10^{-6}) is three-times lower than the affinity of oxytocin (K_m 6 . 10^{-6}). The rate of the breakdown of the enzyme-substrate complex is of course the same in both cases (V_{max} = 0·2 μM/min). The lower affinity of carba^1-oxytocin is most likely caused by the absence of the disulfide bond. Carba^1-oxytocin is inactivated in tissue homogenates approximately at the same rate as deamino-carba^1-oxytocin, that is at a far lower rate than oxytocin[17]. These findings provide in fact evidence of a higher metabolic stability of carba^1-oxytocin compared to oxytocin and of approximately the same stability as that of deamino-carba^1-oxytocin.

The determination of the uterotonic activity is carried out under considerably "unphysiological" conditions[18]. Is is especially the content of ions in the bath which could affect the secondary structure of either the compounds tested or of their complexes with the receptor. We have therefore studied the effect of magnesium ions on the uterotonic activity of carba^1-oxytocin. The degree of potentiation of oxytocin and carba^1-oxytocin was under our experimental conditions (van Dyke–Hasting solution containing 0·5 mM Mg^{2+}) approximately the same and resulted in a shift of the log dose-response curve by approximately 0·3 toward lower concentrations.

We have studied also the biological properties of the last synthetic intermediate of the synthesis of analog *Ic*, *i.e.* of N^{α}-benzyloxycarbonyl-carba^1-oxytocin (*Id*). This product, when tested on the isolated rat uterus, showed no activity up to the dose 2·6 . 10^{-2} mg. Hence, at the given sensitivity of the uterus (the threshold dose for oxytocin was 5 . 10^{-7} mg/ml in this case), the activity of analog *Id* must be lower than 0·05 U./mg. The analogs which are structurally close, N^{α}-acetyloxytocin and N^{α}-carbamoyl-oxytocin, show activities[19] of 1·5 and 0·5 U./mg, respectively. It seems likely that this decrease or even the complete loss of the activity is caused by the increase in size of the substituent on the α-amino group. Analog *Id* showed no inhibitory effect on oxytocin up to a dose ratio of 10 000 : 1 when assayed on the isolated rat uterus.

A comparison of the activities of oxytocin, deamino-oxytocin, deamino-carba^1-oxytocin, and carba^1-oxytocin shows that the great increase in the activities of deamino-carba^1-oxytocin is obviously of more complex character and involves more than the mere additive effect of these two structural changes. On the other hand, the relatively high activities of carba^1-oxytocin definitely eliminate the functional participation of the disulfide bond on the typical oxytocin-like activities. They eliminate namely the possibility that this carba-replacement has led to a strong decrease of the biological activities, a decrease compensated by a great increase of these activities as a result of the absence of the α-amino group. Carba^1-oxytocin will be used as a prototype in the synthesis of additional analogs in which the presence of the α-amino group is absolutely necessary, such as, *e.g.* in the synthesis of hormonogens, analogs with natriuretic effect or in studies on the binding to neurophysin.

EXPERIMENTAL

The melting points were determined on a Kofler block and are corrected. The samples for elemental analysis were dried 12 h at room temperature *in vacuo* (1 Torr). Thin-layer chromatography was carried out on silica gel layer sheets (Kieselgel G, Merck). in the solvent systems 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S_1) and 2-butanol–90% formic acid–water (75 : 13·5 : 13·5) (S_2). Electrophoresis was performed on Whatman No 3 MM paper in the buffers 1M acetic acid (pH 2·4) and pyridine–acetic acid (pH 5·7), 60 min at a potential gradient of 20 V/cm. The R_F- and E-values given characterize the behavior of pure products on chromatography and electrophoresis, respectively. The amino acid analyses were carried out with 20-h hydrolysates (6M-HCl, 105°C) on an automatic amino-acid analyzer (Instrument Development Workshops, Czechoslovak Academy of Sciences, Type 6020). The evaporation was performed on a rotatory evaporator (vacuum of a water aspirator, bath temperature 30—35°C). An oil pump was used for mixtures containing dimethylformamide. The countercurrent distribution was effected in an all glass apparatus (Steady State Distribution Machine, Quickfit and Quartz Ltd, Stone, Stafordshire, England). The Folin-Ciocalteau reagent was used for the localization of the peptide material. Gel filtration was carried out on columns of Bio-Gel (Bio-Rad Laboratories, Richmond, California, U.S.A.). Absorbance measurement at 280 nm was used for the detection in column operations.

$N^{\alpha'}$-Benzyloxycarbonyl-N^{α}-*p*-methoxybenzyloxycarbonylcystathionine α'-Methyl Ester Dicyclohexylammonium Salt (*IIb*)

A solution of 0·5M-$NaHCO_3$ (7·5 ml) and *p*-methoxybenzyloxycarbonyl azide (0·9 g) were added with stirring to a solution of $N^{\alpha'}$-benzyloxycarbonylcystathionine α'-methyl ester[20] (1·1 g) in a mixture of dioxane (40 ml) and water (40 ml). The pH of the reaction mixture was kept at 8·2—8·5 by the addition of 1M-NaOH (total uptake 2·6 ml). An additional portion of *p*-methoxybenzyloxycarbonyl azide (0·2 g) was added to the reaction mixture after 7 h of stirring. The reaction mixture was extracted with ether after 2 additional hours of stirring, then acidified by an aqueous solution of citric acid to pH 3·5, and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried by sodium sulfate, and taken to dryness. The dry residue was dissolved in ethyl acetate and diluted by petroleum ether after the addition of dicyclohexylamine (0·55 ml). The crystals which had separated were crystallized from aqueous methanol.

M.p. 130—132°C, yield 1·35 g (64%). The sample for analysis was recrystallized in the same manner, no change in the melting point was observed; $[\alpha]_D$ −7·3° (*c* 0·5, dimethylformamide). For $C_{37}H_{53}N_3O_9S$ (715·9) calculated: 62·10% C, 7·47% H, 5·87% N; found: 62·10% C, 7·48% H, 5·83% N.

p-Methoxybenzyloxycarbonyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide N$^{\alpha'}$-Benzyloxycarbonyl-α′-hemicystathionine α′-Methyl Ester (*IIIb*)

Dowex 50 (H^+-form, 12 ml) was added to the solution of dicyclohexylammonium salt *IIb* (1·35 g) in a mixture of methanol (40 ml) and water (20 ml). The mixture was stirred for 30 min at room temperature. The resin was filtered off, the filtrate was evaporated, and dried azeotropically (benzene). The dry residue was dissolved in dimethylformamide (20 ml) and prolyl-leucyl-glycine amide[21] (0·56 g) and 1-hydroxybenzotriazole (0·27 g) were added to the solution. The mixture was then treated with dicyclohexylcarbodiimide (0·45 g) at −25°C. The mixture was stirred 1 h at −15°C and 12 h at room temperature and then evaporated. The dry residue was dissolved in ethyl acetate and dicyclohexylurea was filtered off. The solution was extracted with an aqueous solution of citric acid, water, 0·5M-$NaHCO_3$, and again with water. Then it was dried by sodium sulfate, taken to dryness, and the dry residue triturated with ether. The crystalline product was filtered off and washed with ether. Yield 1·5 g (99%), m.p. 103—105°C. The sample for analysis was crystallized from aqueous methanol; m.p. 103—104°C; $[\alpha]_D$ −52·1° (*c* 0·5, dimethylformamide). For $C_{38}H_{52}N_6O_{11}S$ (800·9) calculated: 56·98% C, 6·55% H, 10·50% N; found: 57·25% C, 6·77% H, 10·78 % N.

o-Nitrobenzenesulfenyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide N$^{\alpha'}$-Benzyloxycarbonyl-α′-hemicystathionine α′-Methyl Ester (*IIIa*)

Prolyl-leucyl-glycine amide[21] (0·47 g) and 2·65M-HCl in ether (0·59 ml) were added to the solution of dicyclohexylammonium salt *IIa* (ref.[20]) (1·1 g) in a mixture of dimethylformamide (15 ml) and chloroform (10 ml). The reaction mixture was cooled down to −20°C and dicyclohexylcarbodiimide was added (0·34 g). The mixture was then stirred 1 h at −15°C and 12 h at room temperature and taken to dryness. The dry residue was dissolved in ethyl acetate and this solution was extracted stepwise with 0·2M-H_2SO_4, water, 0·5M-$NaHCO_3$, and again with water. The solution was then dried by sodium sulfate and evaporated. Crystallization from a mixture of methanol and ether afforded 0·50 g (40%) of a product melting at 82—84°C. The sample for analysis was recrystallized in the same manner; m.p. 83—84°C, $[\alpha]_D$ −56·7° (*c* 0·5 dimethylformamide). For $C_{35}H_{47}N_7O_{10}S_2$.0·5 H_2O (798·9) calculated: 52·62% C, 6·06% H, 12·27% N; found: 52·44% C, 5·88% H, 12·42% N. If compound *IIa* was isolated first from its dicyclohexylammonium salt (ethyl acetate, 0·2M-H_2SO_4), a product of the same melting point was obtained in a yield of 34%.

o-Nitrobenzenesulfenylasparaginyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide N$^{\alpha'}$-benzyloxycarbonyl-α′-hemicystathionine α′-Methyl Ester (*IV*)

a) The solution of 2·7M-HCl in ether (0·67 ml) was added to the solution of tetrapeptide *IIIa* (0·65 g) in dimethylformamide (4 ml). The mixture was allowed to stand 3 min and diluted by ether. The crystals which had separated were filtered off, washed with ether, and dried; $E_{5.7}^{His}$ 0·65, $E_{2.4}^{Gly}$ 0·85. The solution of the crystals in dimethylformamide (8 ml) was neutralized by N-ethylpiperidine and *o*-nitrobenzenesulfenyl-asparagine 2,4,5-trichlorophenyl ester[22] (0·5 g) was added. The reaction mixture was stirred 24 h at room temperature and another portion of the active ester (0·2 g) was added. After 24 h the reaction mixture was evaporated and the dry resi-

due was triturated stepwise with light petroleum and ether. The crystalline portion of the product was filtered off and washed with ether, water, 0·5M-$NaHCO_3$, and again with water. The yield was 0·65 g of a product of m.p. 142—145°C. Crystallization from methanol afforded 0·55 g (73%) of a product of m.p. 174—176°C. The sample for analysis was recrystallized in the same manner without a change of the melting point; $[\alpha]_D$ −74·3° (*c* 0·42, dimethylformamide). For $C_{39}H_{53}N_9O_{12}S_2.H_2O$ (922·0) calculated: 50·80% C, 6·01% H, 13·68% N; found: 50·90% C, 5·85% H, 13·74% N. *b*) Tetrapeptide *IIIb* (1·45 g) was dissolved in trifluoroacetic acid (5 ml) in the presence of anisole (0·5 ml). The solution was set aside for 1 h at room temperature, then diluted by toluene (25 ml), and evaporated; $E_{5.7}^{His}$ 0·65, $E_{2.4}^{Gly}$ 0·85. The dry residue was dissolved in dimethylformamide (20 ml), the pH of the solution was adjusted to 9 by N-ethylpiperidine (wet pH-paper strip), and *o*-nitrobenzenesulfenyl-asparagine 2,4,5-trichlorophenyl ester[22] (1 g) was added. The reaction mixture was stirred 24 h at room temperature and another portion of the active ester (0·4 g) was added. After additional 24 h the mixture was evaporated and the dry residue was treated as described under *a*). The yield was 1·25 g of a product of m.p. 143—146°C. Crystallization from methanol afforded 1·10 g (66%) of a product of m.p. 173—175°C, no depression of m.p. when mixed with a sample prepared as described under *a*).

o-Nitrobenzenesulfenylglutaminyl-asparaginyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide $N^{\alpha'}$-Benzyloxycarbonyl-α′-hemicystathionine α′-Methyl Ester (*V*)

The solution of 2·7M-HCl in ether (1·0 ml) was added to the solution of protected pentapeptide *IV* (1·0 g) in dimethylformamide (7 ml). The solution was set aside for 3 min at room temperature and diluted with ether. The hydrochloride which had separated was filtered off and washed with ether; $E_{2.4}^{Gly}$ 0·67, $E_{5.7}^{His}$ 0·33. It was dried and dissolved in dimethylformamide (10 ml) and the pH of the solution was adjusted to 9 by N-ethylpiperidine. *o*-Nitrobenzenesulfenyl-glutamine 2,4,5-trichlorophenyl ester[22] (0·5 g) was then added. The reaction mixture was stirred 24 h at room temperature and another portion of the active ester (0·2 g) was added. After additional 24 h the solution was evaporated and the dry residue was triturated stepwise with light petroleum and ether. The crystals were filtered off, washed with ether, water, 0·5M-$NaHCO_3$, and again with water. Crystallization from a mixture of dimethylformamide and ether afforded 0·95 g (86%) of a product of m.p. 225—226°C. The sample for analysis was recrystallized from aqueous dimethylformamide and no change in m.p. was observed; $[\alpha]_D$ −56·3° (*c* 0·5, dimethylformamide). For $C_{44}H_{61}N_{11}O_{14}S_2$ (1032) calculated: 51·21% C, 5·96% H, 14·93% N; found: 51·13% C, 5·98% H, 14·82% N.

o-Nitrobenzenesulfenylisoleucyl-glutaminyl-asparaginyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide $N^{\alpha'}$-Benzyloxycarbonyl-α′-hemicystathionine α′-Methyl Ester (*VIa*)

The NPS-protecting group was removed from protected hexapeptide *V* (0·75 g) in the same manner as in the case of product *IV* (10 ml of dimethylformamide, 0·55 ml of 2·7M-HCl in ether); $E_{5.7}^{His}$ 0·53, $E_{2.4}^{Gly}$ 0·67. The hydrochloride was dissolved in dimethylformamide (10 ml), the solution was neutralized by N-ethylpiperidine, and *o*-nitrobenzenesulfenylisoleucine N-hydroxysuccinimide ester[11] (0·25 g) was added. The reaction mixture was stirred 24 h, then diluted by dimethylformamide (20 ml), and another portion of the active ester (0·25 g) was added. After additional 24 h the reaction mixture was treated as described for product *V*. Crystallization from the mixture dimethylformamide–ether afforded 0·70 g (83%) of a product of m. p. 245—246°C. The sample for analysis was recrystallized from aqueous dimethylformamide without a change of the melting point; $[\alpha]_D$ −72·1° (*c* 0·5, dimethylformamide). For $C_{50}H_{72}N_{12}O_{15}S_2.H_2O$ (1 163) calculated: 51·63% C, 6·41% H, 14·46% N; found: 51·64% C, 6·22% H, 14·70% N.

Isoleucyl-glutaminyl-asparaginyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide N$^{\alpha'}$-Benzyloxycarbonyl-α'-hemicystathionine(*VIb*)

A suspension of protected heptapeptide *VIa* (0·60 g) in dimethylformamide (20 ml) and 2·7M-HCl in ether (1·0 ml) were shaken until the solid was completely dissolved (approximately 10 min). The solution was diluted by ether, the separated crystals were filtered off and washed with ether; $E_{5.7}^{His}$ 0·40, $E_{2.4}^{Gly}$ 0·68; R_F 0·35 (S2), 0·45 (S1). The dry hydrochloride was dissolved in the mixture methanol (20 ml)-1M-NaOH (2 ml). The solution was stirred 1·5 h at room temperature, methanol was evaporated, and the aqueous solution was passed over a column of Dowex 50 (H^+-form, 25 ml). The column was washed with water and the product was eluted by 10% pyridine. The eluates were freeze-dried and the lyophilisate was triturated with the mixture methanol–ether. The yield was 314·3 mg (61%); $E_{5.7}^{His}$ 0·15, $E_{2.4}^{Gly}$ 0·55; R_F 0·30 (S2), 0·16 (S1). The sample for analysis was reprecipitated from the mixture acetic acid–ether; $[\alpha]_D$ −28·8° (*c* 0·4, dimethylformamide). For $C_{43}H_{67}N_{11}O_{13}S.H_2O$ (996·1) calculated: 51·85% C, 6·98% H, 15·47% N; found: 51·85% C, 6·83% H, 14·94% N. Amino-acid analysis: Asp 1·07, Cyth 0·98, Glu 0·98, Gly 1·00, Ile 0·95, Leu 1·00, Pro 1·06.

Tert-butyloxycarbonyl-O-tert-butyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide N$^{\alpha'}$-Benzyloxycarbonyl-α'-hemicystathionine (*VII*)

N-tert-butyloxycarbonyl-O-tert-butyltyrosine N-hydroxysuccinimide ester[11] (0·11 g) was added to the solution of heptapeptide *VIb* (0·29 g) in dimethylformamide (15 ml) and N-ethylpiperidine (40 μl). The reaction mixture was stirred 24 h at room temperature and then another portion of the active ester (0·06 g) and of N-ethylpiperidine (50 μl) was added. After additional 36 h of stirring the reaction mixture was evaporated and the dry residue was triturated stepwise with light petroleum and ether. The crystalline portion was filtered off and washed with ether, water, an aqueous solution of citric acid (pH 3), and again with water. The yield was 343 mg (90%) of a product of m.p. 212—215°C. The sample for analysis was crystallized from a mixture of dimethylformamide and ether, m.p. 212—214°C; $[\alpha]_D$ −36·4° (*c* 0·5, dimethylformamide). For $C_{61}H_{92}N_{12}O_{17}S$ (1298) calculated: 56·44% C, 7·15% H, 12·95% N; found: 56·04% C, 7·10% H, 12·90% N. Amino-acid analysis: Asp 1·02, Cyth 0·96, Glu 1·01, Ile 0·95, Leu 1·05, Pro 1·08, Tyr 0·96.

N$^{\alpha'}$-Benzyloxycarbonyl-α'-hemicystathionyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide (*Id*)

Bis-*p*-nitrophenyl sulfite[15] (1 g) was added with stirring to a solution of linear octapeptide *VII* (300 mg) in dimethylformamide (10 ml) and pyridine (10 ml) while nitrogen was passed through. Additional portions of the reagent (1 g) and pyridine (5 ml) were added to the reaction mixture after 8 h; 0·5 g of sulfite was added 12 h later. After 6 h, the reaction mixture was taken to dryness and the dry residue was triturated with ether. The crystals were filtered off and washed with ether and water. The dried active ester was dissolved in trifluoroacetic acid (10 ml), the solution was set aside for 45 min at room temperature, then taken to dryness, and the dry residue dissolved in dimethylformamide (10 ml). This solution was added in 4 h to 300 ml of pyridine which was stirred, heated at 50°C, while nitrogen bubbles were passed through. The mixture was allowed to stand 12 h at room temperature under a nitrogen barrier and then evaporated. The dry residue was triturated with ether, filtered, and washed with ether. The dry product was purified by countercurrent distribution in the mixture sec-butanol–0·05% acetic acid. After 100 transfers of the upper phase, the peak corresponding to a distribution coefficient of 9·0 was isolated by evaporation, the dry residue dissolved in methanol, and precipitated by ether. The yield was 152·6 mg (57%)

of a product of R_F 0·45 (S2), 0·35 (S1). The sample was crystallized from aqueous methanol, m.p. 158–160°C; $[\alpha]_D$ −15·0° (*c* 0·5, dimethylformamide) For $C_{52}H_{74}N_{12}O_{14}S.3\,H_2O$ (1177) calculated: 53·06% C, 6·85% H, 14·28% N; found: 53·12% C, 6·47% H, 14·10% N.

α'-Hemicystathionyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-α-hemicystathionyl-prolyl-leucyl-glycine Amide (*Ic*)

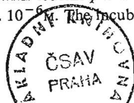
The solution of hydrogen bromide in acetic acid (35%; 7 ml) was added to the solution of product *Id* (124 mg) in acetic acid (3 ml). The mixture was allowed to stand 10 min at room temperature. then diluted by ether (100 ml), the precipitated hydrobromide was filtered off and washed with ether. The dried hydrobromide was dissolved in water (5 ml) and passed over Amberlite IR-4B (OH^--form; 5 ml). The eluate was lyophilized and purified by countercurrent distribution in the system sec-butanol–0·05% acetic acid. Altogether 477 transfers of the upper phase and 92 transfers of the lower phase were made. The tubes corresponding to the peak of a distribution coefficient of 0·32 were pooled, concentrated and lyophilized. The yield was 67·7 mg (57%) of a product showing $E^{Gly}_{2.4}$ 0·70, $E^{His}_{5.7}$ 0·62; R_F 0·17 (S1), 0·09 (S2). The sample for analysis was purified further by gel filtration on Bio-gel P-4 and P-2 in 1M-acetic acid and reprecipitated from its methanolic solution by ether; $[\alpha]_D$ −37·5° (*c* 0·17, 1M-acetic acid). For $C_{44}H_{68}N_{12}O_{12}S.CH_3CO_2H.5\,H_2O$ (1139) calculated: 48·50% C, 7·25% H, 14·78% N; found 48·38% C, 6·50% H, 14·75% N. Amino-acid analysis: Asp 1·10, Cyth 0·81, Glu 1·09, Gly 1·08, Ile 1·05, Leu 1·09, Pro 1·00, Tyr 0·80.

Pharmacological Methods

Uterus strips of adult, virgin rats of the Wistar strain (influenced by estrogens) were used for the activity testing with rat uterus *in vitro*. The isometric contractions were recorded in the solution prepared according to van Dyke-Hastings[18]. A mixture of 95% O_2 and 5% CO_2 was bubbled through the solution kept at 30°C. Oxytocin (Spofa) was used as a standard. The effectiveness of the analog was determined by the two-dose method. The activity test with rat uterus *in situ* was performed according to Pliška[23]. Estrogen-treated rats in ethanol narcosis were laparotomized and one corner of their uterus was tied up. Its contractions were recorded by RCA-5734 transducer. The products were applied intravenously; the effect of the analog was expressed in terms of the area below the curve representing the record of uterus contractions during the action of the analog. The depressor activity on cocks was assayed according to Coon[24]. A comparison with the standard was effected by the two-dose method. The pressor activity was determined with despinalized rats in ether narcosis[25]. The arterial blood pressure was measured in the arteria carotis by a Statham Pressure Transducer and recorded in Model EZ-2 linear recorder. The comparison with the standard was carried out by the four-point test. The activity assayed with an isolated strip of rat mammary gland was determined according to[26]. The isometric contractions of the myoepithelium strip were measured in a bath (5 ml) containing the Tyrod solution at 37°C. The effect of the analog was determined in terms of the contraction peak by the two-dose method. The antidiuretic activity was determined with rats in ethanolic narcosis hydrated up to 8% of their body weight. The effect of the analog was determined according to Pliška and Rychlík[27].

Digestion with Human Serum Oxytocinase

Human serum oxytocinase was prepared by ethanol fractionation and by gel filtrations[28,29]. Its specific activity was 10 U/mg. protein (assayed with leucine-*p*-nitranilide). The concentration of both oxytocin and analog *Ic* was 5 . 10^{-7} to 5 . 10^{-6}M. The incubation was carried out in 40



mM Na-phosphate buffer (pH 7·85) with 0·3 ml (5 μg of protein) of the enzyme solution for 0, 15, 30 and 60 min. The reaction was discontinued by boiling the mixture 3 min and the remaining quantity of the biologically active product was determined by the *in vitro* uterus test. The obtained values (v_0, [S]) were expressed in the double reciprocal plot and the V_{max}- and K_m-values were read. The parameters of the digestion were determined during the initial stage of the inactivation only (up to 10% original concentration of the substrate). At this stage any competitive inhibition by the arising product (*e.g.* by oxytocein in the case oxytocin) does not interfere with the unambiguity of the monosubstrate reaction. This experimental arrangement is necessary since the arising products (*e.g.* the linear peptides with a free amino group) represent potential substrates of oxytocinase[29]. The K_m-value found for oxytocin is in excellent agreement with the value recorded in the literature and obtained with tritiated oxytocin[30].

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