AMINO ACIDS AND PEPTIDES. CV.*

SYNTHESIS OF DEAMINO-[1,6-HOMOLANTHIONINE]-OXYTOCIN, AN ANALOG OF DEAMINO-OXYTOCIN WITH ENLARGED RING, LACKING THE DISULFIDE BOND

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The paper describes the synthesis and certain fundamental pharmacologic data on an analog of deamino-oxytocin in which the disulfide bond has been replaced by the $—CH_2—S—CH_2—$ group. The cyclic part of the molecule of this analog is thus by one member larger than the molecule of the parent hormone. This analog shows a relatively high degree of oxytocin – like activities.

The analogs of deamino-oxytocin** with modified disulfide bond and intact cyclic arrangement of the molecule (*e.g. Ib*) are advantageous – as regards both their high activities and also their relatively easy and well definable preparation[2] – for the investigation of the importance of individual structural elements in the molecule of oxytocin (*Ia*). In the preceding study we prepared[3] two such analogs (*Ic* and *Id*) with rings by one atom smaller (19 atoms) than the ring of deamino-oxytocin (20 atoms). Both these analogs showed the basic oxytocin-like activities, eventhough considerably lower than those of deamino-oxytocin. We were interested in the effect which might have on these carba-analogs the enlargement of the cyclic part of their molecules.

Several oxytocin analogs with enlarged cycle have been reported in the literature. Relatively drastic changes have been performed on endo-Tyr^{2a}-oxytocin (ref.[4,5]) and endo-Ile^{3a}-oxytocin (ref.[6]), as well as on [4-isoglutamine]-oxytocin[7], [4-β-alanine]-oxytocin[8], and [5-isoasparagine]-oxytocin[9], changes which have led to a practically complete loss of the biological activities, or even in some cases gave rise to inhibitory effects. Far more relevant with respect to this study are analogs with their cyclic part larger by one atom, predominantly in the neighborhood of the disulfide bond, *i.e.* [1-hemihomocystine]-oxytocin[10] (*If*) and a similar analog derived from deamino-oxytocin, [1-γ-mercaptopropionic acid]-oxytocin[11] (*Ig*).

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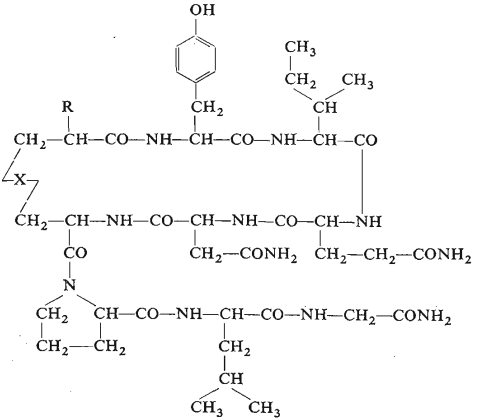
** All amino acids used in this study have L-configuration. The nomenclature and symbols comply with the reported suggestions[1]. HCys stands for homocysteine.

In this paper we report on the synthesis and some biological properties of a deamino-oxytocin analog in which the disulfide bond has been replaced by the group $—CH_2—S—CH_2—$. The cyclic part of this analog is thus enlarged from the original 20 members to 21 members (*Ie*). The synthesis of this product was carried out essentially by the approach which has been found effective in earlier studies on the remaining analogs of this series[2,3,12]. The carboxy-terminal tripeptide, prolyl-leucyl-glycine amide[13,14] (*II*) was acylated by homocysteine derivative *IIIc* which had been prepared by reduction of homocystine[15] by sodium in liquid ammonia and subsequent alkylation *in situ* with γ-bromobutyric acid methyl ester. The amino group of product *IIIa* was protected by the benzyloxycarbonyl group which, unlike in earlier studies[2,3,12], had been introduced into the molecule by means of the Bunte salt in the same yield as by using benzyloxycarbonyl chloride. Homocysteine derivative *IIIc* was condensed in the presence of 1-hydroxybenzotriazole[16] with tripeptide amide *II* by dicyclohexylcarbodiimide and the noncrystalline protected tetrapeptide was decarbobenzoxylated by hydrogen bromide in acetic acid. The free tetrapeptide amide was subsequently acylated by *o*-nitrobenzenesulfenyl-asparagine 2,4,5-trichlorophenyl ester[17], *o*-nitrobenzenesulfenyl-glutamine trichlorophenyl ester[17], and by nitrobenzenesulfenyl-isoleucine N-hydroxysuccinimide ester[2] and products *IV*, *Va*, and *VIa*, respectively, were obtained. The protecting *o*-nitrobenzene-



Ia, X = S—S, R = NH_2
Ib, X = CH_2—S, R = H
Ic, X = S, R = H
Id, X = CH_2, R = H
Ie, X = CH_2—S—CH_2, X = H
If, X = CH_2—S—S, X = NH_2
Ig, X = CH_2—S—S, X = H

sulfenyl group was removed in all cases by hydrogen chloride in ether[18]. The *o*-nitrobenzenesulfenyl protecting group was split off by the same method also from product *VIa*. After alkaline hydrolysis, free peptide *VIc* was isolated on an ion-exchange resin and acylated by N^{α}-tert-butyloxycarbonyl-O-tert-butyltyrosine N-hydroxysuccinimide ester to give product *VII*. As an alternative approach to this product we essayed the acylation of free hexapeptide amide *Vb* (obtained by a similar procedure as product *VIc*) by *o*-nitrobenzenesulfenyl-isoleucine N-hydroxysuccinimide ester, removal of the protecting group from product *VIb*, and acylation by the tyrosine derivative. This approach, however, was less advantageous as regards the yield and easiness of purification of the obtained intermediary products. Protected octapeptide acid *VII* was converted into the active ester by bis-*p*-nitrophenyl sulfite[19] and after the removal of the protecting groups with trifluoroacetic acid cyclization was effected in a pyridine solution. Analog *Ie* was isolated and purified by countercurrent distribution and characterized by amino acid analysis, elemental analysis, thin-layer chromatography, paper electrophoresis, and determination of optical rotation.

In Table I some of the biological activities are given side by side with the activities of oxytocin and of certain closely related analogs. The determination of the activity on uterus *in vitro*[20], of vasodepressor activity[21], of the activity on isolated mammary gland[22] and of the effect on blood pressure of rats[23] were carried out by Dr I. Krejčí, Institute for Pharmacy and Biochemistry, Prague, the determination of antidiuretic activity[24] and of the effect on rat uterus *in situ*[25] by Dr T. Barth of this Institute. The detailed results of these pharmacological analyses will be reported later.

H-Pro-Leu-Gly-NH_2

II

X-HCys($C_3H_6CO_2R$)—OH

IIIa, X = H, R = CH_3
IIIb, X = H, R = H
IIIc, X = Z, R = CH_3

NPS-Asn-HCys($C_3H_6CO_2Me$)-Pro-Leu-Gly-NH_2

IV

X-Gln-Asn-HCys($C_3H_6CO_2R$)-Pro-Leu-Gly-NH_2

Va, X = NPS, R = Me
Vb, X = H. R = H

X-Ile-Gln-Asn-HCys($C_3H_6CO_2R$)-Pro-Leu-Gly-NH_2

VIa, X = NPS, R = Me
VIb, X = NPS, R = H
VIc, X = H, R = H

BOC-Tyr(Bu^t)-Ile-Gln-Asn-HCys($C_3H_6CO_2H$)-Pro-Leu-Gly-NH_2

VII

As can be seen in Table I, analog *Ie* retains a considerable degree of biological activities. While the biological activities of analogs with the smaller ring[3] are of the order of tens of units, analog *Ie* is by one order more active. This finding is even more

TABLE I
Biological Activities (in international units per mg)

Product	Rat uterus isolated	Rat uterus *in situ*	Vaso-depressor	Mammary gland *in vitro*	Antidiuretic	Pressor	Ref.
Ia	486	450	507	533	2·7	3·1	6, 32, 33
Ib	1 898	1 251	1 127	562	21	1·44	12
Ic	9·2	20·9	6·8	42	0·17		3
Id	2·6	0·54	0		0·05		3
Ie	60	170	29	346[a]	0·3	0·025	this paper
If	0·75	—	—	—	—	—	10
Ig	3	—	0	0	—	—	11

[a] In absence of magnesium; the activity in presence of magnesium was 1 680 I.U./mg.

interesting when we consider the fact that the analogs with enlarged ring[4–11] are far less active than analogs whose ring had been made smaller by one member. This observation was also in accordance with the concept of the mechanism of action of these compounds: it could be foreseen that the receptor of the biological tissue would be accessible to a smaller rather than to a larger molecule. The results of our present study show, however, that this concept, at least in this very simple form, is not entirely correct. In spite of the fact that we are still lacking knowledge of the effect of these "carba" replacements on the three-dimensional structure of the molecule, the fact that the "carba" analogs with rings made smaller posses lower activities than analog *Ie* with the enlarged ring, described in this study, provides evidence showing that the receptor of the biological tissue is adaptable to an enlarged molecule rather than to one made smaller. Our present results and conclusions cannot be applied, however, directly on the mechanism of action of oxytocin because *a*) we do not know how these "carba" replacements affect the three-dimensional structure of analog *Ie*, as compared to their effect on the parent hormone, and *b*) we made three replacements which, compared to the molecule of oxytocin, are omission of the α-amino group, a change in the size of the ring, and, finally, the modification of the disulfide bond. We cannot *a priori* eliminate the possibility that in such a complicated molecule as that of oxytocin these replacements may not necessarily be additive.

EXPERIMENTAL

The melting points were determined on a Kofler block and are corrected. Samples for elemental analysis were dried 8—12 hours *in vacuo* (1 Torr) at 40 to 70°C. The thin-layer chromatography was carried out on plates with a silica gel layer (Kieselgel G, Merck) in the solvent systems 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S_1), 2-butanol–90% formic acid–water (75 : : 13·5 : 13·5) (S_2), and 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S_3). Paper eletrophoresis was performed in an apparatus according to Durrum[26] on Whatman No 3MM paper in the following buffers: 1M acetic acid (pH 2·4) and pyridine–acetic acid (pH 5·7), mostly for 60 min at a potential gradient of 20 V/cm. The detection was effected either by ninhydrin or by the chlorination method (on paper[27], on silica gel layer[28]). The R_F and E values if given below are those of pure products. The amino-acid analyses were performed on an automatic analyzer[29] after 20-h hydrolysis in 6M-HCl at 105°C. The reaction mixtures were taken to dryness in a rotatory evaporator at a bath temperature of 30—35°C in the vacuum of a water aspirator. An oil pump was used for mixtures containing dimethylformamide.

S-γ-Methoxycarbonylpropylhomocysteine (*IIIa*)

Homocystine[15] (2·68 g; $[\alpha]_D$ 76·4° (*c* 0·5, 1M-HCl)) was reduced by sodium in liquid ammonia (100 ml). After the solution had been decolorized by ammonium chloride, γ-bromobutyric acid methyl ester (7 g) was added to the reaction mixture and the solution was lyophilized in the vacuum of a water-aspirator. The lyophilisate was dissolved in water (100 ml) at 0°C and the pH of the solution was adjusted to 2 by 1M-HCl. The solution was extracted with ether, evacuated, and passed over a column of Dowex 50 (H^+-form, 100 ml). The column was washed with water, the product was eluted by 10% solution of pyridine (precooled at 0°C) and taken to dryness. (No homocysteic acid was found in the sample oxidized by performic acid by paper electrophoresis under conditions when 0·5% of added homocysteic acid was still detectable). The dry residue was dissolved in water (130 ml) and passed over a column of Amberlite IR-4B (OH^--form, 60 ml). The effluents were taken to dryness and crystallized from water. The yield was 3·75 g (80%) of a product of m.p. 215—226°C. $E^{Gly}_{2.4}$ 0·50 $E^{His}_{5.7}$ 0·31; R_F 0·12 (S_1), 0·60 (S_2). This product was used for subsequent steps of the synthesis. The sample for analysis was recrystallized from water. M.p. 220—225°C; $[\alpha]_D$ −6·2° (*c* 0·25, water). For $C_9H_{17}NO_4S$ (235·3) calculated: 45·97% C, 7·28% H, 5·96% N; found: 45·96% C, 7·34% H, 6·24% N.

S-γ-Carboxypropylhomocysteine (*IIIb*)

The solution of product *IIIa* (0·50 g) in 1M-NaOH (10 ml) was stirred 2 h at room temperature and passed over a column of Dowex 50 (H^+-form, 40 ml). The product was eluted by 10% pyridine, taken to dryness, and crystallized from water. The yield was 0·41 g (87%) of a product of m.p. 229—232°C; $E^{Gly}_{2.4}$ 0·51, $E^{Asp}_{5.7}$ 0·38; R_F 0·00 (S_1), 0·49 (S_2); $[\alpha]_D$ 18·0° (*c* 0·5, 1M-HCl), −5·2° (*c* 0·38, water). The sample for analysis was recrystallized from water and no change in the melting point was observed. For $C_8H_{15}NO_4S$ (221·3) calculated: 43·40% C, 6·83% H, 6·33% N; found: 43·13% C, 6·67% H, 6·45% N. Recorded data[30]: m.p. 225—227°C and $[\alpha]_D$ 17·6° (1M-HCl).

N^{α}-Benzyloxycarbonyl-S-γ-methoxycarbonylpropylhomocysteine Dicyclohexylammonium Salt (*IIIc*)

a) *Using the Bunte salt*[31]: The solution of methyl ester *IIIa* (0·95 g) in water (25 ml) was treated with sodium benzyloxycarbonyl thiosulfate[31] (1·2 g) with stirring. The pH of the reaction mixture was kept constant at 7·6 by the addition of 0·5M-$NaHCO_3$ (total volume added 60 ml).

After 2 h the pH of the reaction mixture was adjusted to pH 3 by the addition of hydrochloric acid, the product was extracted with ether, the ethereal solution was shaken with water, dried by sodium sulfate and taken to dryness. The residue was dried azeotropically (benzene), and dissolved in benzene. After the addition of dicyclohexylamine (0·90 ml), the solution was diluted with light petroleum, cooled down at 0°C, and the separated product was filtered off and washed with light petroleum. The yield was 1·34 g (61%) of a product of m.p. 119—121°C. Crystallization from a mixture of ethyl acetate–light petroleum afforded 1·30 g (59%) of a product of m.p. 121—123°C. R_F 0·71 (S_1), 0·73 (S_3). The sample for analysis was recrystallized from ethyl acetate and no change in the melting point was observed; $[\alpha]_D$ 6·1° (*c* 0·5, dimethylformamide). For $C_{29}H_{46}N_2O_6S$ (550·7) calculated: 63·25% C, 8·44% H, 5·09% N; found: 63·09% C, 8·27% H, 5·10% N.

b) *Using benzyloxycarbonyl chloride*: The solution of ester *IIIa* (0·25 g) in 0·5M-$NaHCO_3$ (5 ml) stirred and cooled by ice was treated with benzyloxycarbonyl chloride (0·35 ml), added in several portions during 10 min. The pH of the reaction mixture was kept constant at 7·0 to 7·5 (by the addition of 0·5M-$NaHCO_3$) 20 min at 0°C and then 1 h at room temperature. The reaction mixture was extracted with ether, acidified with 6M-HCl (to pH 3), and the product was taken into ether by shaking. The ethereal solution was shaken with water, dried by sodium sulfate, and taken to dryness. After azeotropic drying (benzene), the residue was dissolved in benzene and dicyclohexylamine (0·25 ml) was added. The solution was diluted with light petroleum and the separated product was treated as described under *a*). The yield was 0·38 g (65%) of a product of m.p. 116—119°C without depression when mixed with the sample prepared as described under *a*).

o-Nitrobenzenesulfenyl-asparaginyl-S-γ-methoxycarbonylpropylhomocysteinyl-prolyl-leucyl-glycine Amide (*IV*)

The solution of product *IIIc*. DCHA (1·10 g) in a mixture of methanol (20 ml) and water (10 ml) was stirred 0·5 h with Dowex 50 (H^+-form, 10 ml). The resin was filtered off, the filtrate was taken to dryness and dried azeotropically (benzene). The dry residue was dissolved in dimethylformamide (20 ml) and prolyl-leucyl-glycine amide[13,14] (0·60 g) and 1-hydroxybenzotriazol[16] (0·27 g) were added to the solution. The latter was then cooled down to −15°C and dicyclohexylcarbodiimide (0·46 g) was added. The reaction mixture was stirred 1 h at −10°C. After 12 h of standing at 0°C separated dicyclohexylurea was filtered off and the filtrate was taken to dryness. The dry residue was dissolved in ethyl acetate and this solution was subsequently shaken with 0·5M-$NaHCO_3$, water, 2M citric acid, and again with water and then dried by sodium sulfate. After azeotropic drying (benzene) the dry residue was dissolved in acetic acid (5 ml) and to this solution a solution of hydrogen bromide in acetic acid (35%; 7·5 ml) was added. The mixture was set aside for 20 min. at room temperature and then diluted with ether. The tetrapeptide hydrobromide which had separated was dried ($E^{Gly}_{2,4}$ 0·84, $E^{His}_{5,7}$ 0·50; R_F 0·01 (S_1) and 0·14 (S_2)) and then dissolved in dimethylformamide (20 ml). To this solution N-ethylpiperidine (pH 8, wet indicator paper strip) and *o*-nitrobenzenesulfenylasparagine 2,4,5-trichlorophenyl ester[17] (1 g) were added. After 24 h of stirring at room temperature another portion (0·4 g) of the active ester was added to the reaction mixture. After 24 h the reaction mixture was taken to dryness and the dry residue was triturated with light petroleum and ether. The crystalline amount was filtered off and washed with ether, water, 0·5M-$NaHCO_3$ and again with water. The yield was 1·07 g of a product of m.p. 179—185°C. Crystallization from the mixture dimethylformamide–ether afforded 0·93 g (61%) of a product of m.p. 186—190°C. R_F 0·45 (S_1), 0·64 (S_2),

0·73 (S_3). The sample for analysis was recrystallized in the same manner and its m.p. was 187 to 191°C. $[\alpha]_D$ −66·9° (*c* 0·5, dimethylformamide). For $C_{32}H_{48}N_8O_{10}S_2$ (768·8) calculated: 50·02% C, 6·29% H, 14·58% N; found: 50·13% C, 6·54% H, 14·41% N.

o-Nitrobenzenesulfenyl-glutaminyl-asparaginyl-S-γ-methoxycarbonylpropylhomocysteinyl-prolyl-leucyl-glycine Amide (*Va*)

To the solution of protected pentapeptide *IV* (0·385 g) in dimethylformamide (5·0 ml), 2M-HCl in ether (0·8 ml) was added. After 5 min of standing at room temperature the reaction mixture was diluted with ether and the separated oil was triturated with ether. The crystalline amount was filtered off, washed with ether and dried; $E_{2.4}^{Gly}$ 0·81, $E_{5.7}^{His}$ 0·55. The pentapeptide hydrochloride was dissolved in dimethylformamide (20 ml), the pH of the solution was adjusted to 8·0—9·0 by N-ethylpiperidine, and *o*-nitrobenzenesulfenylglutamine 2,4,5-trichlorophenyl ester[17] (0·24 g) was added to the solution. After 24 h stirring at room temperature, another portion (0·12 g) of the active ester was added to the reaction mixture. The latter was stirred for 24 h, then taken to dryness and the dry residue was triturated with light petroleum and ether. The crystalline amount was filtered off, washed on the filter with ether, and immediately recrystallized from a mixture of methanol and ether. The yield was 0·36 g (80%) of a product of m.p. 164—168°C. The sample for analysis was crystallized in the same manner and its m.p. was 170—174°C; $[\alpha]_D$ −20·8° (*c* 0·5, dimethylformamide); R_F 0·31 (S_1), 0·62 (S_2), and 0·70 (S_3). For $C_{37}H_{56}N_{10}O_{12}S_2.0{\cdot}5\ H_2O$ (906·0) calculated: 49·05% C, 6·33% H, 15·46% N; found: 49·03% C, 6·31% H, 15·51% N.

Glutaminyl-asparaginyl-S-γ-carboxypropylhomocysteinyl-prolyl-leucyl-glycine Amide (*Vb*)

To a solution of protected hexapeptide *Va* (1·35 g) in dimethylformamide (6 ml), 2M-HCl in ether (2·25 ml) was added and after 5 min of standing at room temperature the reaction mixture was diluted with ether. The separated hydrochloride was filtered off, washed on the filter with ether, and dried in a desiccator; $E_{2.4}^{Gly}$ 0·72, $E_{5.7}^{His}$ 0·49. The product was dissolved in methanol (15 ml) and 1M-NaOH (6·0 ml) was added to the solution. The latter was set aside for 1 h at room temperature, methanol was then evaporated, and the aqueous solution was applied to a column of Dowex 50 (H^+-form; 45 ml). The column was washed with water and the product was eluted by 10% pyridine. The effluent was acidified to pH 6·5 by acetic acid, concentrated to a small volume, and lyophilized. The lyophilisate was reprecipitated from a mixture of 90% methanol and ether, the oily product was dissolved in water and dried from the frozen state. The lyophilisate was triturated with ether, filtered off, and washed with ether on the filter. The yield of the product was 0·88 g (78%); $E_{2.4}^{Gly}$ 0·70, $E_{5.7}^{His}$ 0·20; R_F 0·01 (S_1), 0·16 (S_2). Amino acid analysis: Gly 1·00, Leu 1·05, Pro 0·95, HCys($C_3H_6CO_2H$) 1·00, Asp 1·01, Glu 1·00; $[\alpha]_D$ −49·2° (*c* 0·5, dimethylformamide). For $C_{30}H_{51}N_9O_{10}S.2\ H_2O$ (765·8) calculated: 47·04% C, 7·23% H, 16·46% N; found: 47·28% C, 7·21% H, 16·48% N.

o-Nitrobenzenesulfenyl-isoleucyl-glutaminyl-asparaginyl-S-γ-methoxycarbonylpropylhomocysteinyl-prolyl-leucyl-glycine Amide (*VIa*)

To a solution of protected hexapeptide *Va* (225 mg) in dimethylformamide (4·0 ml), 2M-HCl in ether (0·5 ml) was added. The reaction mixture was set aside for 5 min at room temperature and then diluted with ether. The oil which had separated was triturated with ether, the crystalline amount was filtered off, washed with ether on the filter, and dried in a desiccator; $E_{2.4}^{Gly}$ 0·72,

$E_{5,7}^{His}$ 0·49. The hexapeptide hydrochloride was dissolved in dimethylformamide (10 ml), the solution was made alkaline (pH 8·0—9·0) by the addition of N-ethylpiperidine, and *o*-nitrobenzenesulfenylisoleucine N-hydroxysuccinimide ester[2] (0·10 g) was added. The reaction mixture was stirred 24 h at room temperature and another portion of the active ester (0·05 g) was added afterwards. After 24 h of stirring, the reaction mixture was taken to dryness. The dry residue was stepwise triturated with light petroleum and ether, the crystalline amount was filtered off and washed on the filter with ether, water, 0·5M-$NaHCO_3$, and water. Crystallization from the mixture dimethylformamide–ether afforded 176 mg (70%) of a product of m.p. 251—254°C. The sample for analysis was recrystallized in the same manner and its m.p. was 252—254°C; $[\alpha]_D$ —64·9° (*c* 0·5, dimethylformamide); R_F 0·36 (S_1), 0·65 (S_2), 0·76 (S_3). For $C_{43}H_{67}N_{11}O_{13}S_2 . 0{\cdot}5\ H_2O$ (1019) calculated: 50·70% C, 6·72% H, 15·14% N; found: 50·70% C, 6·68% H, 15·23% N.

Isoleucyl-glutaminyl-asparaginyl-S-γ-carboxypropylhomocysteinyl-prolyl-leucyl-glycine Amide (*VIc*)

To a solution of protected heptapeptide *VIa* (1·0 g) in dimethylformamide (6·0 ml), 2M-HCl in ether (1·5 ml) was added, the reaction mixture was allowed to stand 5 min at room temperature and was diluted with ether afterwards. The hydrochloride which had separated was filtered off, washed with ether on the filter, and dried in a desiccator: $E_{2,4}^{Gly}$ 0·65, $E_{5,7}^{His}$ 0·44. The product was dissolved in methanol (10 ml) and 1M-NaOH (4·0 ml) was added. After 1 h of standing at room temperature methanol was evaporated and the aqueous solution was applied to a column of Dowex 50 (H^+-form; 30 ml). The column was washed with water and the product was eluted by 10% pyridine. The effluent was acidified to pH 6·5 by acetic acid, concentrated to a small volume, and freeze-dried. The lyophilisate was dissolved in 90% methanol, and the solution was diluted with ether. The product which had separated was filtered off and washed with ether. The yield was 0·67 g (77)%; $E_{2,4}^{Gly}$ 0·65, $E_{5,7}^{His}$ 0·19; R_F 0·02 (S_1), 0·17 (S_2). The sample for analysis was reprecipitated from the mixture dimethylformamide–ether; $[\alpha]_D$ —49·5° (*c* 0·5; dimethylformamide). Amino-acid analysis: Gly 0·96, Leu 0·97, Pro 1·04, Ile + HCys($C_3H_6CO_2$) 2·08, Asp 1·04, Glu 1·00. For $C_{36}H_{62}N_{10}O_{11}S.2\ H_2O$ (879·0) calculated: 49·22% C, 7·57% H, 15·94% N; found: 49·02% C, 7·22% H, 16·09% N.

o-Nitrobenzenesulfenyl-isoleucyl-glutaminyl-asparaginyl-S-γ-carboxypropylhomocysteinyl-prolyl-leucyl-glycine Amide (*VIb*)

To a solution of free hexapeptide *Vb* (0·73 g) in dimethylformamide (20 ml) and N-ethylpiperidine (0·2 ml), *o*-nitrobenzenesulfenylisoleucine N-hydroxysuccinimide ester[2] (0·40 g) was added. The reaction mixture was stirred 24 h at room temperature and another portion of the active ester (0·17 g) was added subsequently. After two more days the reaction mixture was taken to dryness. The dry residue was triturated stepwise with light petroleum and ether, the crystalline amount was filtered off and washed on the filter with ether, water, 0·2M-H_2SO_4 (0°C), and again with water. The yield was 0·44 g (44%) of a product of m.p. 169—178°C. Twice repeated crystallization from the mixture dimethylformamide–ether afforded 0·29 g (29%) of a product of m.p. 190 to 194°C; $E_{2,4}^{Gly}$ 0·65, $E_{5,7}^{His}$ 0·19 (after the removal of the *o*-nitrobenzenesulfenyl protecting group by hydrogen chloride in methanol. The electrophoretic behavior is identical with that of product *VIc*). The sample for analysis was crystallized from the same solvent system. M.p. 186—189°C, $[\alpha]_D$ —57·0° (*c* 0·5, dimethylformamide); R_F 0·10 (S_1), 0·55 (S_2), 0·66 (S_3). For $C_{42}H_{65}N_{11}O_{13} . S_2.1\ H_2O$ (1014) calculated: 49·80% C, 6·66% H, 15·21% N; found: 49·73% C, 6·71% H, 15·08% N.

tert-Butyloxycarbonyl-O-tert-butyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-γ-carboxypropylhomocysteinyl-prolyl-leucyl-glycine Amide (*VII*)

a) To a solution of free heptapeptide *VIc* (0·67 g) in dimethylformamide (20 ml) and N-ethylpiperidine (0·2 ml), tert-butyloxycarbonyl-O-tert-butyltyrosine N-hydroxysuccinimide ester[2] (0·35 g) was added. After 24 h of stirring at room temperature, another portion of the active ester (0·15 g) was added to the reaction mixture. The reaction mixture was taken to dryness 24 h thereafter and the dry residue was triturated stepwise with light petroleum and ether. The crystalline amount was filtered off and washed with ether, water, 3% citric acid, and water on the filter. The yield was 0·74 g (82%) of a product of m.p. 209·5–212°C. The sample for analysis was crystallized from the mixture dimethylformamide–ether and its m.p. was 209–211°C; $[\alpha]_D$ −29·2° (*c* 0·5, dimethylformamide); $E_{2.4}^{Gly}$ 0·56, $E_{5.7}^{His}$ 0·17; R_F 0·14 (S_1), 0·60 (S_2), 0·67 (S_3). (The electrophoretic analysis was carried out after the removing of the protecting groups by hydrogen bromide in acetic acid.) For $C_{54}H_{87}N_{11}O_{15}S.1\,H_2O$ (1180) calculated: 54·90% C, 7·60% H, 13·05% N; found: 54·75% C, 7·59% H, 13·42% N.

b) To a solution of protected heptapeptide *VIb* (0·16 g) in dimethylformamide (3 ml), 2M-HCl in ether (0·25 ml) was added. After 5 min of standing at room temperature the reaction mixture was diluted with ether, the hydrochloride which had separated was filtered off, washed with ether, and dried in a desiccator. The dry residue was dissolved in dimethylformamide (10 ml) and the pH of the solution was adjusted to 8·0–9·0 (wet indicator paper) by N-ethylpiperidine. tert-Butyloxycarbonyl-O-tert-butyltyrosine hydroxysuccinimide ester[2] (0·08 g) was added to the solution. After 24 h of stirring at room temperature, another portion of the active ester (0·03 g) was added. The reaction mixture was stirred for another 24 h and then taken to dryness. The dry residue was triturated with light petroleum and ether, filtered off, and washed stepwise on the filter with ether, water, 3% citric acid, and water. The yield was 0·10 g (54%) of a product of m.p. 210–212°C (no depression of the m.p. was observed when the product was mixed with a sample of product prepared as described under *a*).

Lactam of Tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-γ-carboxypropylhomocysteinyl-prolyl-leucyl-glycine Amide ([Deamino-homolanthionine[1,6]]-oxytocin) (*Ie*)

The preparation of the active ester and its cyclization were carried out in an atmosphere of nitrogen. To a solution of protected octapeptide *VII* (295·5 mg) in a mixture of pyridine (10 ml) and dimethylformamide (10 ml), bis-*p*-nitrophenyl sulfite[19] (1 g) was added. The reaction mixture was stirred 8 h at room temperature, diluted with pyridine (5 ml), and another portion of the reagent (1 g) was added. After 12 h of stirring at room temperature, another 0·5 g portion of bis-*p*-nitrophenyl sulfite was added, the reaction mixture was stirred for 5 h, and then taken to dryness. The dry residue was triturated with ether, filtered off and washed with ether and water, and dried in the open air (286 mg). The active ester was dissolved in trifluoroacetic acid (10 ml), the reaction mixture was allowed to stand 1 h at room temperature, then diluted with toluene (10 ml), and taken to dryness. The dry residue was dissolved in dimethylformamide (10 ml) and this solution was added to 250 ml of pyridine within 4 h with stirring and heating at 50°C. After 12 h the reaction mixture was taken to dryness, the dry residue was triturated with ether, filtered off, and washed thoroughly with ether. After drying the residue was dissolved in 25 ml of the upper phase of the solvent system 2-butanol–0·05% acetic acid, the filtered solution was placed in the second tube of the all-glass Steady State Distribution Machine (Quickfit & Quartz, Ltd., Stone, Staffordshire, England), and 110 transfers of the upper phase were carried out. The peptide material was located by the Folin-Ciocalteau reagent. The peak of distribution coefficient 2·24 (tube No 65–90) was isolated by concentration to a small volume and lyophilization. The yield was 131 mg. (Another peak

of distribution coefficient 0·62 was found.) R_F 0·34 (S_1), 0·29 (S_2). This product was further purified for analysis and biological tests by gel filtration on columns of Bio-gel P-4 and P-2 (100 × 1 cm; 1M acetic acid) and reprecipitated from the mixture methanol–ether; $[\alpha]_D$ −77·7° (*c* 0·25; 1M acetic acid). Amino acid analysis: Gly 1·00, Leu 1·11, Pro 1·02, Ile + HCys ($C_3H_6CO_2H$) 2·11, Asp 0·97, Glu 0·98, Tyr 0·90. For $C_{45}H_{69}N_{11}O_{12}S.4\,H_2O$ (1060) calculated: 50·95% C, 7·32% H, 14·52% N; found: 51·13% C, 6·91% H, 14·34% N.

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