SYNTHESIS AND PROPERTIES OF OXYTOCIN ANALOGUES MODIFIED IN THE TRIPEPTIDE SIDE CHAIN*

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Two oxytocin analogues were synthesized by fragment condensation (6 + 3) in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. In one of the analogues, proline in the position 7 and leucine in the position 8 were substituted by 2-[1-(2-oxo-3-aminopyrrolidinyl)]--4-methylpentanoic acid, in the other proline was replaced by 1-aminocyclopropane-1-carboxylic acid. Biological activities of the first analogue were strongly reduced and dissociation of the uterotonic and galactogogic activities was observed with both the analogues. The structure of 2-(3-tert-butyloxycarbonylaminopyrrolidin-2-on-1-yl)-4-methylpentanoylglycine and its amide was confirmed by mass and ^{1}H NMR spectroscopy.

The number of known oxytocin analogues modified in the sequence position 7 is surprisingly small, particularly if one considers their biological activity. The position 7 should be of importance not only for bonding to the corresponding receptor but also (as a corner amino acid of the assumed β-turn) for keeping the correct spatial arrangement[1]. Also the importance of enzymic cleavage of the tripeptide side chain cannot be neglected[2,3]. An enhanced uterotonic activity has been observed with analogues containing dehydroproline[4] or thiazolidinecarboxylic acid[5,6] which cannot substantially change the spatial orientation of the terminal tripeptide; on the other hand, their higher lipophilicity could improve the hydrophilic interaction with the receptor. Analogues, modified by introduction of glycine[7-10], which is also a suitable amino acid for the β-turn corner position, show a strong dependence of activity on magnesium content in the tested medium, their activity in the presence of magnesium reaching values up to twice higher than that of oxytocin (965 I.U./mg). Introduction of D-proline[11] leads to a substantial decrease in activity, but a far larger decrease is caused by introduction of D-leucine[7]. Reduction in size of the proline ring[12] results in about tenfold reduction of the activity; replacement of one of the hydrogens in the proline ring by hydroxyl[13,14] is accompanied by a complete loss of activity showing again the importance of lipophilicity of the amino acid in the

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position 7. Alanine in this position[9] reduces the activity whereas methylation of its nitrogen atom (N-methylalanine) increases the activity[15], in the case of sarcosine[15] even to the oxytocin level.

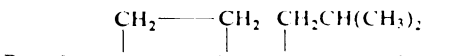
One of the modifications which we effected in position 7 was the so-called proline shift. The ring in the side chain is not closed by bond to the nitrogen atom of the amino acid in position 7 (as in the case of proline) but to nitrogen of the amino acid in the position 8 (*i.e.* to the leucine nitrogen). The ring size is thus the same but the cyclic rigid structure is shifted towards the carboxyl end of the molecule. Such a modification was studied by the American authors[16] and applied to the analogues of LHRH[17] and enkephalin[18] where it fixed the secondary structure. In our second analogue, proline in the position 7 is replaced by 1-aminocyclopropane-1-carboxylic acid. A part of our results has been published as a preliminary communication[19].

Boc–Met–Leu–Gly–OEt

I

[Boc–Met(Me)–Leu–Gly–OEt]$^+$ I^-

II



IIIa, X = OH
IIIb, X = OMe
IIIc, X = NH_2

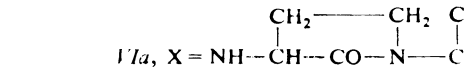
Z–Acc–Leu–Gly–X

IVa, X = OEt
IVb, X = NH_2

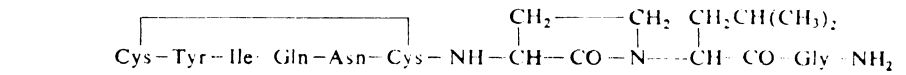
Z–Cys(Bzl)–Tyr(Bu^t)–Ile–Gln–Asn–Cys(Bzl)–OBu^t

V

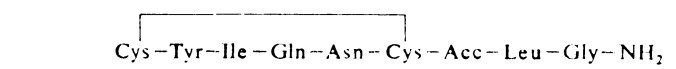
Z–Cys(Bzl)–Tyr–Ile–Gln–Asn–Cys(Bzl)–X



VIa, X = NH–CH–CO–N–CH–CO–Gly–NH_2
VIb, X = Acc–Leu–Gly–NH_2



VIIa



VIIb

Both the analogues* were prepared using the 6 + 3 fragment condensation. In the synthesis of the analogue *VIIa*, the corresponding tripeptide was prepared by treatment of tert-butyloxycarbonylmethionyl-leucyl-glycine ethyl ester[21] (*I*) with methyl iodide to give the sulfonium salt *II*, which reacted with sodium hydride to close the five-membered ring under simultaneous hydrolysis of the ester group. The resulting derivative *IIIa* was esterified with diazomethane to give the ester *IIIb* which on ammonolysis afforded the amide *IIIc*.

The structure of the tripeptide *IIIa* was proved by ^{1}H NMR and mass spectroscopy. For the interpretation of the NMR spectrum see Fig. 1.

The mass spectra of *IIIa* and *IIIc* are very similar and their interpretation provides important structural arguments. The main fragmentation pathway of their molecular ions (M^+ 371 and 370, respectively) starts with β-elimination of the radical CONH $.CH_2COR$ (R = OH or NH_2) to give an important ion *m/z* 269. Loss of C_4H_8

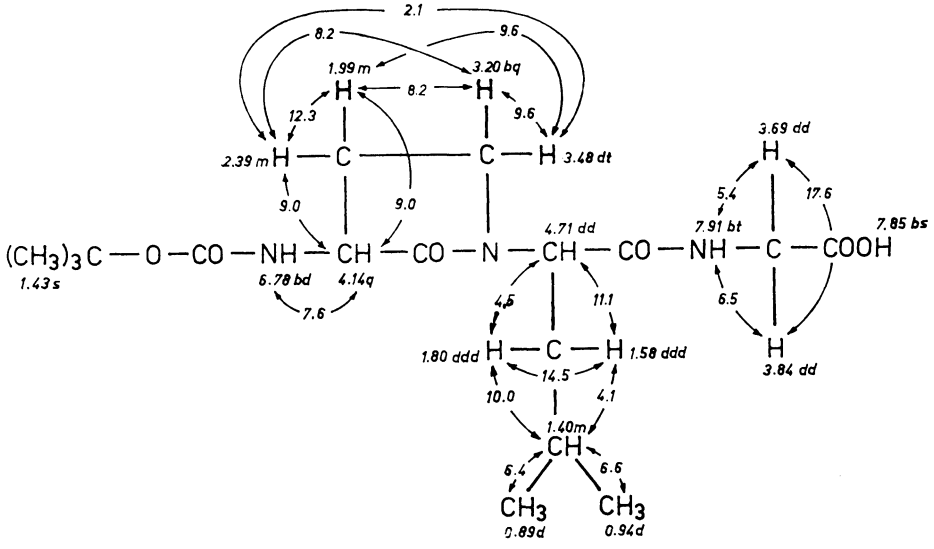


FIG. 1

^{1}H NMR Parameters of compound *IIIa*. For the protons the chemical shifts (δ) and signal multiplicities are given. The coupling constants (in Hz) are given at arrows linking the interacting protons

* Amino acids used in this work are of the L-configuration. The nomenclature and symbols for the amino acids and their derivatives obey the published recommendations[20]; Acc denotes 1-aminocyclopropane-1-carboxylic acid, Thz thiazolidine-4-carboxylic acid, Aze azetidinecarboxylic acid and Pip pipecoline-2-carboxylic acid residues.

from the other end of this ion results in the dominant ion m/z 213, which eliminates H_2O or CO_2 to give ions m/z 195 and 169. The ion m/z 98 is formed in turn by their splitting (Fig. 2). Alternatively, the fragmentation of M^+ proceeds with preservation of its carboxylic (or carboxamidic) end, resulting in formation of characteristic ions which differ by one mass unit for the corresponding processes in the mass spectra of *IIIa* and *IIIc*. Thus, the consecutive elimination of the alkyls C_4H_8 from

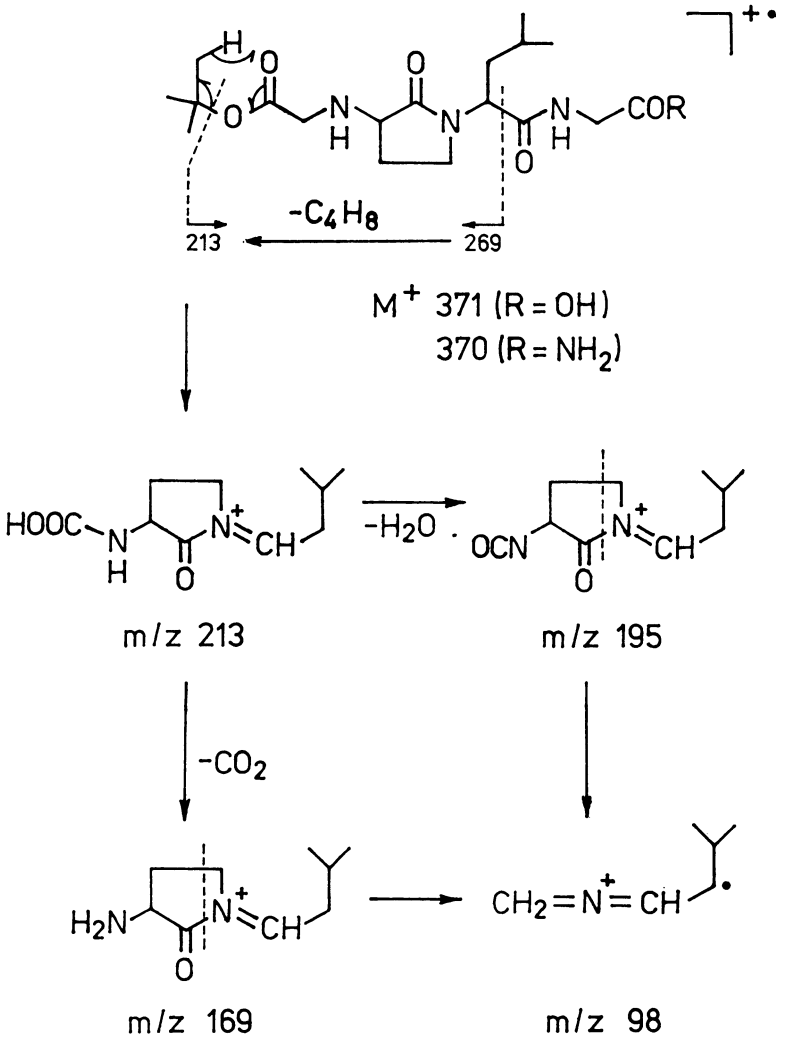


FIG. 2

The main electron impact induced fragmentation pathway for *IIIa* (M^+ 371) and *IIIc* (M^+ 370)

M^+ leads to ions at m/z 315 and 259 (or 314 and 258, respectively), while the loss of C_4H_8O from M^+ yields ions m/z 298 or 297.

The preparation of the analogue *VIIb* started from the carboxy-terminal tripeptide amide *IVb* which was obtained by carbodiimide condensation of 1-benzyloxycarbonylaminocyclopropanecarboxylic acid[22] with leucyl-glycine ethyl ester, followed by ammonolysis of the ester *IVa*.

TABLE I
Biological activities (rat) of oxytocin analogues (I.U./mg)

Compound	Uterotonic (*in vitro*)	Galactogogic (*in situ*)	Pressor	G/U[a]	Ref.
Oxytocin (OT)	450	450	3	1·0	
[Δ^3-Pro^7]OT	1 071	—	3	—	4
[Thz^7]OT	1 180	—	3·46	—	5
	—	—	—	—	6
[Gly^7]OT	68	235	depress.	3·45	7
	330	—	—	—	8
	65	224[b]	0·3	3·45	9
	93	392	0·01	4·21	10
[D-Pro^7]OT	13	3·9[b]	0	0·30	11
[D-Leu^7]OT	0·61	0·51	0	0·83	7
[Aze^7]OT	43	353	0·1	8·20	12
[Pro(4-OH)^7]OT	0	—	—	—	13, 14
[Ala^7]OT	22	—	0·4	—	9
[MeAla^7]OT	62	402	0	6·49	15
[Sar^7]OT	459	564	0	1·23	15
[Nva^7]OT	—	—	—	—	32
[Pip^7]OT	—	—	—	—	13
Tocinamide	3·3	1·1[b]	0	0·33	25
	2·7	5·9	0	2·18	26
VIIa	1·6	8	0·2	5·00	this paper
VIIb	9·8	62	0·2	6·32	this paper

[a] Ratio of galactogogic to uterotonic activity; [b] rabbit.

The carboxy-terminal tripeptide amides *IIIc* and *IVb* were deprotected with trifluoroacetic acid and hydrogen bromide, respectively, and condensed with N-benzyloxycarbonyl-S-benzylcysteinyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteine obtained from the corresponding fully protected hexapeptide *V* (ref.[23]) by treatment with trifluoroacetic acid. The condensation was performed with an excess of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole and the product was purified by chromatography on a column of Sephadex LH-20 in dimethylformamide. The protecting groups in the nonapeptide *VIa* were removed with sodium in liquid ammonia whereas the nonapeptide *VIb* was treated with anhydrous liquid hydrogen fluoride in the presence of anisole because sodium in liquid ammonia opened the three-membered ring in the 1-aminocyclopropanecarboxylic acid moiety (*cf.* the hydrogenolytic ring opening[24]). The sulfhydryl groups were oxidized with potassium ferricyanide and after de-salting both analogues were purified by reversed phase high performance liquid chromatography.

Both analogues have lower biological activities than oxytocin, with dissociation of the uterotonic and galactogogic activities.

This dissociation in favour of the galactogogic activity has been found for all hitherto described oxytocin analogues, modified only in position 7 (considering analogues with only one structural modification), except analogues with a D-amino acid in the position 7, where the uterotonic activity is favoured. The most apparent dissociation in favour of galactogogic activity has been observed for the analogues, containing azetidinecarboxylic acid, 1-aminocyclopropane-1-carboxylic acid, or N-methylalanine as the modifying elements, common feature of the first two being a significant deformation of the N—C^{α}—C′ bond angle.

Oxytocin analogues, modified only in the position 7, can be classified into two groups according to their uterotonic activity: analogues which are more active than tocinamide[25,26] and those with lower activity. In the latter analogues, the spatial arrangement of the cyclic part is for various reasons also affected, which in tocinamide is out of the question. The very low activities of the analogue *VIIa* indicate that such a substantial change in structure of the terminal tripeptide evidently perturbs the spatial arrangement of the whole peptide and thus affects unfavourably its interaction with the receptor.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried at room temperature and 150 Pa for 24 h. Thin-layer chromatography (TLC) was carried out on silica gel coated plates (Silufol, Kavalier, Czechoslovakia) in the following systems: 2-butanol–98% formic acid–water (75 : 13·5 : 13·5) (S1), 2-butanol–25% ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), 1-butanol–acetic acid–water–pyridine (15 : 3 : 6 : 10) (S4). Paper electrophoresis was performed in a moist chamber in 1M acetic acid (pH 2·4) and in pyridine–acetate buffer (pH 5·7) on Whatman 3MM paper at 20 V/cm

for 60 min. Spots in TLC and electrophoresis were detected with ninhydrin or by chlorination method. The reaction mixtures were concentrated on a rotatory evaporator at bath temperatures 30—40°C in vacuum of a water pump (mixtures containing dimethylformamide at 150 Pa). Samples for amino acid analysis were hydrolyzed with 6M-HCl at 105°C for 20 h and analyzed on an automatic two-column instrument (type 6020, Developmental Workshops of Czechoslovak Academy of Sciences) or on a D-500 analyzer (Durrum Corp.). Optical rotations were determined on a Perkin–Elmer instrument type 141 MCA (Norwalk, USA). High performance liquid chromatography (HPLC) was carried out on an SP-8700 instrument equipped with an SP-8400 detector and SP-4100 integrator (all from Spectra Physics, Santa Clara, USA), using a Separon SI-C-18 (25 × 0·4 cm) column (Laboratorní Přístroje, Prague).

^{1}H NMR spectrum of *IIIa* was taken on a Varian XL-200 spectrometer (at 200 MHz) in deuteriochloroform–hexadeuteriodimethyl sulfoxide (2 : 1) with tetramethylsilane as internal standard at 23°C. Electron impact induced mass spectra were measured on a double focussing mass spectrometer AEI MS902 (Associated Electric Industries, Manchester), 70 eV, direct inlet at about 150°C, resolving power 1 000. High resolution measurements were performed at resolving power 10 000 and the exact masses found for the elemental compositions were within ±2 mmu of the theoretical value.

2-(3-Tert-butyloxycarbonylaminopyrrolidin-2-on-1-yl)-4-methylpentanoyl-glycine (*IIIa*)

Methyl iodide (1 ml) was added to the tripeptide *I* (ref.[21]; 1 g) in methanol (30 ml) and the formaton of the sulfonium salt was monitored by reversed phase HPLC. After disappearance of the starting compound *I* (24 h) the mixture was taken down and the residue dried *in vacuo*. The remaining product *II* was dissolved in dimethylformamide (4·6 ml), sodium hydride (164 mg) was added and the mixture was stirred for 10 min at room temperature. After decomposition with water (100 ml) the resulting solution was stirred for 20 min, acidified with solid sodium hydrogen sulfate to pH 3 and extracted with ethyl acetate, The organic layer was washed with water, dried over sodium sulfate and taken down, affording 0·76 g (91%) of the product, melting at 163—170°C. An analytical sample was crystallized from aqueous methanol, m.p. 168—171°C. For $C_{17}H_{29}N_3O_6.1/2\,H_2O$ (380·4) calculated: 53·67% C, 7·95% H, 11·04% N; found: 53·39% C, 8·01% H, 10·77% N. According to the amino acid analysis, the product did not contain leucine or methionine but an amino acid was present which was eluted 6 min before methionine. R_F (in parentheses values for *I*): 0·90 (0·92), (S1), 0·34 (0·72) (S2), 0·78 (0·82) (S3), 0·60 (0·91) (S4): $E_{5.7}^{His}$ 0·00 (0·70), $E_{2.4}^{Gly}$ 1·06 (1·06) (after removal of the Boc group). HPLC: k' 9·1 (methanol–0·05% trifluoroacetic acid 1 : 1). ^{1}H NMR spectrum see Fig. 1. Partial mass spectrum (*m/z* with relative abundance and elemental composition in parentheses): 39 (26), 40 (30), 41 (75), 42 (26), 43 (33), 44 (42), 55 (37), 56 (45), 57 (57), 59 (22), 69 (26), 98 (43, $C_6H_{12}N$), 169 (19, $C_9H_{17}N_2O$), 195 (22, $C_{10}H_{15}N_2O_2$), 213 (100, $C_{10}H_{17}N_2O_3$), 259 (10, $C_9H_{13}N_3O_6$), 269 (19·3, $C_{14}H_{25}N_2O_3$), 298 (3·7, $C_{13}H_{20}N_3O_5$), 315 (5·3, $C_{13}H_{21}N_3O_6$), M^+ 371 (1).

2-(3-Tert-butyloxycarbonylaminopyrrolidin-2-on-1-yl)-4-methylpentanoyl-glycine Amide (*IIIc*)

The compound *IIIa* (0·54 g) in methanol (20 ml) was treated with an ethereal solution of diazomethane. After standing for 5 min the excess diazomethane was destroyed with acetic acid (30 µl) and the solvent was evaporated, leaving *IIIb*, pure according to TLC (R_F 0·64 in S2) and HPLC (k' 14·3; for the starting acid: k' 3·27) in methanol–0·05M ammonium acetate pH 7, 1 : 1. The product *IIIb* was dissolved in a methanolic solution of ammonia (60 ml) and the ammonolysis was followed by HPLC in the above-mentioned system (k' 5·68). After 24 h the mixture was taken down and the product dried *in vacuo*, affording 0·52 g (98%) of *IIIc*, m.p.

168—170°C. An analytical sample was crystallized from water, m.p. 172°C. For $C_{17}H_{30}N_3O_5 . 1/2\ H_2O$ (379·5) calculated: 53·81% C, 8·23% H, 14·76% N; found: 53·59% C, 8·42% H, 14·44% N. $[\alpha]_D$ −85·5° (*c* 0·1; dimethylformamide). R_F 0·66 (S1), 0·62 (S2), 0·65 (S3), 0·73 (S4); $E_{5.7}^{His}$ 0·80, $E_{2.4}^{Gly}$ 1·20 (after removal of the Boc group). Partial mass spectrum: 39 (10), 41 (35), 42 (22), 43 (9), 44 (11), 56 (23), 57 (33), 59 (16), 98 (41, $C_6H_{12}N$), 139 (6·3), 141 (5·8), 153 (7·5, $C_7H_9N_2 . O_2$), 169 (20, $C_9H_{17}N_2O$), 213 (100, $C_{10}H_{17}N_2O_3$), 223 (3·4), 240 (1·8), 241 (1·6), 253 (1·7), 258 (10·3, $C_8H_{14}N_4O_5$), 259 (18·5, $C_{14}H_{25}N_2O_3$), 280 (1·4, $C_{13}H_{18}N_3O_4$), 297 (2·4, $C_{13}H_{22} . N_4O_5$), M^+ 370 (0·2, $C_{17}H_{30}N_4O_5$).

Stability of *IIIc* towards Sodium in Liquid Ammonia

Sodium was added to a solution of *IIIc* (10 mg) in liquid ammonia (15 ml) until the blue colouration persisted for 90 s. After decomposition with acetic acid the mixture was freeze-dried. The residue was dissolved in water and the product extracted with ethyl acetate. The extract was dried over sodium sulfate and taken down, leaving 8·3 mg of a residue, shown by HPLC to be the unchanged starting material.

Benzyloxycarbonyl-1-aminocyclopropane-1-carboxy-leucyl-glycine Ethyl Ester (*IVa*)

Benzyloxycarbonylleucyl-glycine ethyl ester (350 mg) was decarbobenzoxylated in 35% HBr in acetic acid (1 ml), the mixture was diluted with ether and the separated dipeptide hydrobromide was triturated several times with fresh ether and dissolved in chloroform (2 ml). The solution was adjusted to pH 7—8 and benzyloxycarbonyl-1-aminocyclopropane-1-carboxylic acid[22] (235 mg) was added, followed, after cooling to −10°C, by dicyclohexylcarbodiimide (230 mg). The mixture was stirred at −10°C for 1 h and at 0°C overnight. The separated dicyclohexylurea was filtered off, the filtrate was taken down and the residue dissolved in ethyl acetate. After washing with 1M-HCl, water, 0·5M-$NaHCO_3$ and water, the solution was dried over sodium sulfate and taken down. The residue was reprecipitated from ether–light petroleum and the remaining oil crystallized on keeping in a refrigerator overnight. The crystals were filtered and dried, affording 350 mg (77%) of a hygroscopic product; R_F 0·83 (S1), 0·68 (S2), 0·80 (S4), HPLC: *k'* 2·62 (methanol–0·1% trifluoroacetic acid 75 : 25). $[\alpha]_D$ −22·7° (*c* 0·2; methanol). For $C_{22}H_{31}N_3O_6$ (433·5) calculated: 60·95% C, 7·21% H, 9·69% N; found: 60·51% C, 7·35% H, 9·91% N.

Benzyloxycarbonyl-1-aminocyclopropane-1-carboxy-leucyl-glycine Amide (*IVb*)

A solution of the compound *IVa* (320 mg) in methanol (10 ml) was mixed with 50% methanolic ammonia (10 ml). After standing for 4 days in a stoppered flask at room temperature, the mixture was taken down and the remaining foam was dissolved in methanol–water (45 : 55; 8 ml) and purified by HPLC in methanol–water (55 : 45) on Partisil ODS. Yield 180 mg (61%) of amorphous product, pure according to HPLC (*k'* 3·94; methanol–water 60 : 40); $[\alpha]_D$ −33·2° (*c* 0·3; methanol). R_F 0·66 (S1); 0·56 (S2); 0·67 (S3); 0·75 (S4). Amino acid analysis: Gly 1·04, Leu 1·00, Acc 0·86. For $C_{20}H_{28}N_4O_5 . 0·5\ H_2O$ (413·5) calculated: 58·13% C, 7·07% H, 13·55% N; found: 58·45% C, 7·08% H, 13·43% N.

N^{α}-Benzyloxycarbonyl-S-benzylcysteinyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-
-S-benzylcysteinyl-[2-(3-aminopyrrolidin-2-on-1-yl)-4-methylpentanoyl]glycine Amide (*VIa*)

A solution of the protected hexapeptide *V* (ref.[23]; 100 mg) and compound *IIIc* (96 mg) in trifluoroacetic acid (2 ml) was set aside for 1 h at room temperature. The mixture was twice coeva-

porated with toluene (2 ml), the residue dissolved in dimethylformamide (2 ml) and the solution adjusted with N-ethylpiperidine to pH 8 (moist pH indicator paper). After addition of N-hydroxybenzotriazole (35 mg) and cooling to −10°C, dicyclohexylcarbodiimide (85 mg) was added. The mixture was stirred for 1 h at −10°C and for 40 h at room temperature, filtered, concentrated, applied on a column of Sephadex LH-20 (200 × 1 cm) and eluted with dimethylformamide. The pertinent fractions (refractometric detection) were taken down and the residue was triturated with ether and dried *in vacuo*, affording 83 mg (71%) of the product, m.p. 221 to 223°C. R_F 0·64 (S1), 0·39 (S2), 0·62 (S3), 0·82 (S4). $[\alpha]_D$ −53·4° (*c* 0·18; dimethylformamide). Amino acid analysis: Asp 1·05, Glu 1·07, Gly 1·02, Ile 0·93, Tyr 0·94, Cys (Bzl) 1·69. For $C_{64}H_{84}N_{12}O_{14}S_2.2\,H_2O$ (1 346) calculated: 57·13% C, 6·59% H, 12·49% N; found: 57·15% C, 6·27% H, 12·78% N.

Benzyloxycarbonyl-S-benzylcysteinyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteinyl-1-aminocyclopropane-1-carboxy-leucyl-glycine Amide (*VIb*)

A solution of the tripeptide *IVb* (81 mg) in acetic acid (0·2 ml) was mixed with 35% HBr in acetic acid (0·3 ml). After standing at room temperature for 30 min the mixture was diluted with ether. The separated tripeptide hydrobromide was isolated by decanting the supernatant, dissolved in dimethylformamide (1 ml) and the solution was adjusted to pH 8 with N-ethylpiperidine. N-Benzyloxycarbonyl-S-benzylcysteinyl-O-tert-butyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteine tert-butyl ester (*V*; ref.[23]; 117 mg) was dissolved in trifluoroacetic acid (2 ml) and set aside for 1 h at room temperature. After addition of toluene (2 ml) the solution was taken down, the residue was dissolved in dimethylformamide (1 ml) and mixed with solution of the tripeptide amide, prepared as described above. The solution was adjusted to pH 7—8 with N-ethylpiperidine, and 1-hydroxybenzotriazole (40 mg) was added. After cooling to −20°C dicyclohexylcarbodiimide (100 mg) was added and the mixture was stirred at −10°C for 1 h, at 0°C for 1 h and at room temperature overnight. The separated dicyclohexylurea was filtered, the filtrate concentrated to 1·5 ml and applied on a column of Sephadex LH-20 (1 × 200 cm). Elution with dimethylformamide was monitored by differential refractometer. Fractions 61—68 ml after evaporation afforded 68 mg (52%) of the product. R_F 0·78 (S4). $[\alpha]_D$ −61·5° (*c* 0·2; dimethylformamide). Amino acid analysis: Asp 0·95, Glu 1·06, Gly 1·02, Ile + Acc 1·94, Leu 1·02, Tyr 0·92. The analytical sample was precipitated from dimethylformamide and water; m.p. 210—225°C. For $C_{64}H_{84}N_{12}O_{14}S_2.3H_2O$ (1 363·6) calculated: 56·37% C, 6·65% H, 12·33% N; found: 56·30% C, 6·35% H, 12·58% N.

[7,8-2-(3-Aminopyrrolidin-2-on-1-yl)-4-methylpentanoic acid]oxytocin (*VIIa*)

The protected peptide *VIa* (60 mg) was dissolved in liquid ammonia (20 ml) and reduced with sodium. The blue colouration, persisting for 40 s, was destroyed with acetic acid and the solution was lyophilized. The residue was dissolved in 0·1M-HCl (10 ml), diluted with water to 100 ml and adjusted to pH 7 with 0·1M-NaOH. A solution of $K_3Fe(CN)_6$ (35 mg in 7 ml of water) was added during 1 h, the mixture was acidified with acetic acid to pH 4 and applied on a column of Amberlite IRC-50 (15 ml). After washing with 0·25% acetic acid the product was eluted with 50% acetic acid, the eluate was concentrated and freeze-dried. The residue was dissolved in methanol–water (3 : 7; 8 ml) and applied on a column of Partisil ODS (50 × 0·9 cm). Elution with methanol–0·05% trifluoroacetic acid (9 : 11) and lyophilization of the corresponding fractions afforded 9 mg (21%) of the product. R_F 0·17 (S1), 0·10 (S2), 0·09 (S3), 0·58 (S4). $E_{5,7}^{His}$ 0·32, $E_{2,4}^{Gly}$ 0·65. k' 11·6 (methanol–0·05% trifluoroacetic acid, 9 : 11). For $C_{42}H_{64}N_{12}O_{12}S_2.CF_3.COOH.2\,H_2O$ (1 111) calculated: 47·56% C, 6·26% H, 15·12% N; found: 47·13% C, 5·93% H,

14·81% N. $[\alpha]_D$ −24·4° (*c* 0·11; 3M acetic acid). Amino acid analysis: Asp 1·03, Glu 1·07, Gly 1·01, Cys 1·91, Ile 0·99, Tyr 0·98, an amino acid, eluted 6 min before methionine 0·97 (related to the constant of leucine). A compound which did not contain the carboxy-terminal tripeptide was obtained as a side product (12 mg; 28%).

Stability of 1-Aminocyclopropane-1-carboxylic Acid towards Sodium in Liquid Ammonia and Anhydrous Liquid Hydrogen Fluoride

Sodium was added to a solution of 1-aminocyclopropane-1-carboxylic acid (ref.[22]; 2 mg) in liquid ammonia till the blue colouration persisted for 5 min. The excess sodium was destroyed by addition of ammonium chloride and lyophilized. Composition of the lyophilizate was followed by amino acid analysis. In addition to the starting amino acid, a large amount of a compound was found, whose elution time corresponded to that of α-aminobutyric acid or α-aminoisobutyric acid.

A solution of 1-aminocyclopropane-1-carboxylic acid (2 mg) and anisole (0·1 ml) in anhydrous liquid hydrogen fluoride (5 ml) was kept at 20°C for 1 h and evaporated. Amino acid analysis of the residue found only the starting acid.

[7-(1-Aminocyclopropanecarboxylic acid)]oxytocin (*VIIb*)

The protected nonapeptide *VIb* (65 mg) was reduced at 20°C for 1 h with liquid hydrogen fluoride in the presence of anisole (0·5 ml) in an apparatus for work with liquid hydrogen fluoride (Toho Kasei Co., Osaka, Japan). After evaporation of hydrogen fluoride the residue was disolved in acetic acid (2 ml), diluted with water (20 ml) and lyophilized. The lyophilizate was dissolved in water (70 ml), the solution adjusted to pH 3 with 0·1M-HCl and extracted several times with ether. The aqueous layer was degassed, adjusted to pH 7·0 with 0·1M-NaOH and potassium ferricyanide (33 mg) in water (7 ml) was added during 5 min. The pH was kept for 30 min at 7·0 by addition of 0·1M-NaOH and then adjusted to 4 with acetic acid. The solution was applied on a column of Amberlite CG-50I (free acid; 5 ml). After washing with 0·25% acetic acid, the product was eluted with 50% acetic acid. The eluates (30 ml) were freeze-dried, the residue was dissolved in methanol–0·1% trifluoroacetic acid (33 : 67; 12 ml) and applied on a column of Partisil ODS (50 × 0·9 cm). Elution with methanol–0·1% trifluoroacetic acid (40 : 60) afforded 1·52 mg of the lyophylizate, representing the product *VIIb*, pure according to HPLC (*k'* 3·94; methanol–0·1% trifluoroacetic acid 45 : 55). R_F 0·26 (S1), 0·21 (S2), 0·67 (S4). $E_{5,7}^{His}$ 0·30, $E_{2,4}^{Gly}$ 0·75; $[\alpha]_D$ −25·3° (*c* 0·1; 1M acetic acid). For $C_{42}H_{64}N_{12}O_{12}S_2.CF_3COOH.3\,H_2O$ (1 161·2) calculated: 45·51% C, 6·16% H, 14·47% N; found: 45·84% C, 6·32% H, 13·98% N. Amino acid analysis: Asp 1·06, Glu 1·07, Gly 1·00, Cys 1·55, Ile + Acc 2·09, Leu 0·96, Tyr 0·90, Cys(Bzl) 0·00.

Pharmacological Methods

The uterotonic activity was determined on an isolated rat uterus according to Holton[27] in the Munsick's modification[28], the milk-ejecting activity by a modified[30,32] method of Bisset and coworkers[29], and the pressor activity on despinalized rats according to Krejčí[31].

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REFERENCES

1. Walter R.: Fed. Proc., Fed. Amer. Soc. Exp. Biol *36*, 1772 (1977).
2. Walter R. in the book: *Structure-Activity Relationships in Protein and Polypeptide Hormones* (M. Margoulies, F. C. Greenwood, Eds), p. 181. Excerpta Medica, Amsterdam 1971.
3. Walter R., Schlank H.: Endocrinology *89*, 990 (1971).
4. Moore S., Felix A. M., Meienhofer J., Smith C. W., Walter R.: J. Med. Chem. *20*, 495 (1977).
5. Rosamond J. D., Ferger M. F.: J. Med. Chem. *19*, 873 (1976).
6. Barber M., Jones J. J.: Int. J. Peptide Protein Res. *9*, 269 (1977).
7. Bespalova Zh. D., Martynov V. F., Titov M. I.: Zh. Obshch. Khim. *38*, 1684 (1968).
8. Bodanszky M., Bath R. J.: Chem. Commun. *1968*, 766.
9. Walter R., Smith C. W., Roy J., Formento A.: J. Med. Chem. *19*, 822 (1976).
10. Lowbridge J., Manning M., Haldar J., Sawyer W. H.: J. Med. Chem. *20*, 120 (1977).
11. Ferraro J. J., du Vigneaud V.: J. Amer. Chem. Soc. *88*, 3847 (1966).
12. Barber M., Jones J. H., Stachulski A. V., Bisset G. W., Chowdrey H. S., Hudson A. L.: Int. J. Peptide Protein Res. *14*, 247 (1979).
13. Dutta A. S., Anand N., Kar K.: Ind. J. Chem. *4*, 488 (1966).
14. Bespalova Zh. D., Kaurov O. A., Martynov V. F., Natochin Ju V., Titov M. I., Shachmatova E. I.: Vestn. Leningrad. Univ. Fiz. Khim. *22*, 157 (1966).
15. Grzonka Z., Kaszprzykowski F., Lammek B., Gazis D., Schwartz I. L.: *Peptides 1982*; Proc. 17th Eur. Pept. Symp. Prague (K. Bláha, P. Maloň, Eds), p. 445. Walter de Gruyter, Berlin 1983.
16. Freidinger R. M., Perlow D. S., Veber D. F.: J. Org. Chem. *1982*, 104.
17. Freidinger R. M., Veber D. F., Perlow D. S., Brooks J. R., Saperstein R.: Science *210*, 656 (1980).
18. Freidinger R. M.: *Peptides.* Proc. 7th Amer. Pept. Symp. (Rich D. H., Gross E., Eds), p. 673. Pierce Chemical Company, Rockford, Illinois 1981.
19. Procházka Z., Lebl M., Barth T., Hlaváček J., Jošt K.: *Peptides 1982.* Proc. 17th Eur. Pept. Symp. Prague (K. Bláha, P. Maloň, Eds), p. 441. Walter de Gruyter, Berlin 1983.
20. *Biochemical Nomenclature and Related Documents.* International Union of Biochemistry, London 1978.
21. Procházka Z., Jošt K.: This Journal *45*, 1305 (1980).
22. Procházka Z., Budějínský M., Smolíková J., Trška P., Jošt K.: This Journal *47*, 2291 (1982).
23. Hlaváček J., Barth T., Bláha K., Jošt K.: This Journal *44*, 275 (1979).
24. Burroughs L. F.: Nature (London) *179*, 360 (1957).
24. Ressler C.: Proc. Soc. Exp. Biol. Med. *92*, 725 (1956).
26. Zaoral M., Flegel M.: This Journal *37*, 1539 (1972).
27. Holton P.: Brit. J. Pharmacol. *3*, 328 (1948).
28. Munsick R. A.: Endocrinology *66*, 451 (1960).
29. Bisset G. W., Clark B. J., Haldar J., Harris M., Lewis G. P., Rocha e Silva M.: Brit. J. Pharmacol. Chemother. *31*, 537 (1967).
30. Barth T., Jošt K., Rychlík I.: Endocrinol. Exper. *9*, 35 (1974).
31. Krejčí I., Kupková B., Vávra I.: Brit. J. Pharmacol. Chemother. *30*, 497 (1967).
32. Kaurov O. A., Natochin Ju. V., Popernackii O. A., Shachmatova E. I.: Zh. Obshch. Khim. *43*, 2521 (1973).

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