ANALOGUES OF DEAMINO-1-CARBA-OXYTOCIN WITH AN ALIPHATIC AMINO ACID IN POSITION 4: CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITIES*

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Linear octapeptides were prepared by stepwise synthesis in solution. Cyclization by the method of active esters yielded analogues of deamino-1-carba-oxytocin with valine, leucine or isoleucine in position 4 instead of the glutamine residue. The basic pharmacological activities of the analogues were determined; their natriuretic action was lower than that of oxytocin.

The interest in the natriuretic action of oxytocin (*Ia*) increased when it was established that the substitution of glutamine** in position 4 by leucine increased the natriuretic and saluretic activities[2-4]. A number of other analogues was prepared[5-8] in which the amino acids in positions 2 and 4 were replaced by aliphatic amino acids. It was assumed that the enhancement of the lipophilic nature of this part of the oxytocin molecule led to an increase of natriuretic activity accompanied in some cases even by an inhibition of the antidiuretic effect. Furthermore, significantly higher natriuretic activity was observed[9] in the case of carba-analogues of deamino-oxytocin that have one of the sulphur atoms forming the disulphide bond substituted by a methylene group. We tried to combine the two structural changes (*i.e.* the carba-substitution of the disulphide bond and replacement of glutamine in position 4 by an aliphatic amino acid). In this paper we describe the synthesis and basic pharmacological activities of three analogues of deamino-1-carba-oxytocin (*Ib*) that have a valine (*Ic*), isoleucine (*Id*) or leucine (*Ie*) residue in position 4.

We used the protected pentapeptide[10] *II* as the initial material in the synthesis. The amino-protecting *o*-nitrobenzenesulphenyl group[11] was removed by HCl in ether and the free pentapeptide ester was acylated by active esters of the appropriate *o*-nitrobenzenesulphenyl amino acids, thus obtaining protected hexapeptides *IIIa–IIIc*. The amino-protecting group was removed by HCl and the peptides

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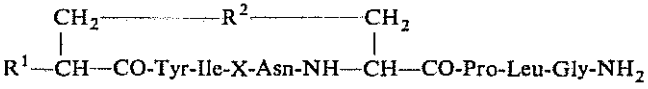
** The amino acids used were of L-configuration. The nomenclature and symbols comply with the published recommendation[1].

were acylated by N-hydroxysuccinimide ester of *o*-nitrobenzenesulphenylisoleucine; protected heptapeptides *IVa–IVc* were thus obtained. The *o*-nitrobenzenesulphenyl group was split off as mentioned above, the methyl ester was subjected to alkaline hydrolysis and free heptapeptides *IVd–IVf* were isolated by means of an ionex. They were acylated by tert-butyloxycarbonyl- (or *o*-nitrobenzenesulphenyl-)-O-tert-butyltyrosine N-hydroxysuccinimide ester and protected octapeptide acids *Va–Vc* were obtained. These compounds were transformed into active esters by the action of bis-(*p*-nitrophenyl) sulphite and, after the removal of the protecting group, subjected to cyclization in pyridine solution. During the last steps of the synthesis, sulphoxides were formed; these were then reduced to sulphides by means of HBr in acetic acid in the presence of acetone[12,13]. The analogues were finally purified by gel filtration on Bio-Gel P–4.



Ia, $R^1 = NH_2$, $R^2 = S—S$, X = Gln
Ib, $R^1 = H$, $R^2 = CH_2S$, X = Gln
Ic, $R^1 = H$, $R^2 = CH_2S$, X = Val
Id, $R^1 = H$, $R^2 = CH_2S$, X = Ile
Ie, $R^1 = H$, $R^2 = CH_2S$, X = Leu
If, $R^1 = H$, $R^2 = CH_2S$, X = Glu

Nps-Asn-Cys($C_3H_6CO_2Me$)-Pro-Leu-Gly-NH_2

II

Nps-X-Asn-Cys($C_3H_6CO_2Me$)-Pro-Leu-Gly-NH_2

IIIa, X = Val, *IIIb*, X = Ile, *IIIc*, X = Leu

R^1-Ile-X-Asn-Cys($C_3H_6CO_2R^2$)-Pro-Leu-Gly-NH_2

IVa, $R^1 = Nps$, $R^2 = CH_3$, X = Val
IVb, $R^1 = Nps$, $R^2 = CH_3$, X = Ile
IVc, $R^1 = Nps$, $R^2 = CH_3$, X = Leu
IVd, $R^1 = H$, $R^2 = H$, X = Val
IVe, $R^1 = H$, $R^2 = H$, X = Ile
IVf, $R^1 = H$, $R^2 = H$, X = Leu

R-Tyr(Bu^t)-Ile-X-Asn-Cys($C_3H_6CO_2H$)-Pro-Leu-Gly-NH_2

Va, R = Boc, X = Val, *Vb*, R = Boc, X = Ile, *Vc*, R = Nps, X = Leu

The biological activities of the analogues prepared are given in Table I. With exception of the galactogogic activity of compound *Id*, all the compounds studied had significantly lower activities in almost all the tests than deamino-1-carba-oxytocin (*Ib*) on the one hand and than the corresponding disulphide analogues[14,15] on the other. The analogues of deamino-monocarba-oxytocin substituted in position 4 (*cf.*[16]) are the first case observed in which the replacement of one sulphur atom by a methylene group combined with the substitution of the α-amino group by hydrogen resulted in a decrease of biological activities as compared with the corresponding compounds containing a disulphide bridge. The natriuretic effect was also much lower than that of oxytocin, regardless of whether the assay was performed on non-anaesthetized rats or on rats of the Brattleboro NDI strain in pentobarbital anaesthesis. Recently, several publications appeared[17–19] that refute the original assumption that an increase of the natriuretic effect can be brought about by introducing an aliphatic amino acid into position 4 (*i.e.* by increasing the lipophility of this part of the molecule). On the contrary, any modifications of this position, even those that leave the carbonyl group of glutamine unchanged, result in a decrease of the natriuretic activity[20].

Therefore, only two structural modifications are so far known to increase the natriuretic action of oxytocin[9,20]: The substitution of the α-amino group of cysteine in position 1 by hydrogen and of one sulphur atom forming the disulphide bond by a methylene group. However, the increase of natriuresis in these cases cannot be classified as specific because it is accompanied by an increase of all the other biological activities.

TABLE I
Biological Activities of Oxytocin Analogues (I.U./mg)

Analogue	Uterotonic (*In vitro*)	Galactogogic	Pressor	Antidiuretic	Natriuretic[a]	
					anaesthetized rat	non-anaesthetized rat
Ib	1 899	604	17·5	24·1	125	193
Ic	2·8	10·9	<0·2	0·003	18	16·5
Id	58·7	295·5	2·8	3·00	45·4	65·4
Ie	0·4	0·5	<0·2	<0·15	33—40	4—7
If[b]	1·0	39·6	<0·2	0·04	—	14

[a] % of the activity of oxytocin; [b] ref.[20].

EXPERIMENTAL

Samples for elemental analysis were dried for 24 h at room temperature and 150 Pa. Melting points were determined on a Kofler block. Thin-layer chromatography was performed either on silica gel sheets (Silufol, Kavalier) or on sheets with Kieselgel G (Merck) in the following systems: 2-butanol-98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4). Electrophoresis was performed in a moist chamber in 1M acetic acid (pH 2·4) and in pyridine–acetate buffer (pH 5·7) at 20 V/cm for 60 min. Detection was performed by means of ninhydrin or the chlorination method. Sulphoxides were detected according to ref.[21]. Reaction mixtures were evaporated on a rotary evaporator (150 Pa, bath temperature 40°C). Counter-current distribution was performed by means of an all-glass Steady State Distribution Machine from Quickfit & Quartz, Stone, Staffordshire, England, with independent shifting of upper and lower phases. The amino acid analyses were carried out on an automatic analyzer (Development Workshops, Czechoslovak Academy of Sciences, Prague; type 6020). When linear and cyclic octapeptides containing isoleucine or valine (compounds *Va*, *Vb*, *Ic*, *Id*) were analysed, 20 h of hydrolysis in 6M-HCl were not sufficient and it was necessary to either prolong hydrolysis to 200 h or use more drastic measures (a mixture of HCl and propionic acid at 160°C, *cf.*[22]). Peptides containing leucine in position 4 (*Vc*, *Ic*) gave good results in amino-acid analysis even when hydrolysis was performed under standard conditions.

o-Nitrobenzenesulphenylvalyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl-prolyl--leucyl-glycinamide (*IIIa*)

To the solution of pentapeptide[10] *II* (2·0 g) in methanol (90 ml), 2·03M-HCl in ether (3·3 ml) was added. The mixture was concentrated and then diluted with ether. The oily substance formed was triturated with ether and the resultant solid matter was filtered off, washed with ether and dried. The pentapeptide-ester hydrochloride was dissolved in dimethylformamide (26 ml), the solution was neutralized with N-ethylpiperidine (pH 8—9, moist pH paper) and *o*-nitrobenzenesulphenylvaline N-hydroxysuccinimide ester[23] (1 g) was added. After 24 h at room temperature, a further portion of active ester was added (0·5 g) and 40 h later the solution was evaporated and the remnant was gradually triturated with light petroleum and ether. The crystalline substance formed was filtered off and washed with water, 0·5M-$NaHCO_3$ and again with water. The yield was 1·86 g (82%) of product, m.p. 210—223°C, which was purified by reprecipitation from dimethylformamide and water (1·80 g, m.p. 230—233°C). R_F 0·65 (S1), 0·60 (S2), 0·57 (S3), 0·77 (S4); $E_{5.7}^{His}$ 0,49· $E_{2.4}^{Gly}$ 0·77 (electrophoresis was performed after the removal of the amino-protecting group); $[\alpha]_D$ —68·8° (*c* 0·3, dimethylformamide). For $C_{36}H_{55}N_9O_{11}S_2$ 853·9) calculated: 50·63% C, 6·49% H, 14·76% N; found: 50·43% C, 6·68% H, 14·53% N. Amino-acid analysis: Asp 1·00, Pro 1·00, Gly 1·00, Val 0·98, Leu 1·02, Cys($C_3H_6CO_2H$) 1·00.

o-Nitrobenzenesulphenylisoleucyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl--prolyl-leucyl-glycinamide (*IIIb*)

The preparation was performed according to the procedure used in the synthesis of compound *IIa*. Crystallization from dimethylformamide and ether and extraction with ethyl acetate yielded 8% of product, m.p. 238—241°C. R_F 0·69 (S1), 0·63 (S2), 0·60 (S3), 0·77 (S4); $E_{5.7}^{His}$ 0·47, $E_{2.4}^{Gly}$ 0·79 after the removal of the amino-protecting group). The sample for analysis was recrystallized from dimethylformamide and water, m.p. 241—243°C; $[\alpha]_D$ —75·1° (*c* 0·4, dimethylformamide).

For $C_{37}H_{57}N_9O_{11}S_2$ (868·0) calculated: 51·20% C, 6·62% H, 14·52% N; found: 51·43% C, 6·83% H, 14·71% N.

o-Nitrobenzenesulohenylleucyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl-prolyl--leucyl-glycinamide (*IIIc*)

As in the case of compound *IIIa*, compound *IIIc* was prepared from compound *II* and *o*-nitrobenzenesulphenylleucine N-hydroxyphthalimide ester. The yield was 80% of product, m.p. 230—232°C. The sample for analysis was crystallized from dimethylformamide and ether, the m.p. did not change. R_F 0·61 (S1), 0·62 (S2), 0·57 (S3), 0·68 (S4); $E_{5.7}^{His}$ 0·54, $E_{2.4}^{Gly}$ 0·74 (electrophoresis was performed after the removal of the amino-protecting group); $[\alpha]_D$ —71·3° (*c* 0·5, dimethylformamide). For $C_{37}H_{57}N_9O_{11}S_2$ (868·0) calculated: 51·20% C, 6·62% H, 14·52% N; found: 50·80% C, 6·49% H, 14·60% N.

o-Nitrobenzenesulphenylleucine N-hydroxyphthalimide Ester

To the solution of *o*-nitrobenzenesulphenylleucine (1·42 g) and N-hydroxyphthalimide (0·81 g) in dimethylformamide (40 ml), cooled to —20°C, dicyclohexylcarbodiimide (1·1 g) was added. The mixture was stirred for 1 h at —20°C, 8 h at 0°C and at room temperature overnight, evaporated, the product was dissolved in ether and undissolved dicyclohexylurea was filtered off. The product was purified by chromatography on a silica gel column (30—60 μ, elution with benzene) and crystallized from a mixture of ethyl acetate and light petroleum. The yield was 1·13 g (53%) of product, m.p. 99—102°C; R_F 0·86 (S1), 0·84 (S3), 0·81 (S4). The sample for analysis was recrystallized in the same way, m.p. 100—101°C; $[\alpha]_D$ —136·5° (*c* 0·3, dimethylformamide). For $C_{20}H_{19}N_3O_6S$ (429·5) calculated: 55·93% C, 4·46% H, 9·79% N; found: 55·39% C, 4·33% H, 9·71% N.

o-Nitrobenzenesulphenylisoleucyl-valyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl--prolyl-leucyl-glycinamide (*IVa*)

To the solution of peptide *IIIa* (0·6 g) in dimethylformamide (5 ml), 2M-HCl in ether (0·84 ml) was added. After 4 min at room temperature, the mixture was diluted with ether, the precipitate was filtered off, washed with ether and dried; $E_{5.7}^{His}$ 0·49, $E_{2.4}^{Gly}$ 0·77. Hydrochloride was dissolved in dimethylformamide (10 ml), pH was adjusted to 8—9 by the addition of N-ethylpiperidine (moist pH paper) and *o*-nitrobenzenesulphenylisoleucine N-hydroxysuccinimide ester[10] (0·3 g) was added to the solution. After 24 h, a further portion of active ester (0·3 g) was added and 42 h later the solution was evaporated and the remnant triturated with light petroleum and ether. The solid product was filtered off and washed with ethyl acetate, ether, water and ether; the yield was 0·63 g of product, m.p. 233—235°C. Recrystallization from dimethylformamide and ether yielded 0·62 g (90%) of product with the same m.p.; R_F 0·67 (S1), 0·56 (S2), 0·62 (S3), 0·85 (S4); $E_{5.7}^{His}$ 0·51, $E_{2.4}^{Gly}$ 0·68 (electrophoresis was performed after the removal of the amino--protecting group by HCl). The sample for analysis was reprecipitated from dimethylformamide and ether, m.p. 233—236°C; $[\alpha]_D$ —56·0° (*c* 0·4, dimethylformamide). For $C_{42}H_{66}N_{10}O_{12}S_2 \cdot 1{\cdot}5\ H_2O$ (994·2) calculated: 50·74% C, 6·99% H, 14·09% N; found: 50·84% C, 6·73% H, 14·00% N.

o-Nitrobenzenesulphenylisoleucyl-isoleucyl-asparaginyl-S-(γ-methoxycarbonylpropyl)-cysteinyl-prolyl-leucyl-glycinamide (*IVb*)

Compound *IVb* was prepared from protected hexapeptide *IIIb* analogously as compound *IVa*. The product was crystallized from a mixture of dimethylformamide, ethyl acetate and ether;

the yield was 94%, m.p. 236—239°C. The sample for analysis was recrystallized in the same way; the m.p. did not change. R_F 0·72 (S1), 0·64 (S2), 0·72 (S3), 0·82 (S4); $E_{5.7}^{His}$ 0·48, $E_{2.4}^{Gly}$ 0·65 (after the removal of the amino-protecting group); $[\alpha]_D$ —53·8 (*c* 0·4, dimethylformamide). For $C_{43}H_{68}N_{10}O_{12}S_2$ (981·2) calculated: 52·64% C, 6·98% H, 14·27% N; found: 52·63% C, 7·02% H, 14·08% N.

o-Nitrobenzenesulphenylisoleucyl-leucyl-asparaginyl-S-(γ-methoxycarbonylpropyl)cysteinyl--prolyl-leucyl-glycinamide (*IVc*)

Peptide *IVc* was prepared from the protected hexapeptide *IIIc* in the same way as compound *IVa*; the yield was 92% and m.p. 220—224°C. After crystallization from a mixture of dimethylformamide and ether, 88% of product was obtained with m.p. 220—222°C. The sample for analysis was recrystallized in the same way; the m.p. did not change. R_F 0·61 (S1), 0·61 (S2), 0·58 (S3), 0·72 (S4); $E_{5.7}^{His}$ 0·51, $E_{2.4}^{Gly}$ 0·71 (electrophoresis was performed after the removal of the amino-protecting group); $[\alpha]_D$ —62·6° (*c* 0·5, dimethylformamide). For $C_{43}H_{68}N_{10}O_{12}S_2$ (981·3) calculated: 52·63% C, 6·99% H, 14·28% N; found: 52·58% C, 7·04% H, 14·30% N.

Isoleucyl-valyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*IVd*)

To the solution of protected heptapeptide *IVa* (0·55 g) in dimethylformamide (17 ml), 2M-HCl in ether (0·69 ml) was added. After 4 min at room temperature, the mixture was diluted with ether, the precipitate was filtered off, washed with ether and dried; $E_{5.7}^{His}$ 0·50, $E_{2.4}^{Gly}$ 0·66. Hydrochloride was dissolved in methanol (25 ml) and 1M-NaOH (2·15 ml) was added to the solution. After 1 h at room temperature, methanol was evaporated, the mixture was diluted with water (3 ml) and applied to a column of Dowex 50 (H^+-cycle, 30 ml). The column was washed with water (until the reaction to chloride ions was negative) and the product was eluted by 10% aqueous pyridine. The eluate was reduced in volume by evaporation and freeze-dried. The lyophilizate (0·42 g) was reprecipitated from methanol and ether; the yield was 0·40 g (88%) of product, m.p. 211—214°C. $E_{5.7}^{His}$ 0·13, $E_{2.4}^{Gly}$ 0·73; R_F 0·24 (S1), 0·02 (S2), 0·48 (S3), 0·66 (S4); $[\alpha]_D$ —56·6° (*c* 0·2, dimethylformamide). For $C_{35}H_{61}N_9O_{10}S.H_2O$ (818·0) calculated: 51·39% C, 7·76% H, 15·41% N; found: 51·68% C, 7·54% H, 15·03% N.

Isoleucyl-isoleucyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*IVe*)

The protecting groups were removed from compound *IVb* as described in the preparation of compound *IVd*; the yield was 47%. $E_{5.7}^{His}$ 0·14, $E_{2.4}^{Gly}$ 0·70; R_F 0·26 (S1), 0·02 (S2), 0·65 (S3), 0·67 (S4). The sample for analysis was recrystallized from 90% aqueous methanol and ether; m.p. 200 to 204°C; $[\alpha]_D$ —58·5° (*c* 0·2, dimethylformamide). For $C_{36}H_{63}N_9O_{10}S.H_2O$ (832·0) calculated: 51·97% C, 7·87% H, 15·15% N; found: 52·04% C, 7·80% H, 15·09% N.

Isoleucyl-leucyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*IVf*)

Free heptapeptide *IVf* was obtained from compound *IVc* by the same procedure that was used for preparing compound *IVd*; the yield was 96%. The sample for analysis was reprecipitated from 90% aqueous methanol and ether, m.p. 176—180°C (decomposition); $[\alpha]_D$ —60·2°(*c* 0·06, dimethylformamide); $E_{5.7}^{His}$ 0·14, $E_{2.4}^{Gly}$ 0·63. For $C_{36}H_{63}N_9O_{10}S.2\,H_2O$ (850·0) calculated: 50·87% C, 7·95% H, 14·83% N; found: 51·13% C, 7·66% H, 14·45% N.

Tert-Butyloxycarbonyl-O-tert-butyltyrosyl-isoleucyl-valyl-asparaginyl-S-(γ-carboxypropyl)-cysteinyl-prolyl-leucyl-glycinamide (*Va*)

To the suspension of *IVd* (0·37 g) in dimethylformamide (45 ml), N-ethylpiperidine (63 μl) and tert-butyloxycarbonyl-O-tert-butyltyrosine N-hydroxysuccinimide ester[10] (0·46 g) were added. After 72 h of mixing at room temperature the solution was evaporated, the residue triturated with light petroleum and ether, the solid part was filtered off and washed with ether, water, once again with ether and dried; the yield was 0·51 g (98%) of product with m.p. 234—236°C; R_F 0·67 (S1), 0·68 (S2), 0·79 (S3), 0·82 (S4); $E_{5.7}^{His}$ 0·15, $E_{2.4}^{Gly}$ 0·46 (electrophoresis was performed after the removal of the amino-protecting group by trifluoroacetic acid). The sample for analysis was crystallized from dimethylformamide–ether without change in the m.p. $[\alpha]_D$ —40·0° (*c* 0·1, dimethylformamide). Amino acid analysis (200 h, 6M-HCl): Asp 0·99, Pro 1·01, Gly 1·05, Val 0·96, Ile 1·04, Leu 1·01, Tyr 0·91, Cys($C_3H_6CO_2H$) 1·05. For $C_{53}H_{86}N_{10}O_{14}S.1·5\,H_2O$ (1146) calculated: 55·52% C, 7·82% H, 12·21% N; found: 55·50% C, 7·51% H, 12·35% N.

Tert-Butyloxycarbonyl-O-tert-butyltyrosyl-isoleucyl-isoleucyl-asparaginyl-S-(γ-carboxypropyl)-cysteinyl-prolyl-leucyl-glycinamide (*Vb*)

The procedure was analogous to that used in the case of compound *Va*. The yield of product *Vb* was 98%, m.p. 235—237°C; R_F 0·68 (S1), 0·68 (S2), 0·80 (S3), 0·81 (S4); $E_{5.7}^{His}$ 0·19, $E_{2.4}^{Gly}$ 0·50 (the sample used for electrophoresis was subjected to treatment with trifluoroacetic acid for 1 h). The sample for analysis was recrystallized from methanol with ether, m.p. 236—239°C; $[\alpha]_D$ —44·2° (*c* 0·2, dimethylformamide). Amino acid analysis (6M-HCl, 20 h 105°C; the values in brackets refer to results obtained after hydrolysis in a mixture of propionic acid and HCl for 3 h at 160°C (*cf.*[22]), in both cases in the presence of phenol): Asp 0·99 (1·01), Pro 0·96 (1·00), Gly 1·03 (1·04), Ile 1·47 (1·99), Leu 1·00 (1·01), Tyr 1·05 (0·90), Cys ($C_3H_6CO_2H$) 0·97 (1·04). For $C_{54}H_{88}$·$.N_{10}O_{14}S.H_2O$ (1151) calculated: 56·33% C, 7·87% H, 12·16% N; found: 56·37% C, 7·69% H, 12·24% N.

o-Nitrobenzenesulphenyl-O-tert-butyltyrosyl-isoleucyl-leucyl-asparaginyl-S-(γ-carboxypropyl)-cysteinyl-prolyl-leucyl-glycinamide (*Vc*)

Octapeptide *Vc* was obtained from free heptapeptide *IVf* in a similar way as described for compound *Va*. The yield was 92%, m.p. 210—213°C (after crystallization from aqueous dimethylformamide); R_F 0·69 (S1), 0·16 (S2), 0·67 (S3), 0·68 (S4); $E_{5.7}^{His}$ 0·35, $E_{2.4}^{Gly}$ 0·63 (electrophoresis was performed after the removal of the amino-protecting group by HCl); the sample for analysis was recrystallized in the same way, m.p. 216—218°C; $[\alpha]_D$ —2·8° (*c* 0·2, dimethylformamide). Amino acid analysis: Asp 1·05, Pro 0·96, Gly 1·00, Ile 1·00, Leu 2·05, Tyr 1·00, Cys($C_3H_6CO_2H$) 0·81. For $C_{55}H_{83}N_{11}O_{14}S_2.H_2O$ (1204) calculated: 54·86% C, 7·12% H, 12·80% N; found: 54·53% C, 6·95% H, 12·70% N.

Lactam of Tyrosyl-isoleucyl-valyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl--glycinamide (*Ic*)

The preparation of the active ester and cyclization were performed under nitrogen. To a solution of protected octapeptide *Va* (250 mg) in dimethylformamide (10 ml) and pyridine (10 ml), bis(*p*-nitrophenyl) sulphite[24] (0·8 g) was added. After 7 h of stirring at room temperature, a further portion of reagent (0·8 g) was added and 15 h later, 0·4 g of sulphite was added. After further 5 h, the solution was evaporated, the residue was triturated with ether, the crystalline residue was filtered off and washed with ether and water. The active ester was dried (0·22 g), dissolved

in trifluoroacetic acid (7 ml); after 1 h at room temperature the solution was diluted with toluene (7 ml) and evaporated. The remnant was dissolved in dimethylformamide (10 ml) and added at a rate of 1·25 ml/h to 220 ml of pyridine while stirring and heating it to 50°C. The mixture was left for 12 h at room temperature. After evaporation, the residue was triturated with ether, filtered off and washed with ether; the yield was 174 mg. The product was purified by counter-current distribution in a system 2-butanol–0·05% aqueous acetic acid (1 : 1); 120 transfers of the upper and 237 transfers of the lower phase were performed. Two peaks with $K = 4·5$ and 8·5 were localized by means of the Folin–Ciocalteau reagent. The peak with the higher distribution coefficient was concentrated and freeze-dried (the yield was 48 mg); part of the material was subjected to further purification by gel filtration on a column of Bio-Gel P—4 (100 × 1 cm; 3M acetic acid). The product was detected by OD_{280} and freeze-dried. R_F 0·59 (S1), 0·50 (S2), 0·61 (S3), 0·75 (S4); $[\alpha]_{250}$ +659° (*c* 0·05, water). Amino acid analysis (6M-HCl, 200 h): Asp 0·98, Pro 1·00, Gly 1·04, Val 1·01, Ile 1·01, Leu 0·96, Tyr 1·01, Cys($C_3H_6CO_2H$) 0·98. For $C_{44}H_{68}N_{10}O_{11}S.2\,H_2O$ (981·1) calculated: 53·86% C, 7·39% H, 14·27% N; found: 54·13% C, 6·95% H, 14·00% N.

The peak with the distribution coefficient 4·5 was concentrated, freeze-dried and purified on Bio-Gel P—4; R_F 0·27 (S1), 0·24 (S2), 0·32 (S3), 0·65 (S4); $[\alpha]_{250}$ +686° (*c* 0·05, water). The test for sulphoxides[21] was positive; vibrations at 1 020 cm^{-1} were observed in the IR spectrum.

Lactam of Tyrosyl-isoleucyl-isoleucyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*Id*)

The preparation of the active ester from protected octapeptide *Vb* and cyclization were performed according to the method described for the preparation of compound *Ic*. Counter-current distribution resulted in two peaks with $K = 5·6$ and 10. The compound with the higher distribution coefficient was not chromatographically homogeneous and had a positive reaction in the test for sulphoxides. A part of the product (11·7 mg) was suspended in acetone (0·25 ml) and a solution of HBr in acetic acid (0·13 ml) was then added to the suspension at 0°C. After 5 min the mixture was freeze-dried, acetone was added to the residue and the solution was evaporated. The remnant was dissolved in 3M acetic acid and filtered through a column of Bio-Gel P—4. After freeze-drying, 8·9 mg of product were obtained with a negative reaction in the test for sulphoxide presence; R_F 0·54 (S1), 0·48 (S2), 0·60 (S3), 0·65 (S4); $[\alpha]_D$ −57·0° (*c* 0·1, 3M acetic acid). Amino acid analysis (6M-HCl, 200 h); Asp 1·05, Pro 0·97, Gly 1·00, Ile 2·06, Leu 1·04, Tyr 1·00, Cys($C_3H_6CO_2H$) 0·94. For $C_{45}H_{70}N_{10}O_{11}S.1·5\,H_2O$ (986·2) calculated: 54·81% C, 7·46% H, 14·20% N; found: 54·83% C, 7·25% H, 14·52% N. The peak with the distribution coefficient of 5·6 was found to have characteristics of a sulphoxide of compound *Id*; R_F 0·39 (S1), 0·36 (S2), 0·44 (S3), 0·65 (S4).

Lactam of Tyrosyl-isoleucyl-leucyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*Ie*)

To a solution of protected octapeptide *Vc* (300 mg) in a mixture of dimethylformamide (10 ml) and pyridine (10 ml), bis(*p*-nitrophenyl) sulphite (1 g) was added while stirring and bubbling through with nitrogen. After 12 h, 1 g more of reagent and 10 ml of pyridine were added and 12 h later another portion of reagent (0·5 g) was added. After 16 h, the solution was evaporated, the residue was triturated with ether, the solid substance was filtered off and washed with ether and water. The active ester was dried and then dissolved in dimethylformamide (4 ml), 2M-HCl in ether (0·3 ml) was added to the solution and after 3 min, the solution was diluted with ether (100 ml).

The precipitate was filtered off, washed with ether and dissolved in dimethylformamide (10 ml). This solution was added to pyridine (300 ml) in the course of 3 h while stirring, heating to 50°C and bubbling with nitrogen. After 12 h at room temperature, the solution was evaporated, the residue was triturated with ether and dissolved in trifluoroacetic acid (15 ml). After 45 min at room temperature the solution was diluted with toluene (20 ml) and evaporated. Further purification and reduction was performed by the same method as used for the preparation of compounds *Ic* and *Id*. The only difference was that during counter-current distribution another solvent system was used, namely 1-butanol–benzene–water (containing 0·5% acetic acid and 0·1% pyridine) (3 : 2 : 5); $K = 0{\cdot}08$ (300 transfers of the upper phase). The test for sulphoxides gave negative results. R_F 0·57 (S1), 0·20 (S2), 0·57 (S3), 0·80 (S4); $[\alpha]_D$ —83·3° (*c* 0·04, 1M acetic acid). Amino acid analysis (6M-HCl, 20 h): Asp 1·01, Pro 0·97, Gly 0·92, Ile 0·96, Leu 2·10, Tyr 1·02, Cys($C_3H_6CO_2H$) 0·84. For $C_{45}H_{70}N_{10}O_{11}S.2\,H_2O$ (995·2) calculated: 54·31% C, 7·49% H, 14·07% N; found: 54·53% C, 7·28% H, 13·43% N.

Pharmacological Methods

The uterotonic activity was assayed on the isolated rat uterus[25,26]. Galactogogic activity[27,28] was determined on anaesthetized lactating rats (9—15 days of lactation). Antidiuretic activity was determined using anaesthetized male rats under 6—8% water load[29,30]. The pressor activity was assayed on pithed male rats[31]. Natriuretic activity was determined both on non-anaesthetized male rats under a mild (4%) water load and on adult male rats of the Brattleboro NDI strain under pentobarbital anaesthesis and 4—6% water load according to ref.[32,33].

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