AMINO ACIDS AND PEPTIDES. C.*

THE EFFECT OF THE PRESENCE OF SULPHUR ATOMS ON THE BIOLOGICAL ACTIVITY OF OXYTOCIN; SYNTHESIS OF DEAMINO-CARBA^6-OXYTOCIN AND DEAMINO-DICARBA-OXYTOCIN

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The synthesis is described of two analogues of deaminooxytocin in which the disulphide linkage has been modified. In one analogue, both sulphur atoms have been replaced by methylene groups, in the other analogue only one sulphur atom of cysteine in position six. Both of these analogues showed typical oxytocin-like activity, and the mono-carba compound was highly active.

Of the two possible functions of the disulphide linkage in the oxytocin molecule – functional and steric – the former has been excluded by the synthesis of oxytocin (*Ia*) analogues, cyclic in molecular arrangement but without a disulphide bond, and the finding that such compounds had biological activity[1,2]. Similar results were later reported for other forms of the neurohypophysical hormones, lysine-vasopressin[3] and arginine-vasopressin[4], as well as with similar analogues of vasotocin[5].** (All of the above analogues with an altered disulphide linkage were, mainly for reasons of simplified synthesis, derived from the de-amino form of the corresponding hormone). In addition, this type of substitution has also been applied to the intrachain bridge in insulin, and it was found that even in this case (despite the fact that "carba"-insulin was not obtained in pure and crystalline form) an insulin-like activity was retained[8]. The functional insignificance of the disulphide linkage is therefore not only valid for

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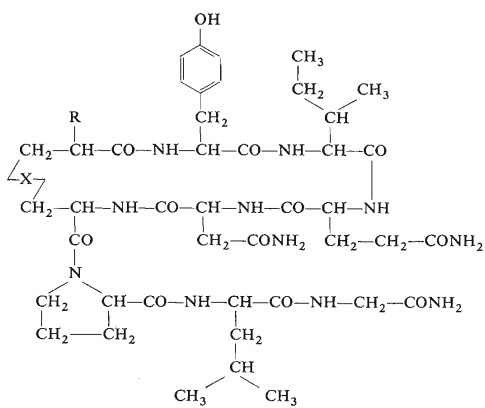
** All amino acids used in this work were L-configuration. Terminology and symbols were according to published suggestions[6]. HCys indicates a residue of homocysteine and Asub a residue of α-aminosuberic acid. The lables attached to analogues, *Ib*, *Ic* and *Id* (lactams of the corresponding linear peptides) would be too long for the purposes of discussion. According to the suggestion of du Vigneaud[7] it would be possible to lable substance *Ib* α-deamino-cystathionine-oxytocin and *Id* β-deamino-cystathionine-oxytocin. In the present work we have kept to the term already used in previous publications, "carba-analogues", *i.e.* deamino-carba^1-oxytocin for substance *Ib*, deamino-carba^6-oxytocin for substance *Id* and deamino-dicarba-oxytocin for substance *Ic*.

the oxytocin molecule but would appear to have a more general validity, certainly for the entire group of neurohypophysial hormones. On the other hand, the disulphide linkage is of critical importance in maintaining cyclic structure, as shown by the high activity of the carba analogues in comparison with the practically null activity of the corresponding straight chain (non-cyclic) compounds (*cf*[2]).

In previous work[2] it was determined that marked differences in biological activities exist with these analogues: deamino-carba[1]-oxytocin (*Ib*) was more than one order of ten more active than the analogue in which both sulphur atoms had been replaced by methylene groups (deamino-dicarba-oxytocin, *Ic*). The compounds described by Jošt and Rudinger[2] were not chemically pure, but practically the same differences are now reported with pure substances. There are two possible explanations for this latter fact: either the polar nature of the sulphur atom contributes to biological activity, or the steric arrangement of the molecule *Ic* is so disturbed as to decrease biological activity. A further open question is the equivalence of the two sulphur



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

HCys($C_2H_4CO_2Me$)
II

Z-HCys($C_2H_4CO_2Me$)-OH
III

Z-HCys($C_2H_4CO_2Me$)-Pro-Leu-Gly-NH_2
IV

Z-Asub(OMe)-OH
V

atoms – *i.e.* the effect is the same when either one or the other sulphur is replaced by a single methylene group. In the present communication we describe the synthesis and some biological characteristics of deamino-carba[6]-oxytocin *Id*, an isomer of the previously[1,2,9] prepared deamino-carba[1]-oxytocin (*Ib*). For comparison we also prepared deamino[1]-dicarba-oxytocin (*Ic*) previously prepared by a different method[2,10].

The synthesis of both of these analogues holds to the general arrangement previously worked out for deamino-carba[1]-oxytocin[9] (*Ib*). The initial homocystine[11] was reduced with sodium in liquid ammonia and in aqueous solution was alkylated with methyl acrylate. The resulting S-β-methoxycarbonylethylhomocysteine (*II*) was transformed into the benzyloxycarbonyl derivative *III* with benzyloxycarbonyl chloride, or even better with sodium benzyloxycarbonyl thiosulphate[12]. After transforming to 5-chloro-8-hydroxyquinoline ester (see[13]) the substance was combined with the amide of prolyl-leucyl-glycine[14,15]. From the resulting protected tetrapeptide *IV* the benzyloxycarbonyl protecting group was removed with hydrogen bromide in acetic acid and the ester of the tetrapeptide-amide was gradually acylated with the 2,4,5-trichlorophenyl esters of *o*-nitrobenzenesulphenyl-asparagine[16] and -glutamine[16]. From protected penta-(*VIa*) and hexa-peptides (*VIIa*) the *o*-nitrobenzenesulphenyl group was split off with hydrogen chloride in ether[17] and the hexapeptide with a free amino group was then acylated with the N-hydroxysuccinimide-ester of *o*-nitrobenzenesulphenylisoleucine[9]. The amino protecting group was removed from heptapeptide *VIIIa* again with hydrogen chloride in ether and after

TABLE I

Biological Activity (International Units/mg)

Substance	Rat uterus		Vasodepressor	Mammary gland	Antidiuretic	Pressor
	isolated	*in situ*		*in vitro*		
Oxytocin	486[a]	450[b] (cat)	507[a]	533[c]	2·7[a]	3·1[a]
Deamino-oxytocin	803[d]	900[e]	975[d]	—	19[d]	1·44[d]
Deamino-carba[1]-oxytocin (*Ib*)	1 898	1 251	1127	562	3·75	0·017
Deamino-carba[6]-oxytocin (*Id*)	929	2 792	571	756	115	1·5
Deamino-dicarba-oxytocin (*Ic*)	93 (96[f])	95	25 (52[f])	374	3·2 (1·78[f])	(0·25[f])

[a] Ref. 21, [b] ref. 23, [c] ref. 25, [d] ref. 22, [e] ref. 24, [f] ref. 10.

alkaline hydrolysis we isolated the free heptapeptide amide *IXa*. Acylation with the N-hydroxysuccinimid ester of tert-butyloxycarbonyl-O-tert-butyltyrosine[9] gave the octapeptide acid *Xa*, which was transformed into the active ester using bis-*p*-nitrophenyl sulphite[18] and after removing the tert-butyl protecting groups with trifluoroacetic acid the molecule was cyclised in pyridine solution. Cyclic analogue *Id* was isolated using counter-current distribution and characterised by elemental analysis, amino acid analysis, paper electrophoresis and thin-layer chromatography.

In a similar manner we prepared deamino-dicarba-oxytocin (*Ic*). Even in this case, as with substance *III*, it proved advantageous to introduce the benzyloxycarbonyl protecting group into the molecule of the β-methyl ester of α-aminosuberic acid by means of sodium benzyloxycarbonyl thiosulphate[12]. We can confirm published reports[19] that introduction of benzyloxycarbonyl protecting groups into ω-esters of dicarboxylic acids with a long side chain proceeds with a low yield and poor reproducibility. In all tested cases – with only one exception[20] – the use of sodium benzyloxycarbonyl thiosulphate[12] removed these difficulties, increased yields and the purity of the product and reproducibility. The resulting ω-methylester of benzyloxycarbonyl-α-aminosuberic acid (*V*) was condensed with the amide of prolyl-leucyl-glycine using dicyclohexylcarbodiimide, and the non-crystalline protected tetrapeptide was not isolated, but after removal of the benzyloxycarbonyl group was used for the next synthetic step. By stepwise addition of protected amino acids as active esters and going through compounds *VIb*, *VIIb*, *VIIIb*, and *IXb* we achieved protected octapeptide *Xb*, which was transformed into its active ester, the protecting groups were split off and the molecule was cyclised in the same manner as with substance *Id*.

In Table I biological activities of the two analogues are shown along with data for deamino-carba[1]-oxytocin (ref.[9]) in comparison to reference molecules oxytocin and deamino-oxytocin. *In vitro* uterotonic activity[26], vasodepressor activity[27], action

Nps-Asn-X(OMe)-Pro-Leu-Gly-NH_2

VI

Nps-Gln-Asn-X(OMe)-Pro-Leu-Gly-NH_2

VII

Nps-Ile-Gln-Asn-X(OMe)-Pro-Leu-Gly-NH_2

VIII

H-Ile-Gln-Asn-X(OH)-Pro-Leu-Gly-NH_2

IX

BOC-Tyr(Bu^t)-Ile-Gln-Asn-X(OH)-Pro-Leu-Gly-NH_2

X

a: X = HCys(C_2H_4CO) *b*: X = Asub

on the isolated mammary gland[25] and action on blood pressure in the rat[28] were studied by I. Krejčí of the Research Institute for Pharmacy and Biochemistry, and antidiuretic activity[29] and the action on the rat uterus *in situ*[30] by T. Barth of this Institute. The detailed results of these studies will be published separately.

The biological activities of analogues *Ib* and *Id* (both mono-carba) were in most cases higher than those shown by oxytocin itself and comparable with those of deamino-oxytocin (which in the present case can be considered as the "mother substance" of the analogues under discussion). Of particular interest is the very high antidiuretic activity of deamino-carba^6-oxytocin, which exceeds by an order of ten the activity of any other known oxytocin analogue. The activities of deamino-dicarba-oxytocin (*Ic*) are very close to those values reported by Japanese investigators[10] and in practically all cases were far lower than values measured for analogues *Ib* and *Id*. In general one might state for the two monocarba-analogues that in *in vivo* tests deamino-carba^6-oxytocin showed the higher activity (compound *Ic*), whereas deamino-carba^1-oxytocin (*Ib*) was more active on isolated tissues. In both cases, however, it is clear that substitution of one or the other sulphur atoms not only did not result in a decrease in activity, but even a slight increase in oxytocin activity in comparison with deamino-oxytocin. For the time being it cannot be decided whether the high oxytocin-like activities of substances *Ib* and *Id* are due to resistance to enzymatic degradation or whether their steric arrangement (we can assume a difference from oxytocin in this regard) favours interaction with the receptor. Detailed biochemical and physical chemical studies of these analogues will be required to that end.

EXPERIMENTAL

Preparation of analytical samples, chromatographic and electrophoretic analyses, amino acid analysis and processing of reaction mixtures were all the same as presented in the introduction of the experimental section of the accompanying article[9].

S-β-Methoxycarbonylethylhomocysteine (*II*)

Homocystine[11] (7·2 g; $[\alpha]_D > 76°$ (*c* 1, 1M-HCl)) was reduced with sodium in liquid ammonia. After evaporation of the ammonia the remainder was dissolved in water (120 ml), pH was adjusted to 8·1 with stirring and bubbling through of nitrogen, and methyl acrylate (12 ml) was added, After 24 h the reaction mixture was filtered through a column of Dowex 50 (H^+ cycle), the column was then washed through with water and the product was eluted with 10% pyridine. The yield was 7·5 g (75%), m.p. 230—233°C (Kofler). $E_{S\cdot7}^{Gly}$ 1·0, $E_{2\cdot4}^{Gly}$ 0·57; R_F 0·12 (S_1), 0·50 (S_2), $[\alpha]_D$ −6·0° (*c* 0·20, water). The literature[31] gives an m.p. of 230—231·5°C, $[\alpha]_D$ −1·63° (1M-HCl) for a product prepared in practically the same manner with a yield of 82%.

S-β-Carboxyethylhomocysteine

A solution of substance *II* (1·1 g) in 1M-NaOH (15 ml) was stirred at room temperature for 2 h and filtered through a column of Dowex 50 (H^+ cycle). The product was eluted with 10% pyridine, the eluate was evaporated, the resulting powder was mixed and ground with ether and then

filtered. The yield was 1·0 g (97%, m.p. 240—243°C (Kofler); $E_{5.7}^{Glu}$ 0·81, $E_{2.4}^{Gly}$ 0·54; R_F 0·48 (S_1), 0·30 (S_3). The product was dissolved in a mixture of hot acetic acid (100 ml) and water (6 ml) and diluted with ether. The crystals which formed were filtered and washed with ether. The yield was 0·9 g (87%), m.p. 242—245°C (Kofler). The sample for analysis was recrystallised in the same manner, with no change in m.p.; $[\alpha]_D$ +16·9° (*c* 0·20, 1M-HCl); —6·4° (*c* 0·20, water). For $C_7H_{13}NO_4S$ (207·3) calculated: 40·56% C, 6·32% H, 6·76% N; found: 40·56% C, 6·32% H, 6·93% N. The literature[32] gives an m.p. of 245—247°C and $[\alpha]_D$ +23·1 (*c* 1, 1M-HCl) for a product prepared by 24 hrs. boiling of methionine and chloroacetic acid in concentrated hydrochloric acid, with a yield of 73%.

The Dicyclohexylammonium Salt of Benzyloxycarbonyl-S-β-methoxycarbonylethylhomocysteine (*III*)

a) *By means of benzyloxycarbonyl chloride*: To a solution of monomethyl ester *II* (1·57 g) in 0·5M-$NaHCO_3$ (27·5 ml) we added over 10 min of stirring and cooling with ice benzyloxycarbonyl chloride (2·21 ml). The pH of the reaction mixture was maintained at 7·5 by addition of 0·5M-$NaHCO_3$ with stirring and cooling (20 min) and was then left for 1 h at room temp. The reaction mixture was then extracted with ether, acidified with 6M-HCl to pH 3, the separated product was taken up by shaking with ether, the latter solution was then shaken with water, dried with sodium sulphate and evaporated. The concentrate was dried azeotropically (benzene), dissolved in benzene and dicyclohexylamine (1·25 ml) and light petroleum were added. After cooling to 0°C crystals separated out and were filtered and washed with light petroleum. Crystallisation from ethyl acetate gave a yield of 2·07 g of product (54%), m.p. 152—154°C (Kofler). The sample for analysis was recrystallised from 90% ethanol, m.p. 156—158°C (Kofler); $[\alpha]_D$ +5·6° (*c* 0·5, methanol). For $C_{28}H_{44}N_2O_6S$ (536·7) calculated: 62·66% C, 8·26% H, 5·22% N; found: 62·84% C, 8·24% H, 5·20% N.

b) *By means of Bunte salt*[12]: Monoester *II* (0·66 g) and sodium benzyloxycarbonyl thiosulphate (0·81 g) were dissolved in 0·5M-$NaHCO_3$ (40 ml). The reaction mixture was stirred at room temp. and pH was maintained at 8·0—8·5 by addition of 1M-NaOH (total added 3·9 ml). After 2 h the pH of the reaction mixture was adjusted to 3 with hydrochloric acid, the product was taken up by shaking into ether, the latter solution was shaken with water, dried with sodium sulphate and evaporated. The concentrate was dried azeotropically (benzene), dissolved in benzene, and dicyclohexylamine (0·59 ml) and light petroleum were added. After cooling to 0°C the separated product was filtered and washed with light petroleum. The yield was 1·40 g (84%), m.p. 150—152°C (Kofler) with no difference from the sample prepared under *a*) above.

The 5-Chloro-8-quinolyl Ester of Benzyloxycarbonyl-S-β-methoxycarbonylethylhomocysteine

To a solution of the dicyclohexylammonium salt of benzyloxycarbonyl-S-β-methoxycarbonylethylhomocysteine (*III*) (4·1 g) and the hydrochloride of 5-chloro-8-hydroxyquinoline (1·7 g) in a mixture of chloroform (180 ml) and dimethylformamide (5 ml) at —20°C we added dicyclohexylcarbodiimide (1·6 g). The reaction mixture was stirred at —10°C for 1 h, left at room temp. for 36 hrs and then concentrated to half its original volume. The dicyclohexylurea which separated out was filtered and the filtrate evaporated to dryness. The dry powder was dissolved in ethyl acetate and this solution was consecutively shaken with water, 1M-HCl, water, 0·5M-$NaHCO_3$ and water, dried with sodium sulphate and evaporated to dryness. The yield was 3·0 g (76%), m.p. 98—100°C (capillary). The sample for analysis was crystallised from ethyl acetate and showed a m.p. of 101—102°C (capillary); $[\alpha]_D$ —31·3° (*c* 0·51, dimethylformamide). For $C_{25}H_{25}ClN_2O_6S$ (517·0) calculated: 58·08% C, 4·87% H, 5·42% N, 6·86% Cl; found: 58·26% C, 5·01% H, 5·65% N, 6·74% Cl.

The Amide of Benzyloxycarbonyl-S-β-methoxycarbonylethylhomocysteinyl-prolyl-leucyl-glycine (*IV*)

To a solution of the amide of prolyl-leucyl-glycine[14,15] (1·75 g) in dimethylformamide (18 ml) we added the 5-chloro-8-quinolyl ester of benzyloxycarbonyl-S-β-methoxycarbonylethylhomocysteine (3·0 g). After two days standing at room temperature, the reaction mixture was evaporated to dryness, the powder was ground several times with 1M-HCl and water, and the final crystalline product was filtered and washed with water. The yield was 2·85 g (79%), m.p. 129—132°C (capillary). The sample for analysis was crystallised from a mixture of ethyl acetate and light petroleum and showed a m.p. of 135—136°C (capillary); $[\alpha]_D$ −49·3° (*c* 0·5, dimethylformamide). For $C_{29}H_{43}N_5O_8S$ (621·4) calculated: 56·02% C, 6·97% H, 11·27% N; found: 55·96% C, 6·99% H, 11·24% N.

The Amide of *o*-Nitrobenzenesulphenylasparaginyl-S-β-methoxycarbonylethylhomocysteinyl-prolyl-leucyl-glycine (*VIa*)

To a solution of protected tetrapeptide *IV* (3·0 g) in acetic acid (10 ml) we added a 35% solution of HBr in acetic acid (30 ml). After 10 min standing at room temp. the reaction mixture was diluted with ether and the hydrobromide which separated out was filtered and washed with ether. $E_{5.7}^{His}$ 0·60, $E_{2.4}^{Gly}$ 0·93. After drying in an exsiccator over NaOH the tetrapeptide hydrobromide was dissolved in dimethylformamide (25 ml), the solution was neutralised with N-ethylpiperidine (2 ml) and we added the 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulphenylasparagine[16] (2·4 g). After 24 h at room temperature we added further active ester (1 g) to the reaction mixture and after a further 24 h standing the mixture was evaporated to dryness, the dry powder was ground with light petroleum and ether, the crystalline product was filtered and washed consecutively with ether, water, 0·5M-$NaHCO_3$ and water. The yield was 3·36 g (93%) m.p. 171—173°C (capillary). The sample for analysis was crystallised from a mixture of dimethylformamide and water with no change in m.p.; $[\alpha]_D$ −70·6° (*c* 0·51, dimethylformamide). For $C_{31}H_{46}N_8O_{10}S_2$ (754·8) calculated: 49·33% C, 6·14% H, 14·85% N; found: 49·30% C, 6·22% H, 15·05% N.

The Amide of *o*-Nitrobenzenesulphenylglutaminyl-asparaginyl-S-β-methoxycarbonylethyl-homocysteinyl-prolyl-leucyl-glycine (*VIIa*)

To a solution of protected pentapeptide *VIa* (3·1 g) in dimethylformamide (15 ml) we added 2·2M-HCl in ether (4·6 ml). After 5 min standing at room temperature the mixture was diluted with ether (200 ml) and the oil which separated out was ground with ether, the crystalline portion was filtered, washed with ether and dried. $E_{5.7}^{His}$ 0·55, $E_{2.4}^{Gly}$ 0·82; R_F 0·35 (S_1), 0·25 (S_2) and 0·37 (S_4). The pentapeptide hydrochloride was dissolved in dimethylformamide (40 ml) and N-ethylpiperidine (0·7 ml) was added along with the 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulphenylglutamine[16] (2·5 g). After 24 h standing at room temperature we added a further 1 g of the active ester and after a further 24 h standing the reaction mixture was evaporated to dryness, the dry powder was ground with light petroleum and ether, the crystalline portion was filtered and washed with ether, water, 0·5M-$NaHCO_3$ and then water (3·5 g, m.p. 145—150°C). Crystallisation from a mixture of dimethylformamide and ether gave a yield of 2·4 g (66%), m.p. 157—160°C (capillary). The sample for analysis was recrystallised in the same manner with no change in m.p.; $[\alpha]_D$ −52·1° (*c* 0·50, dimethylformamide). For $C_{36}H_{54}N_{10}O_{12}S_2 . 1\,H_2O$ (900·9) calculated: 48·00% C, 6·27% H, 15·55% N; found: 48·16% C, 6·15% H, 15·54% N.

The Amide of *o*-Nitrobenzenesulphenylisoleucyl-glutaminyl-asparaginyl-S-β-methoxycarbonyl-ethylhomocysteinyl-prolyl-leucyl-glycine (*VIIIa*)

To a solution of protected hexapeptide *VIIa* (2·05 g) in dimethylformamide (17 ml) we added 2·2M-HCl in ether. The reaction mixture was left standing 4 min at room temperature and was then diluted with ether (250 ml); the crystals which separated out were filtered, washed with ether and dried. $E_{5.7}^{His}$ 0·33, $E_{2.4}^{Gly}$ 0·67; R_F 0·18 (S_1), 0·13 (S_2). The hexapeptide hydrochloride was dissolved in dimethylformamide (35 ml) and to this solution was added N-ethylpiperidine (0·5 ml) and the N-hydroxysuccinimid ester of *o*-nitrobenzenesulphenylisoleucine[9] (1·25 g). After 24 h at room temperature a further 0·6 g of the active ester were added and after a further 24 h standing the mixture was evaporated to dryness, the dry powder was consecutively ground with light petroleum and ether, the crystalline portion was filtered and washed with ether, water, 0·5M-$NaHCO_3$ and again water. Crystallisation from a mixture of dimethylformamide and ether gave a yield of 1·90 g (85%), m.p. 230—233°C (capillary). The sample for analysis was recrystallised in the same manner with no change in m.p.; $[\alpha]_D$ —61·8° (*c* 0·51, dimethylformamide). For $C_{42}H_{65}.N_{11}O_{13}S_2.1\ H_2O$ (1014) calculated: 49·75% C, 6·66% H, 15·20% N; found: 49·90% C, 6·65% H, 15·40% N.

The Amide of Isoleucyl-glutaminyl-asparaginyl-S-β-carboxyethylhomocysteinyl-prolyl-leucyl-glycine (*IXa*)

To a solution of fully protected heptapeptide *VIIIa* (1·70 g) in dimethylformamide (35 ml) we added 2·2M-HCl in ether (2·1 ml) and after 4 min standing at room temp the reaction mixture was diluted with ether (300 ml). The hydrochloride which separated out was filtered, washed with ether, dried and dissolved in methanol (20 ml). To this solution we added 1M-NaOH (6·5 ml) and after 1 h standing at room temperature the methanol was evaporated off and the remaining aqueous solution was filtered through a column of Dowex 50 (65 ml, H^+-cycle). The column was then washed through with water, the product was eluted with 10% pyridine, the latter solution was concentrated by evaporation and the product was precipitated from a mixture of methanol and ether. The yield was 1·25 g (91%); $E_{5.7}^{His}$ 0·28, $E_{2.4}^{Gly}$ 0·63; R_F 0·13 (S_1), 0·43 (S_2). Amino acid analysis showed: Asp 1·00, Glu 1·00, Gly 1·00, HCys($C_3H_5O_2$) 1·25, Ile 1·00, Leu 1·00, Pro 1·08. The sample for analysis was recrystallised from a mixture of dimethylformamide and ether; $[\alpha]_D$ —48·6° (*c* 0·22, dimethylformamide). For $C_{35}H_{60}N_{10}O_{11}S.1\frac{1}{2}\ H_2O$ calculated: 49·11% C, 7·42% H, 16·36% N; found: 49·17% C, 7·69% H, 15·54% N.

The Amide of Tert-butyloxycarbonyl-O-tert-butyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-β-carboxyethylhomocysteinyl-prolyl-leucyl-glycine (*Xa*)

To a solution of the free heptapeptide amide *IXa* (1·20 g) in dimethylformamide (21 ml) and N-ethylpiperidine (0·5 ml) we added the N-hydroxysuccinimid ester of tert-butyloxycarbonyl-O-tert-butyltyrosine[9] (1·28 g). After 24 h standing at room temp. a further 0·2 g of active ester were added to the mixture and after a further 24 h standing the mixture was evaporated to dryness, the dry powder was consecutively ground with petrol ether and ether, the crystalline fraction was filtered and washed with ether, water, 3% citric acid and then with water. The yield was 1·29 g of a product with m.p. 203—206°C (Kofler). Extraction with hot methanol (5 ml) gave 0·50 g of substance with m.p. 210—212°C (Kofler); from mother liquors we obtained 0·55 g of a less pure product of m.p. 203—204°C (Kofler). The sample for analysis was prepared from the material with the higher m.p. by crystallisation from a mixture of dimethylformamide and ether, m.p. 211—213°C (Kofler); $[\alpha]_D$ —34·6° (*c* 0·50, dimethylformamide). For $C_{53}H_{86}N_{11}O_{15}S.1\ H_2O$ (1167) calculated: 54·55% C, 7·60% H, 13·21% N; found: 54·65% C, 7·33% H, 12·99% N.

The Lactam of Tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-β-carboxyethylhomocysteinyl-prolyl-leucyl-glycine Amide ("Deamino-carba^6-oxytocin" *Id*)

Preparation and cyclisation of the active ester were carried out in an atmosphere of nitrogen. To a solution of protected octapeptide *Xa* (302 mg) in a mixture of pyridine (10 ml) and dimethylformamide (10 ml) we added bis-*p*-nitrophenyl sulphite[18] (1 g). The reaction mixture was stirred at room temperature for 8 h, diluted with pyridine (5 ml) and a further 1 g of reagent was added; after 12 h mixing we added a further 0·5 g bis-*p*-nitrophenyl sulphite and after a further 5 h the reaction mixture was evaporated to dryness, the dry powder was ground with ether, filtered and washed with ether, then water, dried in air, showing a yield of 318 mg.

The active ester was dissolved in trifluoroacetic acid (10 ml) and after 1 h standing at room temp the reaction mixture was diluted with toluene (10 ml) and evaporated to dryness. The dry powder was dissolved in dimethylformamide (10 ml) and over 4 h this latter solution was added to 250 ml of pyridine at 50°C with stirring. After 12 h the reaction mixture was evaporated to dryness, the dry powder was ground with ether, filtered and washed well with ether. After drying, the powder was dissolved in 25 ml of the upper phase of the solvent system 2-butanol–0·05% acetic acid, the solution was then transferred to the second tube of the all-glass steady state distribution machine (Quickfit & Quartz, Ltd., Stone, Staffordshire, England) and 108 shifts of the upper phase were carried out. The contents of tubes 62—80 ($K = 1·95$; localisation of the peak with the Folin reaction) were pooled, concentrated to a small volume and freeze-dried. The yield was 118·7 mg (47%); R_F 0·20 (S1), 0·35 (S2), 0·65 (S3); on paper electrophoresis in two different buffers (pH 2·4 and 5·7) the substance did not migrate. For analysis and biological testing this product was further purified by gel filtration on columns of Bio-Gel P-4 and P-2 (100 × 1 cm, 1M acetic acid) and crystallised from a mixture of methanol and ether; $[\alpha]_D$ −84·5° (*c* 0·26, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·00, Gly 1·04, HCys($C_3H_5O_2$) 1·11, Ile 1·01, Leu 1·01, Pro 0·89, Tyr 0·94. For $C_{44}H_{67}N_{11}O_{12}S.1\ H_2O$ (992·1) calculated: 53·26% C, 7·01% H, 15·53% N; found: 53·13% C, 7·09% H, 14·90% N.

The Dicyclohexylammonium Salt of the ω-Methylester of Benzyloxycarbonyl-α-aminosuberic acid (*V*)

To a solution of the hydrochloride of the ω-methylester of α-aminosuberic acid[2] (1·30 g) in 20 ml water and 25 ml 0·5M-$NaHCO_3$ we added sodium benzyloxycarbonyl thiosulphate (1·46 g) and the pH of the reaction mixture was maintained at 8·0—8·5 by addition of 1M-NaOH (about 9 ml in total). After 2·5 hrs the reaction mixture was acidified to pH 3 with hydrochloric acid, the product was shaken and taken up in ether, the latter solution was washed with water, dried with sodium sulphate and evaporated. The powder was dissolved in benzene and to the solution was added dicyclohexylamine (1·06 ml). After dilution with light petroleum the solution was cooled to 0°C. The crystals which separated out were filtered and washed with light petroleum. The yield was 2·36 g (87%), m.p. 118—121°C (capillary), with no difference as compared to the sample prepared with benzyloxycarbonyl chloride[2].

The Amide of the ω-Methylester of *o*-Nitrobenzenesulphenyl-asparaginyl-α-aminosuberyl-prolyl-leucyl-glycine (*VIb*)

To a solution of substance *V*. DCHA (2·0 g) in a mixture of methanol (40 ml) and water (25 ml) we added Dowex 50 W X 4 (H^+-cycle, 30 ml). After 30 min stirring the ion exchanger was filtered, the filtrate was evaporated to dryness and azeotropically (benzene) dried. The dry residue was dissolved in dimethylformamide (20 ml) along with the amide of prolyl-leucyl-glycine[14,15] (1·28 g) and N-hydroxysuccinimide (0·48 g) and at −20°C we added with constant

mixing dicyclohexylcarbodiimide (0·48 g). The reaction mixture was stirred for 1 h at −10°C, 2 h at 0°C and 12 h at room temperature. The dicyclohexylurea which separated out was filtered, washed with dimethylformamide and the pooled filtrates were evaporated to dryness. The dry powder was ground with light petroleum, dissolved in ethyl acetate, the solution was consecutively shaken with 1M-HCl, water, 0·5M-$NaHCO_3$ and again water, dried with sodium sulphate and evaporated. The concentrate was azeotropically dried (benzene), dissolved in acetic acid (8 ml) and to this solution was added 35% HBr in acetic acid (20 ml). After 10 min standing at room temperature the reaction mixture was diluted with ether, the hydrobromide which separated out was washed several times with ether and then dried in an exsiccator over sodium hydroxide. After solution in dimethylformamide (24 ml) the pH of the solution was adjusted to 8·5 with N-ethylpiperidine (2 ml) (moist pH paper measurement) and we then added the 2,4,5-trichlorophenylester of *o*-nitrobenzenesulphenylasparagine[16] (2 g). After 24 h standing at room temp we added a further 1 g of active ester and after a further 24 h standing the mixture was evaporated to dryness, the powder was consecutively ground with light petroleum and ether, the crystalline portion was filtered and washed with ether, water, 0·5M-$NaHCO_3$ and again water. The yield was 1·90 g (66%, calculated on the dicyclohexylammonium salt *V*), m.p. 170–171°C (capillary). The sample for analysis was crystallised from a mixture of dimethylformamide and ether, m.p. 175–177°C (capillary); $[\alpha]_D$ −69·8° (*c* 0·5, dimethylformamide). For $C_{32}H_{48}N_8O_{10}S.\frac{1}{2}H_2O$ (745·8) calculated: 51·53% C, 6·62% H, 15·03% N; found: 51·54% C, 6·47% H, 14·83% N.

The Amide of the ω-Methylester of *o*-Nitrobenzenesulphenylglutaminyl-asparaginyl-α-aminosuberyl-prolyl-leucyl-glycine (*VIIb*)

To a solution of the protected pentapeptide *VIb* (1·9 g) in dimethylformamide (19 ml) we added 2M-HCl in ether (3·15 ml). After 4 min at room temperature the solution was diluted with ether, the hydrochloride which separated out was filtered, washed with ether and dried in an exsiccator; $E_{5.7}^{His}$ 0·33, $E_{2.4}^{Gly}$ 0·81. After dissolving in dimethylformamide (32 ml) and alkalisation with N-ethylpiperidine (0·8 ml) we added the 2,4,5-trichlorophenyl ester of *o*-nitrobenzenesulphenylglutamine[16] (1·45 g) and after 24 h at room temperature we added a further 0·55 g of active ester. After a further 24 h standing at room temperature the mixture was evaporated to dryness and worked up in the same manner as substance *VIb*. Crystallisation from a mixture of dimethylformamide and ether gave a yield of 1·45 g (64%), m.p. 158–163°C (Kofler). The sample for analysis was recrystallised in the same manner, m.p. 159–164°C (Kofler); $[\alpha]_D$ −48·6° (*c* 0·51, dimethylformamide). For $C_{37}H_{56}N_{10}O_{12}S.1\,H_2O$ (882·9) calculated: 50·33% C, 6·62% H, 15·87% N; found: 50·35% C, 6·36% H. 15·92% N.

The Amide of the ω-Methylester of *o*-Nitrobenzenesulphenylisoleucyl-glutaminyl-asparaginyl-α-aminosuberyl-prolyl-leucyl-glycine (*VIIIb*)

To a solution of protected hexapeptide *VIIb* (0·60 g) in dimethylformamide (3 ml) we added 2M-HCl in ether (1 ml). After 5 min at room temperature the mixture was diluted with ether and the hydrochloride which separated out was filtered, washed with ether and dried in an exsiccator; $E_{5.7}^{His}$ 0·50, $E_{2.4}^{Gly}$ 0·74; R_F 0·21 (S_1), 0·15 (S_2), 0·19 (S_4). After dissolving in dimethylformamide (10 ml) the solution was alkalinised with N-ethylpiperidine (0·15 ml) and we then added the N-hydroxysuccinimid ester of *o*-nitrobenzenesulphenylisoleucine[9] (0·33 g). After 24 h at room temperature we added a further 0·15 g of active ester and after a further 24 h the reaction mixture was evaporated to dryness and the dry powder was worked up in the same manner as substance *VIb* (yield 0·55 g, m.p. 210–215°C). Crystallisation from a mixture of dimethylformamide and ether gave a yield of 0·50 g (74%), m.p. 240–242°C (capillary); the sample for elemental analysis was recrystallised in the same manner, m.p. 252–255°C (Kofler); $[\alpha]_D$ −66·4° (*c* 0·51, dimethyl-

formamide). For $C_{43}H_{67}N_{11}O_{13}S.1\,H_2O$ (996·1) calculated: 51·85% C, 6·98% H, 15·47% N; found: 51·66% C, 7·12% H, 15·37% N.

The Amide of Isoleucyl-glutaminyl-asparaginyl-α-aminosuberyl-prolyl-leucyl-glycine (*IXb*)

To a solution of protected heptapeptide *VIIIb* (1 g) in dimethylformamide (25 ml) we added 2M-HCl in ether (1·5 ml). After 4 min at room temperature the mixture was diluted with 250 ml ether, the hydrochloride which separated out was filtered and washed with ether. After drying in an exsiccator the hydrochloride was dissolved in methanol (12·5 ml) and 1M-NaOH was added (5 ml). After 1 h at room temperature the methanol was evaporated off and the aqueous solution was placed on a column of Dowex 50 (H^+ cycle, 75 ml), the column was washed through with water and the product was eluted with 10% pyridine. The eluate was evaporated to dryness and the powder was crystallised from a mixture of methanol and ether. The yield was 0·61 g (73%), R_F 0·12 (S_1), 0·46 (S_2) and $E^{Gly}_{2·4}$ 0·69; $E^{His}_{5·7}$ 0·18; $[\alpha]_D$ −39·1° (*c* 0·50, dimethylformamide). Amino acid analysis: Asp, 0·99, Glu 0·99, Pro 1·08, Gly 1·00 Asub + Ile 2·14, Leu 1·05. The sample for elemental analysis was recrystallised from a mixture of dimethylformamide and ether. For $C_{36}H_{62}N_{10}O_{11}\,.\,2\frac{1}{2}\,H_2O$ (856·0) calculated: 50·51% C, 7·89% H, 16·37% N; found: 50·76% C, 7·44% H, 16·11% N.

The Amide of Tert-butyloxycarbonyl-O-tert-butyltyrosyl-isoleucyl-glutaminyl-asparaginyl-α-aminosuberyl-prolyl-leucyl-glycine (*Xb*)

To a solution of free heptapeptide amide *IXb* (0·50 g) in dimethylformamide (10 ml) and N-ethylpiperidine (0·25 ml) was added the N-hydroxysuccinimidester of tert-butyloxycarbonyl-O-tert-butyltyrosine[9] (0·5 g). After 24 h at room temperature a further 0·25 g of active ester were added and after a further 24 h the mixture was evaporated to dryness, the dry powder was ground successively with light petroleum, aqueous citric acid (pH 3), filtered and washed with citric acid and then water. The yield was 0·47 g (70%) m.p. 201—205°C (Kofler). A sample was dissolved in trifluoroacetic acid and after 1 h we carried out chromatography and electrophoresis: $E^{Gly}_{2·4}$ 0·57, $E^{His}_{5·7}$ 0·23; R_F 0·02 (S_1), 0·21 (S_2). The sample for analysis was crystallised from a mixture of dimethylformamide and ether, m.p. 201—203°C; $[\alpha]_D$ −30·8° (*c* 0·50, dimethylformamide). For $C_{54}H_{87}N_{11}O_{15}.H_2O$ (1148) calculated: 56·45% C, 7·80% H, 13·41% N; found: 56·35% C, 7·98% H, 13·37% N.

The Lactam of Tyrosyl-isoleucyl-glutaminyl-asparaginyl-α-aminosuberyl-prolyl-leucyl-glycine amide (Deamino-dicarba-oxytocin; *Ic*)

To a solution of protected octapeptide-acid *Xb* (301·5 mg) in a mixture of dimethylformamide (10 ml) and pyridine (10 ml) with mixing and in an atmosphere of nitrogen we added bis-*p*-nitrophenyl sulphite (1 g). After 7 h at room temp we added a further 1 g of reagent and 12 h later a further 0·5 g. After a further 6 h stirring and bubbling through with nitrogen we evaporated the mixture to dryness, the dry powder was ground with ether, filtered and washed with ether and then water. After drying the product was dissolved in trifluoroacetic acid (10 ml) and after 1 h at room temp we added 10 ml toluene and evaporated to dryness. The dry powder was dissolved in dimethylformamide (10 ml) and ether 4 h the solution was added to hot (50°C) pyridine (250 ml) under constant mixing and N_2 bubbling. After 12 h at room temp the reaction mixture was evaporated to dryness, the dry powder was triturated with ether, filtered and washed with ether. After drying the product was dissolved in 25 ml of the upper phase of the solvent system 2-butanol–0·05% acetic acid (1 : 1) and 120 transfers of the upper phase in the steady state distribution apparatus were carried out (as with substance *Id*). The peak with partition coefficient 2·15

(tubes 70—87) was pooled, concentrated and freeze-dried. The yield was 80·0 mg (30%) of a chromatographically pure substance; R_F 0·22 (S_1), 0·40 (S_2), 0·67 (S_3). For analysis and biological testing the product was further purified by gel filtration on Bio-gel P-2 and P-4 in 1M acetic acid, and then crystallised from methanol–ether; $[\alpha]_D$ −67·4° (*c* 0·22, 1M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·00, Pro 0·93, Gly 0·98, Ile + Asub 1·92, Leu 1·00, Tyr 0·88. For $C_{45}H_{69}N_{11}O_{12}.3\,H_2O$ (1010) calculated: 53·51% C, 7·48% H, 15·26% N; found: 53·75% C, 7·05% H, 15·13% N.

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