SYNTHESIS AND INHIBITORY PROPERTIES OF OXYTOCIN ANALOGUES MODIFIED IN THE AMINO-TERMINAL REGION OF THE MOLECULE*

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By the acylation of [2-O-methyltyrosine]oxytocin, analogues were obtained having the amino group of cysteine in position 1 substituted by a mesyl, bromoacetyl, or a pivaloyl residue. N^{α}-Glycyl-[2-O-methyltyrosine]oxytocin and N^{α}-sarcosyl-[2-O methyltyrosine]oxytocin were prepared by fragment condensation, reduction of the protected decapeptides by sodium in liquid ammonia, and subsequent oxidation. All the analogues inhibited the action of oxytocin on the uterus *in vitro* and the pressor effect of vasopressin. They had little or no milk-ejecting activity; no inhibition was observed in this test.

[2-O-Methyltyrosine]oxytocin[1-3]** (*Ib*) was one of the first synthetic analogues of oxytocin (*Ia*) found to have inhibitory properties[5]. However, this and other analogues[6,7] with modified amino acids in position 2 inhibited the uterotonic action of oxytocin only under conditions *in vitro*. Analogues that inhibited the uterotonic effect of oxytocin under conditions *in vivo* were obtained when the α-amino group of cysteine in position 1 of [2-O-methyltyrosine]oxytocin was substituted. The properties of some analogues of this type, namely N^{α}-carbamoyl-[2-O-methyltyrosine]-oxytocin[8,9] (*Ic*) and N^{α}-acetyl-[2-O-methyltyrosine]oxytocin[10,11] (*Id*), were already described. In the present paper we describe the synthesis and some biological properties of analogues of [2-O-methyltyrosine]oxytocin with the α-amino group of cysteine substituted by various groups.

Both aminoacyl derivatives *Ie* and *If* were prepared by the reduction of the respective decapeptides *IIa* and *IIb* with sodium in liquid ammonia and subsequent oxidation, and were purified by counter-current distribution and gel filtration. The protected decapeptides *IIa* and *IIb* were obtained by the acylation of heptapeptide amide *III* by tripeptide azides[12] prepared from tripeptide esters *IVa* and *IVc via* hydrazides *IVb* and *IVd*. Tripeptide *IVa* was prepared from benzyloxycarbonyl-

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** The amino acids used in this work had L-configuration. The nomenclature and symbols follow the published rules[4].

glycine and S-benzylcysteinyl-O-methyltyrosine methyl ester using dicyclohexylcarbodiimide, tripeptide *IVc* by acylation of the above-mentioned dipeptide ester by benzyloxycarbonylsarcosine using the method of mixed anhydrides. The other analogues were prepared by treating [2-O-methyltyrosine]oxytocin (*Ib*) with a suitable reagent: in the case of analogues *Ig* and *Ih*, with a chloride of pivalic acid or methanesulphonic acid in a dimethylformamide solution in the presence of a tertiary base[10] and, in the case of the analogue *Ii*, bromoacetyl bromide in aqueous carbonate buffer, by analogy with the synthesis of N^{α}-bromoacetyl-oxytocin[13]. Analogues *Ig* and *Ih* were purified by chromatography on a column of CM-Sephadex in pyridine–acetate buffer and by gel filtration, analogue *Ii* by counter-current distribution and gel filtration. Chromatographic purification cannot be used in the latter case because a quarternary ammonium salt is apparently formed from the analogue in the presence of pyridine, as observed by means of thin-layer chromatography. The properties of analogues *Ic* and *Id* were the same as those described earlier[8,10].

TABLE I
Biological Activity of the Analogues in Rats

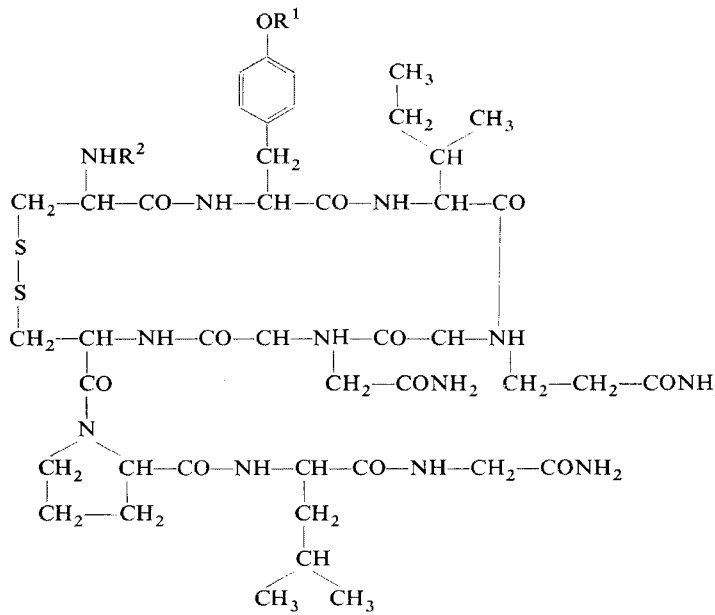
Analogue	Inhibition constants[a] (isolated uterus)			Milk ejection I.U./mg	Inhibition (%) of pressor response[b]
	pA_2		pD_2		
Ic	7·22[c]	(4)	9·07—9·52	inhibition[d]	inhibition[d]
Id	7·58	(5)	9·00—9·33	inhibition[e]	inhibition[f]
Ie	6·85	(4)	9·33—9·37	0·64	50
If	6·52	(6)	9.05—9·31	inactive[g]	100
Ig	7·26	(11)	9·05—9·25	1·13	100
Ih	7·82	(8)	9·05—9·52	inactive[h]	50
Ii	7·33	(6)	9·21—9·23	inactive[h]	50

[a] Ref.[29]; [b] 8-lysine-vasopressin to analogue ratio of 1 : 2000, in the case of analogue *Ih*, 1 : 1000; [c] arithmetic mean, number of experiments is given in parentheses; [d] ref.[9]; [e] ref.[32]; [f] ref.[11]; [g] up to doses of $1 . 10^{-1}$ mg/kg; [h] up to doses of $1 . 10^{-2}$ mg/kg.

The oxytocin-like pharmacological properties of the synthetized analogues were determined in assays of the uterotonic activity *in vitro*, milk-ejecting activity *in vivo*, and of the pressor activity. The results are presented in Table I, together with the values for structurally similar analogues *Ic* and *Id*. All the analogues studied inhibited the pressor activity of lysine-vasopressin; 50—100% inhibition was observed at vasopressin to inhibitor ratio of 1 : 1000 to 1 : 2000. Analogues *Ie* and *Ig* were

slightly active in the milk-ejection assay, the others were inactive. In contrast to the analogues *Ic* and *Id* studied earlier, none of the newly synthetized analogues inhibited the milk-ejection activity of oxytocin.

All the examined analogues acted as inhibitors on the isolated uterus. The inhibition constants were in the range of 10^{-7} to 10^{-8}M and did not differ significantly from those of analogues *Ic* and *Id*. After higher doses of analogues *Ig*, *Ih*, and *Ii* non-competitive inhibition, but no uterotonic activity, was observed. It follows that compounds *Ig*, *Ih* and *Ii* are not partial agonists. By contrast, both amino-acylat-



Ia, $R^1 = H$, $R = H$,
Ib, $R^1 = CH_3$, $R^2 = H$
Ic, $R^1 = CH_3$, $R^2 = NH_2CO$
Id, $R^1 = CH_3$, $R^2 = CH_3CO$
Ie, $R^1 = CH_3$, $R^2 = NH_2CH_2CO$
If, $R^1 = CH_3$, $R^2 = CH_3NHCH_2CO$
Ig, $R^1 = CH_3$, $R^2 = (CH_3)_3CCO$
Ih, $R^1 = CH_3$, $R^2 = CH_3SO_2$
Ii, $R^1 = CH_3$, $R^2 = BrCH_2CO$
Ij, $R^1 = H$, $R^2 = BrCH_2CO$

X-Cys(Bzl)-Tyr(Me)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2

IIa, X = Z-Gly *IIb*, X = Z-Sar

Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2

III

X-Cys(Bzl)-Tyr(Me)-Y

IVa, X = Z-Gly, Y = OMe *IVc*, X = Z-Sar, Y = OMe
IVb, X = Z-Gly, Y = N_2H_3 *IVd*, X = Z-Sar, Y = N_2H_3

ed analogues *Ie* and *If* acted as agonists at higher concentrations, which was observed earlier in the case of analogue *Id*. One possible explanation is the enzymic liberation of the acetyl group and the appearence of [2-O-methyltyrosine]oxytocin, which can manifest either inhibitory or partially agonistic action, depending on the experimental conditions. Such an explanation would seem even more probable in the case of analogues *Ie* and *If*, since these peptides resemble "hormonogens"[14,15]; however, enzymic cleavage would release [2-O-methyltyrosine]oxytocin instead of the hormone oxytocin.

Despite the great differences between the groups substituting the α-amino group of cysteine in position 1, all the analogues *Ic*–*Ii* had similar biological activities, especially their behaviour as antagonists in the assay on the isolated rat uterus. Our results, together with other findings published earlier[8,16,17], show that in order to synthetize analogues that would inhibit the action of oxytocin under a variety of experimental conditions, it is necessary to perform substitutions in the *para*-position of the aromatic amino acid in position 2 and at the same time eliminate the free α-amino group of oxytocin. The chemical character of the group substituting the α-amino group does not seem very important (*cf.* also[18]).

It is interesting to compare the biological action of analogue *Ii* with that of *Ij*, which was described earlier and found to inhibit irreversibly the effect of neurohypophysial hormones on some adenylate cyclase system[13]. The present knowledge of the relation between the chemical structure and biological activity[8] would lead us to expect low oxytocin-like activity of *Ij*, but no inhibitory action. Analogue *Ii* behaved as a competitive inhibitor of the uterotonic effect of oxytocin in our experiments; we did not observe irreversible binding of the analogue to the receptor. It is true that at high concentrations of the inhibitor the inhibition became non-competitive. However, it is highly probable that the interaction is unspecific. Other analogues, namely *Ig* and *Ih*, behaved in a similar way; their chemical structure precludes the formation of a covalent bond between the analogue and the receptor in the target tissue.* The fact that self-inhibition of oxytocin occurs in the adenylate cyclase system at high concentrations of the hormone[22] also seems to prove that irreversible inhibition of the above-mentioned type is not specific.

The search for an irreversible inhibitor that could serve as an "affinity label" for the isolation of the receptor is accompanied by difficulties which did not arise during the formally similar task of determining the active centre of enzymes. The required inhibitor should react with the functional group of the receptor forming a covalent bond (this reaction does not occur between the agonist and the receptor).

* So far, we have not excluded the possible formation of a covalent bond based on the interaction between the sulfhydryl groups of the receptor and the disulphide bridge of the antagonist. This type of bond is apparently not formed between agonists of oxytocin and the receptor in the uterus[19–21]; on the basis of the receptor theory we may assume that these bonds are not formed with antagonists.

The inhibitor should not be bound to other components of the target tissues. The inhibitory effect of analogue *Ii* on the isolated uterus gives evidence of interaction between the inhibitor and receptor but not of covalent binding. We do not wish to imply, however, that the goal, *i.e.* the isolation of receptors using irreversible inhibitors, cannot be reached. New possibilities lie in introducing various reactive groups into the molecule of an analogue and in their suitable placing.

EXPERIMENTAL

Methods and Material

The melting points were determined on a Kofler block and the values were corrected. Samples for elemental analysis were dried above phosphorus pentoxide for 24 or 48 h at room temperature in a vacuum of 1 Torr. Thin-layer chromatography was performed on plates with silica gel (Kieselgel G, Merck) in systems: 2-butanol–90% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% ammonia–water (85 . 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3) and 1-butanol–pyridine–acetic acid–water (30 : 20 : 12 : 6) (S4). Electrophoresis was performed on paper Whatman No 3 MM for 1 h at a potential gradient of 20 *V*/cm in buffers: 1M acetic acid (pH 2·4) and pyridine–acetic acid (pH 5·7). The compounds were detected by ninhydrin and the chlorination method; the values of R_F and *E* are given for those cases when the compounds were pure. Samples for amino-acid analysis were hydrolyzed for 20 h at 105°C in 6M-HCl in ampoules sealed at 1 Torr. Analyses were performed by an automatic apparatus (Development Workshop of the Czechoslovak Academy of Sciences, type 6020). The reaction mixtures were evaporated under vacuum by a water pump in a bath of 30—35°C, mixtures containing dimethylformamide at 1 Torr using an oil pump. Counter-current distribution was effected in an all-glass Steady State Distribution Machine (Quickfit & Quartz Ltd, Stone, Staffordshire, England) in which it was possible to transfer the upper and lower phase. In all cases, the system: 2-butanol–0·05% acetic acid was used, and the peptide material was located by the Folin-Ciocalteau reaction (samples were collected from every other tube). Gel filtration was done in chromatographic columns (100 × 1 cm or 140 × 2·5 cm) filled with Bio-Gel P-2 or P-4 (Bio-Rad Laboratories, Richmond, Ca., U.S.A.) using 1M or 3M acetic acid at flow rates of 7 or 12 ml/h. Peptide material was located by measuring the absorption at 280 nm. Chromatography on CM-Sephadex (Pharmacia Fine Chemicals AB, Uppsala, Sweden) was performed in the buffer: pyridine–acetic acid–water (100 : 3·6 : 900), at a flow rate of 20 ml/h, using a 35 × 1·5 cm column. The peptides were located by measuring the absorption at 280 nm.

o-Nitrobenzenesulphenyl-S-benzylcysteinyl-O-methyltyrosine Methyl Ester

Dicyclohexylcarbodiimide (1·5 g) was added to a solution of the dicyclohexylammonium salt of *o*-nitrobenzenesulphenyl-S-benzylcysteine (2·09 g) and O-methyltyrosine methyl ester hydrochloride (0·92 g) in dimethylformamide (5 ml), cooled to −20°C. The mixture was stirred for 3 h at −15°C and then overnight at room temperature, evaporated and dissolved in ethyl acetate. Dicyclohexylurea was removed by filtration and the filtrate was first washed with water, then with 0·5M-$NaHCO_3$, water, sulphate buffer[23] and water again, dried with Na_2SO_4 and evaporated. Crystallization from a mixture of benzene and light petroleum yielded 2·04 g (96%) of product with a m.p. of 136—138°C. The sample for analysis was recrystallized in the same way, its m.p. was 138—139°C; $[\alpha]_D$ +17·0° (*c* 0·36, dimethylformamide). For $C_{27}H_{29}N_3O_6S_2$ (555·7) calculated: 58·36% C, 5·26% H, 7·56% N; found: 58·59% C, 5·64% H, 7·80% N.

Benzyloxycarbonylglycyl-S-benzylcysteinyl-O-methyltyrosine Methyl Ester (*IVa*)

A 2·06M solution of HCl in methanol (1·71 ml) was added to a suspension of *o*-nitrobenzenesulphenyl-S-benzylcysteinyl-O-methyltyrosine methyl ester (1·96 g) in methanol. The cleavage of the amino-protecting group was followed by thin-layer chromatography (benzene with 2% of ethanol). The mixture was evaporated, the residue was triturated with light petroleum, dried and then dissolved in dimethylformamide (20 ml). Benzyloxycarbonylglycine (0·74 g) was added to the solution, the mixture was chilled to −30°C, and N-ethylpiperidine (0·49 ml) and dicyclohexylcarbodiimide (0·8 g) were added. The mixture was stirred overnight, evaporated and the residue was dissolved in chloroform. Dicyclohexylurea was removed by filtration, the solution was washed with water, sulphate buffer (pH 2), 0·5N-$NaHCO_3$, water, dried with Na_2SO_4 and evaporated. Crystallization from a mixture of methanol and ether yielded 1·27 g (60%) of product with m.p. of 138·5–141·5°C. The sample for analysis was recrystallized from methanol; the m.p. was 142–143°C; $[\alpha]_D$ −21·4° (*c* 0·57, dimethylformamide). A benzyloxycarbonyl group was split off the sample: $E_{2.4}^{His}$ 0·59, $E_{5.7}^{His}$ 0·60. For $C_{31}H_{35}N_3O_7S.0{\cdot}5\,H_2O$ (602·7) calculated: 61·78% C, 6·02% H, 6·97% N; found: 62·11% C, 5·86% H, 7·22% N.

Benzyloxycarbonylglycyl-S-benzylcysteinyl-O-methyltyrosine Hydrazide (*IVb*)

Hydrazine hydrate (0·26 ml) was added to a solution of ester *IVa* (1·0 g) in methanol (19 ml). After 60 h at room temperature, the crystallized product was separated by filtration and washed with methanol, water and ether. Thus, 0·69 g (69%) of product was obtained which had a m.p. of 179–180·5°C. The sample for analysis was crystallized from a mixture of dimethylformamide and methanol; the m.p. did not change; $[\alpha]_D$ −25·8° (*c* 0·47, dimethylformamide). For $C_{30}H_{35}N_5O_6S$ (593·7) calculated: 60·69% C, 5·94% H, 11·80% N; found: 60·65% C, 5·98% H, 11·99% N.

Benzyloxycarbonylglycyl-S-benzylcysteinyl-O-methyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-leucyl-glycine Amide (*IIa*)

A solution of 2·6M-HCl in tetrahydrofurane (2·4 ml) was added to a solution of hydrazide *IVb* (0·50 g) in dimethylformamide (4 ml), the mixture was chilled to −30°C and n-butylnitrite (0·143 ml) was added while stirring. After 4 min at the same temperature, an addition was made of a chilled solution of heptapeptide-amide[24] *III* (0·70 g) in dimethylformamide (20 ml) and N-ethylpiperidine (0·9 ml). The mixture was kept for 3 days at 0°C and then evaporated; the residue was triturated with 1M-HCl, filtered and washed with 1M-HCl and water; 1·1 g, m.p. 243 to 247°C. Crystallization from a mixture of dimethylformamide and methanol yielded 0·92 g (79%) of product with a m.p. of 245–249°C; $[\alpha]_D$ −39·3° (*c* 0·12, dimethylformamide). For $C_{68}H_{91}N_{13}O_{15}S_2.H_2O$ (1413) calculated: 57·82% C, 6·64% H, 12·89% N; found: 57·55% C, 6·54% H, 13·09% N.

N^{α}-Glycyl-[2-O-methyltyrosine]oxytocin (*Ia*)

Decapeptide *IIa* (890 mg) was reduced with sodium in liquid ammonia in the usual way. The evaporation of ammonia and oxidation with $K_3Fe(CN)_6$ were performed under nitrogen[25]. The aqueous solution was filtered on a column of Amberlit IR-4B (Cl^- cycle, 180 ml). The eluate was concentrated and freeze-dried, and then purified by counter-current distribution (462 transfers of the upper and 65 transfers of the lower phase). The peak with the distribution coefficient K = 0·33 was isolated; 313 mg (45%) of product was obtained. The sample for analysis was further purified by gel filtration on Bio-Gel P-2 in 1M acetic acid. $E_{2.4}^{Gly}$ 0·56, $E_{5.7}^{His}$ 0·39; R_F 0·34 (S1),

0·33 (S2), 0·28 (S3), 0·69 (S4), $[\alpha]_D$ −55° (*c* 0·24, 3M acetic acid). Amino acid analysis: Asp 1·00, Glu 1·06, Pro 0·98, Gly 1·87, Cys 1·63, Ile 1·00, Leu 1·02, Tyr(Me) 0·24, Tyr 0·75. For $C_{46}H_{71}N_{13}O_{13}S_2.CH_3CO_2H.2\,H_2O$ (1174) calculated: 49·09% C, 6·78% H, 15·51% N; found: (corrected for 0·87% of ash) 48·82% C, 6·43% H, 15·62% N.

Benzyloxycarbonylsarcosyl-S-benzylcysteinyl-O-methyltyrosine Methyl Ester (*IVc*)

A solution of benzyloxycarbonylsarcosine (1·12 g) in chloroform (6 ml) was cooled to 0°C, and N-ethylpiperidine (0·685 ml) and 2-butyl chloroformate (0·66 ml)were added to it. The mixture was stirred for 5 min at 0°C. Then, N-ethylpiperidine (0·685 ml) was added together with a solution of S-benzylcysteinyl-O-methyltyrosine methyl ester hydrochloride (prepared from *o*-nitrobenzenesulphenyl derivative (2·78 g) by splitting off the amino-protecting group with HCl in methanol) in dimethylformamide (10 ml). The mixture was stirred for 10 min at 0°C, then for 1 h at room temperature, and afterwards evaporated. The residue was dissolved in chloroform and the solution was washed with water, 1M-HCl, 0·5M-$NaHCO_3$, dried and evaporated. Crystallization from a mixture of benzene, ether and light petroleum yielded 2·60 g (85%) of product with a m.p. of 114—117°C. The sample for analysis was crystallized from a mixture of methanol and ether, m.p. 117—119°C; $[\alpha]_D$ −24·6° (*c* 0·56, dimethylformamide). For $C_{32}H_{37}N_3O_7S$ (607·7) calculated: 63·25% C, 6·14% H, 6·91% N; found: 62·75% C, 6·07% H, 6·89% N.

Benzyloxycarbonylsarcosyl-S-benzylcysteinyl-O-methyltyrosine Hydrazide (*IVd*)

Hydrazine hydrate (1·0 ml) was added to a solution of ester *IVc* (2·6 g) in methanol (20 ml). After 60 h at room temperature, the mixture was filtered and the crystalline product was washed with methanol and water. After drying (70°C, 1 Torr), the product was crystallized from a mixture of dimethylformamide and ether; the yield was 2·18 g (83%), m.p. 191—194°C; $[\alpha]_D$ −26·8° (*c* 0·47, dimethylformamide). For $C_{31}H_{37}N_5O_6S$ (607·7) calculated: 61·26% C, 6·14% H, 11·52% N; found: 61·18% C, 5·88% H, 11·80% N.

Benzyloxycarbonylsarcosyl-S-benzylcysteinyl-O-methyltyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-leucyl-glycine Amide (*IIb*)

A 2·0M solution of HCl in tetrahydrofurane (3·2 ml) was added to a solution of hydrazide *IVd* (0·56 g) in dimethylformamide (6 ml), the mixture was chilled to −30°C, n-butyl nitrite (0·15 ml) was added, and 4 min later a cold solution of heptapeptide *III* (0·70 g) in dimethylformamide (20 ml). The pH of the reaction mixture was adjusted to 8·5 with N-ethylpiperidine (0·8 ml). After 3 days at 0°C, the mixture was evaporated, the residue was triturated with 1M-HCl, the crystalline substance was isolated by filtration and washed with 1M-HCl and water. Crystallization from a mixture of dimethylformamide, methanol and ether yielded 0·93 g (71%) of product with a m.p. of 249—251°C; $[\alpha]_D$ −42·3° (*c* 0·33, dimethylformamide). Amino acid analysis: Asp 0·96, Glu 1·01, Pro 0·99, Gly 0·93, Ile 0·96, Leu 1·00, Sar 1·01, Cys(Bzl) 1·85, Tyr 0·73, Tyr(Me) 0·20. For $C_{69}H_{93}N_{13}O_{15}S_2.H_2O$ (1427) calculated: 58·09% C, 6·71% H, 12·76% N; found: 58·04% C, 6·45% H, 12·93% N.

Sarcosyl-[2-O-methyltyrosine]oxytocin (*If*)

Decapeptide *IIb* (890 mg) was reduced and oxidized similarly to decapeptide *IIa*. Counter-current distribution (381 transfers of the upper and 82 transfers of the lower phase) resulted in the isolation of 227 mg (33%) of product with $K = 0{\cdot}38$. The sample for analysis was purified by gel

chromatography on Bio-Gel P-4 and P-2 in 1M acetic acid. $E^{Gly}_{2.4}$ 0·57, $E^{His}_{5.7}$ 0·23; R_F 0·26 (S1), 0·37 (S2), 0·20 (S3), 0·68 (S4); $[\alpha]_D$ −62·2° (*c* 0·24, 3M acetic acid). Amino-acid analysis: Asp 0·98, Glu 1·00, Pro 1·01, Gly 0·95, Cys 1·70, Ile 0·94, Leu 1·00, Sar 0·90, Tyr 0·66, Tyr(Me) 0·26. For $C_{47}H_{73}N_{13}O_{13}S_2.CH_3CO_2H.3\ H_2O$ (1206) calculated: 48·78% C, 6·93% H, 15·09% N; found: 48·70% C, 6·65% H, 15·05% N.

N$^{\alpha}$ Pivaloyl-[2-O-methyltyrosine]oxytocin (*Ig*)

Pivaloyl chloride (0·014 ml) was added to a solution of compound *Ib* (100 mg) in dimethylformamide (1·2 ml), the mixture was chilled to −50°C and N-ethylpiperidine (0·015 ml) then added. After 16 h at 0°C the mixture was diluted with ether, the product isolated by centrifugation, dried and purified by chromatography on CM-Sephadex (55 mg), Bio-Gel P-4 (3M acetic acid) (33 mg) and P-2 (26·5 mg). $[\alpha]_D$ −90·2° (*c* 0·24, 3M acetic acid); R_F 0·65 (S1), 0·49 (S2), 0·69 (S3), 0·75 (S4). Amino-acid analysis: Asp 1·00, Glu 1·05, Pro 1·12, Gly 0·99, Cys 1·57, Ile 1·00, Leu 1·04, Tyr(Me) 0·31, Tyr 0·65. For $C_{49}H_{76}N_{12}O_{13}S_2.3{\cdot}5\ H_2O$ (1168) calculated: 50·37% C, 7·16% H, 14·39% N; found: 50·26% C, 6·97% H, 14·51% N.

N$^{\alpha}$-Methanesulphonyl-[2-O-methyltyrosine]oxytocin (*Ih*)

A solution of compound *Ib* (59 mg) in dimethylformamide (0·2 ml) was chilled to −30°C; a solution of methanesulphonyl chloride (67·6 mg) in dimethylformamide (1·2 ml) and a solution of N-ethylpiperidine (67·5 mg) in dimethylformamide (1·2 ml) were then added. After 16 h at 0°C the mixture was diluted with ether, centrifuged and the isolated product was purified by chromatography on CM-Sephadex (36 mg) and Bio-Gel P-2 in 1M acetic acid (24·7 mg). $[\alpha]_D$ −88·5° (*c* 0·19, 3M acetic acid); R_F 0·51 (S1), 0·34 (S2), 0·51 (S3), 0·72 (S4). Amino acid analysis: Asp 1·03, Glu 1·00, Pro 1·07, Gly 0·97, Cys 0·88, Ile 1·03, Leu 0·98, Tyr(Me) 0·27, Tyr 0·66. For $C_{45}H_{70}N_{12}O_{14}S_3.3{\cdot}5\ H_2O$ (1162) calculated: 46·50% C, 6·68% H, 14·46% N; found: 46·79% C, 6·18% H, 13·98% N.

N$^{\alpha}$-Bromoacetyl-[2-O-methyltyrosine]oxytocin (*Ii*)

A solution of compound *Ib* (150 mg) in 1N-$NaHCO_3$ (15 ml) was cooled to 0°C and a solution of bromoacetyl bromide (3 g) in dioxane (15 ml) was added; the pH of the solution was maintained at 8—9 (1·5N-Na_2CO_3). After adding acetic acid (pH 4·5), the solution was freeze-dried. The product was purified by counter-current distribution (80 transfers of the upper phase, $K = 4{\cdot}0$, 152 mg), gel filtration on Bio-Gel P-4 (1M acetic acid, 80·5 mg) and P-2 (3M acetic acid, 68·5 mg). $[\alpha]_D$ −88·0° (*c* 0·21, 3M acetic acid): R_F 0·16 (S1), 0·36 (S2), 0·66 (S3). Amino acid analysis: Asp 1·00, Glu 1·03, Pro 1·01, Gly 0·97, Cys 1·78, Ile 0·98, Leu 1·02, Tyr(Me) 0·32, Tyr 0·57. For $C_{46}H_{69}.BrN_{12}O_{13}S_2.4\ H_2O$ (1214) calculated: 45·50% C, 6·39% H, 13·85% N; found: 45·88% C, 5·87% H, 13·44% N.

A sample of product *Ii* was dissolved in pyridine–acetate buffer (pH 5·7) and, after 4 h at room temperature, freeze-dried. In all chromatographic systems, two spots were observed: R_F 0·61 and 0·16 (S1), 0·36 and 0·12 (S2), 0·66 and 0·13 (S3), 0·68 and 0·53 (S4).

Pharmacologic Methods

Isolated rat uterus: The assay was performed on uterine strips obtained from adult virgin estrogen-treated rats of the Wistar strain. The uterine strips were placed in van Dyke-Hastings

solution[26] aerated with 95% O_2 + 5% CO_2 at 30°C. The Ca^{2+} concentration in the solution was 0·5 mM. Isometric contractions were registered by means of a magnetoelectric transducer[27]. The activity of the peptides was determined by the four-point test[28] or by comparing threshold doses; oxytocin was used for reference. The inhibitory effect of the analogues was estimated by measuring the shift of the log dose — response curves, and expressed by the inhibition constant according to[29].

Rat blood pressure: Pithed male rats were used for the assay[30]. The pressor activity of the peptides was compared with that of 8-lysine-vasopressin or oxytocin and determined by the four-point test or by comparing threshold doses. When inhibitory properties of the analogues were studied, they were applied before the dose of hormone. The amount of analogue necessary to decrease the submaximal pressor response to the hormone by 50% was determined.

Milk-ejecting activity was determined on lactating rats (5—14 days after parturition) according to[31]. The activity (with reference to oxytocin) was determined by the four-point test or by comparing threshold doses.

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