ANALOGUES OF OXYTOCIN WITH ESTERS OF GLUTAMIC ACID INSTEAD OF GLUTAMINE IN POSITION 4: SYNTHESIS OF A COMPOUND WITH HIGH AND SPECIFIC GALACTOGOGIC ACTIVITY*

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The reaction of [4-glutamic acid]deamino-1-carba-oxytocin (*Ib*) with diazoalkanes yielded the corresponding methyl ester *Ic* and ethyl ester *Id*. Alternatively, the methyl ester was prepared by means of carbodiimide or thionyl chloride, or by treating the caesium salt of compound *Ib* with methyl iodide. [2-O-Methyltyrosine, 4-glutamic acid methyl ester]deamino-1-carba-oxytocin (*Ih*) was also synthetized. The structure of the analogues was verified and their galactogogic and uteronic activities tested. Compound *Ic* had the highest galactogogic potency observed as yet (1307 I.U./mg).

A number of attempts have been made[1-3] to obtain oxytocin analogues** with high and specific galactogogic activity. All the modifications we performed in position 4 of the deamino-1-carba-oxytocin molecule resulted in compounds with lower biological activities[5]. Nevertheless, it seemed possible that this approach could lead to the preparation of a compound with a specific galactogogic effect. The highest specificity of the galactogogic effect was observed in the case of [4-glutamic acid]-deamino-1-carba-oxytocin[5] (*Ib*), whose ratio of galactogogic activity (assayed on rats *in vivo*) to uterotonic activity (*in vitro*) was equal to 39. However, the absolute value of galactogogic activity was rather low (39 I.U./mg; this represented about 6% of the activity of deamino-1-carba-oxytocin). We made an attempt to prepare a compound that would be selective as well as potent but did not find one with these properties in the series of [4-glutamic acid]deamino-1-carba-oxytocin amides[5]. We therefore decided to prepare the corresponding esters. In this case, the conformation of the peptide or its interaction with the receptor should not be influenced by the fact that the glutamic acid residue is easily ionised, as in compound *Ib*. The esters also have both the oxygen atoms in position γ of glutamic acid.

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** The nomenclature and symbols of amino acids and peptides were used according to published suggestions[4]. The amino acids used in this work were of L-configuration.

For the synthesis of the esters, as in the case of amides, we used compound *Ib* (ref.[6]). The quickest and mildest method of preparing esters is the reaction of a carboxylic acid with diazoalkanes. However, this procedure could be complicated in our case by the parallel or consecutive reaction of diazoalkane with the tyrosine hydroxyl[7] which would lead to the formation of a compound with lower biological activities and a different spectrum of effects[8] than could be expected if only the methylation of the carboxyl occurred. Nevertheless, we found that practically no product of two-fold methylation was formed if the reaction was run for a short period in methanol in the presence of phenol. After simple gel filtration, analogue *Ic* was obtained in good yield. On the other hand, if the yellow solution was left for 45 min at room temperature, a mixture of five substances was formed, none of which was identical with the product of the short-term reaction.

O-Methyltyrosine is demethylated to a certain degree during acid hydrolysis. However, after 20 h of hydrolysis, we never observed total regeneration of tyrosine. Therefore, the absence of O-methyltyrosine in the hydrolysate can be taken as proof that it was not present in the initial molecule. The absence of O-methyltyrosine in compound *Ic* was also checked by transforming it into the known compound deamino-1-carba-oxytocin[9] (*Ia*) by means of liquid ammonia or a methanolic solution of ammonia. In the first case, a number of side products were formed and in the second, the reaction was very slow; nevertheless, no [2-O-methyltyrosine]deamino--1-carba-oxytocin was formed, as could be seen by chromatographical comparison with the authentic sample[10]. The uterotonic activity of crude mixture after ammonolysis is slightly lower than that of deamino-1-carba-oxytocin; this can be attributed to the presence of side products in the mixture tested. The biological activity of an analogue containing O-methyltyrosine would be considerably lower. Moreover, the treatment of compound *Ic* with sodium hydroxide transforms it into acid *Ib*, and the dependence of the position of maximum extinction in the UV spectrum of compound *Ic* on the pH value of the solution indicates that a free hydroxyl group is present in the tyrosine residue.

We obtained the same results when we prepared ethyl ester *Id* by means of diazoethane. By contrast, if diphenylmethyl ester was prepared by means of diphenyldiazomethane, the reaction with carboxyl and hydroxyl proceeded at a comparable rate. After 3 h, a certain amount of the initial acid remained unchanged, and the remainder was transformed into two products in approximately equivalent proportions. Preliminary biological tests showed that neither of the products had significant activity, so that further attempts to prepare diphenylmethyl ester were not made.

We experimented with other possible ways of preparing compound *Ic*. Dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole was found to be sufficiently effective and mild. In the absence of the additive, the reaction resulted in an unidentified mixture of substances, in which the product *Ic* represented only

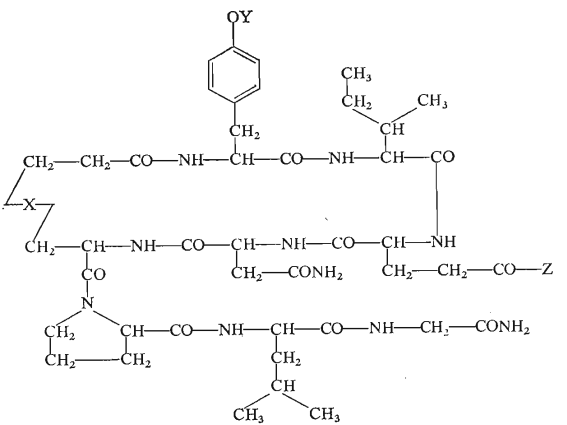
about 30% of the total amount. The reaction of acid *Ib* with methanol in the presence of thionyl chloride gave a mixture of substances in which product *Ic* represented 50%. A relatively mild method for preparing esters was the transformation of compound *Ib* into a caesium salt, which then reacted with methyl iodide, providing a methyl ester[11]. This procedure can be applied to compounds containing thioether grouping and it was used in the preparation of certain methionine peptides[12]. It is important not to exceed the equivalent amount of alkyl iodide used in the reaction. We anticipated that the carboxyl group would not react readily and that a concurrent reaction could occur, resulting in the formation of a sulfonium salt. We therefore performed the reaction by adding the alkyl iodide gradually to the solution of the caesium salt of compound *Ib*. Notwithstanding, the chromatographic analysis of the reaction product detected the presence of unchanged acid *Ib*, methyl ester *Ic* and another substance. By performing chromatography on Sephadex LH – 20 in dimethylformamide, we isolated all the components in pure state and found that the last compound contained iodide ions. Since the amount of the compound was small, we could not determine whether it was a sulfonium salt of acid *Ib* or of ester *Ic*.

We therefore investigated the behaviour of thioether sulfur in compounds of this type in the presence of methyl iodide in a model experiment, namely during the reaction of methyl iodide with deamino-1-carba-oxytocin (*Ia*). After 3 h the reaction mixture contained about 70% of the initial analogue *Ia*, after 20 h approximately 60% of the analogue was converted, and complete transformation into the methylsulfonium salt *Ie* was achieved only when the dimethylformamide solution of the two components (the alkyl iodide was present in a 15-fold molar excess) was kept for three days at room temperature. It follows that the formation of sulfonium salts is not very significant in the case of rapid reactions; moreover, the side product can be easily removed by gel filtration.

Our previous results showed[15] that the oxidation of the sulfur atom to a sulfoxide selectively increased the galactogogic effect of analogues of the 1-carba-oxytocin type. We therefore prepared sulfoxide *Ii* from methyl ester *Ic* by using sodium periodate in a water–methanol solution.

The O-methyltyrosine analogue *Ig* (or *Ih*) was prepared by acylating heptapeptide *IIa* (ref.[6]) with the active ester of tert-butyloxycarbonyl-O-methyltyrosine and the resulting octapeptide *IIb* was transformed into a *p*-nitrophenyl ester by means of bis(*p*-nitrophenyl) sulfite. After removing the protecting groups with trifluoroacetic acid, cyclization was performed in the usual way. The cyclization product was purified by two methods. One portion was filtered through a cation exchange column; the product contained about 20% of a compound reacting positively to ninhydrin and morin. Both compounds gave a sulfoxide-negative reaction[13]. The other portion was subjected to counter-current distribution. The product differed chromatographically from the two compounds obtained by the first purification procedure (it had lower R_F values in most solvent systems), and from the crude mixture. It was

found to contain sulfoxides and was therefore reduced by hydrogen bromide and acetone[14,15] and purified by gel filtration. The analogue *Ig* thus obtained was transformed into compound *Ih* by means of diazomethane.



Ia, X = CH_2—S, Y = H, Z = NH_2
Ib, X = CH_2—S, Y = H, Z = OH
Ic, X = CH_2—S, Y = H, Z = OCH_3
Id, X = CH_2—S, Y = H, Z = OC_2H_5
Ie, X = CH_2—$S^{(+)}(CH_3)I^{(-)}$, Y = H, Z = NH_2
If, X = CH_2—SO, Y = CH_3, Z = OH
Ig, X = CH_2—S, Y = CH_3, Z = OH
Ih, X = CH_2—S, Y = CH_3, Z = OCH_3
Ii, X = CH_2—SO, Y = H, Z = OCH_3

X—Ile—Glu(OBu^t)—Asn—Cys(C_3H_6COOH)—Pro—Leu—Gly—NH_2

IIa, X = H, *IIb*, X = Boc—Tyr(Me)

The analogues were tested for uterotonic activity *in vitro* and galactogogic activity *in vivo* (Table I). Methyl ester *Ic* had high galactogogic potency and relatively low uterotonic activity; the specificity of its action was higher than that of acid *Ib*. The activities of the ethyl ester *Id* were lower by one order of ten and its specificity was also lower. [4-Glutamic acid methyl ester]oxytocin[16], which contains a disulfide bond, had lower uterotonic activity; its synthesis or galactogogic activity were not

described. We determined the antidiuretic and pressoric potencies of analogue *Ic*; both values were very low (0·045 and 0·1 I.U./mg, respectively). Our results indicate that in order to obtain an analogue with high and specific galactogogic action, it is necessary to have a polar group in the region of the γ-carboxyl of glutamic acid in position 4; its ionization must be prevented by the presence of as small as possible group. A larger substituent probably affects the conformation of the molecule unfavourably, thus decreasing the activity. Considering that the galactogogic response to methyl ester *Ic* lasted only a short time, we made an attempt to prepare a compound with protracted action. One modification, which is known to produce this type of compound[8], is the substitution of the hydrogen atom of the tyrosine hydroxyl group by a methyl group. In the case of analogues *Ig* and *Ih*, protracted galactogogic action was observed, but the absolute values of the activities were low. The other two analogues, one with the sulfur atom oxidized to a sulfoxide (*Ii*) and the other transformed into a methylsulfonium salt (*Ie*), had considerably lower activities; nevertheless, the galactogogic activity was higher than uterotonic. The ratio of the two activities was different in the case of each analogue; for the methylsulfonium salt *Ie*, the ratio was equal to 2, whereas for the sulfoxide *Ii* it amounted to 55. Our hypothesis[15] was therefore confirmed within certain limits.

The results show that other modifications of this type could provide compounds with sufficiently high, selective and protracted action which could be used for clinical purposes.

TABLE I

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

Analogue	Rat Uterus (isolated)	Galactogogic (rat *in vivo*)
Ia	1 899	604
Ib	1·0	39·6
Ic	26·0	1 307
Id	5·7	132·7
Ie	20·0	47·0
Ig	0·1	0·5
Ih	0·2	2·0
Ii	0·2	11·3

EXPERIMENTAL

Samples for elemental analysis were dried for 24 h *in vacuo* (150 Pa) at room temperature. Silica gel sheets (Silufol, Kavalier) were used for thin layer chromatography in the following solvent systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% ammonium hydroxide–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), and pyridine–1-butanol–acetic acid–water (10 : 15 : 3 : 6) (S4). Electrophoresis was performed in a moist chamber apparatus on Whatman 3MM paper, using 1M-acetic acid (pH 2·4) or pyridinium acetate (pH 5·7) buffers. In most cases, the electrophoresis was run 1 h at a potential gradient of 20 V/cm. The substances were detected by ninhydrin or by the chlorination method. Amino acid analyses were performed after 20 h of hydrolysis (6M-HCl, 105°C, 150 Pa), using an automatic analyser (Development Workshops Czechoslovak Academy of Sciences; type 6020). The reaction mixtures were evaporated using a rotary evaporator at bath temperature of 30—40°C. When the mixtures contained dimethylformamide, they were evaporated by means of an oil pump. The melting points were determined on a Kofler block and the values were not corrected. Counter-current distribution was performed in an all-glass apparatus in which it was possible to transfer the upper and lower phases (Counter-current Distribution Machine, Quickfit & Quartz, Stone Staffordshire, England). Peptide material was detected according to the Folin-Ciocalteau method. Gel filtration was performed on columns of Bio-Gel P—2 and P—4 (Bio-Rad Laboratories, Richmond, USA) and Sephadex LH—20 (Pharmacia, Uppsala, Sweden). Peptide material was detected by its absorption at 280 nm. Optical rotation was measured by a Perkin Elmer 141 MCA apparatus.

[4-γ-Glutamic Acid Methyl Ester]deamino-1-carba-oxytocin (*Ic*)

a) To the solution of [4-glutamic acid]deamino-1-carba-oxytocin[6] (*Ib*) (23 mg) and phenol (10 mg) in methanol (0·4 ml), a freshly prepared solution of diazomethane in ether was added by drops until the yellow colour remained stable for 1 min. The mixture was then decolourized by adding acetic acid and the product was precipitated with ether. After precipitation from methanol with ether, the product was dissolved in methanol (0·3 ml) and 3M acetic acid (3 ml) and applied to a column of Bio-Gel P—4 (100 × 1 cm). Lyophilization of the fraction containing the product yield the pure compound (19 mg; 80%); R_F 0·32 (S1), 0·29 (S2), 0·41 (S3) and 0·67 (S4); $[\alpha]_D$ —64·3° (*c* 0·14, 1M acetic acid). For $C_{45}H_{68}N_{10}O_{13}S.\ C_2H_4O_2.3\ H_2O$ (1103) calculated: 51·15% C, 7·12% H, 12·70% N; found: 51·23% C, 6·84% H, 12·90% N. Amino acid analysis: Asp 1·03, Glu 1·00, Pro 1·02, Gly 0·98, Ile 1·00, Leu 1·02, Tyr 0·98, Cys(C_3H_6COOH) 0·97.

b) To the solution of analogue *Ib* (5 mg) and 1-hydroxybenzotriazole (13·5 mg) in methanol (0·3 ml) at 0°C, dicyclohexylcarbodiimide (20·6 mg) was added. The mixture was kept for 1 h at 0°C and for 20 h at room temperature, filtered, diluted by 3M acetic acid (2 ml) and applied to a column of Bio-Gel P—4. Lyophilization of the fraction containing peptide material produced 4 mg of a compound chromatographically identical with the product of reaction *a*), together with approximately 10% of a compound with a lower R_F value (0·22 (S1) and 0·22 (S2)).

c) The reaction was performed as sub *b*) but without adding 1-hydroxybenzotriazole. The resultant mixture contained four compounds, out of which methyl ester *Ic* represented about 30%. Approximately the same amount was identical with the side product of reaction *b*).

d) Analogue *Ib* (5 mg) was dissolved in a mixture of methanol (0·2 ml) and water (0·1 ml) and the pH value of the solution was adjusted by 0·16M caesium hydroxide (31 µl) to 7. After evaporation, the product was dissolved twice in dimethylformamide (0·2 ml) and evaporated.

To the solution of the caesium salt in dimethylformamide (0·25 ml), a dimethylformamide solution of methyl iodide (71 mg/ml) was added in 3 min intervals in 1 μl portions (total amount 10 μl). The mixture was then left for 90 min at room temperature. After evaporation and precipitation from methanol and ether, the product was dissolved in dimethylformamide (1 ml) and transfered to a column of Sephadex LH—20 (200 × 1 cm). After the evaporation of the fractions containing a compound with the smallest elution volume and precipitation from methanol and ether 2·3 mg of pure methyl ester *Ic* was obtained. Apart from this, two compounds with a larger elution volume were obtained; one of which was the initial acid *Ib* and the other was characterized by semiquantitative determination of iodide ions, as a sulfonium salt of compound *Ib* or *Ic*.

e) Analogue *Ib* (5 mg) was suspended in thionyl chloride (0·15 ml) and after 5 min the suspension was diluted with methanol (0·3 ml). After a further 16 min, the product was precipitated with ether and reprecipitated several times from methanol and ether. Chromatographic analysis of the product showed that it contained only 5% of methyl ester *Ic*, and that the rest was identical with the side product of reaction *b*).

f) The analogue *Ib* (5 mg) was dissolved in methanol (0·3 ml) and at —60°C thionyl chloride (0·1 ml) was added by drops. During 30 min the mixture was warmed to room temperature, the solution was treated as stated under *e*). TLC revealed the presence of the same compounds as described sub *e*), but in a 1 : 1 ratio.

g) Analogue *Ib* (0·6 mg) was dissolved in methanol (0·2 ml), a solution of diazomethane in ether (2 ml) was added and the resulting yellow solution was kept at room temperature for 45 min. The chromatography of the reaction product revealed the presence of five compounds none of which were identical with methyl ester *Ic* obtained by reaction *a*).

[4-Glutamic Acid γ-Ethyl Ester]deamino-1-carba-oxytocin (*Id*)

To the solution of compound *Ib* (7 mg) and phenol (10 mg) in methanol (0·4 ml) we added a solution of diazoethane in ether until the yellow colour persisted for 1 min. The solution was decolourized by the addition of acetic acid and the product was precipitated with ether. After the precipitation from methanol with ether the analogue was purified by gel-filtration analogously to methyl ester *Ic*. The yield was 5·1 mg, R_F 0·43 (S1), 0·35 (S2), 0·45 (S3) and 0·68 (S4). For $C_{46}H_{70}N_{10}O_{13}S.C_2H_4O_2.2\,H_2O$ (1099) calculated: 52·44% C, 7·15% H, 12·74% N; found: 52·13% C, 6·82% H, 12·83% N. Amino-acid analysis: Asp 1·05, Glu 1·03, Pro 0·98, Gly 1·03, Ile 1·01, Leu 1·06, Tyr 0·91, Cys(C_3H_6COOH) 0·96.

Deamino-1-carba-oxytocin (*Ia*)

a) The solution of methyl ester *Ic* (1 mg) in liquid ammonia (0·3 ml) was kept for 20 min at room temperature in a stoppered test-tube. Ammonia was evaporated and the product was precipitated from methanol and ether. TLC analysis revealed the presence of required product *Ia* (~60%), unreacted ester *Ic* (~10%) and five more unidentified compounds. Chromatographical comparison with [2-O-methyltyrosine]deamino-1-carba-oxytocin[10] demonstrated the absence of a methylated compound in *Ia*. The uterotonic activity of this unpurified product amounted to about 40% of oxytocin activity.

b) Methyl ester *Ic* (2 mg) was dissolved in methanol saturated with ammonia (at 20°C) (0·2 ml). After two days of standing at room temperature, about 20% conversion to compound *Ia* (TLC analysis) occurred. Glycerol (2 μl; *cf.*[17]) was added to the mixture and after 4 days of standing at room temperature the reaction mixture was evaporated and precipitated from methanol

with ether. TLC analysis showed the presence of 60% of methyl ester *Ic* and 40% of the amide *Ia*. The uterotonic activity of this mixture was nearly the same as that of oxytocin.

Tert-butyloxycarbonyl-O-methyltyrosyl-isoleucyl-γ-tert-butyl-glutamoyl-asparaginyl-
-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycine Amide (*IIb*)

To the suspension of free heptapeptide[6] *IIa* (0·4 g) in dimethylformamide (15 ml), N-ethylpiperidine (90 μl) and N-tert-butyloxycarbonyl-O-methyltyrosine 2,4,5-trichlorophenyl ester (0·35 g) were added. After 20 h of stirring all went in the solution and further portions of N-ethylpiperidine (60 μl) and active ester (0·2 g) were added. After 25 h of stirring the mixture was evaporated, triturated with ether, the solid part was collected and washed with ether and water. The yield was 0·51 g (97%) of the title compound, m.p. 212—213°C, R_F 0·64 (S1), 0·47 (S2), 0·70 (S3), 0·79 (S4); $E_{5.7}^{His}$ 0·00, $E_{2.4}^{Gly}$ 0·53 (electrophoresis was performed with a sample which had been treated for 1 h with trifluoroacetic acid). The sample for analysis was reprecipitated from dimethylformamide with water, without a change in the m.p.; $[\alpha]_D$ —40·8° (*c* 0·2, dimethylformamide). For $C_{54}N_{86}N_{10}O_{16}S.3\,H_2O$ (1217) calculated: 53·29% C, 7·62% H, 11·51% N; found: 53·09% C, 7·23% H, 11·16% N. Amino-acid analysis: Asp 1·05, Glu 0·99, Pro 1·00, Gly 1·04 Ile 0·95, Leu 1·06, Tyr + Tyr(Me) 0·95, Cys(C_3H_6COOH) 1·09.

Lactam of O-Methyltyrosyl-isoleucyl-glutamoyl-asparaginyl-
-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*Ig*)

To the solution of octapeptide *IIb* (350 mg) in dimethylformamide (13 ml) and pyridine (13 ml) bis(*p*-nitrophenyl) sulfite (1 g) was added under nitrogen. After 8 h at room temperature 1 g of sulfite and after 16 h another 0·5 g portion of sulfite were added; 4 h later the mixture was evaporated, the residue was triturated with ether, collected, washed with ether, water and dried (347 mg). This compound was dissolved in trifluoroacetic acid (10 ml) and after standing for 1 h at room temperature the solution was diluted with toluene (10 ml) and evaporated. The residue was triturated with ether, dried and dissolved in dimethylformamide (10 ml). The solution was added, at a rate of 3·2 ml/h, to the mixture of pyridine (250 ml) and N-ethylpiperidine (50 μl), at 50°C under nitrogen. The solution was stirred 3 h at 50°C and 15 h at room temperature, evaporated and the residue was precipitated from dimethylformamide with ether. For further working up the product was divided in two parts:

a) One part (100 mg) was dissolved in a mixture of methanol (0·5 ml) and water (3 ml) and transferred to a column of Dowex 50 (H^+-cycle; 5 ml). The product was eluted with water and freeze-dried. An oily product was obtained which was precipitated from methanol with ether (56 mg). According to TLC it contained about 20% of ninhydrin- and morin-positive compound, with lower R_F. The test for the presence of sulfoxides[13] was negative.

b) Another part (180 mg) was purified by means of counter-current distribution in a solvent system of 2-butanol–0·05% aqueous acetic acid (1 : 1). After 100 transfers of the upper phase two peaks were found. One, with $K = 1·3$ (tubes 48—66) contained only ninhydrin-positive compounds and was not studied further. The peak with $K = 6$ (tubes 75—99) was concentrated to a small volume and lyophilized. The product which was obtained (98 mg) gave a positive test for sulfoxides; R_F 0·23 (S1), 0·05 (S2), 0·21 (S3), 0·63 (S4). Part of this compound (75 mg) was suspended in acetone (2 ml) and HBr in acetic acid (35%, 3 ml) was added. After 5 min at room temperature the solution was evaporated, the residue dissolved in acetone (2 ml) and evaporated. The remnant was dissolved in a mixture of methanol (0·5 ml) and 3M acetic acid (4 ml) and purified on a Bio-Gel P—4 column. Lyophilization of the main peak afforded 62 mg

of the product, identical with the main product from procedure *a*) and negative in the sulfoxide-test. R_F 0·31 (S1), 0·06 (S2), 0·34 (S3), 0·63 (S4). $[\alpha]_D$ −60·5° (*c* 0·1, 3M acetic acid). For $C_{45}H_{68}N_{10}O_{13}S.C_2H_4O_2.2\,H_2O$ (1085) calculated: 52·02% C, 7·06% H, 12·91% N; found: 52·23% C, 6·71% H, 12·94% N. Amino-acid analysis: Asp 1·01, Glu 0·99, Pro 0·95, Gly 1·03, Ile 0·99, Leu 1·03 Tyr + Tyr(Me) 1·02, Cys(C_3H_6COOH) 0·99.

Lactam of O-Methyltyrosyl-isoleucyl-γ-methylglutamoyl-asparaginyl-S-(γ-carboxypropyl)cysteinyl-prolyl-leucyl-glycinamide (*Ih*)

To the solution of cyclopeptide *Ig* (25 mg) in methanol (1·3 ml), a solution of diazomethane in ether was added until the yellow colour persisted for 1 min. The solution was decolourized by the addition of acetic acid and the product was precipitated with ether. Reprecipitation from methanol with ether afforded 21 mg of the title compound with R_F 0·32 (S1), 0·28 (S2), 0·40 (S3) 0·67 (S4). $[\alpha]_D$ −77·3° (*c* 0·1, 1M acetic acid). For $C_{46}H_{70}N_{10}O_{13}S.C_2H_4O_2.2\,H_2O$ (1099) calculated: 52·44% C, 7·15% H, 12·74% N; found: 52·19% C, 6·76% H, 12·80% N. Amino-acid analysis: Asp 1·00, Glu 0·96, Pro 1·00, Gly 1·01, Ile 1·01, Leu 1·06, Tyr + Tyr(Me) 1·05, Cys(C_3H_6COOH) 0·87.

Sulfoxide of [4-Glutamic Acid γ-Methyl Ester]deamino-1-carba-oxytocin (*Ii*)

To the solution of compound *Ic* (5 mg) in a mixture of methanol (20 μl) and water (400 μl), sodium periodate (2·5 mg) was added. After 3 h at room temperature the mixture was applied to a column of Bio-Gel P—4 in 3M acetic acid. The main peak of peptide material was freeze-dried; the yield was 2·8 mg of the product with R_F 0·24 (S1), 0·16 (S2). $[\alpha]_D$ −45·9° (*c* 0·1, 1M acetic acid). For $C_{45}H_{68}N_{10}O_{14}S.4\,H_2O$ (1077) calculated: 50·17% C, 7·11% H, 13·00% N, found: 49·88% C, 6·79% H, 12·95% N. Amino-acid analysis: Asp 1·02, Glu 0·99, Pro 0·82 Gly 1·05, Ile 0·98, Leu 1·02, Tyr 0·87, Cys(C_3H_6COOH) 0·52.

Methoiodide of Deamino-1-carba-oxytocin (*Ie*)

To the solution of deamino-1-carba-oxytocin (*Ia*) (19 mg) in dimethylformamide (1 ml), methyl iodide (50 μl) was added. After standing for 3 days at room temperature the product was precipitated by the addition of ether and reprecipitated from methanol with ether. This material was dissolved in dimethylformamide and purified on a column of Sephadex LH—20. The peak containing peptide material was evaporated and precipitated from methanol with ether. Yield, 17 mg; R_F 0·35 and 0·05 (S1), 0·32 and 0·00 (S2), 0·41 and 0·09 (S3) and 0·54 (S4). For $C_{45}H_{70}IN_{11}O_{12}S.6\,H_2O$ (1223) calculated: 44·15% C, 6·75% H, 12·59% N; found: 43·80% C, 5·98% H, 12·76% N. Amino-acid analysis: Asp 1·01, Glu 0·99, Pro 1·09, Gly 1·01, Ile 0·99, Leu 0·99, Tyr 0·92, Cys(C_3H_6COOH) 0·25.

Pharmacological Methods

Uterotonic activity was determined on an isolated strip of rat uterus[18,19] placed in Mg^{2+}-free solution. Galactogogic activity[3,20] was assayed on lactating rats (5—10 days after delivery).

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Note added in proof: The synthesis of [4-glutamicacid methyl ester]oxytocin and its deamino derivative has been described: Photaki I., Tzougraki C., Kotsira-Engonopoulos C.: Int. J. Peptide Protein Res. *13*, 426 (1979).