SOLID PHASE SYNTHESIS AND BIOLOGICAL ACTIVITIES OF OXYTOCIN CARBA ANALOGUES CONTAINING THREONINE IN POSITION 4*

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Solid phase methodology was developed for the synthesis of carba-analogues of oxytocin. Two known compounds (deamino-1-carba-oxytocin (*I*) and deamino-6-carba-oxytocin (*II*)) and two new analogues ([4-threonine]deamino-1-carba-oxytocin (*III*) and [4-threonine]deamino-6-carba-oxytocin (*IV*)) were synthesized using different approaches. The latter two compounds were found to possess high biological activity in the rat uterotonic (*in vitro* and *in vivo*) and galactogogic (*in vivo*) assays.

Carba-analogues of neurohypophyseal hormones** were designed twenty years ago[2] to prove that the disulfide bridge of these hormones is not important as a functional group for biological activity, but rather as a structural element holding the proper spatial arrangement of the molecules. Since that time many carba-analogues of the neurohypophyseal hormones (vasopressin, oxytocin, vasotocin) have been synthesized, and in addition carba-analogues also have been found to be highly active for a number of other hormones (somatostatin, calcitonin, insulin) (for the list of references see[3,4]). Syntheses of these compounds were performed by solution phase techniques and in comparison with solid-phase methodology are much more time consuming. In order to improve the accessibility of carba-analogues of peptide hormones we have decided to develop the solid phase method for their synthesis, and report here the solid phase synthesis of carba analogues of oxytocin[5].

As standards for the trial syntheses we used deamino-1-carba and deamino-6-carba-oxytocin (*I* and *II*) because their solution syntheses have been well established[6-8] and we had the purified compounds for comparison. Basically, two approaches can be chosen for the syntheses of carba-cyclic analogue on the polymer support – *a*) cyclization in solution after the cleavage of the peptide from the resin, and *b*) cyclization on the polymeric support. Additional options can be developed

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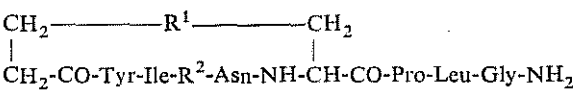
** All the chiral amino acids, mentioned in this work, are of the L-series. The nomenclature and symbols of the amino acids and peptides obey the published recommendations[1].

by using different protecting groups for the amino groups of the growing peptide chain, different side chain carboxylic acid protecting groups and different kinds of attachment to the solid support.

In the synthesis of deamino-1-carba-oxytocin (*I*) we utilized benzhydrylamine resin and Boc protecting groups for α-amino protection. The side chain carboxylic acid group on the modified cysteine residue (S-carboxypropylcysteine) was protected as methyl ester. Protected amino acids were coupled by N,N'-dicyclohexylcarbodiimide (DCC) with the addition of N-hydroxybenzotriazole (HOBt). For the coupling of Boc-Asn and Boc-Gln, we used *p*-nitrophenyl esters with the addition of HOBt as catalyst. After assembling the linear resin-peptide, we tried to hydrolyze the methyl ester protecting group using 10% 1 mol l^{-1} KOH in dimethylformamide. Samples were removed after 2 and 20 min and 2 and 20 h, and the peptide was cleaved from the resin by treatment with liquid hydrogen fluoride. This study showed that even after 20 h hydrolysis, the methyl ester was no more than 10% hydrolysed; however, a large number of side products appeared (according to thin-layer chromatographic and electrophoretic evaluation). Therefore, all the product was cleaved by hydrogen fluoride and hydrolysis of methyl ester was performed in aqueous solution. The free peptide obtained was cyclized according to the method of Krojidlo and coworkers[9] and purified by gel filtration and reversed-phase high performance liquid chromatography. Its identity with the standard was proven by comparing its chromatographical (TLC, HPLC), spectroscopical (^{1}H NMR) and pharmacological (uterus *in vitro*) properties.



I, $R^1 = CH_2—S$, R^2 = Gln *II*, $R^1 = S—CH_2$, R^2 = Gln
III, $R^1 = CH_2—S$, R^2 = Thr *IV*, $R^1 = S—CH_2$, R^2 = Thr

In the synthesis of deamino-6-carba-oxytocin (*II*) we utilized the Merrifield resin and Fmoc group[10] for the protection of α-amino group from the fourth to seventh step of the synthesis. The side chain carboxylic group was protected as tert-butyl ester. Boc-Leu, Boc-Pro, Fmoc-Hcy($C_2H_4COOBu^t$), Fmoc-Asn, Fmoc-Gln, Fmoc-Ile and Boc-Tyr(Bu^t) were coupled to Boc-Gly-resin by the DCC/HOBt procedure. Fmoc groups were cleaved by 50% piperidine in dichloromethane and after the last step all protecting groups were removed by treatment with trifluoroacetic acid and cyclization was performed on the resin with the use of DCC and HOBt. The cyclic peptide was cleaved from the resin by ammonolysis and purified by reversed-phase high performance liquid chromatography. The product was identical with deamino-6-carba-oxytocin.

A modification of the first synthesis was used in the preparation of [Thr^4]deamino-1-carba-oxytocin (*III*). In this case the peptide was cleaved from the resin at the heptapeptide stage by the action of hydrogen fluoride, and after hydrolysis of the methyl ester, the free peptide was acylated by an active ester of protected tyrosine. Cyclization was performed in methanolic solution after transformation of the free carboxylic group to the N-hydroxybenzotriazolyl ester. After the purification by gel filtration and liquid chromatography an analogue was obtained, the activities of which are given in Table I.

Synthesis of [Thr^4]deamino-6-carba-oxytocin (*IV*) was accomplished with the free carboxylic acid group on the side chain of the modified homocysteine residue, and using symmetrical anhydrides for coupling. Boc-Gly, Boc-Leu, Boc-Pro and Boc-Hcy($C_2H_4COOBu^t$) (ref.[4]), were coupled to the benzhydrylamine resin by the DCC/HOBt procedure, then the α-amino and side chain protecting groups were cleaved simultaneously by trifluoroacetic acid, and Boc-Asn-ONp was coupled with HOBt catalysis. Boc-Thr(Bzl), Boc-Ile and Boc-Tyr were coupled as symmetrical anhydrides formed immediately prior to use. The resin octapeptide was divided in two parts. One part was treated with trifluoroacetic acid, and cyclization was performed on the resin by the action of DCC and HOBt. Then the product was cleaved from the resin by liquid hydrogen fluoride. The other part was first treated with hydrogen fluoride and the peptide was cyclized in methanolic solution as described above. The first approach to cyclization (on the polymeric support) gave a slightly higher yield (12·6% *vs* 9·7%), and the product was homogeneous even after a single gel filtration. The product of the solution cyclization was slightly contaminated by the sulfoxide and had to be purified by liquid chromatography. Both methods also were used for the synthesis of deamino-6-carba-oxytocin (*II*).

These results suggest that the method of choice for the synthesis of carba-analogues of neurohypophyseal hormones is *via* symmetrical anhydrides and an unprotected side chain carboxylic acid group, followed by cyclization on the resin.

TABLE I

Values of capacity factor for carba analogues (k'_S) and respective sulfoxides (k'_{SO}) on Vydac C 18 in 25% acetonitrile in 0·1% trifluoroacetic acid

Compound	k'_S	k'_{SO}
I	4·45	3·19
II	2·90	2·25
III	5·36	2·47 + 3·26
IV	5·15	2·53 + 2·89

In the case of cyclo carba-analogues of MSH this approach led to a much lower yield[4]. Thus it would appear that the method of choice will depend on the nature of the cyclic peptide to be prepared.

During the chromatographic analysis of these carba analogues of oxytocin we observed quite an unusual behavior of analogues *III* and *IV* in comparison with compounds *I* and *II* (Table I). When analogues contain threonine in position 4 (*III* and *IV*), the difference between 1-carba and 6-carba analogues is much less pronounced than is the case for the glutamine containing compounds (*I* and *II*). The separation of diastereoisomeric sulfoxides has been described for 1-carba analogues, but has not been observed in the case of 6-carba analogues[11]. In the case of the sulfoxide of analogue *I*, separation of the diastereoisomers was not achieved in an acidic mobile phase[11]. The diastereomeric sulfoxide derivatives of *III* and *IV* can be chromatographically separated, but the difference between the diastereoisomers is in the case of *IV* much less pronounced then in the case of sulfoxides made from *III*. These results suggest that [4-threonine]carba analogues interact with the stationary phase differently. In this regard, the unusual temperature dependence of the ^{1}H NMR chemical shift of the threonine peptide amide[12] has been explained as caused by conformational differences in solution of Thr^4-containing analogues of oxytocin.

The biological activities of the oxytocin analogues are given in Table II. Substitution of glutamine by threonine in position 4 of oxytocin led to substantial increases

TABLE II

Biological activities (I.U./mg) of some oxytocin analogues determined in rats

Compound[a]	Uterotonic		Milk ejecting	Vasopressor
	in vitro	*in vivo*		
Oxytocin	450	450	450	3
[Mpa^1]oxytocin[b]	795	900	266·5	1·4
[Thr^4]oxytocin[c]	923	—	543[h]	0·43
[Mpa^1, Thr^4]oxytocin[c]	149	—	385[h]	0·1
[Hmp^1]oxytocin[d]	1 275	—	694	14·7
[Hmp^1, Thr^4]oxytocin[d]	4 179	—	808	4·92
1-Carba-oxytocin[e,f]	734	120	142	2·95
Deamino-1-carba-oxytocin[f,g] (*I*)	1 898	1 250	604	17·5
Deamino-6-carba-oxytocin[f,g] (*II*)	929	2 792	456	1·5
[Thr^4]deamino-1-carba-oxytocin (*III*)	272	556	409	1·3
[Thr^4]deamino-6-carba-oxytocin (*IV*)	695	1 057	692	1·1

[a] Mpa 3-mercaptopropanoic acid, Hmp L-2-hydroxy-3-mercaptopropanoic acid; [b] ref.[15]; [c] ref.[16]; [d] ref.[17]; [e] ref.[18]; [f] ref.[19]; [g] ref.[20]; [h] rabbit.

in the uterotonic activity and the same was true in the case of oxytocin analogues containing an α-hydroxy group instead of a primary amino group. In the case of deamino-oxytocin the introduction of threonine had the opposite effect. However, this substitution can improve the selectivity of oxytocin-like activities *vs* vasopressin-like activities. The effect of combining the Thr^4 substitution with other structural modifications is somewhat unpredictable and therefore we decided to examine the effect of simultaneous introduction of a carba bridge and deamination in position 1. As can be seen (Table II), the new analogues are highly potent in typical oxytocin assays, but the introduction of Thr^4 led to a lowering of the potency relative to deamino-1-carba- (*I*) and deamino-6-carba-oxytocin (*II*). The only exception is the milk ejecting activity of [Thr^4]-deamino-6-carba-oxytocin (*IV*) which is higher than that of the Gln^4-containing compound *II*. The difference between 1-carba and 6-carba substitution is evident again in that the decrease of the *in vitro* uterotonic activity resulting from Thr^4 substitution is much more pronounced in the 1-carba than in the 6-carba series. The pressor activity was influenced in the same manner. Milk ejecting activity was decreased in the 1-carba analogue *III* but increased in the 6-carba analogue *IV*. In examining the *in vivo* uterotonic activity we observed an unusual time course for the biological response of the 6-carba analogue *IV*. All carba analogues of this series have protracted activity[13], but analogue *IV* shows a completely normal time course of response. The reason for the prolonged activity of 6-carba analogues is not clear[13], but it had been shown in some cases that it has nothing to do with stability towards enzymatic cleavage[14].

EXPERIMENTAL

General methods: Capillary melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Silufol plates (Kavalier, Czechoslovakia) using the following solvent systems: A) 1-butanol–acetic acid–water (4 : 1 : 5, upper phase only); B) 1-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12); C) 1-butanol–pyridine–acetic acid–water (5 : 5 : 1 : 4); D) 2-propanol–25% aqueous ammonia–water (3 : 1 : 1). Detection was by iodine vapors and ninhydrin. ^{1}H NMR spectra were recorded on a Bruker 250 MHz spectrometer. Electrophoresis was performed on Whatman 1 chromatography paper using an instrument by Gelman with 0·1 mol l^{-1} pyridinium acetate pH 5·3 as the solvent and a potential drop of 20 V/cm. Detection utilized ninhydrin and chlorination methods. Optical rotations were obtained on a Perkin–Elmer 241 MC polarimeter at the sodium yellow line (529 nm). Amino acid analysis was obtained with an automatic analyzer (Development Workshops, Czechoslovak Academy of Sciences, Prague; type 6020) following hydrolysis for 20 h at 105°C in 6 mol l^{-1} HCl. No corrections were made for destruction of amino acids during hydrolysis. High pressure liquid chromatography (HPLC) was performed on a Spectra-Physics SP-8700 instrument equipped with an SP-8400 detector, using a Vydac C 18 column (25 × 0·4 cm) for analytical analysis and a Waters RCM column (15 × 0·8 cm) for preparative work. We used 0·1% trufluoroacetic acid as the buffer and acetonitrile as the organic modifier. N^{α}-Boc-protected amino acids and amino acid derivatives were purchased from Vega Biochemicals (Tucson, U.S.A.) or Bachem (Torrence, U.S.A.). Before use, all amino-acid derivatives were subjected

to a ninhydrin test[21] to establish their purity. The *p*-methylbenzhydrylamine resin (1% divinylbenzene cross-linked polystyrene) was prepared by previously reported methods[22].

Solid-phase peptide synthesis: A cycle for incorporating each amino acid residue into the growing peptide chain consisted of the following: *1*) washing with dichloromethane (4 . 25 ml, 1 min/wash); *2*) cleaving the Boc group by adding 25 ml of 50% trifluoroacetic acid in dichloromethane containing 2% anisole, one treatment for 2 min, a second for 20 min; *3*) washing with dichloromethane (4 . 25 ml, 1 min/wash); *4*) neutralizing by adding 10% diisopropylethylamine in dichloromethane (2 . 25 ml, 2 min/wash); *5*) washing with dichloromethane (3 . 25 ml, 1 min/wash); *6*) adding the Boc-protected amino acid derivative in 20 ml of dichloromethane followed by HOBt (dissolved in a minimal amount of dry dimethylformamide), followed by DCC and shaking for 3·5—12 h; *7*) washing with dichloromethane (3 . 25 ml, 1 min/wash); *8*) washing with ethanol (3 . 25 ml, 1 min/wash); *9*) washing with dichloromethane (4 . 25 ml, 1 min/wash). Between steps *6* and *7*, several milligrams of the resin were removed and used in a ninhydrin test to determine the progress of the coupling. In some cases symmetrical anhydrides or active esters were used in step *6*. When Fmoc-protecting group was used the procedure published by Colombo[23] was followed. Peptides were cleaved from the resin with anhydrous liquid hydrogen fluoride (0°C for 60 min) containing 10% anisole and 5% 1,2-ethanedithiol[24].

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

Benzhydrylamine resin (Pierce, 2% divinylbenzene, 0·4 mmol/g, 4·88 g) was suspended in dichloromethane and Boc-amino acids were coupled to the resin by the DCC/HOBt procedure. All reagents were used in 3 molar excess and coupling was monitored by the ninhydrin test[21]. For coupling of Boc-Asn and Boc-Gln, *p*-nitrophenyl esters with addition of N-hydroxybenzotriazole were employed. Amino acids were coupled in the following order: Boc-Gly, Boc-Leu, Boc-Pro, Boc-Cys($C_3H_6COOCH_3$), Boc-Asn (peptide-resin A).

At this point the resin was dried and additional couplings were made with only half of the resin. After the coupling of Boc-Gln and Boc-Ile, the resin was split again (peptide-resin B) and Boc-Tyr(2,6-Cl_2-Bzl) was coupled (peptide-resin C). Part of this peptide-resin (0·38 g) was treated with 10% 1 mol l^{-1} KOH in dimethylformamide. Approximately 10 mg of the resin was taken out after 2, 22, 142, and 1 253 min. Samples were washed by dimethylformamide, dichloromethane and dried. The peptide was then cleaved from the resin by liquid hydrogen fluoride (5 ml, 45 min, 0°C) in the presence of 1,2-ethanedithiol (0·1 ml) and anisole (0·2 ml). The product was extracted by 30% acetic acid, lyophilized and analyzed by thin-layer chromatography (5 systems) and electrophoresis (pH 5·4). Only for the sample treated with base for the longest time did we observe traces (< 10%) of compound with a hydrolyzed methyl ester group.

Peptide-resin C (0·29 g) was treated with liquid hydrogen fluoride (10 ml, 45 min, 0°C) in the presence of 1,2-ethanedithiol (0·4 ml) and anisole (0·8 ml). The product was extracted by 30% acetic acid and lyophilized. The lyophilizate (36 mg) was dissolved in water (4 ml) and the pH of the solution was brought to 12·5 by addition of 1 mol l^{-1} KOH. After 30 min mixing at room temperature, the pH of the solution was brought to 2·3 by 5% HCl and solution was transferred to a Dowex 50 (3 ml, H^+ cycle) column. The column was washed with water until the reaction for Cl^- ions was negative and the product was eluted with 15% pyridine. After lyophilization, the peptide was dissolved in 1 mol l^{-1} HCl and lyophilized again. The hydrochloride was dissolved in dimethylformamide (1 ml), the solution cooled to 0°C, and N-hydroxybenzotriazole (50 mg) and dicyclohexylcarbodiimide (52 mg) were added. The mixture was stirred for 1 h at 0°C and 1 h at room temperature and filtered to the stirred solution of 50 µl of diisopropylethylamine in methanol (40 ml). After 30 min mixing at room temperature, the bulk of the solvent

was evaporated *in vacuo* and product was precipitated by addition of ether, filtered, dried, dissolved in 0·2 mol l^{-1} acetic acid and applied to a Sephadex G-25 column. Elution was performed by 0·2 mol l^{-1} acetic acid and 28 mg (34% overall yield) of product was obtained. Part of the product was further purified by HPLC (Vydac C-18, 25 × 0·4 cm, gradient from 30% methanol in 0·05% trifluoroacetic acid to 79% methanol in 30 min, flow 1 ml/min) with a recovery of 44%. The product was compared with the peptide prepared previously by solution techniques. It was found identical by thin-layer chromatography (R_F 0·27 (A), 0·71 (B), 0·46 (C), 0·42 (D), HPLC ($k' = 4·76$; Vydac C-18 38% methanol in 0·1% trifluoroacetic acid), and 250 MHz ^{1}H NMR spectroscopy (to be published separately).

[4-Threonine]deamino-1-carba-oxytocin (*III*)

Peptide-resin A (2 g) was treated with trifluoroacetic acid and Boc-Thr(Bzl) and Boc-Ile were coupled by the standard procedure. The heptapeptide was cleaved from the resin by liquid hydrogen fluoride (20 ml, 0°C, 45 min) in the presence of 1,2-ethanedithiol (1·5 ml) and anisole (3 ml). After washing with ethyl acetate, the peptide was extracted by 30% acetic acid and lyophilized. The lyophilizate (233 mg; 36% yield) was dissolved in water (10 ml) and the pH of the solution was made 12·5 by addition of 1 mol l^{-1} NaOH. After 30 min of mixing at room temperature, the pH was made 3 by addition of 5% HCl and solution was introduced on Dowex 50 (10 ml, H^+ cycle) column. The column was washed with water, and the product eluted by 15% pyridine and lyophilized.

Boc-Tyrosine (168 mg) was dissolved in dimethylformamide (2 ml), and, after addition of N-hydroxybenzotriazole (81 mg) and cooling to 0°C, dicyclohexylcarbodiimide (103 mg) was added. After mixing for 1 h at 0°C and 2 h at room temperature, the mixture was filtered and the solution added to the lyophilizate which had been dissolved in dimethylformamide (2 ml). The solution was stirred for 35 h at room temperature and the product was precipitated by addition of ether, filtered, washed with ether, dried *in vacuo* and dissolved in a mixture of trifluoroacetic acid, dichloromethane and anisole (49 : 49 : 2, 5 ml). After 40 min at room temperature, 1 ml of 3 mol l^{-1} HCl (ether) was added and the solution was evaporated. The residue was washed with ether, dried and dissolved in dimethylformamide (3 ml). N-Hydroxybenzotriazole (240 mg) was added, the solution was cooled to 0°C, and after addition of dicyclohexylcarbodiimide (265 mg), it was stirred for 0·5 h at 0°C and 2 h at room temperature. The product was filtered into a stirred solution of diisopropylethylamine (300 µl) in methanol (200 ml), and after 2 h of mixing at room temperature, the solution was concentrated *in vacuo* and the product was precipitated by ether, filtered and dried *in vacuo* (150 mg). Part of this product (30 mg) was dissolved in 1 mol l^{-1} acetic acid and introduced on to a Bio-gel P-4 column (100 × 1 cm). The fraction containing monomeric material was lyophilized (4 mg) and further purified by HPLC on a Vydac C-18 (25 × 0·4 cm) column (gradient from 0 to 35% of acetonitrile in 0·1% trifluoroacetic acid during 35 min; flow rate 1 ml/min). The pure compound (2·6 mg) was subjected to an oxidation test[11] and a mixture of diastereoisomeric sulfoxides was observed. R_F 0·27 (A), 0·70 (B), 0·82 (C), 0·85 (D). $[\alpha]_D$ −44·9° (*c* 0·07; 3 mol l^{-1} acetic acid). Amino acid analysis: Asp 0·98, Thr 0·89, Pro 1·00, Gly 1·00, Ile 0·98, Leu 1·01, Tyr 1·04, Cys(C_3H_6COOH) 0·92. For $C_{43}H_{66}N_{10}O_{12}S.3\,H_2O$ (1 001) was calculated: 51·59% C, 7·25% H, 13·99% N; found: 51·28% C, 7·56% H, 13·72% N.

Deamino-6-carba-oxytocin (*II*)

a) The following amino acids were coupled to *p*-methylbenzhydrylamine resin (2 g, 0·6 mmol/g) by the standard DCC/HOBt procedure: Boc-Gly, Boc-Leu, Boc-Pro and Boc-Hcy-($C_2H_4COOBu^t$). Then Boc-Asn-ONp was coupled in the presence HOBt to the growing peptide

chain, the resin was dried (peptide resin B) and divided in half. Boc-Gln-ONp, in the presence of N-hydroxybenzotriazole, was used in the next step and, additional amino acids were coupled as symmetrical anhydrides prepared from 3 mmol of Boc-amino acid (Ile and Tyr) and 1·25 mmol of DCC in dimethylformamide solution (1 h at 0°C and 30 min at room temperature). After cleaving the terminal amino protecting group, cyclization was performed by DCC and HOBt (40 h). The peptide was cleaved from the resin by liquid hydrogen fluoride (15 ml, 1 h, 0°C) in the presence of anisole (1 ml) and 1,2-ethanedithiol (0·5 ml), and the product was extracted by 30% acetic acid and lyophilized. Purification was performed by gel filtration on Bio-gel P-4 (100 × 1 cm) and 34 mg (5·6%) of the product was obtained. This compound was found identical with a previously prepared (by solution methods) standard by TLC, HPLC and ^{1}H NMR spectroscopy (250 MHz).

Merrifield's resin (120 mg, 2% DVB) with Boc-Gly attached to it (0·45 mmol/g) was used in a second approach. Boc-Leu, Boc-Pro, Fmoc-Hcy($C_2H_6COOBu^t$), Fmoc-Asn, Fmoc-Gln, Fmoc-Ile and Boc-Tyr(Bu^t) were subsequently coupled to the resin by the standard DCC/HOBt procedure (3 molar excess of all reagents). Boc groups were cleaved by a trifluoroacetic acid–dichloromethane–anisole (49 : 49 : 2) mixture and Fmoc groups were cleaved by 50% piperidine in dichloromethane. Cyclization was performed on the resin with the use of DCC and HOBt. The peptide was cleaved from the resin by ammonolysis and purified by HPLC (Vydac C-18, 25 × 0·4 cm, gradient from 30 to 79% of methanol in 0·05% trifluoroacetic acid in 30 min, flow 1·5 ml/min). The purified product (5·1 mg; 12% yield) was compared with a standard and the product from *a*), and was found identical.

N$^{\alpha}$-Fluorenylmethoxycarbonyl-S-(β-tert-butoxycarbonylethyl)homocysteine

A solution of S-(β-tert-butoxycarbonylethyl)homocysteine (2·63 g) (ref.[4]) in 10% Na_2CO_3 (20 ml) and dioxane (10 ml) was cooled to 0°C and treated with a solution of fluorenylmethoxycarbonyl chloride (2·6 g) in dioxane (15 ml). After 1 h mixing at 0°C, the solution was poured into H_2O (0°C, 300 ml), the mixture was extracted by ether (2 × 80 ml), and the aqueous layer was acidified by HCl to pH 2. The product was extracted with ether, dried over Na_2SO_4 and the oil obtained after evaporation was reprecipitated from dichloromethane by light petroleum. The oil was dissolved in dichloromethane, placed on a silica gel column (40 × 2·5 cm), and eluted with 2% methanol in dichloromethane. Fractions were monitored by TLC (8% ethanol in dichloromethane) and the compound with R_F 0·35 was collected. After evaporation, 2·19 g (45%) of a slowly crystallizing compound was obtained. M.p. 87–89°C, $[\alpha]_D$ −17·8° (*c* 1·8; dimethylformamide). For $C_{26}H_{31}NO_6S$ (485·6) was calculated: 64·31% C, 6·43% H, 2·88% N; found: 64·06% C, 6·64% H, 2·86% N.

[4-Threonine]deamino-6-carba-oxytocin (*IV*)

Peptide-resin D (1·27 g) and symmetrical anhydrides of Boc-Thr(Bzl), Boc-Ile and Boc-Tyr were used in the same manner as in the preparation of *II*. The octapeptide resin was dried and divided in two parts: *a*) one part (0·67 g) was subjected to cyclization on the resin as previously described. The peptide was cleaved by liquid hydrogen fluoride (10 ml, 1 h, 0°C) in the presence of anisole (1 ml) and 1,2-ethanedithiol (0·5 ml). After extraction by 30% acetic acid and lyophilization, 139 mg of crude product was obtained. Purification by gel filtration on Bio-gel P-4 (100 × × 1 cm, 4 batches) afforded 39 mg (12·6%) of pure peptide. R_F 0·26 (A), 0·70 (B), 0·82 (C), 0·85 (D). $[\alpha]_D$ −54·2° (*c* 0·14; 3 mol l^{-1} acetic acid). Amino acid analysis: Asp 1·03, Thr 0·92, Pro 0·96, Gly 1·00, Ile 0·97, Leu 1·04, Tyr 0·98, Hcy(C_2H_4COOH) 0·91. For $C_{43}H_{66}N_{10}O_{12}S.2H_2O$ (983·1) was calculated: 52·53% C, 7·18% H, 14·25% N; found: 52·10% C, 7·42% H,

13·96% N. *b*) The other portion (0·58 g) of the resin was subjected to hydrogen fluoride cleavage (10 ml, 0°C, 1 h) in the presence of anisole (1 ml) and 1,2-ethanedithiol (0·5 ml). The free octapeptide hydrofluoride (89 mg) was converted to its N-hydroxybenzotriazolyl ester and cyclized in the same manner as in the case of *III*. The product was purified by gel filtration on Bio-gel (100 × 1 cm) in 1 mol l^{-1} acetic acid, and 26 mg (9·7%) of *IV* contamined with sulfoxide (25% according to HPLC analysis) was obtained.

Pharmacological Methods

The *in vitro* uterotonic assay was carried out on isolated rat uterine strips[25,26]. For determination of the *in vivo* activity, oestrogenized rats in ethanol anaesthesia were used[27]. Galactogogic activity was determined on ethanol-anaesthetized rats (4—15 days after delivery)[19,28], and pressor activity on despinalized rats[29].

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