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Conformation–Activity Studies of Oxytocin. Effects of Structural Modifications at Corner Positions of the β-Turns on the Uterotonic Activity[1]

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Replacement of the aliphatic isoleucine residue in position 3 of oxytocin (the first corner position of the β-turn in the 20-membered ring of the solution conformation of the hormone) by phenylalanine has been shown to result in analogues with reduced affinity and intrinsic activity when tested by the individual dose–response procedure on the isolated rat uterus. Studies of effects of structural modifications have been extended to include two additional β-turn corner positions. First, the dose–response behavior of [Leu[4]]oxytocin and [Phe[4]]oxytocin, two analogues in which the Glu[4] side chain in the second corner position of the β-turn in the 20-membered ring has been substituted by hydrophobic and bulky groups, was compared with that of oxytocin. Second, the solid-phase synthesis and biological properties of [Phe[3],Leu[4],Met[8]]oxytocin and [Phe[3,4],Met[8]]oxytocin are described. The presence of leucine or phenylalanine in position 4 evokes a drastic reduction in both the affinity and intrinsic uterotonic activity of the resulting analogues, with phenylalanine significantly more effective in reducing intrinsic activity than leucine ($p < 0.001$).

In the structural model proposed for oxytocin bound to the uterine receptor (Figure 1), the Tyr[2] and Asn[5] side chains are key moieties in the "active site" of the hormone,[2,3] while residues in positions 3, 4, 7, and 8 at the corner positions of the β-turns of the solution conformation of oxytocin[4] are thought to be important for receptor binding.[2,3] With respect to the Tyr[2] it is not sufficient that the hydroxyl group is present as such—as opposed to being alkylated or deleted (for a summary see ref 5)—but for optimal uterotonic activity it appears that the hydroxyl group must be able to assume a particular orientation. Any number of chemical modifications, which introduce bulky groups in the vicinal position 1 that are expected to "dislodge" the Tyr[2] side chain,[5,6] result in partial agonists or competitive inhibitors.[7-12] Likewise, substituents in the aromatic ring of Tyr[2] itself ortho to the hydroxyl group could change the preferred orientation of the Tyr[2] side chain and again give rise to reduced intrinsic activity.[11,13-15] Similar steric interference is more difficult or may be impossible to introduce by modification of the other vicinal residue (position 3) to Tyr[2], since the side chain of the residue in position 3 in neurohypophyseal hormones occupies the first corner position of the type II β-turn and hence an equatorial position. However, with the introduction of the aromatic phenylalanine residue into position 3, the intrinsic uterotonic activity is descreased, possibly because of a change in the relative population of the tyrosine side-chain rotamers due to enhanced van der Waal interactions of the Tyr[2] and Phe[3] side chains.[5,16-18]

In the present study these investigations were extended to include two additional corner positons (residues 4 and 8) of the β-turns present in oxytocin. Specifically, the effects on the biological activities—with particular emphasis on the intrinsic uterotonic activity—of substituting Gln[4] in oxytocin by a bulky and hydrophobic group either aliphatic ([Leu[4]]oxytocin)[19] or aromatic ([Phe[4]]oxytocin)[20] was investigated. By way of extending our earlier studies with oxypressin ([Phe[3]]oxytocin)[18] to positions 4 and 8, [Phe[3],Leu[4],Met[8]]oxytocin and [Phe[3,4],Met[8]]oxytocin also were prepared and similarly biologically evaluated.

The partially protected peptide intermediates required for the synthesis of [Phe[3,4],Met[8]]oxytocin and [Phe[3],Leu[4],Met[8]]oxytocin were prepared by the solid-phase technique[21] using a schedule of washing, N^{α}-deprotection, and coupling as previously described.[22] In general, a chloromethylated polystyrene copolymer–1% divinylbenzene resin was used as a support to which Boc-Gly-OH was esterified using the cesium salt method of Gisin.[23] The *tert*-butyloxycarbonyl group[24] was used throughout for the temporary protection of the N^{α} group and was removed by treatment with 50% CF_3CO_2H in CH_2Cl_2 to which 2% 1,4-butanedithiol was added as an antioxidant after the incorporation of the methionine residue. Coupling was affected by dicyclohexylcarbodiimide[25] (DCC) or DCC mediated with 1-hydroxybenzotriazole[26] (HBT). The completeness of each coupling reaction was monitored by the ninhydrin test[27] and repeat couplings or acetylations[21,28] were performed when necessary. The benzyl group was used for the protection of the sulfhydryl and tyrosine hydroxyl moieties. The completed, partially protected peptide was removed from the resin by ammonolysis in methanol.[29] The remaining protecting groups were removed by treatment with sodium in anhydrous liquid NH_3.[30] The disulfide bond was formed by oxidation with diiodoethane.[31] The products were purified by gel filtration[32] and partition chromatography[33] on Sephadex columns. [Phe[4]]oxytocin was resynthesized by the solid-phase procedure. The product obtained agreed in its physical, chemical, and biological properties with those reported by Nestor et al.[20] A different partition chromatographic system was employed, which may explain the higher yield of purified [Phe[4]]oxytocin in the current study. [Leu[4]]oxytocin[19] was kindly supplied by Dr. V. J. Hruby.

The new analogues, [Phe[3],Leu[4],Met[8]]oxytocin and [Phe[3,4],Met[8]]oxytocin, were tested for some of the biological activities characteristic of neurohypophyseal hormones and the results are summarized in Table I. Table II lists the maximum of the in vitro uterotonic response, obtained by determining the dose–response relationship of the analogue using the individual injection technique of Chan and Kelly,[35] as compared to oxytocin. A dose–response pattern typical for the compounds tested is shown for [Phe[3,4],Met[8]]oxytocin in Figure 2.

In comparing agonists a diminished maximum in the attainable response and/or a change in the slope of the dose–response curve reflects a reduced intrinsic activity.[36,37] All of the compounds in this study exhibited a diminished ability to maximally stimulate the contraction of the isolated rat uterus as compared to oxytocin. [Phe[3,4],Met[8]]oxytocin consistently gave values during four-point determinations[38] which were statistically not parallel with those of oxytocin and no potency could be determined. The data in Table II show that the presence in position 4 of an amino acid residue with a bulky lipophilic side chain causes a reduction in the intrinsic activity of the resulting analogue. Both [Leu[4]]oxytocin and [Phe[4]]oxytocin exhibited a greater loss of intrinsic activity than did [Phe[3]]oxytocin ($p < 0.001$). When comparing [Leu[4]]oxytocin and [Phe[4]]oxytocin as well as [Phe[3],Leu[4],Met[8]]oxytocin and [Phe[3,4],Met[8]]oxytocin, it is ap-

Table I. Biological Activities of Oxytocin Analogues Possessing Amino Acid Substitutions in Positions 3, 4, and 8[a]

Peptide	Uterotonic (rat)	Vasodepressor (fowl)	Antidiuretic (rat)	Pressor (rat)
[Phe[3],Leu[4],Met[8]]oxytocin	0.62 ± 0.07	1.05 ± 0.17	0.33 ± 0.09	0.92 ± 0.03
[Phe[3,4],Met[8]]oxytocin	n.d.[b]	pA_2 = 6.29 ± 0.11[c]	0.43 ± 0.12	n.d.[b]

[a] Agonist activities are expressed in U/mg ± SEM. [b] In the uterontonic assay nonparallel four-point determinations were obtained; in the pressor assay the analogue behaved as a weak agonist which caused attenuation of the response to subsequent injections of either the standard or the analogue and potencies could not be determined. [c] Inhibitory potencies were determined and expressed as pA_2 values as defined by Schild.[34] This represents the negative logarithm to base 10 of the average molar concentration of the antagonist which will reduce the appropriate biological response to $2x$ units of pharmacologically active compound (agonist) to the level of x units of agonist. Specific details of the antivasodepressor assay are described by Vavrek et al.[9]

Table II. Maximum Uterotonic Responses of Oxytocin Analogues

Compd	% max response[a]	Specific uterotonic potency
[Phe[3]]oxytocin	90 ± 3[b] (12)[c]	39 ± 3[b]
[Leu[4]]oxytocin	81 ± 4 (8)	13 ± 1[d]
[Phe[4]]oxytocin	75 ± 4 (11)	0.75[e]
[Phe[3],Leu[4],Met[8]]oxytocin	66 ± 8 (8)	n.d.[f]
[Phe[3,4],Met[8]]oxytocin	54 ± 9 (7)	n.d.[f]

[a] Maximum response to synthetic oxytocin was taken as 100%. [b] Reported by Walter et al., ref 18. [c] Number of individual determinations. [d] Hruby et al., ref 19. [e] Nestor et al., ref 20. [f] Not determined, see Table I.

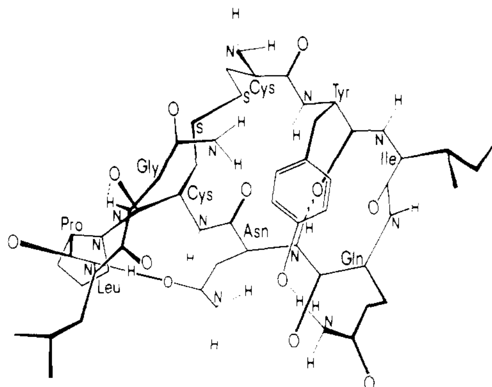

Figure 1. The three-dimensional model proposed for oxytocin bound to the uterine receptor. See ref 2.

parent that the phenylalanine side chain in position 4 is slightly more effective in reducing intrinsic activity than is the aliphatic leucine side chain ($p < 0.001$) in both the [Leu[8]]oxytocin and [Met[8]]oxypressin series studied.

By way of interpretation of the above results on the basis of the biologically active conformation of oxytocin,[2,3] it appears that bulky side chains in position 4 cause the observed reduction of intrinsic uterotonic activity by sterically interfering with the proper orientation of one or both of the active elements Tyr[2] and Asn[5] of the peptide. These data also imply that when the peptide is bound to the uterotonic receptors, the orientation of the side chain at position 4 is *toward* the active elements and reaches into the active cavity.

In this study methionine, with a sulfur atom which contains deformable electron orbitals, was introduced into position 8 in hopes of enhancing receptor binding of the resultant analogue; however, with such low affinity analogues as described in this study it is not certain that any benefit was achieved. As expected, the Met[8] residue did not have any detectable influence on the intrinsic activity of the analogues when tested on the isolated uterus.

Experimental Section

Melting points were determined in open capillary tubes and are reported uncorrected. Optical rotations were measured in a

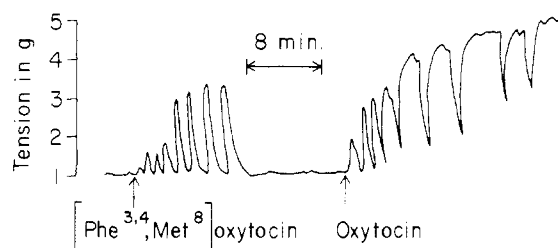

Figure 2. Typical pattern of the effects of [Phe[3,4],Met[8]]oxytocin and oxytocin on the isolated rat uterus by the individual injection dose-response procedure. Doses were increased geometrically according to a 1/2 log 10 schedule. Arrows indicate injection of the first dose of either 5.2 × 10^{-9} M [Phe[3,4],Met[8]]oxytocin or 4.9 × 10^{-11} M oxytocin. When the response to a dose of peptide began to subside, the tissue was drained and rinsed five times before the next higher dose was administered.

Zeiss circle polarimeter (0.01°). For thin-layer chromatography, loads up to 50 μg were applied to precoated plates of silica gel 60 F-254. Chromatograms were developed for 100–150 mm in the following systems: (A) BuOH–HOAc–H_2O (4:1:1); (B) BuOH–pyridine–HOAc–H_2O (15:10:3:6); (C) BuOH–pyridine–H_2O (20:10:11). Visualization was made with Cl_2 followed by 4,4-tetramethyldiaminodiphenylmethane–KI spray.[39] Amino acid analysis[40] was performed on a Durrum D-500 amino acid analyzer in duplicate, following hydrolysis for 24 h in constant boiling HCl at 110 °C in vacuo.[41] In some cases, for the protection of Tyr, a crystal of phenol[42] was added. Where elemental analyses appear only as the symbols of the elements, data fall within ±0.4% of theory.

Rat uterine assays were performed on isolated horns from virgin rats in natural estrus according to the method of Holton[43] as modified by Munsick,[44] with the use of Mg^{2+}-free van Dyke–Hastings solution as bathing fluid. Avian vasodepressor assays were performed on conscious chickens by the method of Coon,[45] as described in the U.S. Pharmacopeia,[46] as modified by Munsick et al.[47] Pressor assays were carried out on anesthetized male rats as described in the U.S. Pharmacopeia.[48] Antidiuretic assays were performed on anesthetized male rats according to the method of Jeffers et al.[49] as modified by Sawyer.[50] Either the four-point assay design of Schild[38] or matches were used to obtain specific activities as compared to U.S.P. posterior pituitary reference standard. For the determination of the dose–response relationships on the rat uterus, the same experimental conditions of temperature and bathing fluid composition as employed for the determination of the uterotonic potency were used. Isometric contractions were recorded with a Grass polygraph in connection with a Grass force-displaced transducer (FT03C, springs removed). The tissue was subjected to a baseline tension of 1 g. The dose–response behavior of the peptides was determined by the individual injection method of Chan and Kelly,[35] which minimizes the exposure time of the tissue to the test compound—as opposed to the cumulative dose method of van Rossum.[36] The doses were increased geometrically according to 1/2 log 10 procedure until a maximum response was reached. The tissue was drained and rinsed five times after each injection. The order for testing the oxytocin standard and the analogue on the same uterine horn was randomized. The values presented are an average of at least seven determinations on a minimum of six uterine horns. Following the completion of a determination, the tissue was rinsed 20 or more times until a smooth baseline was established. The

maximum response to oxytocin was determined for each preparation and taken as 100%; this defined response was the standard reference point for comparison of all other responses.

Boc-Asn-Cys(Bzl)-Pro-Met-Gly-*O*-resin (1) was prepared from polystyrene copolymer–1% divinylbenzene (Lab Systems, Inc., 0.75 mmol/g substituted as Cl). Boc-Gly was attached according to the method of Gisin[23] and, following hydrolysis for 24 h in propionic acid–HCl (1:1) at 132 °C in vacuo, amino acid analysis yielded 0.57 mmol of Gly/g of resin. Standard solid-phase techniques[21] as previously described[22] were employed throughout the synthesis using 10 g (5.7 mmol) of Boc-Gly-*O*-resin with a solvent volume of 120 mL per step. Removal of the Boc group was accomplished in 50% CF_3CO_2H in CH_2Cl_2, containing 2% anisole. After incorporation of the methionine residue, 2% 1,4-butanedithiol was added to the CF_3CO_2H solution. Couplings were performed with 2.1–2.2 equiv of *tert*-butyloxycarbonylamino acid and DCC[25] in CH_2Cl_2 for 2 h. When HBT[26] was included in the coupling mixture, 4.5 equiv of HBT was employed. Repeat couplings, when performed, used 0.5 equiv of *tert*-butyloxycarbonylamino acid and DCC and 1 equiv of HBT. For all HBT mediated couplings, *tert*-butyloxycarbonylamino acid and HBT were added in dimethylformamide (DMF), followed by DCC in CH_2Cl_2. HBT mediated coupling mixtures were agitated for 1.5–12 h, with the addition of 1 equiv of diisopropylethylamine (i-Pr_2NEt) after 1 h. A coupling was judged complete (better than 99.4%) by a ninhydrin-negative test.[27] Boc-Met was fully attached in a 2-h DCC coupling. After the coupling of Boc-Pro a small percentage of free amine remained (~1%) and the resin was acetylated[21,28] to give a ninhydrin-negative test. Following a 2-h DCC coupling of Boc-Cys(Bzl) and a 1.5-h HBT–DCC coupling of Boc-Asn, the pentapeptide-resin was washed in methanol and DMF and dried in vacuo at 40 °C overnight: yield, 13.65 g. Amino acid analysis following hydrolysis at 130 °C in propionic acid–HCl (1:1) in vacuo gave the following values in mmol/g of pentapeptide-resin (molar ratios based on Gly in parentheses): Asp, 0.37 (0.90); Pro, 0.41 (1.00); Gly, 0.41 (1.00); Met, 0.39 (0.95); Cys(Bzl), 0.38 (0.93); NH_3, 0.40 (0.98). Gly content is 93% of the possible 0.43 mmol/g, calculated from the initial substitution and corrected for 100% theoretical weight gain.

Cys(Bzl)-Tyr(Bzl)-Phe-Phe-Asn-Cys(Bzl)-Pro-Met-Gly-NH_2 (2). This synthesis proceeded as described above; beginning with 1 (4 g) and using DCC couplings, each residue coupled successfully in 2 h. Following the Boc-Cys(Bzl) coupling an additional deprotection cycle was executed for removal of the NH_2-terminal Boc group. The product was washed in EtOH and dried overnight. The entire batch was suspended in anhydrous MeOH [distilled from $Mg(OCH_3)_2$] and the suspension was saturated at 0 °C with anhydrous NH_3 (distilled from Na). The system was sealed and allowed to stir at room temperature for 4 days. Following removal of the NH_3 and MeOH by evaporation in vacuo, the product was extracted with warm (55 °C) DMF. The resin was removed by filtration, the filtrate evaporated to dryness by rotary evaporation, and the residue redissolved in DMF (25 mL, 100 °C). The product was precipitated by the addition of 100 mL of H_2O, collected, washed with H_2O, EtOH, and Et_2O, and dried in vacuo: 1.58 g (67% yield from Boc-Gly-*O*-resin); mp 215–216 °C dec; $[\alpha]^{24}_D$ −41° (*c* 1.0, DMF); TLC (A) R_f 0.61 with several trace impurities. Amino acid analysis: Asp, 1.04; Pro, 1.01; Met, 0.85; Tyr, 0.94; Phe, 2.01; Cys(Bzl), 1.90; NH_3, 2.00. Anal. ($C_{70}H_{83}N_{11}S_3 \cdot H_2O$)C, H, N.

[3,4-Diphenylalanine,8-methionine]oxytocin (3). The remaining protecting groups from 2 (136 mg, 0.10 mmol) were removed by treatment with Na in anhydrous liquid NH_3.[30] The faint blue color of a slight excess of Na was allowed to persist for just 10–15 s and then discharged with 1 drop of HOAc. The NH_3 was removed by lyophilization and the flask opened under N_2. The residue was dissolved in 1-propanol (90 mL), and H_2O (100 mL) was added. A solution of ICH_2CH_2I (29.3 mg, 0.1 mmol) in 1-propanol (10 mL) was added and the disappearance of sulfhydryl groups was monitored by the method of Ellman.[51] No sulfhydryl remained after 5 min; HOAc (5 mL) was added, and the solvents were rotary evaporated to 3 mL and finally diluted with HOAc (3 mL). This solution was applied to a 2.15 × 108 cm column of Sephadex G-15 (fine) preequilibrated with 50% HOAc. The column was eluted with 50% HOAc and collected in 2.8-mL fractions. Peptide material was detected by reading the absorbancy at 280 nm. The major peak appeared with a maximum at an elution volume of 182 mL (46% of column volume). Fractions 58–71 containing the main peak were pooled, their volume was reduced, and the product was lyophilized from glacial HOAc: yield 93 mg. The entire batch was dissolved in 3 mL of the upper phase of the solvent system BuOH–C_6H_6–HOAc–1.5% aqueous pyridine (5:5:4:6) and subjected to partition chromatography[33] through a 2.82 × 69 cm column of Sephadex G-25 block polymerizate (100–200 mesh) which had been preequilibrated to both lower and upper phases of the system. The column was eluted with the upper phase at 21 mL/h and collected in 5.2-mL fractions. The major peak, identified by the method of Lowry et al.,[52] emerged with a maximum at R_f 0.49 followed by and resolved from a trace impurity, R_f 0.29. Fractions 37–50 were pooled, their volume was reduced, and the product was lyophilized from H_2O: yield, 70 mg. The entire product was further purified by partition chromatography on a 2.15 × 118 cm column of Sephadex G-25 block polymerizate (100–200 mesh) which had been preequilibrated to both phases of the solvent system BuOH–C_6H_6–HOAc–1.5% aqueous pyridine (10:15:12:18). The column was eluted with the upper phase at 20 mL/h and the product emerged with a peak maximum at R_f 0.19. Fractions 78–103 were pooled, the organic solvents were removed by rotary evaporation, and the aqueous phase was lyophilized after addition of H_2O: 59 mg (55% yield from 2); $[\alpha]^{24}_D$ −26° (*c* 0.5, 1 N HOAc). TLC (A) R_f 0.38. Amino acid analysis: $Cys(O_3H)$,[53] 1.98; Asp, 1.01; Pro, 1.02; Gly, 1.00; Met, 0.94; Tyr, 0.90; Phe, 1.99; NH_3, 2.12. Anal. ($C_{49}H_{65}N_{11}O_{11}S_3 \cdot C_2H_4O_2 \cdot 2H_2O$) C, H, N.

Cys(Bzl)-Tyr(Bzl)-Phe-Leu-Asn-Cys(Bzl)-Pro-Met-Gly-NH_2 (4). Solid-phase synthesis proceeded as described for 2 from 1 (4 g). Following a 2-h DCC coupling cycle, Boc-Phe required one 4-h HBT recoupling. After the final deprotection and ethanol wash, the peptide resin was dried in vacuo: yield, 4.55 g. Ammonolysis and product isolation was performed as for 2: 1.68 g (75% yield from Boc-Gly-*O*-resin). The entire batch was reprecipitated from hot HOAc (15 mL) with hot EtOH (170 mL). The precipitate was collected, washed with EtOH and Et_2O, and dried: 1.34 g as acetate salt (54% yield from Boc-Gly-*O*-resin); mp 215–216 °C dec; $[\alpha]^{24}_D$ −33° (*c* 1.0, DMF); TLC (A) R_f 0.59, with three trace impurities. Amino acid analysis: Asp, 1.03; Pro, 1.04; Gly, 1.00; Met, 0.89; Leu, 0.98; Tyr, 0.89; Phe, 0.98; Cys(Bzl), 1.83; NH_3, 2.02. Anal. ($C_{67}H_{85}N_{11}O_{11}S_3 \cdot 0.5C_2H_6O$) C, H, N.

[3-Phenylalanine,4-leucine,8-methionine]oxytocin (5). Reduction by Na in liquid NH_3 and formation of the disulfide was performed as for the preparation of 3 using 4 (134 mg, 0.1 mmol). The crude product was isolated by lyophilization from HOAc and subjected to gel filtration in 50% HOAc as described for 3. The major peak emerged at 54% of column volume; fractions 59–72 comprising it were pooled and the product was lyophilized. Further purification was affected by partition chromatography in the system BuOH–C_6H_6–HOAc–1.5% aqueous pyridine (10:15:12:18) on a 2.82 × 68 cm column of Sephadex G-25 block polymerizate (100–200 mesh). The major product emerged with a peak maximum at R_f 0.17. Fractions 65–89 were pooled and the product was isolated by evaporation and lyophilization: 56 mg (54% yield from 4); $[\alpha]^{24}_D$ −44° (*c* 0.5, 1 N HOAc); TLC (A) R_f 0.38, (B) R_f 0.64. Amino acid analysis: $Cys(O_3H)$,[53] 2.01; Asp, 1.01; Pro, 1.02; Gly, 1.00; Met, 0.95; Leu, 1.00; Tyr, 0.91; Phe, 0.98; NH_3, 2.08. Anal. ($C_{46}H_{67}N_{11}O_{11}S_3 \cdot 2C_2H_4O_2 \cdot H_2O$) C, H, N.

Z-Cys(Bzl)-Tyr(Bzl)-Ile-Phe-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (6). Using 3.4 g of the Boc-Gly-*O*-resin employed for the synthesis of 1 this fully protected nonapeptide was synthesized in the same manner as described for 1 and 3 with Boc-Leu·H_2O replacing Boc-Met and Boc-Ile·0.5H_2O replacing the second Boc-Phe coupling. Z-Cys(Bzl) was used in the last coupling step so that 6 would be identical with the protected nonapeptide prepared by solution techniques.[20] The peptide was removed from the resin with NH_3 as previously described and extracted with warm dimethyl sulfoxide. The dimethyl sulfoxide solution was rotary evaporated to dryness (50 °C, 0.01 mmHg) and the residue was dissolved in DMF (20 mL, 100 °C). The product precipitated after the addition of H_2O (50 mL) and cooling. The precipitate was washed with H_2O, EtOH, and Et_2O and dried in vacuo: 1.54 g (77% yield based on Gly content of resin); mp 253–255 °C (lit. mp 255.5 °C); $[\alpha]^{27}D$ −47° (*c* 0.6, DMF) [lit. $[\alpha]_D$ −49° (*c* 0.4, DMF)]. The product proved too insoluble to utilize TLC and,

in fact, was not hydrolyzed in 6 N HCl after 24 h. Amino acid analysis after 3 h of hydrolysis in propionic acid–12 N HCl (1:1) at 130 °C gave the following ratios: Asp, 1.02; Pro, 1.00; Gly, 1.00; Ile, 0.77; Leu, 1.01; Tyr, 0.78; Phe, 0.91; Cys(Bzl), 2.01; NH_3, 2.00. Apparently the Tyr-Ile sequence is not completely hydrolyzed under these conditions.

4-Phenylalanine-oxytocin (7). Reduction by Na in liquid NH_3 and formation of the disulfide bond was performed as described for the preparation of 3 using 6 (330 mg, 0.23 mmol). The crude product was purified on the same column as described for the partition chromatography of 5 in the system used for 5. The major component emerged as a sharp symmetrical peak with a maximum at R_f 0.26. The appropriate fractions were pooled and the product was isolated by evaporation and lyophilization: 136 mg (58% yield from the nonapeptide). TLC showed a single spot: R_f (A), 0.38; R_f (C) 0.61 [lit. R_f (C) 0.61]. Amino acid analysis gave the following molar ratios: Asp, 1.00; Pro, 1.04; Gly, 1.00; $^1/_2$Cys, 1.90; Ile, 0.96; Leu, 1.02; Tyr, 0.91; Phe, 1.01; NH_3, 1.81. The product had a uterotonic activity of approximately 0.8 U/mg and was an inhibitor in the vasodepressor assay with a pA_2 of ~7.0.[26]

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