then rinsed with 150 mL of MeOH–H_2O (1:1).

A 1.5-g (2.91 mmol) sample of 1 was then dissolved in 10 mL of MeOH and diluted with 10 mL of H_2O. This solution was added to the above column and 2-mL fractions were collected at a flow rate of 1.5 mL/min. A total of 20 fractions was collected. Fractions 3–14 were combined and evaporated to a solid which was recrystallized from 2-propanol and dried at 60 °C for 8 h under high vacuum to give 0.96 g (69%) of **19**, mp 240–242 °C.

***N*-[2-(Triphenylphosphonium bromide)ethyl]isoindoline Hydrobromide Monohydrate (20).** To 171 mL of 0.8 M BH_3 in THF (0.14 mol) at 0–5 °C under N_2 atmosphere was added dropwise a solution of 5.0 g (0.034 mol) of phthalimide in 130 mL of THF. The mixture was warmed gradually, then refluxed 1 h, and stirred overnight at room temperature. It was then cooled to 5 °C and 6 N HCl was added dropwise until pH 2. The mixture was warmed gradually, then refluxed 1 h, and filtered (solid discarded), and the solvent was removed under vacuum. To the residue was added 100 mL of H_2O and the solution was basified with NH_3(g) to pH 9, then extracted with $CHCl_3$, and dried over K_2CO_3. Evaporation gave a solid–liquid mixture which was filtered. The filtrate was then vacuum distilled to give 1.8 g (44%) of isoindoline, bp 75–82 °C (7.5 mm) [reported[6] bp 96–97 °C (10 mm)].

To a solution of 6.4 g (14 mmol) of **12** in 60 mL of DMF was added a solution of 1.7 g (14 mmol) of the above isoindoline in 10 mL of DMF. The reaction mixture was heated at 64 °C under N_2 for 4 h, and the solvent was then evaporated to an oil. This was triturated with EtOAc to a solid which was recrystallized from EtOH and then H_2O and dried under high vacuum at 60 °C for 10 h. This gave 5.0 g (61%) of **20**, mp 256–260 °C dec.

2-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)ethyldiphenylphosphine Oxide (24). To a solution of 4.92 g (19 mmol) of *N*-(2-bromoethyl)phthalimide in 10 mL of DMF under N_2 atmosphere was added 5.0 g (19 mmol) of *n*-butoxydiphenylphosphine. The reaction mixture was heated on a steam bath for 10 h and then refluxed for 12 h. The solvent was evaporated to an oil which was treated with benzene and filtered. The filtrate was evaporated to another oil which was chromatographed over silica gel Woelm (dry column grade, activity III) with benzene, followed by $CHCl_3$. The $CHCl_3$ fractions were combined and evaporated to a white solid which was recrystallized from EtOAc and dried at 60 °C under high vacuum for 6 h. This gave 0.85 g (12%) of **24**, mp 164–167 °C.

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A Ureido Group Containing Analogue of Oxytocin Comprising Eight Amino Acid Residues

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A new analogue of oxytocin was constructed from L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-lysyl-L-prolyl-L-leucylglycinamide. Reaction of this 8-peptide amide with di-*p*-nitrophenyl carbonate yielded a cyclic compound, in which the $-CH_2SSCH_2-$ bridging portion of oxytocin formed by the oxidative linking of the two cysteine side chains was replaced by the $-CH_2CH_2CH_2CH_2-$ group of lysine, while the ϵ-NH_2 group of the same residue took the place of the α-CH of cysteine-1. The N-terminal amino group of oxytocin, which is not necessary for its hormonal activities, was omitted. The new analogue, referred to as [1,6-*N*'-carbonyl-L-lysine]oxytocin, possessed a rat uterotonic activity in vitro of 3.9 ± 0.3 units/mg, less than 0.5 unit/mg of rat antidiuretic activity, and caused a marked tachyphylaxis in the rat pressor assay. Moreover, the analogue was a strong competitive inhibitor, with a pA_2 value of 7.27 ± 0.13 of the oxytocin induced vasodepressor response in chickens.

In the paper[1] announcing the first synthesis of a peptide hormone, du Vigneaud and his associates considered oxytocin an octapeptide. Yet, both in biosynthesis and in the laboratory procedures two cysteine residues are incorporated rather than the disulfide cystine. Therefore it is probably more appropriate to call oxytocin a cyclic nonapeptide (or a 9-peptide[2]). A numbering system has been proposed[3] accordingly (Figure 1). The recognition by Rudinger and his collaborators[4] that the disulfide bridge is not an essential feature of the molecule opened the way to the construction of new analogues in which the sulfur atoms were replaced by CH_2 groups.[5,6] An inspection of the structure of deamino-dicarbaoxytocin[6] suggested to us that if the cysteine residue in position 6 of the C-terminal 8-peptide of oxytocin would be replaced by lysine (compound I in Figure 2), then the insertion of a –CO– group could close a ring of 20 atoms. The resulting compound, II, would be quite analogous to deamino-dicarbaoxytocin,[6] except that in II an –NH– group takes the place of a $-CH_2-$ group in the potent oxytocin analogue. The possible perturbations caused by a urea grouping in the ring of II on the conformation of the molecule and/or the influence on its interaction with specific oxytocin receptors has not been overlooked, but it seemed to be worthwhile to prepare compound II and examine its biological activities.

Synthesis of [6-lysine]oxytocin$_{2-9}$ (compound I) has been described earlier.[7] In our first attempts to prepare compound II we tried to acylate one of the two free amino groups of I with *p*-nitrophenyl chlorocarbonate[8] in the

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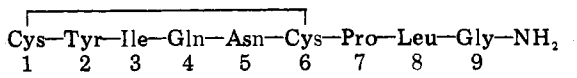
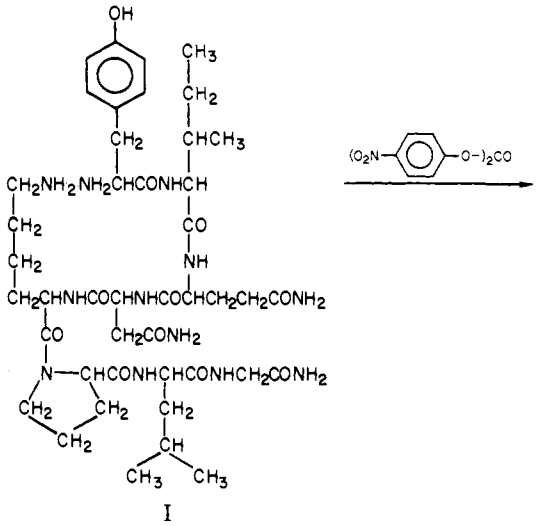
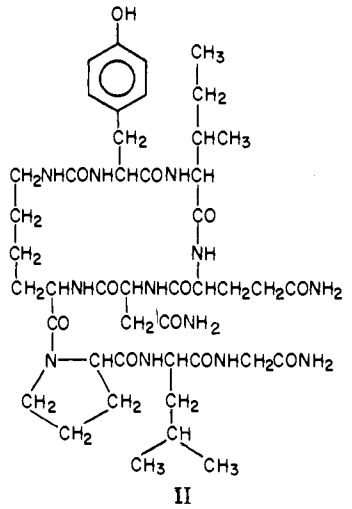


Figure 1. The structure and numbering system of oxytocin.



I



II

Figure 2. Conversion of [6-lysine]oxytocin$_{2-9}$ to compound II, [1,6-N^{ϵ}-carbonyl-L-lysine]oxytocin.

expectation that the acid chloride part of the reagent would readily react with either one of the two amino groups and the resulting urethane would undergo—after appropriate dilution—intramolecular aminolysis with the second amino group. These experiments failed, probably because the chlorocarbonate was decomposed by trace amounts of water in the solvent (dimethylformamide). Therefore we selected the less reactive di-*p*-nitrophenyl carbonate.[9] By monitoring on thin-layer chromatograms the reaction of this reagent with compound I, a fairly fast reaction could be observed, yielding two spots with strong UV absorption, positive fluorescamine[10] reaction, and turning yellow on exposure to ammonia. These were, presumably, monosubstituted derivatives of I. The solution was diluted to suppress intermolecular reactions and was then allowed to stand at room temperature for about 1 day. After this time period, no more fluorescamine-positive material was present, and the major component gave no yellow color when exposed to ammonia. The crude product, obtained upon removal of the solvent in vacuo, was chromatographed on a Sephadex LH-20 column with methanol as solvent. The gel-permeation chromatography removed part of the material that, because of both its elution pattern and its slow migration on thin-layer chromatograms, was considered to consist of oligomers and polymers of II. Further purification was achieved by countercurrent distribution, followed by a second gel-permeation chromatography.

The analogue was tested for some of the biological activities characteristic of neurohypophyseal hormones (rat uterotonic,[11] avian vasodepressor,[12] rat pressor,[13] and rat antidiuretic[14]) as compared to U.S.P. posterior pituitary reference standard. It was found to possess in vitro rat uterotonic potency of 3.9 ± 0.3 units/mg (mean ± SEM) or about 0.8% of the potency of oxytocin. In the avian vasodepressor assay compound II behaved as a competitive inhibitor of the vasodepressor response to oxytocin when tested according to the method of Vavrek et al.,[15] with a pA_2[16] of 7.27 ± 0.13 (mean ± standard deviation). Thus its inhibitory potency in the vasodepressor assay is comparable to that of [1-L-penicillamine]oxytocin (pA_2 = 7.50 ± 0.47).[15] A dose of 0.04 mg/100 g was sufficient to give a detectable rat pressor response; however, this dose caused a marked tachyphylaxis which attenuated the response to subsequent doses of standard or analogue, and no specific potency could be determined. The analogue possesses less than 0.05 unit/mg of rat antidiuretic potency.

These results reveal that the pharmacological properties of compound II are quite different from those of oxytocin or 1-deamino-dicarbaoxytocin which has a similar primary structure. The biological effect of the ureido group, which is part of the ring in II, is considerable, although it is apparent that II retains a high degree of affinity for the oxytocin receptor in the chicken.

Experimental Section

Uncorrected capillary melting points are reported. The solvents used were reagent grade; dimethylformamide (DMF) was dried over a 4A molecular sieves (Davison). For amino acid analysis, samples were hydrolyzed with constant boiling hydrochloric acid in evacuated sealed ampules at 110 °C for 16 h and analyzed by the Spackman–Stein–Moore method[17] on a Beckman Spinco 120 C amino acid analyzer. Thin-layer chromatograms (TLC's) were run on glass plates precoated with silica gel (Brinkman) in the following solvent systems: A, 1-butanol–AcOH–pyridine–H_2O (30:6:24:20); B, 1-butanol–AcOH–H_2O (4:1:1); C, 1-butanol–pyridine–AcOH–H_2O (30:24:6:11); D, 95% EtOH–H_2O (7:3); E, $CHCl_3$–MeOH (9:1).

Conversion of [6-Lysine]oxytocin$_{2-9}$ (I) to Compound II. The protected 8-peptide amide *N*-Z-*O*-Bzl-Tyr-Ile-Gln-Asn-Z-(NO_2)-Lys-Pro-Leu-Gly-NH_2[7] (I) [50 mg, 0.037 mmol; $[\alpha]^{25}_D$ −29.3° (*c* 1, DMF containing 1% AcOH); mp 226–233 °C] was dissolved in AcOH (1 mL) and a 5 M solution of HBr in AcOH (1 mL) was added. After 8 h at room temperature ether (7 mL) was added. The precipitate was collected by centrifugation, washed with ether (2 × 7 mL), and dried in vacuo over NaOH: wt 36 mg; TLC R_f (B) 0.11, R_f (C) 0.27.

The 8-peptide hydrobromide (0.26 g, 0.24 mmol) was dissolved in DMF (5 mL), and di-*p*-nitrophenyl carbonate (0.08 g, 0.26 mmol) and diisopropylethylamine (DIEA) (0.08 mL, 0.5 mmol) were added. After about 5 min the following spots were observed on TLC: R_f (B) 0.82, UV positive and yellow after exposure to NH_3 (di-*p*-nitrophenyl carbonate); R_f (B) 0.61, UV positive, yellow after exposure to NH_3 and fluorescamine negative; R_f (B) 0.52, UV positive, yellow after NH_3 and fluorescamine positive; R_f (B) 0.40, UV positive, yellow after NH_3 and fluorescamine positive; R_f (B) 0.33, UV positive, yellow after NH_3 and fluorescamine positive; R_f (B) 0.15, UV positive and fluorescamine positive (starting material). The reaction mixture was diluted with DMF (250 mL); after about 16 h at room temperature, the mixture was fluorescamine negative. The solvent was removed in vacuo, and MeOH was added and evaporated in vacuo twice. The residue was thrice precipitated from MeOH (1 mL) with EtOAc (15 mL). The precipitate was collected and dried in vacuo over NaOH: wt 217 mg. On TLC there were three spots: one minor with R_f (B) 0.41, a major spot at R_f (B) 0.34, and an elongated spot at about

R_f (B) 0.16 (probably oligomers). None of the spots turned yellow when exposed to NH_3. A part of the product gradually became insoluble in MeOH. Therefore the crude material was suspended in MeOH (4 mL), the solvent evaporated in vacuo, and this process repeated. Finally the residue was suspended in MeOH, the solid collected by centrifugation, and the supernatant applied to a column (2 × 60 cm) of LH-20 Sephadex (50 g). The insoluble material was washed with an additional amount of MeOH (2 mL) which was also applied to the column. The MeOH-insoluble material (oligomers) weighed 61 mg. Methanol was used as eluent and 140 fractions (1.5 mL each) were collected. The desired material was located by UV absorption and TLC in tubes 60–80. Evaporation of the solvent left 107 mg; TLC R_f (B) 0.38 with impurities at R_f (B) 0.45 and R_f (B) 0.26. The product (112 mg, from two experiments) was dissolved in both layers (3 mL each) of the system 1-butanol–AcOH–H_2O (4:1:5) and distributed in an automatic Craig apparatus through 60 transfers. The distribution pattern was determined by TLC and by evaporating the solvent from individual tubes and weighing the residues. The contents of tubes 42–48 were combined and the solvents were removed in vacuo to yield a solid: 42 mg (17% based on compound I); TLC R_f (B) 0.37, R_f (C) 0.7, R_f (D) 0.79. Along with the desired material a trace amount of impurity with R_f (B) 0.45, R_f (C) 0.73, and R_f (D) 0.82 was also found which could not be separated from the product. Analytical chromatography was carried out by reverse phase HPLC on a Varian instrument (μ Bondapak C-18; 2 mL/min; 2000 psi; 0.50 AUFS/0.1 mV; λ = 274 nm; 80:20 0.01 M NH_4OAc-acetonitrile). In addition to the major peak (3 min) a very small peak (10 min) was also observed. Compound II had no well-defined melting point: $[\alpha]^{25}_D$ −72° (c 1, AcOH); λ_{max} (alcohol) 278 nm (ϵ 1420, 0.0005 M, 2 mg/4 mL). Amino acid analysis of compound I gave Asp, 1.03; Glu, 1.02; Pro, 0.92; Gly, 1.00; Ile, 0.99; Leu, 1.04; Tyr, 0.89; Lys, 0.99; NH_3, 3.40. The ratio of amino acids after hydrolysis of compound II for 16 h was Asp, 1.05; Glu, 1.00; Pro, 1.02; Gly, 1.00; Ile, 0.92; Leu, 1.00; Tyr, 0.14; Lys, 0.27; NH_3, 3.09. Hydrolysis of compound II produced also an additional peak at 181-min elution time on the long column. The structure of this species is probably the following: $H_2NCH(COOH)CH_2CH_2CH_2CH_2NHCONHCH(CH_2$–$C_6H_4$–O-H)COOH. After 128 h of hydrolysis, amino acid analysis gave Asp, 1.00; Glu, 1.05; Pro, 1.05; Gly, 1.00; Ile, 0.87; Leu, 0.95; Tyr, 0.34; Lys, 0.54; NH_3, 3.2; the 181-min peak still present.

Anal. Calcd for $C_{44}H_{68}N_{12}O_{12}$: C, 55.2; H, 7.2; N, 17.6. Found: C, 51.8; H, 6.6; N, 16.3. Since the values of elemental analysis suggested the presence of inorganic impurities, a sample (19 mg) was rechromatographed on a Sephadex LH-20 column (2 × 60 cm) in methanol. The recovered material had unchanged elemental composition. Chemical ionization mass spectra failed to provide useful data, but in field desorption mass spectra a peak with mass 957 corresponded to M + H (calcd 957). A much stronger peak, however, with mass of 979 was that of a sodiated derivative. The presence of the latter explains the low C, H, and N values in the elemental analysis. This compound II behaves like an ionophor; it has strong affinity to Na ions and could take them up from the environment, e.g., glass. (Cf. the similar ion binding properties of [α-glutamic acid]oxytocin.[18])

Biological Tests. Rat uterotonic assays were performed on six isolated horns from virgin rats in natural estrus according to the method of Holton, as modified by Munsick, with the use of Mg^{2+}-free van Dyke–Hastings solution as bathing fluid.[11] Avian vasodepressor assays were performed on conscious chickens by the method of Coon, as described in the U.S. Pharmacopeia, as modified by Munsick et al.[12] Pressor assays were carried out on four anesthetized male rats as described in the U.S. Pharmacopeia.[13] Antidiuretic assays were performed on four anesthetized male rats according to the method of Jeffers et al., as modified by Sawyer.[14] When agonistic activity was detected, the four-point assay design of Schild[19] was used to obtain specific activities as compared to U.S.P. posterior pituitary reference standard. The inhibitory activity of the analogue against synthetic oxytocin was determined by the application of the normal conditions of the avian vasodepressor assay[12] and is expressed as pA_2 values as defined by Schild.[16] [pA_2 values represent the negative logarithm to the base 10 of the average molar concentration of an antagonist which will reduce $2X$ units of pharmacologically active compound (agonist) to the response of X units of agonist.] Ten individual determinations on three animals were performed. For details of the experimental method see Vavrek et al.[15]

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