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Synthesis of Three Oxytocin Analogs Related to [1-Deaminopenicillamine]oxytocin Possessing Antioxytocic Activity†

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[1-Mercaptodimethylacetic acid]oxytocin, [1-β-mercapto-α,α-dimethylpropionic acid]oxytocin, and [1-β-mercapto-β,β-diethylpropionic acid]oxytocin, analogs of deaminopenicillamine-oxytocin, have been synthesized from a common protected octapeptide resin intermediate and purified by sequential gel filtration on Sephadex G-15 in 50% AcOH and 0.2 *N* AcOH. The 3 compounds were devoid of oxytocic and avian vasodepressor activities, but all showed a significant degree of inhibition of the effects of oxytocin on the isolated rat uterus and on avian blood pressure. Each compound showed approximately the same inhibitory potency in both biological systems when compared to that of deaminopenicillamine-oxytocin. [1-Mercaptodimethylacetic acid]oxytocin and [1-β-mercapto-α,α-dimethylpropionic acid]oxytocin had about 20% and 33 to 47%, respectively, of the inhibitory potency of deaminopenicillamine-oxytocin. [1-β-Mercapto-β,β-diethylpropionic acid]oxytocin had approximately twice the inhibitory potency of deaminopenicillamine-oxytocin ([1-β-mercapto-β,β-dimethylpropionic acid]oxytocin in both systems.

In a series of studies bearing on the specificity of the half-cystine residue in position 1 of oxytocin (Figure 1) in relation to the pharmacol behavior of the hormone, this residue was formally replaced with an L-penicillamine (L-β,β-dimethylcysteine) residue.[1] The resulting [1-L-penicillamine]-oxytocin was devoid of oxytocic activity but instead turned out to be a potent inhibitor of the oxytocic activity of oxytocin.[1-3] [1-D-Penicillamine]oxytocin[1-3] was also found to possess antioxytocic activity, but to a lesser extent. Both diastereoisomers also showed an inhibitory effect on the avian vasodepressor (AVD) effect of oxytocin.

Following the unexpected finding that the presence of two Me groups on the β-carbon of the half-cystine residue converts oxytocin to an antioxytocic agent, studies were initiated on related analogs to see what modifications in the 1 position would enhance or diminish the antioxytocic activity. Since the formal replacement of the free amino group of oxytocin with H gives an analog, deamino-oxytocin ([1-β-mercaptopropionic acid]oxytocin), that is even more potent in its oxytocic activity than oxytocin,[4,5] the deamino analog of penicillamine-oxytocin was prepared.[1] Deaminopenicillamine-oxytocin ([1-β-mercapto-β,β-dimethylpropionic acid]oxytocin) had as potent an antioxytocic effect as L-penicillamine-oxytocin and likewise showed an anti-AVD effect.[1-3] It is of interest that [1-deaminopenicillamine-d_8]oxytocin, in which the deaminopenicillamine residue has been replaced with a fully deuterated deaminopenicillamine residue, has the same degree of antioxytocic activity as the protio analog.[6]

To ascertain whether both β-Me groups are necessary to produce antioxytocic activity, [1-L-β-mercapto-β-methylpropionic acid]oxytocin and [1-D-β-mercapto-β-methylpropionic acid]oxytocin were studied.[7] Neither of these diastereoisomers having only one β-Me substiuent showed antioxytocic or anti-AVD activity, but both possessed 5–10% of the oxytocic and AVD potencies of deamino-oxytocin.

With the demonstration that two β-Me substituents are required for antioxytocic activity, we became interested in whether two such substituents on the α-C would have a like effect. We have therefore synthesized [1-β-mercapto-α,α-dimethylpropionic acid]oxytocin, as described in the Experimental Section. The compd, which had no detectable oxytocic# or AVD** activity, was found to possess about half of the antioxytocic potency and about one-third of the anti-AVD potency of deaminopenicillamine-oxytocin. Although this compd is not as potent as the β-substitued analog, the results demonstrate that the antioxytocic activity is not confined to substitution on the β-C.

It is to be noted that the analogs so far discussed all possess the 20-membered cyclic disulfide ring present in oxytocin and deamino-oxytocin. It has been found that the 19-membered ring analog of deamino-oxytocin, namely [1-mercaptoacetic acid]oxytocin, possesses 25 units/mg of oxytocic and 4 units/mg of AVD activity.[13] Deamino-oxytocin possesses 803 and 975 units/mg of oxytocic and AVD activity, resp.[5] To find out whether substitution of Me groups

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#The oxytocic response to oxytocin of isolated uteri from Sherman albino rats (200–250 g) in natural estrus was measured by the method of Holton,[8] as modified by Munsick,[9] with the use of Mg-free van Dyke–Hastings soln as the bathing fluid. Isotonic contractions were recorded with a Harvard heart/smooth muscle transducer and Grass polygraph Model 5.

**Avian vasodepressor responses were measured on conscious roosters (~2500 g) by the method of Coon,[10] as described in "The Pharmacopeia of the United States of America,"[11] as modified by Munsick, *et al.*[12]

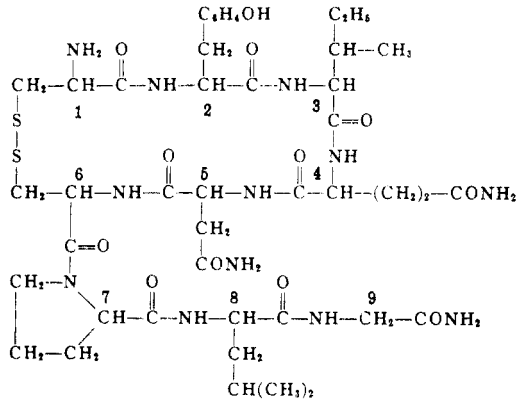

Figure 1. Structure of oxytocin, with numbers indicating the position of the individual amino acid residues.

for the hydrogens in the 1 position of the 19-membered ring analog would convert the latter to an antioxytocic agent, we have synthesized [1-mercaptodimethylacetic acid]-oxytocin. This analog, which had no detectable oxytocic or AVD activity, turned out to be only 20% as potent as deaminopenicillamine-oxytocin as an inhibitor of the oxytocic and AVD activities of oxytocin. Thus, the results with this compd demonstrate that antioxytocic activity is not confined to 20-membered ring analogs.

Returning, then, to a consideration of substituents in the β position, we decided to see whether increasing the size of the substituents would influence the degree of antioxytocic activity. We therefore synthesized the diethyl analog, [1-β-mercapto-β,β-diethylpropionic acid]oxytocin. This compd, which was devoid of oxytocic and AVD activities, possessed an enhanced antioxytocic activity which was twice that of deaminopenicillamine-oxytocin. The anti-AVD activity was enhanced to about 70% more than that of deaminopenicillamine-oxytocin. It is obvious that further increase in the size of the substituents on the β-C might prove interesting, and furthermore that substituents on both α- and β-carbons would warrant investigation.

Detailed data on the inhibitory properties of the analogs under consideration are given in Table I, along with the corresponding data for L- and D-penicillamineoxytocin and deaminopenicillamine-oxytocin, which have not previously been evaluated by the method used in this paper.

The inhibitory activities of the analogs were detd and expressed as pA_2 values as defined by Schild[14] (see footnote *a* to Table I) in the antioxytocic and anti-AVD systems. Isolated rat uterus and conscious chicken prepns were used, under the normal conditions of the oxytocic# and AVD** assays, resp. A dose of synthetic oxytocin (x units) which would give a moderate, reproducible response (R) was first selected. Then $2x$ units were administered immediately following an injection of antagonist. The latter procedure was repeated until two levels of antagonist were found, one of which would reduce the effect of $2x$ units of oxytocin to slightly more than and the other to slightly less than R. Concns of antagonist were calcd on the basis of a 10-ml tissue bath in the antioxytocic studies and on the basis of an assumed blood vol of 150 ml in the AVD studies. After plotting, according to Schild, the 2 concns of antagonist on a logarithmic scale against response, one interpolates between them to the molar concn (M) which corresponds to the response R. The negative logarithm of this concn to the base 10 is termed pA_2. Average M ($\bar{M}$) from a given series of assays and the corresponding pA_2 values are listed in Table I for each analog.

Also presented in Table I are 2 columns ($\bar{M}_{DAP}/\bar{M}_{analog}$) which relate the antioxytocic and anti-AVD potencies of the different analogs to those of deaminopenicillamine-oxytocin. These columns provide a profile of the group of analogs as inhibitors and make it evident that in most cases the ratios show a close parallelism between the two inhibitory systems studied. It is interesting that the only analogs in Table I which do not fit in with the parallelism shown are the two (L- and D-penicillamine-oxytocin) which bear a free α-NH_2 group.

As earlier reported,[15] the 22-membered ring analog of deamino-oxytocin, namely [1-γ-mercaptovaleric acid]oxytocin, possessed no oxytocic or AVD activity, but did possess antioxytocic activity. This compd has now been found to have anti-AVD properties also, and $\bar{M}$ values (expressed as in Table I) have been measured for both inhibitions: 3.22 (6), σ = 1.14 (antioxytocic); 3.97 (7), σ = 0.87 (anti-AVD). The corresponding pA_2 values are 6.49 and 7.40, resp. Thus for this analog also we find that its antioxytocic and anti-AVD potencies relative to the corresponding potencies of deaminopenicillamine-oxytocin are very nearly equal ($\bar{M}_{DAP}/\bar{M}_{analog}$ = 0.36 and 0.33, resp).

The marked parallelism between the antioxytocic and anti-AVD profiles of the compds studied may be significant. One may speculate that there is some basic similarity in the receptors involved in the two tissues. Possibly further comparisons of the relative inhibitory potencies of groups of compds in 2 or more biol systems may yield important information that can be related to the types of receptors involved.

Table I. Inhibitory Properties of Oxytocin Analogs Related to [1-Deaminopenicillamine]oxytocin

Analog	antioxytocic			anti-AVD		
	pA_2[a]	$\bar{M} \times 10^7$[a]	$\bar{M}_{DAP}/\bar{M}_{analog}$[b]	pA_2	$\bar{M} \times 10^8$	$\bar{M}_{DAP}/\bar{M}_{analog}$
[1-Deaminopenicillamine]oxytocin (DAP)	6.94	1.16 (36) σ = 0.51	1.00	7.88	1.31 (27) σ = 0.61	1.00
[1-L-Penicillamine]oxytocin	6.86	1.39 (7) σ = 0.40	0.83	7.50	3.18 (8) σ = 1.05	0.41
[1-D-Penicillamine]oxytocin	6.32	4.81 (6) σ = 1.87	0.24	6.78	16.8 (7) σ = 4.0	0.08
[1-α-Mercapto-α,α-dimethylacetic acid]oxytocin	6.16	6.91 (10) σ = 3.56	0.17	7.21	6.18 (6) σ = 1.71	0.21
[1-β-Mercapto-α,α-dimethylpropionic acid]oxytocin	6.60	2.49 (9) σ = 0.98	0.47	7.41	3.93 (6) σ = 1.09	0.33
[1-β-Mercapto-β,β-diethylpropionic acid]oxytocin	7.24	0.58 (9) σ = 0.12	2.0	8.11	0.78 (8) σ = 0.17	1.68

[a] pA_2 values (see Schild[14]) represent here the negative log to the base 10 of the av molar concn ($\bar{M}$) of an antagonist which will reduce the response of the uterine horn or the chicken to $2x$ units of a pharmacologically active compd (agonist) to the response to x units of the agonist. In these studies synt oxytocin was the agonist used. The number of individual detns is given in parentheses, and σ is the std deviation. [b] Ratio of molar concn of [1-deaminopenicillamine]oxytocin to molar concn of other analog.

Experimental Section††

S-Bzl-mercaptodimethylacetic Acid (I). Benzylmercaptan (6.2 g) was added to a warm soln of Na (1.15 g) in anhyd EtOH (100 ml) and stirred under N_2 for 15 min. Ethyl α-bromoisobutyrate[17] (9.75 g) was added dropwise in anhyd EtOH (40 ml) and the soln was refluxed for 1 hr under N_2. A soln of NaOH (2 g) in a mixt of H_2O (20 ml) and EtOH (50 ml) was added to the hot soln, and hydrolysis was carried out at reflux for 6 hr. The solvents were removed *in vacuo*, and the residue was dissolved in H_2O (150 ml). The aq soln was extd with toluene and then acidified with HCl. The ppt was collected, washed on the filter with H_2O, and air-dried. Crystn from hexane give 4.4 g (needles), mp 95–96°. *Anal.* ($C_{11}H_{14}O_2S$) C, H. Ir and nmr data were consistent with I.

Boc-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly Resin (II). This compd was synthesized by the solid-phase method of Merrifield.[18] Boc-glycyl resin[19] (4 g) contg 0.76 mmole of glycine per g of resin was swelled overnight in CH_2Cl_2 (40 ml) in a 100-ml Merrifield reaction flask. The Boc-glycyl resin was then subjected to seven 21-step cycles of deblocking, neutralization, and coupling as described in the synthesis of [8-phenylalanine]oxytocin.[20] The AcOH was reagent grade which was distd through a 1.2 × 30-cm column packed with glass helices. Three equivs each of Boc-amino acids and DCI were used in the coupling steps. Boc-glutamine and Boc-asparagine were added as their *p*-nitrophenyl esters in 3 times the equiv amts and coupled in DMF. The DCI couplings were allowed to procede for 4 hr and the *p*-nitrophenyl ester reactions were allowed to procede overnight. After the Boc-*O*-benzyltyrosine was coupled to the peptide, the resin was washed with CH_2Cl_2, EtOH, AcOH, and finally with several portions of anhyd EtOH. The resin was sucked dry, then dried overnight *in vacuo* over KOH. The wt gain of the resin was 2.66 g, which represents 88% of the theoretical wt gain based on the amt of Boc-glycine esterified to the resin.

S-Bzl-mercaptodimethylacetyl-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (III). The protected octapeptide–resin compd II (1 g) was swelled overnight in CH_2Cl_2 (10 ml) and subjected to one 21-step solid-phase cycle as described previously. Three equivs each of I and DCI were used in the coupling step, and after the first 3-hr coupling period the peptide–resin was subjected to an addnl treatment with 3 equivs of the acid and of DCI, with coupling allowed to procede overnight.

Before cleavage with methanolic NH_3,[21] the substituted resin was subjected to an addnl deblocking and neutralization sequence, dried *in vacuo* over KOH, and then swelled for 3 hr in cold (0°) anhyd MeOH. Anhyd NH_3, distd from Na, was bubbled into the suspension for 3 hr at 0°. The flask was stoppered and the suspension was stirred at 0° overnight. The MeOH and NH_3 were removed *in vacuo*, and the residue was stirred with DMF for 3 hr. The suspension was filtered, the resin was rinsed with several portions of DMF, and the combined DMF soln was concd to a small vol. This was added dropwise to a large vol of H_2O with stirring, and the resulting milky suspension was stored overnight at 5°. The ppt was collected by centrifugation and washed several times with H_2O in the centrifuge tube, the H_2O being removed by centrifugation and decantation. The moist, packed ppt was frozen and lyophilized, the lyophilisate was dissolved in AcOH and filtered, and the AcOH soln was lyophilized to give 402 mg of compd III, mp 217–218°, $[\alpha]^{22}D$ −32.7° (*c* 1, DMF). Amino acid analysis of an acid-hydrolyzed sample gave the correct ratios of amino acids and NH_3. *Anal.* ($C_{65}H_{87}N_{11}O_{12}S_2 \cdot H_2O$) C, H, N. The material moved as one spot on silica gel tlc in S_a and S_b.

[1-Mercaptodimethylacetic Acid]oxytocin. The protected nonapeptide amide III (100 mg) was dissolved in anhyd NH_3 (25 ml) and reduced with Na at the boiling point of NH_3,[22] allowing the blue color to persist for 15 sec before destroying excess Na with NH_4Cl. The NH_3 was removed *in vacuo*, and the residue was dissolved in 0.03% trifluoroacetic acid (300 ml). The soln was adjusted to pH 6.8, and oxidn with ferricyanide[4] was carried out keeping the pH at 6.8. The ferri- and ferrocyanide ions were removed with AG3-X4 resin (trifluoroacetate cycle). The clear, colorless filtrate was lyophilized, and the residue was dissolved in 50% AcOH (2 ml). This soln was applied to a 1.10 × 103-cm column of Sephadex G-15 which had been equilibrated with 50% AcOH.[23] The analog was eluted at a flow rate of 9 ml/hr. Examn of the fractions by uv absorption (275 mμ) showed a sym peak centered at 46 ml. The tubes corresponding to the peak were pooled, dild with 1 vol of H_2O, concd to a small vol, and lyophilized. The lyophilisate was dissolved in 50% AcOH (2 ml) and applied to a 2.20 × 110-cm column of Sephadex G-15 which had been equilibrated with 0.2 *N* AcOH.[23] The analog was eluted at a flow rate of 40 ml/hr and emerged as a sym peak centered at 400 ml, preceded by a small shoulder of uv_{275}-absorbing material. The contents of the tubes corresponding to the main fractions were pooled, concd to 5 ml, and lyophilized to give 40 mg, $[\alpha]^{23}D$ +17.8° (*c* 1, DMF). An acid-hydrolyzed sample of this analog gave the expected ratios of amino acids and NH_3. *Anal.* ($C_{44}H_{67}N_{11}O_{12}S_2$) C, H, N.

β-(*S*-Bzl-mercapto)-α,α-dimethylpropionic Acid (IV). Benzyl mercaptan (12.3 g, 0.1 mole) was added to a soln of NaOEt (0.2 mole) in abs EtOH. After 15 min, chloropivalic acid (β-chloro-α,α-dimethylpropionic acid) (13.6 g, 0.1 mole) was added; the soln was refluxed for 18 hr under N_2 and evapd to dryness. The residue was dissolved in H_2O, and the basic aq layer was washed with Et_2O (50 ml). The soln was adjusted to pH 7, and the aq layer was washed with Et_2O (100 ml). The soln was acidified (pH 2), and the product was extd with Et_2O (160 ml). The Et_2O layer was dried (Na_2SO_4) and evapd to dryness *in vacuo*. Crystn from hexane gave 13.6 g (62%) of the acid IV; mp 51–51.5° (cor). Recrystn (1.8 g) from pentane (25 ml) gave analytically pure IV (1.5 g). *Anal.* ($C_{12}H_{16}O_2S$) C, H, S. Ir and nmr data were consistent with the acid IV.

β-(*S*-Bzl-mercapto)-α,α-dimethylpropionyl-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (V). The protected octapeptide–resin II (1 g) was swelled overnight in CH_2Cl_2 (10 ml) and subjected to a sequence of deblocking, neutralization, and coupling using 3 equivs each of the acid IV and DCI as described earlier. At the end of the 4-hr coupling period, the resin was washed with CH_2Cl_2 and a second coupling with 3 equivs of IV and DCI was allowed to procede overnight. Subsequent steps for washing, deblocking, and ammonolytic cleavage of the peptide from the resin were carried out as described for the synthesis of III to give 357 mg of the protected nonapeptide amide V, mp 225°, $[\alpha]^{22}D$ −31.8° (*c* 1, DMF). Amino acid analysis gave the correct ratios for the amino acids and NH_3. The material moved as one spot on silica gel tlc plates in solvents S_a and S_b. *Anal.* ($C_{66}H_{89}N_{11}O_{12}S_2$) C, H, N.

[1-β-Mercapto-α,α-dimethylpropionic Acid]oxytocin. The protected nonapeptide amide V (100 mg) was reduced with Na in liq NH_3, the resulting product was oxidized with $K_3Fe(CN)_6$, and the analog was isolated and purified as described earlier, wt 49.5 mg, $[\alpha]^{23}D$ −44.9° (*c* 1, DMF). The analog gave the correct ratios of amino acids and NH_3 on amino acid analysis. It was homogenous on silica gel tlc in solvents S_a and S_b. *Anal.* ($C_{45}H_{69}N_{11}O_{12}S_2$) C, H, N.

β-(*S*-Bzl-mercapto)-β,β-diethylpropionic Acid (VI). A mixt of 3-pentanone (43 g), ethyl bromoacetate (83.5 g), C_6H_6 (200 ml), and $PhCH_3$ (175 ml) was added dropwise to granular Zn (33 g) with heating. After the initial reaction had subsided, the mixt was refluxed for 4 hr, cooled, and 10% H_2SO_4 (200 ml) was added. The org layer was dried ($MgSO_4$), and the solvent was evapd. The residue was distd at reduced pressure to give ethyl β-hydroxy-β,β-diethylpropionate (60.2 g), bp 75–76° (4 mm). This ester (46 g) was dehydrated by refluxing with P_2O_5 (30 g) in C_6H_6 (200 ml) for 24 hr. H_2O (100 ml) was added to the cooled soln and the org layer was sepd and dried ($MgSO_4$). Distn of the residue after evapn of the solvent gave a mixt of 25% of the α,β-unsaturated ester and 75% of the β,γ-unsaturated ester, as detd by nmr. A portion of this mixt (15.6 g) was refluxed with benzylmercaptan (21 g) in piperidine (40 ml) for 24 hr. The soln was cooled, acidified with HCl, and extd with Et_2O. The Et_2O was removed from the dried soln, and the residue was refluxed with K_2CO_3 (25 g) in a mixt of MeOH (300 ml) and H_2O (75 ml) for 24 hr. The solvent was distd from the reaction mixt until the vapors registered 100° and the distillate was clear. The residue in the flask was extd with Et_2O, the org soln was dried ($MgSO_4$), and the Et_2O was removed. Distn of the residue gave 17.3 g of a yellow oil, bp 158–167° (0.3 mm), which was crystd from hexane, mp 75–76°. *Anal.* ($C_{14}H_{20}O_2S$) C, H, S. Ir and nmr data were consistent with the structure VI.

β-(*S*-Bzl-mercapto)-β,β-diethylpropionyl-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (VII) was synthesized by condensation of II (1 g) with VI, followed by ammonolysis and purification, all as described earlier; wt 370 mg, mp 235°, $[\alpha]^{22}D$ −43.3° (*c* 1, DMF). Amino acid analysis gave the correct ratios for amino acids and NH_3. The protected nonapeptide amide VII moved as one spot on silica gel tlc plates in solvents S_a and S_b. *Anal.* ($C_{68}H_{93}N_{11}O_{12}S_2$) C, H, N: Calcd, 11.7; found, 11.2.

[1-β-Mercapto-β,β-diethylpropionic Acid]oxytocin. The pro-

††Melting points were detd in open capillary tubes and are uncorrected. Solvent systems: S_a, CH_3OH–$CHCl_3$ (3:8); S_b, *n*-BuOH–AcOH–H_2O (4:1:1). Amino acid analyses were performed on a Beckman/Spinco amino acid analyzer Model 116 by the method of Spackman, *et al.*[16] Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for the elements, were within ±0.4% of the theoretical values.

tected nonapeptide amide VII was reduced with Na in liq NH_3, the resulting product was oxidized with $K_3Fe(CN)_6$, and the analog was isolated and purified as described earlier; wt 31 mg; $[\alpha]^{23}D$ −37.6° (*c* 1, DMF). Amino acid analysis gave the correct ratios of amino acids and NH_3. The analog moved as 1 spot on silica gel tlc plates in the above solvents. *Anal.* ($C_{47}H_{73}N_{11}O_{12}S_2$) C, H, N.

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Further Studies of the Role of the Asparagine Residue in Oxytocin. Synthesis and Biological Properties of [5-α,γ-Diaminobutyric acid]oxytocin†‡

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The synthesis of [5-α,γ-diaminobutyric acid]oxytocin, an analog of oxytocin which contains an α,γ-diaminobutyric acid residue in place of the asparagine residue in position 5, is reported. On assay for biological activity this analog was found to possess 0.03 unit/mg of oxytocic activity, approximately 0.03 unit/mg of avian vasodepressor activity, and less than 0.01 and 0.001 unit/mg, respectively, of mammalian pressor and antidiuretic activities. The analog was incapable of inhibiting the oxytocin-induced responses in the 4 biological systems tested. It is concluded that [5-α,γ-diaminobutyric acid]oxytocin possesses a low affinity for the neurohypophyseal hormone receptors, and that this effect is a result of an increase in the conformational flexibility of this analog as compared with oxytocin.

All naturally occurring neurohypophyseal hormones of known amino acid sequence possess an Asp(NH_2) residue in position 5. Oxytocin analogs which bear a Me,[1,2] β-carboxamideethyl,[3] hydroxymethyl,[1] γ-aminopropyl,[4] and *i*-Pr[5] side chain in position 5 instead of the carboxamidemethyl moiety all exhibit an exceedingly low potency with respect to the activities characteristic of oxytocin—a finding which led us to focus on the importance of position 5 for the conformational stability of the hormonal molecule.[6-8] The elucidation of the conformation of oxytocin[9] confirmed the central role of the Asp(NH_2) residue. It is critical for the maintenance of both β-turns comprised of the sequences -Tyr-Ile-Glu(NH_2)-Asp(NH_2)- and -Cys-Pro-Leu-Gly- (Figure 1).

In the present communication we report on the synthesis of [5-α,γ-diaminobutyric acid]oxytocin and on the determination of its biological properties in order to assess the capability of α,γ-diaminobutyric acid (Dbu) to replace successfully the Asp(NH_2). The free tetrapeptide, *S*-Bzl-Cys-Pro-Leu-Gly-NH_2,[10-13] was elongated with BOC-Glu(NH_2)-N^{γ}-Pht-Dbu (**5**) in the presence of 1.4 equiv of DCI and 2.0 equiv of *N*-hydroxysuccinimide according to the procedure of Weygand, *et al.*,[14] to yield BOC-Glu(NH_2)-N^{γ}-Pht-Dbu-*S*-Bzl-Cys-Pro-Leu-Gly-NH_2 (**6**).

The dipeptide **5** was secured from N^{γ}-Pht-Dbu·H_2O (**1**), obtained after phthalylation of the dihydrochloride salt of the free acid by the general procedure of Nefkens, *et al.*,[15] in the following manner: the acid **1** was converted to its *p*-toluenesulfonate salt (**2**), which in turn was esterified with Ph_2CHN_2;[16-18] the resultant ester **3** was allowed to react with BOC-Glu(NH_2)-ONp[19] to give BOC-Glu(NH_2)-N^{γ}-Pht-Dbu diphenylmethyl ester (**4**), which was subsequently deesterified to give **5**. The hexapeptide (**6**) was elongated stepwise to yield the fully protected nonapeptide, *N*-Z-*S*-Bzl-Cys-*O*-Bzl-Tyr-Ile-Glu(NH_2)-N^{γ}-Pht-Dbu-*S*-Bzl-Cys-Pro-Leu-Gly-NH_2 (**9**). Peptide **9** was successfully dephthalylated with either hydrazine hydrate[20,21] or hydrazine acetate[22] to yield **10**, which was fully deprotected by treatment with

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‡Abbreviations used have been suggested in *J. Med. Chem.*, 13(5), 8A (1970). The amino acids (except glycine) are of the L configuration.