(21) E. L. Bravo, M. C. Khosla, and F. M. Bumpus, *J. Clin. Endocrinol. Metab.*, **40**, 530 (1975).
(22) W. Koenig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(23) S. Visser, J. Raap, K. E. T. Kerling, and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **89**, 865 (1970).
(24) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).
(25) R. K. Olsen, *J. Org. Chem.*, **35**, 1912 (1970).
(26) V. J. Hruby, F. Muscio, C. M. Groginsky, P. M. Gitu, and D. Saba, *J. Med. Chem.*, **16**, 624 (1973).
(27) J. S. Morley, *J. Chem. Soc. C*, 2410 (1967).
(28) M. C. Khosla, R. R. Smeby, and F. M. Bumpus in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 227.
(29) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 3102 (1972).
(30) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Science*, **156**, 253 (1967).
(31) W. Koenig, R. Geiger, and H. Wissman, German Offen 2202613 (1973); *Chem. Abstr.*, **79**, 137510 (1973).
(32) H. C. Beyerman and R. A. int'l Veld, *Recl. Trav. Chim. Pays-Bas*, **88**, 1019 (1969).
(33) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 6165 (1972).
(34) J. M. Stewart and J. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969.
(35) M. J. Peach, Symposium on the Pharmacology of Angiotensin Antagonists, FASEB Proceedings, Atlantic City, N.J., March 1975, in press.

Synthesis and Some Pharmacological Properties of Five Analogs of Oxytocin Having L-Homocysteine in Position 6[1]

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Five analogs of oxytocin have been synthesized with a homocysteine residue in position 6 and 2-, 3-, or 4-carbon residues in position 1. The compounds, which contain 20-, 21-, and 22-membered disulfide rings, respectively, were [1-α-mercaptoacetic acid,6-homocysteine]oxytocin, [6-homocysteine]oxytocin, [1-β-mercaptopropionic acid,6-homocysteine]oxytocin, [1,6-homocystine]oxytocin, and [1-γ-mercaptobutyric acid,6-homocysteine]oxytocin. The appropriate protected polypeptide intermediates were prepared by the solid-phase method of peptide synthesis. The protecting groups were removed by treatment with Na in NH_3 and the disulfide bond was formed by oxidation with ICH_2CH_2I in aqueous MeOH. Purification was effected by partition chromatography followed by gel filtration. The pharmacological activities of all five analogs are reported for the oxytocic, avian vasodepressor, and rat pressor assays. Compared to oxytocin, these analogs exhibited sharply reduced agonist potencies, and several exhibited antagonist activity.

The importance of the 20-membered disulfide ring to the biological activities of oxytocin and the vasopressins has been an object of continued investigation in this and other laboratories. Several studies have been done on analogs of oxytocin (Figure 1) in which the ring size has been increased in various ways.[3-12] None of these compounds showed more than a slight degree of biological activity.

One of the ways in which a change in the ring size has been accomplished without interrupting the α-amino acid sequence is by the formal insertion of a methylene group at position 1 to form the 21-membered ring analog [1-L-homocysteine]oxytocin.[6] This analog showed only a slight oxytocic activity and no avian vasodepressor (AVD) or rat pressor activity. In this analog the substitution of the homocysteine for the cysteine residue, while increasing the size of the ring, leads simultaneously to a change in the separation of the free amino group from the disulfide bond. The synthesis of the highly potent 1-deamino-oxytocin[13] ([1-β-mercaptopropionic acid]oxytocin), which possesses almost twice the oxytocic and AVD potency of oxytocin, showed that the free amino group is not requisite for these activities. The study of the effect of ring size without the involvement of the amino group was undertaken with the synthesis of several analogs of 1-deamino-oxytocin by the formal introduction of one or more methylene groups or the deletion of one methylene group at the β-mercaptopropionic acid residue at position 1.[7,8,11,12,14] In this manner analogs having 19-, 21-, 22-, and 28-membered rings were prepared and showed drastic reduction or total loss of activity.

Another possible way to study the effect of ring size without involvement of the free amino group at position 1 is to expand the ring size by the formal insertion of a methylene group at position 6. We report here the synthesis of five analogs of oxytocin in which the Cys[6] residue has been replaced by an L-homocysteine residue. Combining this modification with various residues in position 1, the following analogs of oxytocin have been prepared: [6-homocysteine]oxytocin (**3**)[15] ([Hcy[6]]oxytocin), [1,6-homocystine]oxytocin (**5**)[15] ([Hcy[1,6]]oxytocin), [1-α-mercaptoacetic acid,6-homocysteine]oxytocin (**7**)[16] ([α-Maa[1],Hcy[6]]oxytocin), [1-β-mercaptopropionic acid,6-homocysteine]oxytocin (**9**) ([β-Mpa[1],Hcy[6]]oxytocin), and [1-γ-mercaptobutyric acid,6-homocysteine]oxytocin (**11**) ([γ-Mba[1],Hcy[6]]oxytocin). The common intermediate, Boc-Tyr(Bzl)-Ile-Gln-Asn-Hcy(Bzl)-Pro-Leu-Gly-O-resin

Table I. Solid-Phase Procedure

Step no.	Reagent	Repetitions	Time, min
1	CH_2Cl_2	3	2
2	CF_3CO_2H-anisole-CH_2Cl_2 (48:2:50)	1	25
3	CH_2Cl_2	4	2
4	*i*-Pr_2NEt-CH_2Cl_2 (7:93)	2	2
5	CH_2Cl_2	4	2
6	Boc-amino acid in CH_2Cl_2 or DMF[a]	1	
7[b]	DCC in CH_2Cl_2	1	Variable[a]
8	CH_2Cl_2 or DMF[a]	3	2
9	EtOH	3	2

[a] See Experimental Section. DMF was the solvent whenever HBt was used. Different reaction times were employed depending on the use of HBt and on the amino acid residue. [b] This step is added to the reagent present from step 6. Only half of the normal solvent volume is used in steps 6 and 7, so that the total volume is the same as in all other steps.

Table II. Pharmacological Properties

Compound	Ring size	Oxytocic[a]	AVD[a]	Pressor[a]
Oxytocin	20	546 ± 18[b]	507 ± 23[c]	3.1 ± 0.1[c]
[Hcy^6]oxytocin	21	12.0 ± 0.5	0.2–0.3	No activity
[Hcy^{1,6}]oxytocin	22	$\bar{M} = 9.6 \times 10^{-7}$ (9), $\bar{\sigma} = 1.7 \times 10^{-7}$, $pA_2 = 6.02$	$\bar{M} = 3.3 \times 10^{-7}$ (7), $\bar{\sigma} = 0.6 \times 10^{-7}$, $pA_2 = 6.48$	No activity
Deamino-oxytocin ([β-Mpa^1]oxytocin)	20	803 ± 36[d]	975 ± 24[d]	1.44 ± 0.06[d]
[α-Maa^1,Hcy^6]oxytocin	20	37 ± 1	0.3–0.4	No activity
[β-Mpa^1,Hcy^6]oxytocin	21	22 ± 1	$\bar{M} = 2.7 \times 10^{-8}$ (9), $\bar{\sigma} = 1.4 \times 10^{-8}$, $pA_2 = 7.57$	No activity
[γ-Mba^1,Hcy^6]oxytocin	22	Agonist 0.3–0.5 Antagonist $\bar{M} = 1.8 \times 10^{-7}$ (8), $\bar{\sigma} = 0.4 \times 10^{-7}$, $pA_2 = 6.74$	$\bar{M} = 4.6 \times 10^{-8}$ (12), $\bar{\sigma} = 1.5 \times 10^{-8}$, $pA_2 = 7.34$	$\bar{M} = 6.8 \times 10^{-6}$ (7), $\bar{\sigma} = 1.4 \times 10^{-6}$, $pA_2 = 5.17$

[a] Agonist activity is expressed in units per milligram as mean potencies ± standard error. Inhibitory potencies were determined and expressed as pA_2 values as defined by Schild.[27] This represents the negative logarithm to the base 10 of the average molar concentration ($\bar{M}$) of the antagonist which will reduce the appropriate biological response to $2x$ units of pharmacologically active compounds (agonist) to the level of x units of agonist. Specific details of the antioxytocic and anti-AVD assays are described by Vavrek et al.[28] and of the antipressor assay by Dyckes et al.[29] [b] W. Y. Chan et al.[30] [c] W. Y. Chan and V. du Vigneaud.[31] [d] B. M. Ferrier et al.[13c] The number of individual determinations is given in parentheses and σ is the standard deviation.

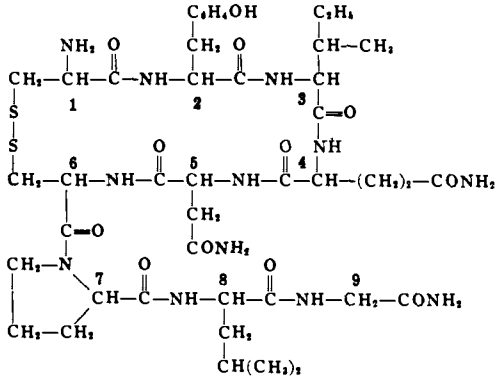

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues. In deamino-oxytocin the NH_2 at position 1 is replaced with H.

(1), was synthesized by the general procedure of the Merrifield solid-phase method[17] as outlined in Table I with the modifications described in the Experimental Section. Five different portions of 1 were used for the attachment of the five different N-terminal residues. The partially protected polypeptide amide intermediates were obtained by ammonolysis of the appropriate peptide resins. The remaining protecting groups were removed by treatment with Na in refluxing NH_3,[18] and the disulfide bond was formed by oxidation with diiodoethane[19] in aqueous MeOH. The analogs were purified by partition chromatography[20] and gel filtration.[21] During the purification of compounds 7, 9, and 11, a by-product was isolated in the gel filtration step. In one case (9) the amino acid analysis of the by-product contained Tyr, Ile, Asp, Glu, and NH_3 in the ratio of 1:1:1:1:2 as the only identifiable components (see Experimental Section). This result would seem to indicate that the Hcy-Pro bond was easily cleaved by the Na–NH_3 treatment. This is a well-documented event for other X-Pro bonds[22] but does not seem to be as troublesome for the Cys-Pro bond present in most oxytocin analogs.

The purified analogs were tested for oxytocic,[23] AVD,[24] and rat pressor[25] activities against U.S.P. posterior pituitary reference standard. The four-point assay design[26] was used whenever measurable activity was detected. When little or no activity was detected in the above assays, the compounds were checked for inhibition of the oxytocic and AVD responses to synthetic oxytocin for inhibition of the pressor response to synthetic lysine-vasopressin (LVP).

The pharamcological activities of the compounds are shown in Table II. In all three pharmacological assays all of the analogs exhibited a drastic reduction in agonist activity or the appearance of inhibitory activity. One analog, [γ-Mba^1,Hcy^6]oxytocin, was found to possess extremely weak agonist as well as potent antagonist activity. In order to measure the antagonist potency, the injection of [γ-Mba^1,Hcy^6]oxytocin was given 1.5–2 min prior to the injection of synthetic oxytocin. This allowed sufficient time for any agonist response to be complete. After the first injection of [γ-Mba^1,Hcy^6]oxytocin, a potentiation of the response to subsequent injections of the oxytocin standard was observed. However, a given amount of [γ-Mba^1,Hcy^6]oxytocin inhibited the potentiated response to the same degree as it inhibited the original response.

The drastic differences in activities between deamino-oxytocin and [α-Maa^1,Hcy^6]oxytocin, which still possesses a 20-membered ring, seem to indicate that an effect more complex than the change in ring size is being measured. In the remaining four analogs reported here one or two additional methylene groups have been inserted at position 1 in addition to the one at position 6, and further reduction in agonist potency or enhancement of inhibitory potency is observed. In previous studies where alkyl groups have been added in position 1 without expanding the ring size (e.g., [1-β,β-diethyl-β-mercaptopropionic acid]oxytocin), analogs of oxytocin were produced which are inhibitors of oxytocin in the oxytocic and AVD assays and of LVP in the pressor assay.[28,32] In fact, the results observed here may reflect the effect of introducing more steric bulk in the form of the extra methylene groups in the region of the disulfide bridge rather than an effect of increased ring size per se.

Experimental Section

All melting points were determined in open capillary tubes and are corrected. Thin-layer chromatography was performed on precoated glass plates of silica gel GF 254 (0.25 mm, E. Merck) in the following solvent systems: (A) $CHCl_3$–MeOH–HOAc (18:2:1); (B) BuOH–HOAc–H_2O (4:1:1); (C) BuOH–pyridine–HOAc–H_2O (15:10:3:6); (D) acetone–HOAc–H_2O (8:1:1). The load size was 10–30 μg, and chromatogram lengths were 100–150 mm.

Final visualization was made by chlorination followed by NaI–starch treatment. In all cases unless otherwise noted, single symmetrical spots were observed for purified materials. Amino acid analysis[33] was performed on a Beckman Model 116 amino acid analyzer using a single column system[34] following hydrolysis in deareated 6 *N* HCl at 110°. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Where analyses are indicated only by the symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values. Where compounds are formulated as containing solvent this does not necessarily imply that they are defined solvates; particularly in the case of amorphous products, solvent retention may be due to mild drying conditions [24 hr at 22° (0.005 Torr) over P_2O_5].

N-tert-**Butyloxycarbonyl**-*S*-**benzyl**-L-**homocysteine.** This compound [Boc-Hcy(Bzl)] was prepared by the method of Schnabel[35] from *S*-benzyl-L-homocysteine[36] (5.62 g, 25 mmol) and *tert*-butyl azidoformate (4.0 ml, 28 mmol). The pH of the dioxane–H_2O solution was maintained at 10.2 for 20 hr. The product was isolated by adjusting the pH of the ether-extracted reaction solution to 2 with concentrated HCl and extracting with EtOAc. The oil remaining after removal of the EtOAc crystallized from Et_2O (10 ml): 4.70 g (59%); mp 103–104°; $[\alpha]^{20}D$ −13.3° (*c* 1.2, MeOH); TLC (A) 0.62. Anal. ($C_{16}H_{23}NO_4S \cdot 0.25C_4H_{10}O$) C, H, N.

Boc-Tyr(Bzl)-Ile-Gln-Asn-Hcy(Bzl)-Pro-Leu-Gly-O-resin (1). Boc-Gly-O-resin was prepared by standard methods[37] from polystyrene crosslinked with 2% divinylbenzene containing 2 mmol/g of chloromethyl groups. A small portion of the Boc-Gly-O-resin was treated with CF_3COOH (steps 1–5 of Table I) to remove the Boc-protecting groups and any Boc-Gly attached to the resin at quaternized sites. A substitution of 0.76 mmol/g was determined by amino acid analysis following 24-hr hydrolysis in 12 *N* HCl–dioxane (1:1) at 115°. The same substitution (0.76 mmol/g) was also found by the aldimine test of Esko et al.[38] as modified by Ehler.[39] A 5.00-g portion of Boc-Gly-O-resin was used. A complete coupling cycle is shown in Table I. A volume of 50 ml of each reagent or solvent was used for each step. The number of equivalents of each reagent referred to below is based on the number of millimoles of glycine esterified to the resin. When 1-hydroxybenzotriazole (HBt) was added to the protected amino acid in step 6 to mediate the coupling reaction,[40] the solvent in steps 6 and 8 was changed from CH_2Cl_2 to dimethylformamide (DMF). The ninhydrin test[41] was performed after each complete cycle or recoupling cycle, and a successful attachment was better than 99.4% complete. Unless otherwise noted the first coupling employed 2.5 equiv of protected amino acid and 2.5 equiv of dicyclohexylcarbodiimide (DCC). If HBt was also used in the first coupling (see below), 5 equiv were used. A repeat coupling (execute steps 5–9 of Table I) employed 0.5 equiv of protected amino acid and DCC and 1 equiv of HBt. Boc-Leu was attached in a single 2-hr coupling. The attachment of Boc-Pro required a 2-hr recoupling following the normal 2-hr first coupling. Boc-Hcy(Bzl) was attached using a 1.5-hr coupling employing 1.5 equiv of Boc-Hcy(Bzl) and DCC and 3 equiv of HBt, followed by two repeat couplings of 1 and 4 hr. Boc-Asn,[42] Boc-Gln, and Boc-Tyr(Bzl) were successfully coupled in single 4-hr cycles employing HBt. Boc-Ile was coupled using a standard 2-hr first coupling and a 14-hr second coupling. The peptide resin was dried in vacuo: 8.30 g (85% of the theoretical weight gain). A small portion was subjected to steps 1–5 (Table I), and the amount of free amino groups was found to be 0.27 mmol/g (70% of theoretical adjusted for observed weight gain).

H-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Hcy-(Bzl)-Pro-Leu-Gly-NH_2 (2). A 1.60-g portion of 1 was employed for this synthesis. It was coupled with Boc-Cys(Bzl) (2.5 equiv, 0.57 g, 1.83 mmol) in the manner described for the coupling of Boc-Asn and Boc-Gln. The number of equivalents of each reagent was based on the amount of glycine originally esterified to this fraction of the total amount of peptide resin (i.e., 0.73 mmol), and the volume of each reagent or wash was 15 ml. Steps 1–5 of Table I were performed to remove the Boc protection. In addition, three washes with anhydrous MeOH (distilled from $Mg(OCH_3)_2$ were performed. The peptide resin was transfered from the solid-phase reaction vessel to a one-neck 300-ml round-bottom flask with anhydrous MeOH (75 ml). The suspension was saturated with anhydrous NH_3 (distilled from Na) at 0°. The flask was stoppered securely and placed in a desiccator where the suspension was stirred magnetically at room temperature for 3 days. The NH_3 and MeOH were removed by evaporation and the crude product was extracted from the resin by stirring in warm (50°) DMF. The product was precipitated from DMF with H_2O, collected, washed with H_2O, EtOAc, and Et_2O, and dried in vacuo: 520 mg (55% based on Gly); mp 219.5–221.5°. TLC (B) showed a single uv and ninhydrin positive spot, R_f 0.47. Visualization with Cl_2 followed by NaI–starch, however, revealed three small impurities. This product was used for the synthesis of **3** without further purification. For analytical purposes a 50-mg portion was precipitated from DMF–EtOH (1:1) with EtOAc and then reprecipitated from DMF with H_2O with 50% recovery for both steps: TLC (B) 0.50 with two trace impurities; mp 220.5–222.5°; $[\alpha]^{21}D$ −30.6° (*c* 0.95, DMF). Anal. ($C_{65}H_{88}N_{12}O_{12}S_2 \cdot H_2O$) C, H, N.

[6-Homocysteine]oxytocin (3). A solution of **2** (130 mg, 0.10 mmol) in anhydrous liquid NH_3 (distilled from Na) was treated with a freshly cut glass-encased stick of Na until the blue color of excess Na persisted for 30 sec. The excess Na was discharged with HOAc, and the NH_3 was removed by evaporation and lyophilization. The reaction vessel was opened under N_2 and a N_2-flushed mixture of H_2O (100 ml) and MeOH (90 ml) was used to dissolve the residue. A solution of ICH_2CH_2I (29 mg, 0.10 mmol) in 10 ml of MeOH was added and the disappearance of the sulfhydryl groups was monitored by the Ellman method.[43] The reaction was complete in 5 min and the pH was adjusted from 11.2 to 3.5 with HOAc (4 ml). The solvents were removed by rotary evaporation and the residue was dissolved in 0.5 ml of the lower phase and 3 ml of the upper phase of the system BuOH–H_2O (1:1, aqueous phase 1.5% pyridine and 3.5% HOAc). This solution was applied to a 2.20 × 52 cm column of Sephadex G-25 (100–200 mesh), which had been equilibrated with both phases of the solvent system, and subjected to partition chromatography.[20] The product was eluted from the column with upper phase at a flow rate of 12.5 ml/hr and collected in fractions of 2.5 ml. Peptide material was detected by the Folin–Lowry method.[44] The main peak emerged with a maximum at R_f 0.24 preceded by and resolved from two smaller peaks at R_f 0.83 and 0.42. Fractions 65–90 were pooled and diluted with two volumes of H_2O. The volume of solvent was reduced to 25 ml by rotary evaporation and 0.2 *N* HOAc (30 ml) was added. The product was isolated by lyophilization: 48.5 mg. The entire batch was further purified by gel filtration[21] on a 2.82 × 68 cm column of Sephadex G-25 (200–270 mesh) in 0.2 *N* HOAc. The product was eluted at a flow rate of 35 ml/hr and collected in fractions of 4.3 ml. The peptide material was detected by reading the absorbency of the eluate at 280 nm. The product emerged as a single sharp symmetrical peak with a maximum at 79% of the column volume. Fractions 73–82 were pooled and the product was isolated by lyophilization: 46.4 mg (46%); $[\alpha]^{20}D$ −65.8° (*c* 0.48, 1 *N* HOAc); TLC (B) 0.37, (C) 0.66. Anal. ($C_{44}H_{68}N_{12}O_{12}S_2 \cdot CH_3CO_2H \cdot 2H_2O$) C, H, N. Amino acid analysis following 21-hr hydrolysis gave the following molar ratios: Asp, 0.99; Glu, 1.01; Pro, 1.00; Gly, 0.96; Leu, 1.04; Tyr, 1.00; NH_3, 3.26. $(Cys)_2$ and $(Hcy)_2$ were present in approximately equal amounts. A peak presumed to be the mixed disulfide Cys–Hcy appeared as an unresolved peak with Ile. Amino acid analysis of a performic acid oxidized sample[45] hydrolyzed as above gave the following molar ratios: $Cys(SO_3H)$ + $Hcy(SO_3H)$, 1.70; Asp, 1.00; Glu, 1.02; Pro, 0.97; Gly, 1.00; Ile, 1.00; Leu, 1.04. The peaks for $(Cys)_2$ and $(Hcy)_2$ and the peak with Ile were absent from this analysis.

H-Hcy(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Hcy(Bzl)-Pro-Leu-Gly-NH_2 (4). This compound was prepared and isolated in the same manner as described for **2** using Boc-Hcy(Bzl) (2.5 equiv, 0.60 g, 1.83 mmol): yield 520 mg (55% based on glycine); mp 223.5–226.5°; TLC (B) showed a single uv-positive spot at R_f 0.53. Visualization with ninhydrin revealed a second faint spot at R_f 0.47, and further treatment with Cl_2 followed by NaI–starch showed two additional trace impurities. The entire batch was reprecipitated from a boiling solution of HOAc–EtOH (1:4) with 73% recovery: mp 225.5–228.5°; $[\alpha]^{24}D$ −36.9° (*c* 1.05, DMF); TLC (B) 0.55 with two trace impurities. Anal. ($C_{66}H_{90}N_{12}O_{12}S_2 \cdot 0.5CH_3CO_2H$) C, H, N.

[1,6-Homocystine]oxytocin (5). This compound was prepared

from 4 (137 mg, 0.10 mmol) in the manner described for 3. The crude product was purified by partition chromatography in the same system as for 3, but using a 6.22 × 69 cm column of Sephadex G-25 and fractions of 6.5 ml. The product emerged as a sharp symmetrical peak with a maximum at R_f 0.30, well resolved from a smaller peak which appeared at the void volume of the column. Fractions 41–52 were pooled, the solvents were removed by rotary evaporation, and the residue was lyophilized from distilled HOAc: 49.4 mg. The entire product was subjected to further purification by gel filtration as described for 3 and emerged at 78% of the column volume. Fractions 67–76 were pooled with two volumes of H_2O and lyophilized: 43.9 mg (42%); $[\alpha]^{24}D$ +65.7° (c 0.55, 1 N HOAc); TLC (B) 0.38, (C) 0.66. Anal. ($C_{45}H_{70}N_{12}O_{12}S_2 \cdot CH_3CO_2H \cdot 2H_2O$) C, H, N. Amino acid analysis following 48-hr hydrolysis gave the following molar ratios: Asp, 0.98; Glu, 1.03; Pro, 1.00; Gly, 1.00; Ile, 1.03; Leu, 1.04; Tyr, 0.85; 1/2(Hcy)2 1.65; NH_3, 2.98. Amino acid analysis of a performic acid oxidized sample hydrolyzed as above but for 17 hr gave the following molar ratios: Hcy(SO_3H), 1.93; Asp, 1.00; Glu, 1.05; Pro, 1.00; Gly, 1.01; Ile, 0.93; Leu, 1.00.

α-Maa(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Hcy(Bzl)-Pro-Leu-Gly-NH_2 (6). Another 1.60 g of 1 was used with 4 equiv (0.88 g, 2.92 mmol) of p-nitrophenyl S-benzyl-α-mercaptoacetate.[14] Referring to Table I, the total volume of step 6 was 15 ml of CH_2Cl_2–DMF (1:1), step 7 was omitted, and DMF was used in step 8. The coupling step was allowed to proceed for 20 hr, then 1 equiv of i-Pr_2NEt was added, and the reaction continued for 2 hr. The remainder of the work-up is as described for 2, omitting the steps required for the removal of the Boc group of the Cys(Bzl) residue: 585 mg (63% based on glycine); mp 235–238°, TLC (B) showed a single uv-positive, ninhydrin-negative spot at R_f 0.59. However, further visualization with Cl_2 followed by NaI–starch revealed the presence of four impurities. The product crystallized from HOAc–EtOH (1:6) with 83% recovery: mp 235–238°; $[\alpha]^{24}D$ –29.4° (c 0.9, DMF); TLC (B) 0.60 with two trace impurities. Anal. ($C_{64}H_{85}N_{11}O_{12}S_2$) C, H, N.

[1-α-Mercaptoacetic acid,6-homocysteine]oxytocin (7). This compound was prepared from 6 (126 mg, 0.10 mmol) in the manner described for 3. Purification was effected by partition chromatography as described for 3 on the column described for 5 in the system BuOH–C_6H_6–H_2O (1:1:2, the aqueous phase 1.5% pyridine and 3.5% HOAc). The eluate was collected in fractions of 4.8 ml. The product emerged as a single peak (R_f 0.24) with a shoulder on the leading edge. TLC (D) of fractions across the peak area showed a major component at R_f 0.68 with a minor component, concentrated in the earlier fractions, at R_f 0.78. Fractions comprising the entire peak area (63–90) were pooled and the product was isolated as described for 5: 59.4 mg. The entire product was subjected to gel filtration as described for 3. The product emerged as a sharp symmetrical peak with a maximum at 82% of the column volume (fractions 75–81) followed by and well resolved from a small broad peak at 100% of the column volume (fractions 88–100). The material in fractions 75–81 was isolated by lyophilization: 43.8 mg (43%); $[\alpha]^{22}D$ –105.3° (c 0.52, N HOAc); TLC (B) 0.41, (D) 0.67. Anal. ($C_{43}H_{65}N_{11}O_{12}S_2 \cdot 2.5H_2O$) C, H, N. Amino acid analysis following 22-hr hydrolysis gave the following molar ratios: Asp, 1.04; Glu, 1.05; Pro, 1.04; Gly, 1.01; Ile, 0.94; Leu, 0.98; Tyr, 0.96; NH_3, 2.93. A peak for (Hcy)2 was present, and one presumed to be the mixed disulfide Maa Hcy appeared just prior to Ile. Amino acid analysis of a performic acid oxidized sample hydrolyzed as above gave the following molar ratios: Hcy(SO_3H), 1.04; Asp, 1.04; Glu, 1.04; Pro, 1.00; Gly, 0.99; Ile, 0.94; Leu, 0.99. The peaks for (Hcy)2 and Maa Hcy were absent from this sample.

β-Mpa(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Hcy(Bzl)-Pro-Leu-Gly-NH_2 (8). This compound was prepared and isolated in the same manner as described for 2 from S-benzyl-β-mercaptopropionic acid (2.5 equiv, 0.36 g, 1.83 mmol), omitting the steps required for the removal of the Boc-protecting group from the Cys(Bzl) residue: 573 mg (61% based on glycine); mp 243–244°. TLC (B) showed a single uv-positive spot at R_f 0.62. Further visualization with Cl_2 followed by NaI–starch revealed three trace impurities. This product was used without further purification for the preparation of 9. For analytical purposes a 50-mg sample was reprecipitated from HOAc–EtOH (1:3) with 73% recovery: mp 243–246°; $[\alpha]^{20}D$ –29.2° (c 0.73, DMF); TLC (B) 0.62 with two trace impurities. Anal. ($C_{65}H_{87}N_{11}O_{12}S_2 \cdot H_2O$) C, H, N.

[1-β-Mercaptopropionic acid,6-homocysteine]oxytocin (9). This compound was prepared from 8 and purified by partition chromatography in the manner described for 7. Fraction size was 6.2 ml and the product emerged with a peak maximum at R_f 0.24 preceded by and overlapping a smaller peak at R_f 0.34. TLC (B), (D) showed approximately the same pattern of impurities as obtained for 7. Material from the entire peak area (fractions 35–64) was isolated in the manner as described for 5: 64.2 mg. The entire product was further purified by gel filtration as described for 3. The product emerged as a sharp symmetrical peak (fractions 69–75) at 80% of the column volume, followed by and resolved from a smaller broader peak (fractions 82–97) with a maximum at 102% of the column volume. The material in fractions 69–75 was isolated by lyophilization: 46.9 mg (45%); $[\alpha]^{25}D$ –117.2° (c 0.53, N HOAc); TLC (B) 0.41, (D) 0.62. Anal. ($C_{44}H_{67}N_{11}O_{12}S_2 \cdot 2H_2O$) C, H, N. Amino acid analysis following 24-hr hydrolysis gave the following molar ratios: Asp, 0.98; Glu, 1.01; Pro, 1.01; Gly, 0.97; Ile, 0.99; Leu, 1.05; Tyr, 0.99; NH_3, 3.15. A peak for (Hcy)2 and a peak presumed to be the mixed disulfide Mpa Hcy appeared between Leu and Tyr. Amino acid analysis of a performic acid oxidized sample hydrolyzed as above gave the following molar ratios: Hcy(SO_3H), 1.01; Asp, 1.00; Glu, 1.01; Pro, 1.00; Gly, 0.99; Ile, 0.96; Leu, 1.04. The peak for (Hcy)2 and the one presumed to be the mixed disulfide of Mpa Hcy were absent from this sample.

The material in fractions 82–97 from the gel filtration was also isolated: 9.2 mg; TLC (B) 0.53, (D) 0.73. Amino acid analysis following 22-hr hydrolysis gave the following molar ratios: Asp, 1.01; Glu, 1.03; Ile, 0.98; Tyr, 0.99; NH_3, 2.11. No detectable amount of Pro, Leu, or Gly was found. No peak for (Hcy)2 or the mixed disulfide Mpa Hcy was detected, both of which were present in the analysis of the analog. In addition, an unknown peak in the basic region appeared before NH_3.

γ-Mba(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Hcy(Bzl)-Pro-Leu-Gly-NH_2 (10). This compound was prepared and isolated in the manner described for 6 using p-nitrophenyl S-benzyl-γ-mercaptobutyrate[8] (4 equiv, 0.97 g, 2.92 mmol): 503 mg (53% based on glycine); mp 240–242°; TLC (B) 0.66 with three impurities, (D) 0.80 with two impurities. This product was used without further purification for the synthesis of 11. For analytical purposes a 40-mg portion was reprecipitated from HOAc–EtOH (1:3) with 75% recovery: mp 244–246°; $[\alpha]^{22}D$ –32.7° (c 1.13, DMF); TLC (B) and (D) as above. Anal. ($C_{66}H_{89}N_{11}O_{12}S_2 \cdot H_2O$) C, H, N.

[1-γ-Mercaptobutyric acid,6-homocysteine]oxytocin (11). This analog was prepared from 10 (129 mg, 0.10 mmol) in the manner described for 7. Initial purification by partition chromatography was also as described for 7, with 4.6-ml fractions. The product emerged as a sharp peak with a maximum at R_f 0.58, resolved from a small peak at R_f 0.82 and another small peak at R_f 0.39. Fractions 24–39 were pooled and the material was isolated as described for 5: 70 mg. TLC (D) showed two trace impurities in addition to the product spot at R_f 0.72. The entire product was subjected to gel filtration as described for 3. The product emerged as a slightly asymmetric peak with a maximum at 79% (fractions 80–90) of the column volume followed by and resolved from a small peak at 102% of the column volume. The material in fractions 80–90 was isolated by lyophilization. TLC (D) showed an elongated spot at R_f 0.68. TLC (B) showed a major spot at R_f 0.48 with trace impurities overlapping both the leading and trailing edge and a third impurity at R_f 0.39. The entire product was further purified by partition chromatography in the system BuOH–C_6H_6–HOAc–H_2O (1:2:2:2) using the column described for 3. The product emerged as a single peak with a maximum at R_f 0.29 (tube 46) followed by a very small peak at R_f 0.24 (tube 60). Fractions 37–55 (2.9-ml fractions) were pooled and the product was isolated as described for 5: 60.2 mg. TLC (B) showed that the impurity overlapping the leading edge of the product spot was gone, but the two others (see above) remained. The entire product was further purified by partition chromatography in the system BuOH–C_6H_6–pyridine–H_2O (2:4:1:7), using the column described for 5. The product emerged as a broad peak (fractions

59–87, 4.0-ml fractions) with a maximum at R_f 0.29 (fraction 78). TLC (B) showed a single round symmetrical spot at R_f 0.50 for aliquots withdrawn across the entire peak area. The material in fractions 59–87 was isolated as described for **5**: 50.0 mg. TLC (B) showed a single spot at R_f 0.48 plus a small new impurity (apparently a pyridinium salt) at the origin. Finally, this entire product was subjected to a second gel filtration. It emerged as a single sharp symmetrical peak with a maximum at 80% of the column volume and was isolated as previously described: 48.5 mg (48%); $[\alpha]^{22}D$ +9.4° (*c* 0.64, 1 *N* HOAc); TLC (B) 0.48. Anal. ($C_{45}H_{69}N_{11}O_{12}S_2 \cdot 3H_2O$) C, H, N. Amino acid analysis following 22-hr hydrolysis gave the following molar ratios: Asp, 1.03; Glu, 1.05; Pro, 1.05; Gly, 1.01; Ile, 0.95; Leu, 0.97; Tyr, 0.96; NH_3, 3.01. The peak for $(Hcy)_2$ was overlapped by a peak presumed to be the mixed disulfide Mba Hcy. Amino acid analysis of a performic acid oxidized sample hydrolyzed as above gave the following molar ratios: Hcy(SO_3H), 0.98; Asp, 1.06; Glu, 1.06; Pro, 0.99; Gly, 1.01; Ile, 0.89; Leu, 0.99. The peaks for $(Hcy)_2$ and Mba Hcy were absent from this sample.

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References and Notes

(1) The symbols α-Maa, β-Mpa, γ-Mba, and Hcy are used to indicate the α-mercaptoacetic acid, β-mercaptopropionic acid, γ-mercaptobutyric acid, and homocysteine residues, respectively. All other symbols follow the recommendations (1971) of the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). The optically active amino acids are of the L configuration.
(2) Correspondence should be sent to the Department of Physiology, University of Illinois at the Medical Center, Chicago, Ill. 60680.
(3) C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **79**, 4511 (1957); C, Ressler and J. R. Rachele, *Proc. Soc. Exp. Biol. Med.*, **98**, 170 (1958).
(4) W. B. Lutz, C. Ressler, D. E. Nettleton, Jr., and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 167 (1959).
(5) St. Guttmann, P. A. Jaquenoud, R. A. Boissonnas, H. Konzett, and B. Berde, *Naturwissenshaften*, **44**, 632 (1957); R. A. Boissonnas, St. Guttmann, B. Berde, and H. Konzett, *Experientia*, **17**, 377 (1961).
(6) D. Jarvis, M. Bodanszky, and V. du Vigneaud, *J. Am. Chem. Soc.*, **83**, 4780 (1961).
(7) D. Jarvis and V. du Vigneaud, *Science*, **143**, 545 (1964).
(8) D. Jarvis, B. M. Ferrier, and V. du Vigneaud, *J. Biol. Chem.*, **240**, 3553 (1965).
(9) M. Manning and V. du Vigneaud, *Biochemistry*, **4**, 1884 (1965).
(10) D. Jarvis, M. Manning, and V. du Vigneaud, *Biochemistry*, **6**, 1223 (1967).
(11) W. Fraefel and V. du Vigneaud, *J. Am. Chem. Soc.*, **92**, 1030 (1970).
(12) W. Fraefel and V. du Vigneaud, *J. Am. Chem. Soc.*, **92**, 4426 (1970).
(13) (a) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *J. Biol. Chem.*, **235**, PC64 (1960); (b) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962); (c) B. M. Ferrier, D. Jarvis, and V. du Vigneaud, *ibid.*, **240**, 4264 (1965).
(14) D. Jarvis and V. du Vigneaud, *J. Biol. Chem.*, **242**, 1768 (1967).
(15) M. Bodanszky, during his work with Professor du Vigneaud, synthesized these analogs by solution techniques but encountered difficulty in obtaining acceptable analytical results. The bioassay (AVD) results obtained at that time agree closely with those reported here. M. Bodanszky, personal communication.
(16) D. Jarvis, during his work with Professor du Vigneaud, synthesized this compound by solution techniques. Due to an unfortunate loss of experimental data this synthesis was never published, but an oxytocic potency of 16 units/mg was reported (see ref 7).
(17) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964); G. R. Marshall and R. B. Merrifield, *ibid.*, **4**, 2394 (1965).
(18) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
(19) F. Weygand and G. Zumach, *Z. Naturforsch. B*, **17**, 807 (1962).
(20) D. Yamashiro, *Nature (London)*, **201**, 76 (1964); D. Yamashiro, D. Gillessen, and V. du Vigneaud, *J. Am. Chem. Soc.*, **88**, 1310 (1966).
(21) J. Porath and P. Flodin, *Nature (London)*, **183**, 1657 (1959).
(22) P. G. Katsoyannis, C. Zalut, A. Tometsko, M. Tilak, S. Johnson, and A. C. Trakatellis, *J. Am. Chem. Soc.*, **93**, 5871 (1971), and references cited therein.
(23) Oxytocic assays were performed on uteri from rats in natural estrus, according to the method of P. Holton, *Br. J. Pharmacol. Chemother.*, **3**, 328 (1948), as modified by R. A. Munsick, *Endocrinology*, **66**, 451 (1960), with the use of Mg-free Van Dyke–Hastings solution as the bathing fluid.
(24) Avian vasodepressor assays were performed on conscious chickens by the method of J. M. Coon, *Arch. Int. Pharmacodyn. Ther.*, **62**, 79 (1939), as described in "The Pharmacopeia of the United States of America", 18th revision, Mack Publishing Co., Easton, Pa., 1970, p 469, as modified by R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *Endocrinology*, **66**, 860 (1960).
(25) Pressor assays were carried out on anethesized male rats as described in "The Pharmacopeia of the United States of America", 18th revision, Mack Publishing Co., Easton, Pa., 1970, p 771.
(26) H. O. Schild, *J. Physiol. (London)*, **101**, 115 (1942).
(27) H. O. Schild, *Br. J. Pharmacol. Chemother.*, **2**, 189 (1947).
(28) R. J. Vavrek, M. F. Ferger, G. A. Allen, D. H. Rich, A. T. Blomquist, and V. du Vigneaud, *J. Med. Chem.*, **15**, 123 (1972).
(29) D. F. Dyckes, J. J. Nestor, Jr., M. F. Ferger, and V. du Vigneaud, *J. Med. Chem.*, **17**, 250 (1974).
(30) W. Y. Chan, M. O'Connell, and S. R. Pomeroy, *Endocrinology*, **72**, 279 (1963).
(31) W. Y. Chan and V. du Vigneaud, *Endocrinology*, **71**, 977 (1962).
(32) J. J. Nestor, Jr., M. F. Ferger, and V. du Vigneaud, *J. Med. Chem.*, **18**, 284 (1975).
(33) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(34) C. W. Smith, M. F. Ferger, and W. Y. Chan, *J. Med. Chem.*, **18**, 822 (1975).
(35) E. Schnabel, *Justus Liebigs Ann. Chem.*, **702**, 188 (1967).
(36) M. D. Armstrong and J. D. Lewis, *J. Org. Chem.*, **16**, 749 (1951).
(37) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969, p 32.
(38) K. Esko, S. Karlsson, and J. Porath, *Acta Chem. Scand.*, **22**, 3342 (1968).
(39) K. W. Ehler, Ph.D. Dissertation, University of Arizona, 1972.
(40) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(41) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(42) V. J. Hruby, F. Muscio, C. M. Groginsky, P. M. Gitu, D. Saba, and W. Y. Chan, *J. Med. Chem.*, **16**, 624 (1973).
(43) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(44) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(45) S. Moore, *J. Biol. Chem.*, **238**, 235 (1963).