NH in the molecule is or is not hydrogen bonded. Thus, the simultaneously observed isotope frequency shifts in nmr and ir spectra give more information than is available from adding together the information obtained separately from ir and nmr spectra.

This method then avoids the problems previously encountered in the separate ir or nmr spectroscopic approaches to determining the bonding character of NH groups since values of exchange rates ($t_{1/2}$) or temperature dependence of the chemical shift ($d\delta/dT$) obtained by nmr are no longer needed as criteria for NH bonding. Now that a more definite way of assigning hydrogen bonds has been established, the $t_{1/2}$ and $d\delta/dT$ values can be examined for the additional information which they contain. For example, if an NH group which is shown to be nonhydrogen bonded by the ir/nmr method also has a long $t_{1/2}$, then it may be in a hydrophobic pocket or shielded from the D_2O in the solvent. Further, if an NH group which has a large $d\delta/dT$ value is shown to be intramolecularly hydrogen bonded by the ir/nmr method, this may indicate that a backbone or side chain conformational change is occurring which affects the chemical shift.

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Synthesis and Some Pharmacological Properties of [1-α-Mercaptoacetic acid]-8-lysine-vasopressin and [1-γ-Mercaptobutyric acid]-8-lysine-vasopressin[1,2]

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Abstract: [1-α-Mercaptoacetic acid]-8-lysine-vasopressin and [1-γ-mercaptobutyric acid]-8-lysine-vasopressin, the 19- and 21-membered ring analogs of [1-deamino]-8-lysine-vasopressin, were prepared from two protected polypeptide precursors which had been prepared by the stepwise active ester method. Removal of the protecting groups was accomplished in NH_3 with Na, and oxidative cyclization was performed with CH_2ICH_2I. Both the 19- and 21-membered ring analogs showed significant changes in pharmacological activity as compared to [1-deamino]-8-lysine-vasopressin. [1-α-Mercaptoacetic acid]-8-lysine-vasopressin possessed 1.1 ± 0.1 units/mg of pressor activity, was inhibitory to oxytocin in the avian vasodepressor assay at $\bar{M} = 8.29 \times 10^{-8}$ ($pA_2 = 7.08$), and possessed 3.3 ± 0.3 units/mg of activity in the rat uterus assay. [1-γ-Mercaptobutyric acid]-8-lysine-vasopressin possessed 1.7 ± 0.3 units/mg of pressor activity and was inhibitory to oxytocin in the avian vasodepressor and rat uterus assays at $\bar{M} = 1.28 \times 10^{-8}$ ($pA_2 = 7.89$) and $\bar{M} = 1.80 \times 10^{-7}$ ($pA_2 = 6.74$), respectively.

A striking feature of the various octapeptide hormones which have been isolated from the posterior pituitary gland of mammals, birds, amphibians, and fish is the 20-membered disulfide ring. Several studies have been done on analogs of oxytocin (Figure 1) in which the ring size has been increased in various ways.[3-11] None of these compounds showed more than a slight degree of biological activity. One analog with a decreased ring size of 19 atoms showed a moderate degree of activity.[12] The synthesis of a 21-membered ring directly pertinent to the present communication was accomplished by the formal insertion of a methylene group at position 1 to form [1-hemi-L-homocystine]oxytocin.[6] This analog showed no avian vasodepressor (AVD) or rat pressor activity and only a slight oxytocic activity. In this analog the substitution of the hemihomocystine for the hemicystine residue leads simultaneously to an increase in the size of the ring and in the separation of the free amino group from the disulfide bond. However, work with [1-hemi-D-cystine]oxytocin[13] which possesses extremely low activities had shown that the steric relationship of the free amino group at position 1 to the rest of the molecule may be critical. The synthesis of the highly potent 1-deamino-oxy-

Table I. Pharmacological Activities

Compd[a]	Vasopressin-like activity Pressor[b]	Oxytocin-like activity AVD[b]	Oxytocic[b]	Ref
Deamino-LVP (β-Mpa-LVP)	126 ± 2	61 ± 2	12 ± 0.5	15
		Anti	Anti	
γ-Mba-LVP	1.7 ± 0.3	$\bar{M} = 1.28 \times 10^{-8}$	$\bar{M} = 1.80 \times 10^{-7}$	
		$pA_2 = 7.89$	$pA_2 = 6.74$	
		Anti		
α-Maa-LVP	1.1 ± 0.1	$\bar{M} = 8.29 \times 10^{-8}$	3.3 ± 0.3	
		$pA_2 = 7.08$		
Deamino-oxytocin (β-Mpa-oxytocin)	1.44 ± 0.06	975 ± 24	803 ± 36	14c
γ-Mba-oxytocin		Nil	3	7
α-Maa-oxytocin	0.01	4	25	9
	Anti[c]	Anti	Anti	
[1-Deaminopenicillamine]oxytocin	$\bar{M} = 5.42 \times 10^{-7}$	$\bar{M} = 1.31 \times 10^{-8}$	$\bar{M} = 1.16 \times 10^{-7}$	29
	$pA_2 = 6.27$	$pA_2 = 7.88$	$pA_2 = 6.94$	

[a] For an explanation of the abbreviations see ref 2. [b] Activities are in units/mg unless otherwise noted. [c] Previously unpublished data.

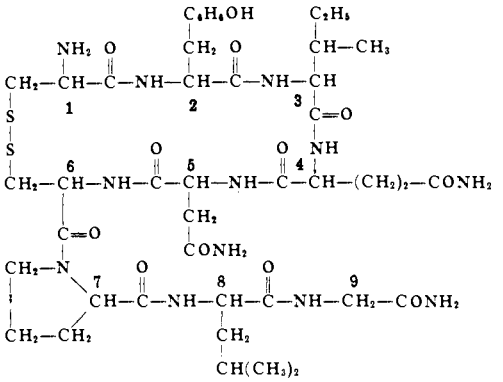

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues. Lysine-vasopressin has a phenylalanine residue instead of an isoleucine residue at position 3 and a lysine residue instead of a leucine residue at position 8. In the deamino analogs, the NH_2 at position 1 is replaced with H.

tocin[14] ([1-β-mercaptopropionic acid]oxytocin), which possessed almost twice the AVD and oxytocic activity 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 deletion of one methylene group at the β-mercaptopropionic acid residue at position 1.[7,10-12] In this manner analogs having 19-, 21-, 22-, and 28-membered rings were prepared and the drastic reduction or total loss of activity indicated that ring size was extremely important for activity. The 22-membered ring analog even possesses a slight inhibitory activity against oxytocin in the oxytocic assay.[10]

In this paper we wish to report an extension of these ring size studies to lysine-vasopressin (LVP) (Figure 1). Using the same considerations that had been employed in the choice of deamino-oxytocin analogs, we undertook the synthesis of [1-α-mercaptoacetic acid]-8-lysine-vasopressin (α-Maa-LVP) and [1-γ-mercaptobutyric acid]-8-lysine-vasopressin (γ-Mba-LVP). These compounds are, respectively, the 19- and 21-membered ring analogs of the potent [1-deamino]-8-lysine-vasopressin[15,16] (deamino-LVP) representing the formal deletion or insertion of a methylene group at position 1 adjacent to the disulfide bond.

The intermediate protected polypeptides α-Maa(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (I) and γ-Mba(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (II) were prepared from the protected octapeptide Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys-(Tos)-Gly-NH_2[17] (III) by the *p*-nitrophenyl ester method[18] using either *p*-nitrophenyl *S*-benzylmercaptoacetate[12] or *p*-nitrophenyl *S*-benzyl-γ-mercaptobutyrate,[7] respectively. In each case removal of the protecting groups was accomplished by treatment with Na in liquid NH_3,[19,17] and the disulfide bond was formed by oxidation of the resulting sulfhydryl peptide with diiodoethane.[20] α-Maa-LVP was purified by ion exchange chromatography[15,21] followed by gel filtration.[22] γ-Mba-LVP was purified by partition chromatography[23,14d] followed by gel filtration. The compounds were tested for rat pressor,[24] AVD,[25] and oxytocic[26] activities against U.S.P. posterior pituitary reference standard. The four point assay design[27] was used whenever measurable activity was detected. When no activity was detected in the above assays, the compounds were checked for inhibition of the oxytocic and AVD responses to synthetic oxytocin. Inhibitory potencies were determined and expressed as pA_2 values as defined by Schild.[28] This represents the negative logarithm to the base 10 of the average molar concentration ($\bar{M}$) of antagonist which will reduce the appropriate biological response to *2X* units of a pharmacologically active compound (agonist) to the level of response to *X* units of the agonist. Specific details of the antioxytocic and anti-AVD assays are described by Vavrek, *et al.*[29]

The behavior of γ-Mba-LVP and α-Maa-LVP in the pressor, AVD, and oxytocic assays is given in Table I. The 21-membered ring analog (γ-Mba-LVP) exhibits a striking reduction in pressor activity as compared to deamino-LVP (to 1.7 from 126 units/mg). In the AVD and oxytocic assays it acts as a potent inhibitor of the response to oxytocin. As shown in the table, the inhibitory potency in both assays is comparable to that of [1-deaminopenicillamine]oxytocin.[29,30] The 19-membered ring analog (α-Maa-LVP) also exhibits a striking reduction in pressor activity as compared to deamino-LVP (to 1.1 from 126 units/mg). This analog exhibits inhibitory activity in the AVD assay but retains 3.3 units/mg in the oxytocic assay. The corresponding 19- and 21-membered ring analogs of deamino-oxytocin do not show inhibition in these assays. The drastic reduction in potency in the vasopressin-like (pressor) activity is comparable to that found in the oxytocin-like (AVD and oxytocic) activities for the corresponding deamino-oxytocin analogs. Thus the size of the ring is important to the activity of deamino-LVP, as it is to the activity of deamino-oxytocin.

Experimental Section[31]

α-Maa(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (I). Compound III[17] (417 mg, 0.30 mmol) was dissolved in 1 ml of ice cold redistilled trifluoroacetic acid (TFA). After 10 min the solution was allowed to warm to room temperature and stand an additional 60 min. The TFA was removed by vacuum rotary evaporation and the residual oil was triturated to a solid in Et_2O. The solid was collected, washed with Et_2O, and dried *in vacuo*.

The entire product was dissolved in 1.5 ml of dimethylformamide (DMF) with α-Maa(Bzl)-ONp[12] (110 mg, 0.36 mmol). The solution was cooled in an ice bath and diisopropylethylamine (*i*-Pr_2NEt) (0.052 ml) was added. The solution was stirred at room temperature and the progress of the reaction was followed by quantitative ninhydrin tests.[32,17] After 30 min an additional 0.02 ml of *i*-Pr_2NEt was added and the mixture was stirred overnight. The solidified reaction mixture was triturated with 95% EtOH and the precipitate was collected, washed with EtOH and EtOAc, and dried *in vacuo:* wt 0.38 g (87%); mp 236.5–238°; $[\alpha]^{24}D$ −28.7° (*c* 1, DMF); homogeneous to tlc (A). This product was used directly for the preparation of α-Maa-LVP. A 25-mg sample was reprecipitated from DMF/H_2O with 83% recovery. *Anal.* Calcd for $C_{73}H_{88}N_{12}O_{14}S_3$: C, 60.31; H, 6.10: N, 11.56. Found: C, 60.14; H, 6.30; N, 11.58.

[1-α-Mercaptoacetic acid]-8-lysine-vasopressin. Compound I (200 mg, 0.138 mmol) was dissolved in 200 ml of anhydrous liquid NH_3 which had been freshly distilled from Na. The stirred solution was treated with Na[17,19] until the blue color persisted for 25 sec without further addition of Na. The excess Na was discharged with HOAc, and the NH_3 was removed by evaporation and lyophilization under vacuum. The residue was dissolved in 133 ml of deionized H_2O and 123 ml of acetone, to which CH_2ICH_2I (40 mg, 0.14 mmol) dissolved in 10 ml of acetone was added. The disappearance of the sulfhydryl groups was followed by the Ellman test.[33] After 5 min the reaction was judged complete. The acetone was removed by rotary evaporation and the pH adjusted to 3.5 with HOAc. The product was desalted on a 1.5 × 15 cm column of IRC-50 (H^+)[34] and recovered by lyophilization (wt 145 mg). A 66-mg portion was subjected to ion-exchange chromatography on a 1.06 × 108 cm column of IRC-50 in 0.5 *M* NH_4OAc at pH 5.85.[15,21] The eluate was collected in 3.0-ml fractions at 8.3 ml/hr and the product was detected by reading the absorbency at 280 nm. The major peak, preceded by a few very small peaks, emerged in fractions 30–60. These fractions were pooled and the product was recovered by desalting and lyophilization (wt 43 mg). This material was dissolved in 1.5 ml of 0.2 *N* HOAc and further purified by gel filtration[19] on a 2.82 × 69.6 cm column of Sephadex G-25 (200–270 mesh) equilibrated with the same solvent. The product was eluted with 0.2 *N* HOAc and collected in fractions averaging 5.1 ml at a flow rate of 35 ml/hr. The peptide material was detected by reading the absorbency of the eluate at 280 nm. It emerged as a single symmetrical peak at 91% of the column volume and was recovered by lyophilization: wt 38.5 mg; $[\alpha]^{25}D$ −45.8° (*c* 0.57, 1 *N* HOAc); homogeneous to tlc (A,B). Amino acid analysis[35] following 20 hr of hydrolysis in 6 *N* HCl at 110° gave the following molar ratios: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; Cys, 1.0; Tyr, 0.8; Phe, 0.9; Lys, 1.1; NH_3, 3.0. *Anal.* Calcd for $C_{45}H_{62}N_{12}O_{12}S_2 \cdot C_2H_4O_2 \cdot 4H_2O$: C, 48.69; H, 6.43; N 14.50. Found: C, 48.29; H, 6.04; N, 14.20.

γ-Mba(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (II). This compound was prepared by treatment of III (417 mg, 0.30 mmol) with TFA followed by γ-Mba(Bzl)-ONp[7] (120 mg, 0.36 mmol) mediated by *i*-Pr_2NEt as described for I: wt 0.38 g (86%); mp 219–221.5°; $[\alpha]^{23}D$ −33.7° (*c* 1.1, DMF); homogeneous to tlc (A). This product was used directly for the preparation of γ-Mba-LVP. A 25-mg portion was reprecipitated from DMF/H_2O with 78% recovery. *Anal.* Calcd for $C_{75}H_{92}N_{12}O_{14}S_3$: C, 60.79; H, 6.26; N, 11.34. Found: C, 60.90; H, 6.55; N, 11.15.

[1-γ-Mercaptobutyric acid]-8-lysine-vasopressin. Compound II (203 mg, 0.137 mmol) was treated with Na/NH_3, oxidized with CH_2ICH_2I, and desalted as described for α-Maa-LVP (wt 144.5 mg). A 62.4-mg portion was subjected to partition chromatography[23,14d] on a 2.82 × 67 cm column of Sephadex G-25 (100–200 mesh) in the system 1-BuOH/H_2O (1:1, the aqueous phase 1.5% pyridine and 3.5% HOAc). The product was eluted with upper phase and the eluate was monitored by the Folin–Lowry method.[36] The product emerged as a sharp symmetrical peak at R_f 0.16 well resolved from several very small preceding peaks. The fractions comprising the main peak were pooled and diluted with three volumes of deionized H_2O. The organic phase was removed by vacuum rotary evaporation and the aqueous phase was lyophilized to dryness (wt 45.0 mg). This material was further purified by gel filtration as previously described. It emerged as a single sharp peak at 94% of the column volume: wt 43.0 mg; $[\alpha]^{24}D$ −40.2° (*c* 0.5, 1 *N* HOAc); homogeneous to tlc (A,C). Amino acid analysis following 18 hr of hydrolysis in 6 *N* HCl at 110° gave the following molar ratios: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; Cys, 1.0; Tyr, 0.9; Phe, 1.0; Lys, 1.1; NH_3, 3.1. *Anal.* Calcd for $C_{47}H_{66}N_{12}O_{12}S_2 \cdot C_2H_4O_2 \cdot 4H_2O$: C, 49.56; H, 6.62; N, 14.16. Found: C, 49.94; H, 6.26; N, 13.83.

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References and Notes

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(2) The symbols α-Maa, β-Mpa, and γ-Mba are used to indicate the α-mercaptoacetic acid, β-mercaptopropionic acid, and γ-mercaptobutyric acid 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.
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