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Synthesis and Properties of 1-Deamino-8-L-homolysine-vasopressin†

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An analog of lysine-vasopressin with β-mercaptopropionic acid replacing the cysteine residue in position 1 and with L-homolysine replacing lysine was synthesized. In the rat, the new analog exhibits significantly higher antidiuretic and pressor activities than the parent hormone.

The recent synthesis of 8-L-homolysine-vasopressin[1] (I) and the biological activity exhibited by this hormone analog together with the enhanced and prolonged effects found in the 1-deamino analogs of the pituitary hormones (*e.g.*, ref 2, 3) prompted the synthesis of 1-deamino-8-L-homolysine-vasopressin (II). In this analog the 1-cysteine residue of the parent hormone is replaced by β-mercaptopropionic acid and the 8-lysine moiety by L-homolysine (Figure 1).

Synthesis of the new hormone analog followed the pattern established for deamino-oxytocin[2] and is described in the Experimental Section. Compared with lysine-vasopressin, II proved to be exceptionally active both in the pressor and in the antidiuretic assay. The pharmacological potencies of II are compared with some selected analogs of vasopressin in Table I. These analogs were chosen to illustrate (1) the effect of the length of the side chain of the basic amino acid residue in position 8, (2) the role of the amino group of the cysteine residue in position 1, and (3) the comparison with other very active compounds reported to date.

Table I demonstrates that the pressor potency of II, about 1000 IU/μmole, is significantly higher than that of the other analogs listed. Since, previously, omission of the amino group in position 1 either hardly changed or else slightly suppressed the pressor activity, this high value was unexpected.

The antidiuretic potency of II, about 10,000 IU/μmole, is significantly higher than that reported for any other analog, with the possible exception of 1-deamino-8-D-arginine-vasopressin. In the latter case, however, the activity is extremely dose dependent and cannot be well represented by a single number.[3] In the dose region investigated, the dose-response curve of II is similar to that of lysine-vasopressin.

The reason for the enhanced biological activities of II are not known at the present time. The synthesis of 8-L-homolysine-vasopressin[1] was undertaken in order to explore the role of the length of the side chain of the basic amino acid in position 8. If the length of this side chain plays a significant role in the interaction between the hormone and the antidiuretic receptor site, I should have shown distinctly higher activity than lysine-vasopressin. As shown in Table I, it did not. Nevertheless, it is still possible that increased chain length intrinsically would lead to higher activity, but the required molecular fit with the receptor can only be obtained when the point of attachment through the amino group is absent. The potencies of II are significantly higher than those of 1-deamino-8-lysine-vasopressin. Alternately, it may be that the absence of the amino group extends the lifetime of the compound *in vivo* by making it less susceptible to aminopeptidases (*cf.* ref 4). This would lead to higher response with time. We hope that our further studies can clarify these questions.

Experimental Section‡

Synthesis. β-Benzylmercaptopropionyl-L-Tyr-L-Phe-L-Gln-L-Asn-(Bzl)-L-Cys-N^{ζ}-tosyl-L-homolysyl-Gly-NH_2. Z-(Bzl)-L-Tyr-L-Phe-L-Gln-L-Asn-(Bzl)-L-Cys-L-Pro-N^{ζ}-tosyl-L-homolysyl-Gly-NH_2[1] (346 mg) was dissolved in AcOH (3.5 ml) with gentle heating. After cooling to room temp, 5 *M* HBr in AcOH (2.5 ml) was added. One hr later, ether (75 ml) was added and the ppt was collected and washed with Et_2O (100 ml). It was dried *in vacuo* over NaOH for 2.5 hr and then dissolved in DMF (2 ml). The soln was filtered and the filter rinsed with DMF (2 × 1 ml). After cooling to 0°, diisopropylethylamine was added dropwise until the vapors gave a slightly alk reaction with a moistened indicator paper. *p*-Nitrophenyl *S*-benzyl-β-mercaptopropionate[5] (93 mg) was added and the soln was stirred at room temp for 1 day when a second portion (47 mg) of the active ester was added. The reaction mixt was kept slightly basic by occasional addns of diisopropylethylamine. After 3 more days, 95% EtOH (45 ml) was added, and the mixt was kept in an ice bath for 5 hr. The pptd solid was filtered and washed with EtOH (2 × 10 ml) and EtOAc (2 × 10 ml). The dried product weighed 239 mg; mp 210–213°, $[\alpha]^{25}D$ −39.5° (*c* 0.52, DMFA). Tlc on silica gel gave single spots in the solvent systems: *n*-PrOH-H_2O (7:3), R_f 0.62, and *n*-BuOH–AcOH–H_2O (3:1:1), R_f 0.60. *Anal.* ($C_{68}H_{86}N_{12}O_{15}S_3$) C, H, N, S.

1-Deamino-8-L-homolysine-vasopressin (II). A portion of the protected nonapeptide (100 mg) was dissolved in boiling liquid NH_3

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‡Capillary melting points were taken and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values. For tlc, precoated plates (E. Merck AG, Darmstadt) were used and peptides were detected by use of the uv, ninhydrin, and chlorination techniques.

Table I. Pharmacological Potencies[a] of Selected Vasopressin Analogs

Vasopressin[b]	Amino compound		1-Deamino compound	
	Blood pressure	Antidiuresis	Blood pressure	Antidiuresis
8-Dab	200[c]	150[c]		
8-Orn	370[d]	90[d]	360[e]	200[e]
Lys	280[f]	260[f]	130[g]	310[g]
8-Homolysine	250[h,i]	300[h,i]	990	10,200
Arg	440[f]	440[f]	400[j]	1,400[j]
8-D-Arg	4[k]	100[k]	10[l,i]	870[l,i]
4-Decarboxamido-8-Arg	40[m,i]	760[m,i]	10[m,i]	1,020[m,i]
4-Decarboxamido-8-Lys	10[g,i]	710[g,i]	4[g,i]	730[g,i]

[a]Potencies were determined on rats and are expressed in International Units per micromole. In most cases, the mean value found in the literature has been rounded to the nearest ten. [b]Unless otherwise indicated, all amino acids are of L configuration. [c]See ref 9. [d]See ref 10. [e]See ref 11. [f]See ref 12. [g]See ref 13. [h]See Bodanszky and Lindeberg.[1] [i]Values are in units per milligram. [j]See ref 14. [k]See ref 15. [l]See Vávra, *et al.*[3] [m]See ref 16.

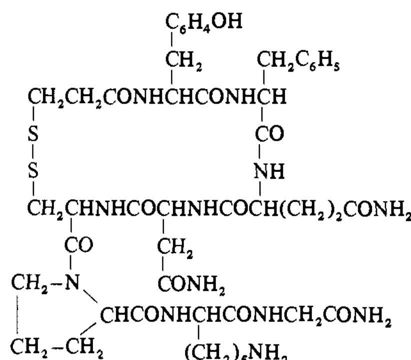

Figure 1. 1-Deamino-8-L-homolysine-vasopressin (II).

(*ca.* 100 ml) and treated with small pieces of Na until the blue color persisted for 3 min. A few drops of AcOH were added, and the NH_3 was allowed to evap spontaneously. The last 20 ml were removed by lyophilization. The residue was dried *in vacuo* for 3 hr and then was dissolved in O_2-free H_2O (150 ml). The soln was neutralized to pH 6.8 and extd with Et_2O (5 × 50 ml). It gave a positive reaction with sodium nitroprusside. $K_3Fe(CN)_6$ (0.011 *M*, 17.5 ml) was added dropwise with stirring. A yellow color developed after addn of 10 ml of the oxidizing agent. The pH was readjusted to 6.8 with 1 *M* NH_4OH and the soln was stirred at room temp for 15 min. The sodium nitroprusside test was no longer positive. The soln was acidified to pH 5.8 by addn of 1 *M* AcOH and passed through a column (1 × 10 cm) of Dowex 1-X2 ion-exchange resin (200–400 mesh, trifluoroacetate form). The colorless eluate (220 ml) was concd *in vacuo* at 30° to *ca.* 100 ml and lyophilized. The product was dissolved in H_2O (8 ml) and chromatogd on a column (3.2 × 94 cm) of Sephadex G-25 fine in 0.1 *M* AcOH. The uv absorption at 254 nm was recorded continuously and the fractions collected were tested with the Folin–Lowry reaction.[5] The eluate from 650 to 835 ml was concd and freeze-dried, thus discarding the fractions corresponding to a small shoulder on the front edge of the peak. A colorless semisolid material (253 mg) was obtained. It was dissolved in H_2O (5 ml) and applied to a column (1.4 × 16 cm) of Whatman CM-52 carboxymethylcellulose equilibrated with 0.025 *M* NH_4OAc, pH 5.5. Elution was performed with the same buffer (350 ml), and the uv absorption at 254 nm was recorded. No more material was eluted when the ionic strength was increased linearly from 0.025 to 0.25 *M*. The fractions from 60 to 140 ml were combined, concd and lyophilized. A shoulder on the back edge of the main peak and a small peak preceeding it were omitted. The resulting white fluffy powder weighed 101 mg. A part of this material (46 mg) was dissolved in 0.1 *M* AcOH (2 ml) and chromatogd on a column of Sephadex G-15 (1.5 × 62 cm) in the same medium. A single peak was obtained when measuring the uv absorption at 254 nm. The corresponding fractions (68 to 102 ml) were combined and lyophilized. As detd from the uv spectrum, the soln contd 0.19 μmole of the hormone analog; yield, 30 mg, $[\alpha]^{25}D$ −54.6° (*c* 1, 1 *M* AcOH). Tlc of this material in the solvent system *n*-BuOH–AcOH–H_2O (4:1:5, upper phase) on cellulose and silica gel gave single spots with R_f values 0.58 and 0.13. Amino acid analysis[6] gave the following molar ratios: Cys, 0.98; Asp, 1.03; Glu, 1.04; Pro, 1.08; Gly, 0.98; Tyr, 0.94; Phe, 0.95; homolysine, 1.05§ (Cys determined as cysteic acid).

Pharmacology. Rat pressor activity was detd in the Dekanski assay system.[7] Lysine-vasopressin concentrate (Sandoz Pharmaceuticals 99601, 100 IU/ml) was used as standard for comparison. A pressor potency of 990 ± 60 IU/μmole was found for II.

Rat antidiuretic activity was detd according to the modified method of Burn.[8] Unanesthetized Sprague-Dawley rats of 160–210 g were used. The response was measured as the delay of time necessary for excretion of 50% of H_2O load (5 ml/100 g of body wt). The same lysine-vasopressin as above was used as standard. The log dose–response lines were established for both standard and analog given sc to groups of 4 rats. Each point on the lines was an average of at least five parallel trials. An antidiuretic potency of 10,200 ± 83 I.U./μmole was found for II. Direct comparison with I was made at 1 dose level. The response expected on the basis of the antidiuretic potency of about 300 IU/mg previously reported[1] was observed.

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§In the quantitative amino acid analysis of the protected intermediates, tosylhomolysine, incompletely hydrolyzed, interfered with the determination of NH_3. Therefore, no values are reported for these two constituents. In the hydrolysate of 8-L-homolysine-vasopressin, homolysine was determined on the short column of the Beckman–Spinco Amino Acid Analyzer, but with the "B" buffer (pH 4.28) as eluent.

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Chemical Modifications of Erythromycin Antibiotics. 3.[1] Synthesis of 4″ and 11 Esters of Erythromycin A and B

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A number of mono-, di-, and trisubstituted alkyl esters of the macrolide antibiotic erythromycins A and B were prepared by selective esterification and hydrolysis. This series of esters was valuable for determining antibacterial activities and absorption characteristics of substituted erythromycins wherein the aminoglycoside moiety remained unesterified. The compounds also provide useful protected intermediates for further chemical studies. The biological activities of the esters and a structure–activity analysis are discussed in the succeeding paper.

The structures of erythromycin A[2] and B[3] were determined by the Lilly group as macrocyclic lactones with two 6-deoxy sugars attached. Erythromycin A and B† (Figure 1) differ only by a tertiary hydroxyl group at C-12. Some of the additional common structural features include a second tertiary OH at C-6 and 3 secondary OH groups, one on each sugar and one at C-11 on the aglycone. Many papers have been written which discuss the esterification of the basic sugar, desosamine, at the 2′ position.[4] Esterification at this position is greatly enhanced by the neighboring Me_2N group which behaves as an intramolecular catalyst for the acylation. When taken orally these esters are well absorbed imparting high serum levels of the erythromycin ester.[4a,4d] These increased serum levels are caused by 2 factors: (1) the increased lipophilicity of the ester and (2) the decreased basicity of the amino group.[5] Recent work of Tardrew, *et al.*,[6] has shown that the 2′-esters are antimicrobially inactive and must first hydrolyze *in vivo* back to the parent erythromycin to be therapeutically effective. The Me_2N group again plays an important role by enhancing the rate of ester hydrolysis. Nevertheless the hydrolysis rate of the 2′-acetate or propionate ester is slow relative to the rate of excretion and a major portion of the antibiotic circulates as the inactive ester form.‡ Thus the observed higher blood levels of erythromycin esters are offset by the low availability of the active base.

When it became apparent that the 2′-esters were devoid of antibacterial activity, our attention turned to the esterification of the other 2 secondary OH groups. If the increased absorption was partly due to their increased lipophilicity, then esters of these groups might also exhibit enhanced serum levels. It was therefore our intention to synthesize erythromycin derivatives in which the 4″ and 11 positions were individually esterified and to study the effect of these substituents on the antibacterial activity and absorption.

Celmer had earlier described a parallel study with the related macrolide antibiotic oleandomycin in which he prepared mono-, di- and triesters and studied their antimicrobial activities and absorption characteristics.[7] Flynn, *et al.*, reported the synthesis of an erythromycin diacetate which was not further characterized.[5]

Recently a group from the Polish Academy of Sciences reported their work on the esterification of erythromycin with acetic and propionic anhydride.[8] Their work paralleled that part of our study which was devoted to the synthesis of the di- and triesters. However, our work was primarily directed toward the synthesis of monoesters at each of the secondary hydroxyl groups. This paper describes our results.

Synthesis. The methods of preparing the various esters of erythromycin A and B are outlined in Scheme I. The synthesis of the 4″ esters was straightforward using methods similar to those previously described.[5,8] The 2′- and 4″-OH groups were both acylated using the appropriate anhydride in pyridine followed by hydrolysis of the 2′ ester with MeOH to produce the 4″ ester. The 3′-dimethylamino group behaves as an intramolecular catalyst making possible the facile hydrolysis of the 2′ esters under neutral conditions in the presence of the 4″ ester. The 4″,11 diesters where both groups are the same were prepared by a prolonged acylation in pyridine to produce the 2′,4″,11 triester followed by a methanolic hydrolysis to the 4″,11 diester. In the case of a majority of the 4″,11 diesters the hydrolysis of the 4″ ester was too slow to permit the direct synthesis of the 11 ester by this procedure. Thus to prepare the 11 esters a new method of protecting the 4″ position had to be developed.

The major side reaction which occurs during the more drastic basic conditions necessary to remove a 4″-acyl group is the hydrolysis of the lactone group of the macrolide ring, a reaction first recognized by the Lilly group. This problem could be overcome by introducing a more base-labile ester at C-4″. Thus by the synthesis of the appropriate triester, a mild base treatment would produce the desired 11 ester. Our attention then was focused on esters of formic acid for it is well recognized that they are more base labile than the corresponding acetates.

The 2′-formates 7 were prepared by the usual method used for 2′ esters, whereby the antibiotic was treated with anhydride and an acid acceptor such as K_2CO_3. We used formic acetic anhydride as the formylating reagent since it is more stable than $(HCO)_2O$[9] and is known to produce formate rather than acetate esters.[10] The 2′-formates were found to be very unstable and in fact were converted to the

†The position numbering system shown in Figure 1 was chosen for this study. The compounds are numbered as shown in Scheme I. The letters A and B following the numbers indicates the erythromycin A or erythromycin B series, respectively.

‡Unpublished human blood level data, Abbott Laboratories.