(7) D. Perlman and G. P. Peruzzotti, *Advan. Appl. Microbiol.*, 12, 277 (1970).
(8) H. G. Floss in "Fermentation Technology Today," Proceedings of the IVth International Fermentation Symposium, G. Terui, Ed., Society of Fermentation Technology, Japan, 1972, pp 407-412.

[1-β-Mercapto-β,β-diethylpropionic acid]-8-lysine-vasopressin, a Potent Inhibitor of 8-Lysine-vasopressin and of Oxytocin†

Douglas F. Dyckes, John J. Nestor, Jr., Martha F. Ferger, and Vincent du Vigneaud*

Department of Chemistry, Cornell University, Ithaca, New York 14850. *Received July 11, 1973*

In earlier work it was found that the replacement of the half-cystine residue in position 1 of oxytocin (Figure 1) with that of L-penicillamine (L-β,β-dimethylcysteine) led to an analog, [1-L-penicillamine]oxytocin, which is a highly potent inhibitor of both the oxytocic and avian vasodepressor (AVD) activities of oxytocin.[1] The deamino analog, [1-deaminopenicillamine]oxytocin ([1-β-mercapto-β,β-dimethylpropionic acid]oxytocin),[1] was found to be more potent than the [L-penicillamine]oxytocin analog by about 20% with respect to antioxytocic activity and about 140% with respect to anti-AVD activity.[2] A further series of analogs has been prepared incorporating modifications in position 1 of [1-deaminopenicillamine]oxytocin.[2-4] Of the various analogs which were prepared,‡ [1-β-mercapto-β,β-diethylpropionic acid]oxytocin[2] ([1-β-Mpa(β-Et_2)]oxytocin) was the most potent inhibitor, possessing about twice the antioxytocic and anti-AVD activity of [deaminopenicillamine]oxytocin. We therefore decided to incorporate the β-mercapto-β,β-diethylpropionyl residue [β-Mpa(β-Et_2)] into lysine-vasopressin (Figure 1) to see whether the resulting analog, [1-β-mercapto-β,β-diethylpropionic acid]-8-lysine-vasopressin ([1-β-Mpa(β-Et_2)]LVP), would exhibit antihormonal properties.

This analog was prepared from the protected polypeptide β-Mpa(β-Et_2)(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 by removal of all protecting groups with Na-NH_3[5] followed by cyclization with ICH_2CH_2I.[6] The protected polypeptide just mentioned was synthesized from Boc-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2[7] by the stepwise *p*-nitrophenyl ester method.[8] N^{α}-Boc protection was used throughout and treatment with anhydrous trifluoroacetic acid was used for Boc removal. The hormone analog was purified by ion-exchange chromatography[9] followed by gel filtration chromatography.[10]

[1-β-Mpa(β-Et_2)]LVP was found to be inactive when tested for oxytocic, AVD, and pressor activities against the U.S.P. posterior pituitary reference standard§ but produced inhibition of the oxytocic and AVD responses to synthetic oxytocin and of the pressor response to synthetic LVP. Inhibitory potencies were determined and expressed as pA_2 values as defined by Schild.[16] This represents the negative logarithm to the base 10 of the average molar concentration ($\bar{M}$) of an 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.*[2] Antipressor assays were performed in an exactly analogous manner, with the concentration of antagonist calculated on the basis of a blood volume of 6.7 ml per 100 g of body weight of the individual rats. The values of $\bar{M}$ for [1-β-Mpa(β-Et_2)]LVP as an antagonist of oxytocin were 1.43×10^{-7} ($\sigma = 0.37$) in the uterus and 2.34×10^{-8} ($\sigma = 0.98$) in the chicken, or $pA_2 = 6.84$ and 7.63, respectively. This means that the compound is roughly equal to [L-penicillamine]oxytocin in inhibitory potency in the oxytocic and AVD assays and one-half to one-third as potent as [1-β-Mpa(β-Et_2)]oxytocin. As an antagonist of LVP rat pressor activity, [1-β-Mpa(β-Et_2)]LVP had a value of $\bar{M} = 7.01 \times 10^{-8}$ ($\sigma = 1.15$), or $pA_2 = 7.15$. This potency is approximately eight times as great as that of [1-β-Mpa(β-Et_2)]oxytocin, which was found in the present study by identical assay techniques to have $\bar{M} = 5.73 \times 10^{-7}$ ($\sigma = 2.72$), or $pA_2 = 6.24$ in the pressor system.

Thus the substitution of the β-mercapto-β,β-diethylpropionic acid residue for the half-cystine residue in the 1 position of not only oxytocin but also of lysine-vasopressin leads to an analog possessing pronounced inhibitory properties.

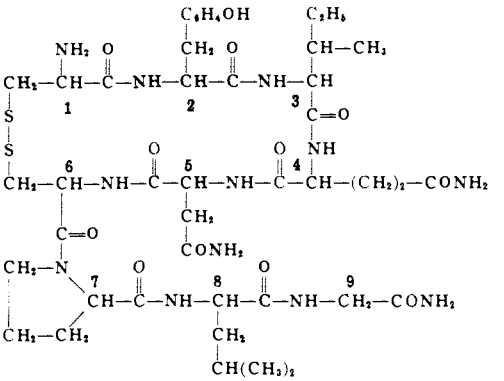

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues. Lysine-vasopressin has a phenylalanine residue instead of an isoleucine residue at position 3 and a lysine residue instead of a leucine residue at position 8. In the deamino analogs, the NH_2 at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

Experimental Section

Intermediate N^{α}-Boc-protected peptides were deblocked by dissolving them in cold, redistilled trifluoroacetic acid and allowing them to react at room temperature for 1 hr. The solvent was then removed *in vacuo* and the residual oil triturated with Et_2O. The resultant white powder was collected, washed with Et_2O, and dried *in vacuo*.

The disappearance of the free amine group during coupling reactions was followed by a quantitative ninhydrin color test.# The presence of excess diisopropylethylamine (*i*-Pr_2NEt) in the reaction mixture was maintained by the addition of further aliquots (10–20% of theoretical amount) of *i*-Pr_2NEt as needed. The atmosphere of the reaction vessel was periodically checked for *i*-Pr_2NEt by means of moist litmus paper.**

†This work was supported in part by Grant HL-11680 from the National Heart and Lung Institute, U. S. Public Health Service. All optically active amino acid residues are of the L configuration. The symbols for the amino acid residues follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

‡In ref 2, all of these analogs are discussed and a table of the antioxytocic and anti-AVD pA_2 values of some of them is given, as well as a detailed presentation of the method used to determine the pA_2 values.

§Oxytocic assays were performed on isolated uteri from virgin rats in natural estrus according to the method of Holton,[11] as modified by Munsick,[12] with the use of Mg-free van Dyke–Hastings solution as the bathing fluid. Avian vasodepressor assays were performed on conscious chickens by the method of Coon,[13] as modified by Munsick, Sawyer, and van Dyke.[14] Pressor assays were carried out on anesthetized male rats as described in the U. S. Pharmacopeia.[15]

#A standard curve for the quantitative ninhydrin color test is prepared using a 0.002 *M* DMF solution (not 0.002 m*M* as erroneously reported[17]) of the trifluoroacetate salt of the free amine compound. Aliquots (5–35 μl) of this solution are incubated for 5 min at 100° with 3 drops each of three Kaiser test reagents,[18] then diluted with 95% EtOH (1.00 ml), and read after 10 min at 590 nm against a reagent blank. During the course of the condensation reactions, appropriate aliquots of the reaction mixture are removed, tested in similar fashion, and compared to the standard curve for estimation of the amount of the free amine peptide remaining in solution.

Precoated plates of silica gel F-254 (0.25 mm, E. Merck) were used for thin-layer chromatography (tlc) of spots containing 5–20 μg of compound and were developed with the solvent systems: (A) BuOH–HOAc–H_2O (3:1:1), (B) BuOH–pyridine–H_2O) (20:10:11), and (C) $CHCl_3$–MeOH (9:1). Dicyclohexylcarbodiimide (DCCI) and *p*-nitrophenol were obtained from Research Plus Laboratories. Melting points were determined in open capillaries and are corrected.

***p*-Nitrophenyl β-(*S*-Benzylmercapto)-β,β-diethylpropionate** (1). Solid DCCI (3.3 g, 16.0 mmol) was added to a cold solution of β-(*S*-benzylmercapto)-β,β-diethylpropionic acid[2] (3.8 g, 16.0 mmol) and *p*-nitrophenol (2.7 g, 19.4 mmol) in EtOAc (15 ml). The reaction mixture stood at 0° for 1 hr and room temperature for 3 days. Glacial HOAc (1 ml) was added. The dicyclohexylurea was removed by filtration and washed with EtOAc (4 × 5 ml). The filtrate was diluted with EtOAc and washed with 0.5 *N* NH_4OH (6 × 50 ml) and saturated NaCl (25 ml). After drying ($MgSO_4$), filtration, and concentration of the solution, the resultant straw-colored oil crystallized as long needles. The crystals were collected, washed with EtOH (5 × 5 ml), and dried *in vacuo* to yield 4.27 g (74%) of pale yellow needles, mp 67–67.5°. Recrystallization from EtOH (8 ml/g) yielded 90% recovery of white needles: mp 67–67.5°; homogeneous to tlc (C, R_f 0.69). *Anal.* ($C_{20}H_{23}NO_4S$) C, H, N, S.

Boc-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (2). Boc-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2[7] (4.69 g, 5.45 mmol) was deprotected as described above. A cold solution of the deprotected amine salt and Boc-Gln-ONp (2.40 g, 6.53 mmol) in DMF (28 ml) was treated with *i*-Pr_2NEt (0.94 ml, 5.45 mmol). After standing for 24 hr at room temperature the reaction mixture was diluted with 95% EtOH (125 ml). The amorphous precipitate was collected on a glass frit, washed with EtOH and EtOAc, and dried *in vacuo* to yield 4.66 g (86.5%) of product: homogeneous to tlc (A, R_f 0.62); mp 187–190° dec; $[\alpha]^{24}D$ −37.1° (*c* 1.7, DMF). The product could be crystallized from 80% EtOH. *Anal.* ($C_{44}H_{64}N_{10}O_{12}S_2$) C, H, N.

Boc-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (3). The hexapeptide 2 (1.97 g, 2.0 mmol) was deprotected as above. A cold solution of the deprotected amine salt and Boc-Phe-ONp (0.91 g, 2.36 mmol) in DMF (10 ml) was treated with *i*-Pr_2NEt (0.32 ml, 1.85 mmol). After standing 48 hr at room temperature the reaction mixture contained a thick, crystalline precipitate. After dilution of the reaction mixture with EtOH (50 ml), the product was collected, washed with EtOH and EtOAc, and air-dried to yield 1.91 g (84%) of material homogeneous to tlc (A, R_f 0.60): mp 185–188° dec; $[\alpha]^{24}D$ −31.8° (*c* 1.3, DMF). An analytical sample was recrystallized from HOAc by dilution with EtOH. *Anal.* ($C_{53}H_{73}N_{11}O_{13}S_2 \cdot C_2H_4O_2$) C, H, N.

Boc-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (4). The heptapeptide 3 (1.11 g, 0.98 mmol) was deprotected as above. A cold solution of the deprotected amine salt and Boc-Tyr(Bzl)-ONp (0.54 g, 1.1 mmol) in DMF (4.5 ml) was treated with *i*-Pr_2NEt (0.16 ml, 0.93 mmol). The reaction mixture was stirred at room temperature for 2 days, diluted with 95% EtOH (25 ml), and filtered. The collected precipitate was washed with EtOH and EtOAc. Drying *in vacuo* yielded 1.24 g (91%) of product: homogeneous to tlc (A, R_f 0.64); mp 204–206° dec; $[\alpha]^{23}D$ −28.7° (*c* 1.7, DMF). An analytical sample was crystallized from HOAc by dilution with EtOH. *Anal.* ($C_{69}H_{88}N_{12}O_{15}S_2 \cdot 2C_2H_4O_2$) C, H, N.

β-Mpa(β-Et_2)(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (5). The octapeptide 4 (0.34 g, 0.25 mmol) was deprotected as above. A cold solution of the deprotected amine salt and *i*-Pr_2NEt (0.039 ml, 0.23 mmol) in DMF (1.0 ml) was treated with the nitrophenyl ester 1 (97.5 mg, 0.26 mmol) and 1-hydroxybenzotriazole[19] (33 mg, 0.24 mmol). After 24 hr the homogeneous reaction mixture was added dropwise to 95% EtOH (7.5 ml). The amorphous precipitate was collected, washed with 95% EtOH and EtOAc, and dried *in vacuo* to yield 0.32 g (87%) of slightly off-white product: homogeneous to tlc (B, R_f 0.71); mp 199–206° dec; $[\alpha]^{24}D$ −37.7° (*c* 1, DMF). *Anal.* ($C_{78}H_{102}N_{12}O_{16}S_3 \cdot 2H_2O$) C, H, N.

[1-β-Mercapto-β,β-diethylpropionic acid]-8-lysine-vasopressin. The protected polypeptide 5 (140 mg) was reprecipitated from hot HOAc (1.3 ml) by addition of 2 vol of 95% EtOH (76% recovery). A sample of reprecipitated 5 (101 mg, 0.066 mmol) was dried *in vacuo* and dissolved in 150 ml of boiling liquid NH_3 (freshly distilled from Na). A fresh stick of Na in a glass tube was introduced intermittently into the solution until a blue color persisted for 20 sec.[5] Excess Na was discharged with HOAc. The solvent was evaporated to a small volume, and the final few milliliters were removed by lyophilization. The lyophilizate was dissolved in deaerated water (66 ml) and acetone (56 ml), and the solution was adjusted to pH 6.5 with HOAc. This solution was treated with ICH_2CH_2I[6] (18.3 mg, 0.066 mmol) in acetone (10 ml), and the disappearance of sulfhydryl groups was followed by the quantitative Ellman test.[20] Oxidation was complete within 2.5 hr. Acetone was removed *in vacuo*, and the aqueous solution was adjusted to pH 4.0 with HOAc. The product was desalted on a column (1.2 × 15 cm) of IRC-50 (H^+)[21] and recovered by lyophilization: 61.5 mg (80%).

A sample (61.5 mg) of this crude product was chromatographed on a column (1.06 × 116 cm) of Bio-Rex 70 in 0.5 *M* NH_4OAc (pH 5.85).[9] Fractions of 2.7 ml each were collected at a flow rate of 6.5 ml/hr, and the eluted materials were detected by their uv absorption at 280 nm. The major product, preceded by a few very small components, emerged in fractions 35–63, with the peak at fraction 38. Fractions 35–43, comprising 63% of the area under the peak, were pooled and adjusted to pH 4, and the product was recovered by desalting as before. The recovered material was further purified by gel filtration chromatography[10] on a column (2.8 × 67 cm) of Sephadex G-25 in 0.2 *N* HOAc at a flow rate of 23 ml/hr. The product peak emerged at 89% of the total column volume. Fractions comprising the peak were pooled and lyophilized: 21.0 mg; $[\alpha]^{26}D$ −76.6° (*c* 0.5, 1 *N* HOAc); homogeneous to tlc (A, R_f 0.29; B, R_f 0.36). Amino acid analysis[22] in a Beckman 116 analyzer after a 20-hr hydrolysis in 6 *N* HCl gave the following molar ratios: Asp, 1.0; Glu, 1.0; Pro, 1.0; Gly, 1.0; Tyr, 1.0; Phe, 1.0; Lys, 0.9; NH_3, 3.0. A sample hydrolyzed identically after a performic acid oxidation by the method of Moore[23] had a cysteic acid to Gly ratio of 1:1. *Anal.* ($C_{50}H_{72}N_{12}O_{12}S_2 \cdot C_2H_4O_2 \cdot 4H_2O$) C, H, N.

Acknowledgment. The authors thank Mrs. Linda Mercer, Mrs. Eileen Suffern, and Mrs. Renée Brown for the bioassays performed in the laboratories of Dr. Louis L. Nangeroni of the New York State Veterinary College at Cornell University.

References

(1) H. Schultz and V. du Vigneaud, *J. Med. Chem.*, 9, 647 (1966); W. Y. Chan, H. Schultz, and V. du Vigneaud, III International Pharmacology Congress, Sao Paulo, Brazil, 1966, abstract 449; W. Y. Chan, R. Fear, and V. du Vigneaud, *Endocrinology*, 81, 1267 (1967).
(2) 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).
(3) V. du Vigneaud, J. D. Meador, M. F. Ferger, G. A. Allen, and A. T. Blomquist, *Bioorg. Chem.*, 1, 123 (1971).
(4) H. Schultz and V. du Vigneaud, *J. Amer. Chem. Soc.*, 88, 5015 (1966).
(5) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, 108, 753 (1935).
(6) F. Weygand and G. Zumach, *Z. Naturforsch., B*, 17, 807 (1962).
(7) D. F. Dyckes, M. F. Ferger, V. du Vigneaud, and W. Y. Chan, *J. Med. Chem.*, 16, 843 (1973).
(8) M. Bodanszky, *Nature (London)*, 175, 685 (1955); M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 81, 5688 (1959).
(9) J. Meienhofer and V. du Vigneaud, *J. Amer. Chem. Soc.*, 82, 2279 (1960); R. D. Kimbrough, Jr., W. D. Cash, L. A. Branda, W. Y. Chan, and V. du Vigneaud, *J. Biol. Chem.*, 238, 1411 (1963).
(10) J. Porath and P. Flodin, *Nature (London)*, 183, 1657 (1959).
(11) P. Holton, *Brit. J. Pharmacol. Chemother.*, 3, 328 (1948).
(12) R. A. Munsick, *Endocrinology*, 66, 451 (1960).
(13) J. M. Coon, *Arch. Int. Pharmacodyn.*, 62, 79 (1939).
(14) R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *Endocrinology*, 66, 860 (1960).
(15) "The Pharmacopeia of the United States of America," 18th rev, Mack Printing Co., Easton, Pa., 1970, p 771.
(16) H. O. Schild, *Brit. J. Pharmacol. Chemother.*, 2, 189 (1947).
(17) M. F. Ferger, W. C. Jones, Jr., D. F. Dyckes, and V. du Vigneaud, *J. Amer. Chem. Soc.*, 94, 982 (1972).
(18) E. Kaiser, R. L. Colescott, C. D. Boissinger, and P. I. Cook, *Anal. Biochem.*, 34, 595 (1970), and references cited therein.

**M. Bodanszky, personal communication.

(19) W. König and R. Geiger, *Chem. Biochem., Proc. Amer. Peptide Symp., 3rd*, 343 (1972).
(20) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(21) H. B. F. Dixon and M. P. Stack-Dunne, *Biochem. J.*, **61**, 483 (1955).
(22) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(23) S. Moore, *J. Biol. Chem.*, **238**, 235 (1963).

Synthesis and Some Pharmacological Properties of 8-ε-Hydroxynorleucine-vasopressin†

Patricia Dreyfuss

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received August 2, 1973*

In both lysine-vasopressin and arginine-vasopressin, the eighth position in the chain is occupied by a basic acid. *A priori*, it seems obvious that the cation-forming group in the side chain of these basic amino acids is involved either in binding to the (unknown) receptor or in triggering hormonal action. Earlier work has shown that replacement of the basic guanido group of arginine-vasopressin by the neutral ureido group led to an analog, 8-L-citrulline-vasopressin[2] that was still active although less potent than the natural hormone. Similarly, some light was shed on the role of the side-chain amino group in the pharmacological properties of lysine-vasopressin in the studies involving 8-N^{ϵ}-formyllysine-vasopressin.[3] The moderate hormonal activities reported for this analog did not permit firm conclusions. While the formylamino group cannot be readily protonated in this compound, the decrease in potency could be due to reasons other than lack of ionic bond formation; *e.g.*, the protecting group might prevent the necessary molecular fit between hormone and receptor. There is a possibility that the amino group in question participates in the hormone-receptor interaction by hydrogen bonding rather than by an ionic bond. In the work reported here, this question was examined by replacing L-lysine in the hormone with an ε-hydroxynorleucine residue.

Recently, in a similar study Hope and Walti[4] replaced the single free amino group of the N-terminal cysteine residue of oxytocin with a hydroxyl group and obtained an oxytocin analog of remarkable potency. Their considerations also involve hydrogen bonding with the receptor.

Synthesis. The new analog, 8-ε-hydroxynorleucine-vasopressin (I), was synthesized by the stepwise approach[5] first applied in the synthesis of oxytocin[6] and lysine-vasopressin.[7] The ε-hydroxynorleucine (II) was prepared and resolved by the methods of Gaudry[8] and Berlinguet and Gaudry[9] with the modifications described in the Experimental Section.

An attempt toward a synthesis using an unprotected hydroxyl group of the ε-hydroxynorleucine residue failed. On exposure to the relatively mild acidolytic conditions required for the removal of the *tert*-butyloxycarbonyl groups, the partially protected dipeptide derivative, *N*-benzyloxycarbonylhydroxynorleucylglycine ethyl ester, was cleaved with the liberation of glycine ethyl ester. This not quite unexpected[10] intramolecular N → O acyl migration rendered the protection of the ε-hydroxyl group mandatory. With ε-acetoxynorleucine, the synthesis proceeded to the completely protected nonapeptide without difficulties. The *O*-acetyl group was removed by hydrazinolysis, and the N-terminal benzyloxycarbonyl group and the two *S*-benzyl groups were removed by reduction with sodium in liquid ammonia. The cyclic disulfide was formed by air oxidation. After desalting on a Sephadex G25 column, the hormone analog was purified by countercurrent distribution through 1000 transfers. Two peaks were obtained; the one with higher distribution coefficient corresponded to the desired hormone analog I. The slower migrating material was identified as the dimer of I by its lower solubility and diffusion rate, and particularly by its high specific rotation.[11,12] When the hydrazinolysis was omitted, I was also obtained as the major product but then, in addition to the dimer peak, a smaller, faster moving peak was also found on analysis of the countercurrent distribution fractions. The material isolated from this peak had the properties to be expected of 8-ε-acetoxynorleucine-vasopressin (III).

Biological Activities. Vasopressor activity was measured by the Dekanski[13] method in rats under urethane anesthesia pretreated with phenoxybenzene. Eight groups of four rats were used. No significant deviation of slope compared to the standard was observed. 8-ε-Hydroxynorleucine-vasopressin had vasopressor activity equivalent to 31 ± 1 U/mg. Antidiuretic activity estimated by intravenous injection into hydrated rats under ethanol anesthesia[14] was about 75 U/mg. The USP posterior pituitary reference standard was used in both assays.

Conclusion. The pressor and antidiuretic effects exhibited by the new analog demonstrate that the amino group in the side chain of lysine-vasopressin is not essential for biological activity. The fact that the analog is less potent than the parent hormone indicates that a hydrogen bond either is not involved or is less effective than an ionic bond between hormone and receptor.

Experimental Section

General Comments. Melting points were taken in capillary tubes and are uncorrected. Where analyses are indicated by only the 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 the use of uv, ninhydrin, chlorination,[15] and charring[16] techniques. The following solvent systems were used: A, *n*-BuOH–AcOH–H_2O (4:1:1); B, EtOAc–pyridine–AcOH–H_2O (60:20:6:11); C, $CHCl_3$–MeOH (9:1). Circular paper chromatograms were run on Whatman No. 1 paper in the upper phase of *n*-BuOH–AcOH–H_2O (4:1:5) and the peptides were detected with ninhydrin. Proton nmr spectra were recorded in a Varian A-60 analytical spectrometer. Chemical shifts are downfield from the standard Me_4Si.

For quantitative amino acid analysis, samples were hydrolyzed with 6 *N* HCl in evacuated, sealed ampoules at 110° for 16 hr and analyzed by the Spackman–Stein–Moore method[17] on a Beckman Spinco 120C amino acid analyzer. II is unstable under hydrolysis conditions. A variable mixture consisting of 10–30% amino acid and the rest another compound, presumably the lactone, is obtained. (Lactone is known to form from the acid on treatment with HCl.[9]) The lactone appeared as a distinct peak just before tyrosine but well separated from it. A constant for this product was calculated after subjecting known amounts of free acid to hydrolysis conditions in which the time of hydrolysis was varied from 16 to 48 hr before amino acid analysis. The constant for the acid itself was determined by analyzing a sample of known weight without prior hydrolysis. In addition, the ninhydrin product of II coincided with that of glycine. This was demonstrated in known mixtures of standard and II. A composite constant determined for mixtures of glycine and I was used in all subsequent determinations.

The properties of the compounds whose preparation is described below are summarized in Tables I and II.

ε-Hydroxy-DL-norleucine (IV). The method of Gaudry[8] was followed through the preparation of 5-ε-hydroxybutylhydantoin. Subsequent hydrolysis was carried out by refluxing for 1–2 days with either concentrated NaOH or concentrated $Ba(OH)_2$. When

†Unless otherwise indicated, all optically active amino acid residues have the L configuration. The symbols for the amino acid residues follow the tentative rules of the IUPAC-IUB Commission on Biomedical Nomenclature.[1] The symbol, Hyn, is used to stand for ε-hydroxynorleucine.