The Synthesis and Pharmacological Properties of 4-Decarboxamido-8-lysine-vasopressin, 5-Decarboxamido-8-lysine-vasopressin, and Their 1-Deamino Analogues*

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SUMMARY

Analogues of 8-lysine-vasopressin in which the carboxamide groups on the glutamine and asparagine residues at positions 4 and 5 are replaced individually by hydrogen have been synthesized by the p-nitrophenyl ester method of peptide synthesis and tested for some of the pharmacological activities characteristic of this pressor-antidiuretic hormone of the posterior pituitary gland. The 4-decarboxamido-8-lysine-vasopressin ([4-α-aminobutyric acid]-8-lysine-vasopressin) has an antidiuretic potency of approximately 700 units per mg, nearly 3 times the potency of 8-lysine-vasopressin. It also possesses approximately 25% of the avian vasodepressor activity, 20% of the oxytocic activity, and 4% of the pressor activity of 8-lysine-vasopressin, whereas 5-decarboxamido-8-lysine-vasopressin ([5-alanine]-8-lysine-vasopressin) does not exhibit an appreciable degree of any of these activities. Thus the carboxamide group of the asparagine residue at position 5 of 8-lysine-vasopressin appears to play an important role in determining the pharmacological effects of the hormone. The corresponding 1-deamino analogues of the 4- and 5-decarboxamido-8-lysine-vasopressins, in which the free amino group on the half-cystine residue at position 1 is replaced by hydrogen, have also been synthesized. 1-Deamino-4-decarboxamido-8-lysine-vasopressin possesses extremely high antidiuretic activity (730 units per mg), low pressor activity (3.5 units per mg), and the same oxytocic and avian vasodepressor activities as 4-decarboxamido-8-lysine-vasopressin, whereas 1-deamino-5-decarboxamido-8-lysine-vasopressin is practically inactive. The successive replacement of the carboxamide and amino groups on the glutamine and half-cystine residues at positions 4 and 1, respectively, of 8-lysine-vasopressin thus results in a striking enhancement of antidiuretic activity coupled with a marked decrease in pressor activity.

Study of the contribution of the various chemical functional groups of the posterior pituitary hormone, oxytocin, to its biological activity has led to the synthesis of a series of analogues of oxytocin in which these functional groups have been replaced by hydrogen (1). As part of these investigations 4-decarboxamido-oxytocin ([4-α-aminobutyric acid]-oxytocin) and 5-decarboxamido-oxytocin ([5-alanine]-oxytocin) were synthesized (2). When these two analogues were tested for some of the biological activities characteristic of the posterior pituitary hormones, it was found that the presence of the carboxamide group of the asparagine residue at position 5 is essential for the exhibition of appreciable activity, in contrast to the carboxamide group of the glutamine residue at position 4.

We thought it would be of interest to extend these structural modifications to 8-lysine-vasopressin (Fig. 1) to determine whether the same striking differences would be found in the contribution of the carboxamide groups at positions 4 and 5 to biological activity as had been found in the case of oxytocin. We have therefore synthesized 4-decarboxamido-8-lysine-vasopressin ([4-α-aminobutyric acid]-8-lysine-vasopressin) and 5-decarboxamido-8-lysine-vasopressin ([5-alanine]-8-lysine-vasopressin). Since the replacement of the free amino group in position 1 of 8-lysine-vasopressin by hydrogen increases the antidiuretic potency and decreases the pressor potency (3), we have also synthesized 1-deamino-4-decarboxamido-8-lysine-vasopressin ([1-β-mercapto-propionic acid]-4-α-aminobutyric acid]-8-lysine-vasopressin). The nomenclature used here for our analogues follows tentative rules of the IUPAC-IUB Commission on Biochemical Nomenclature.

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**FIG. 1. Structure of S-lysine-vasopressin. Numbers indicate the positions of the individual amino acid residues.**

All the protected peptide intermediates for the synthesis of the analogues were prepared by use of the p-nitrophenyl ester method (4) in a stepwise fashion as employed in the synthesis of 1-deamino-8-lysine-vasopressin (5) and l-deamino-8-lysine-vasopressin (3), and in each case the analogues were isolated and purified by partition chromatography in the solvent system butanol-ethanol-pyridine-acetic acid (10). The 4-decarboxamido-8-lysine-vasopressin was obtained from the protected compounds to the cyclic disulfides by means of potassium ferricyanide (8, 9) and subsequent oxidation of the resulting disulfhydryl peptides similar to those used for synthesis of the 1-decarboxamido compounds, with a glutamine residue in place of the α-amino butyric acid residue and an alanine residue in place of the asparagine residue. The 5-decarboxamido analogue was purified by chromatography in two solvent systems, butanol-pyridine-0.2 N acetic acid (10:7:24) and butanol-ethanol-pyridine-0.2 N acetic acid (4:1:1:7). For chromatography of 1-deamino-5-decarboxamido-8-lysine-vasopressin, the ratios of the components of these systems were changed to butanol-pyridine-0.2 N acetic acid (6:1:8) and butanol-ethanol-pyridine-0.2 N acetic acid (6:1:1:8).

The four highly purified analogues appeared to be homogeneous when subjected to thin layer chromatography, paper electrophoresis, and gel filtration on Sephadex G-25. Elementary analysis and analysis for amino acids and ammonia gave the expected values in all cases.

The analogues were tested for some of the biological activities exhibited by the posterior pituitary hormones. Four-point assay design was used in the bioassays, which were carried out under the direction of Dr. W. Y. Chan of this laboratory. Assays for antidiuretic activity were performed on male rats according to the method of Jeffers, Livezey, and Austin (11) as modified by Sawyer (12). Rat pressor assays were carried out on anesthetized male rats as described in the United States Pharmacopeia (13). Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by Muniesia, Sawyer, and van Dyke (14). Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of Holton (15) as modified by Muniesia (16) with the use of magnesium-free van Dyke-Hastings solution as the bathing fluid. All of the assays were carried out against the U.S.P. Posterior Pituitary Reference Standard. The potencies of the lysine-vasopressin analogues with respect to these pharmacological activities are reported in Table I along with the potencies of 8-lysine-vasopressin and 1-deamino-8-lysine-vasopressin.

### Table I

**Pharmacological activities of lysine-vasopressin analogues**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean activities and standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antidiuretic (rat)</td>
</tr>
<tr>
<td></td>
<td>units/mg</td>
</tr>
<tr>
<td>8-Lysine-vasopressin*</td>
<td>240 ± 13</td>
</tr>
<tr>
<td>1-Deamino-8-lysine-vasopressin*</td>
<td>301 ± 11</td>
</tr>
<tr>
<td>4-Decarboxamido-8-lysine-vasopressin</td>
<td>707 ± 107</td>
</tr>
<tr>
<td>1-Deamino-4-decarboxamido-8-lysine-vasopressin</td>
<td>729 ± 26</td>
</tr>
<tr>
<td>5-Decarboxamido-8-lysine-vasopressin</td>
<td>~0.2</td>
</tr>
<tr>
<td>1-Deamino-5-decarboxamido-8-lysine-vasopressin</td>
<td>~0.05</td>
</tr>
</tbody>
</table>

* These values are those obtained on a sample of synthetic lysine-vasopressin purified by partition chromatography on Sephadex G-25 (17). The value for pressor activity has already been reported (17). The milk-ejecting activity of this material was found to be approximately 60 units per mg.

† See Kimbrough, Cash, Branda, Chan, and du Vigneaud (3).

‡ No measurable activity, but had potentiating effect on the response to the U.S.P. Posterior Pituitary Reference Standard.
**EXPERIMENTAL PROCEDURE**

N-Carbobenzoxy-L-α-aminobutyryl-L-asparaginy1-S-benzyl-L-cysteiny1-L-proly1-N'-tosyl-L-lysylglycinamide — N-Carbobenzoxy-L-asparaginy1-S-benzyl-L-cysteiny1-L-proly1-N'-tosyl-L-lysylglycinamide

The protected hexapeptide described in the preceding section, 1.0 g, was dissolved by being heated in 15 ml of glacial acetic acid. The solution was cooled to room temperature, and 15 ml of HBr in glacial acetic acid (32%, w/w) were added. After being dried over P2O5, in a vacuum the product weighed 1.1 g; m.p. 227–229° (sintering at 236°); [α]D420 — 37.4° (c, 1, in dimethylformamide).

C17H16N3O3S2

Calculated: C 58.7, H 6.34, N 12.3
Found: C 58.3, H 6.35, N 12.6

N-Carbobenzoxy-S-benzyl-L-cysteiny1-L-(γ-lysyl-L-phenylalany1-L-α-aminobutry1-L-asparaginy1-S-benzyl-L-cysteiny1-L-proly1-N'-tosyl-L-lysylglycinamide — The preceding protected octapeptide, 0.9 g, was suspended in 25 ml of trifluoroethanol, and HBr was bubbled through the suspension for 1 hr. The solvent was evaporated in a vacuum, and the residue was washed four times with ether, dried over NaOH, and then dissolved in 5 ml of dimethylformamide. The pH was adjusted to 8 with triethylamine, and 0.35 g of p-nitrophenyl N-carbobenzoxy-S-benzyl-L-cysteinamide (20) was added. After 3 days, the reaction mixture was added to 50 ml of a mixture of ethanol and water (1:1). The precipitate was collected by filtration and washed twice with 25-ml portions of ethanol and three times with 25-ml portions of ethyl acetate. After being dried over P2O5, in a vacuum the compound weighed 0.8 g; m.p. 205–208°; [α]D420 — 39.6° (c, 1, in dimethylformamide).

C21H19N5O3S2

Calculated: C 59.9, H 6.11, N 11.3
Found: C 59.6, H 6.11, N 11.1

4-Deoxyamide-8-lysine-vasopressin ([4-L-α-Aminobutyric acid]-8-lysine-vasopressin) — The protected nonapeptide described in the preceding section, 101 mg, was dissolved in approximately 150 ml of liquid ammonia (freshly distilled from sodium) and treated at the boiling point with sodium until a blue color persisted throughout the solution for about 30 sec. The ammonia solution was subsequently concentrated, and the final 25 ml were removed by lyophilization. The loose powder was dissolved in 150 ml of water. The pH was adjusted to 6.8 with acetic acid and kept between 6.5 and 7.0 by the addition of NaOH while the solution was titrated with 12.6 ml of a 0.01 m potassium ferricyanide solution. After 4 hours the sulphydryl content of the solution was tested by the Ellman method (22) and found to be zero. The solution was acidified with 0.5 ml of acetic acid and was kept between 6.5 and 7.0 by the addition of NaOH while the solution was titrated with 12.6 ml of a 0.01 m potassium ferricyanide solution. After 4 hours the sulphydryl content of the solution was tested by the Ellman method (22) and found to be zero. The solution was acidified with 0.5 ml of acetic acid and the ferrocyanide and ferricyanide ions were removed by passage through a column of Rexyn CGS (Fisher) in the chloboide form. After the solution had been concentrated to a volume of approximately 10 ml, butanol-ethyl-pyridine (4:1:1) was added dropwise until a second phase started to separate.
This solution was applied to a Sephadex G-25 column (2.15 × 120 cm) that had been equilibrated with the aqueous phase of the system butanol-ethanol-pyridine-1 N acetic acid (4:1:1:7). The chromatogram was developed with the organic phase at a flow rate of 17 ml per hour, and 95 fractions containing 5.6 ml each were collected. The plot of the Folin-Lowry color values (23) of aliquots from every second fraction indicated a major peak with a maximum at Fraction 63 in addition to two smaller peaks with maxima at Fractions 30 and 85, respectively. The central fractions of the main peak were combined, diluted with twice the volume of water, and concentrated to a volume of 20 ml. Addition of 100 ml of water and subsequent concentration to a volume of approximately 20 ml were repeated three times, and the solution was finally lyophilized to yield 36.7 mg of a fluffy white powder. Material, 1.5% mg, from several experiments was dissolved in 10 ml of the organic phase of butanol-ethanol-pyridine-1 N acetic acid (4:1:1:7) and applied to a Sephadex column (2.83 × 58 cm) that had been equilibrated with both phases of this system. Elution with the organic phase was performed, and fractions were collected at a flow rate of 28 ml per hour. The Folin-Lowry color values showed a single symmetrical peak having an Rf of 0.27. The amount of 1-decarboxamido-S-lysine-vasopressin isolated from this peak was 130.9 mg; [α]D25 +33.2° (c, 0.5, in 1 N acetic acid). When the analogue was subjected to paper electrophoresis in a pyridine-acetic acid buffer (pH 5.6) and to thin layer chromatography on Silica Gel G in the upper phase of the system butanol-acetic acid (4:1:5), it traveled as a single spot. Upon gel filtration of the compound on Sephadex G-25 in 0.2 N acetic acid it emerged as a single peak. For analysis a sample was dried in a vacuum at 100° over P2O5 for 6 hours, and a loss of weight of 8.6% was observed.

C61H12N12O11S2.C4H4O4
Calculated: C 52.6, H 6.39, N 15.7
Found: C 52.2, H 6.34, N 15.6

A sample was hydrolyzed in 6 N HCl at 110° for 22 hours and then analyzed by the method of Spackman, Stein, and Moore (24) in the 50°-50° and 30°-50° systems on a Beckman/Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained, with the value of glycine taken as 1.0: aspartic acid, 1.0; proline, 1.0; glycine, 1.0; α-amino-n-butyric acid, 1.0; cystine, 1.0; tyrosine, 0.8; phenylalanine, 1.0; lysine, 0.9; ammonia, 2.0.

S-Benzyl-β-mercapto-pro-pionyl-L-tyrosyl-L-phenylalanyl-L-α-amino-n-butyryl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyn-N-tosyl-L-lysylglycinamide—N-Carbobenzyloxyl-O-benzyl-L-tyrosyl-L-phenylalanyl-1-L-α-amino-n-butyryl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyn-N-tosyl-L-lysylglycinamide was suspended in 35 ml of trifluoroethanol, and HBr was bubbled through the mixture for 1 hour. The solvent was removed in a vacuum, and the residue was washed four times with 80-ml portions of ether, dissolved in 20 ml of methanol, precipitated by addition of 200 ml of ether, and washed twice with small portions of ether. After being dried over NaOH the material was dissolved in 4 ml of dimethylformamide. The pH was adjusted to 8.5 with triethylamine, and 0.32 g of β-nitrophenyl S-benzyl-β-mercapto-propionate (10, 25) was added. After 2 days, the material was precipitated by addition of 50 ml of a mixture of ethanol and water (1:1), collected, and washed three times with 25-ml portions of ethanol and three times with 25-ml portions of ethyl acetate. After being dried over P2O5 in a vacuum the compound weighed 0.75 g; m.p. 217°-219°; [α]D25 +53.4° (c, 1, in dimethylformamide).

C61H12N12O11S2
Calculated: C 59.4, H 6.27, N 11.5
Found: C 59.3, H 6.34, N 11.4

1-Deamino-4-decarboxamido-5-lysine-vasopressin ([1-β-Mercapto-propionic acid]4-2-α-amino-n-butyric acid]-5-lysine-vasopressin)—The removal of the protecting groups from the preceding protected polypeptide and the oxidation of the resulting disulf-hydryl compound to the cyclic disulfide were carried out by the procedures described for the synthesis of 4-decarboxamido-S-lysine-vasopressin. After removal of the ferricyanide and ferrocyanide ions, the aqueous solution of the 1-deamino-4-decarboxamido-5-lysine-vasopressin prepared from 105 mg of the protected polypeptide was concentrated to a volume of 10 ml, equilibrated with the organic phase of the solvent system butanol-ethanol-pyridine-1 N acetic acid (6:1:1:8), and applied to a Sephadex G-25 column (2.15 × 120 cm) that had been equilibrated with the aqueous phase. Elution with the organic phase was performed, and 4.5-ml fractions were collected at a flow rate of 10 ml per hour. Folin-Lowry color determinations on aliquots of selected fractions indicated a major peak with a maximum at Fraction 46. The material isolated from Fractions 42 through 50 weighed 22.6 mg, and 140.2 mg of such material obtained from several experiments was submitted to chromatography in the solvent system butanol-ethanol-pyridine-0.2 N acetic acid (6:1:1:8). The sample was dissolved in 5 ml of the organic phase of this system and applied to a column (2.83 × 58 cm) that had been equilibrated with both phases. Elution with the organic phase was performed, and fractions were collected at a flow rate of 24.5 ml per hour. The Folin-Lowry color values showed a single peak, from which 115.8 mg of 1-deamino-4-decarboxamido-5-lysine-vasopressin, having an Rf of 0.48 and [α]D25 +111.2° (c, 0.5, in 1 N acetic acid), were isolated. When a sample of this compound was subjected to electrophoresis and thin layer chromatography under conditions already described, it traveled as a single spot. It also emerged as a single peak from a gel filtration column. For analysis a sample was dried in a vacuum at 100° over P2O5 for 6 hours, and a loss of weight of 7.6% was observed.

C61H12N12O11S2
Calculated: C 54.1, H 6.39, N 15.4
Found: C 54.8, H 6.38, N 15.2

A sample was hydrolyzed in 6 N HCl at 110° for 22 hours and then analyzed in the 50°-50° and 30°-50° systems on a Beckman/Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained, with the value of glycine taken as 1.0: aspartic acid, 1.0; proline, 1.0; glycine, 1.0; α-amino-n-butyric acid, 1.0; cystine, 1.0; tyrosine, 0.8; phenylalanine, 1.0; lysine, 0.9; ammonia, 2.0.

N-Carbobenzyloxyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyn-N-tosyl-L-lysylglycinamide—A solution of 11.3 g of N-carbobenzyloxyl-S-benzyl-L-cysteinyl-L-prolyn-N-tosyl-L-lysylglycinamide (5) in 50 ml of glacial acetic acid was treated with 50 ml of a solution of...
HBr in glacial acetic acid (40%, w/w). After 1 hour the hydrobromide of the free tetrapeptide was precipitated by addition of 1 liter of dry ether, washed several times with ether, and dissolved in 1 liter of water. The pH of the solution was adjusted to 7 with 0.5 M KHCO₃ solution. The mixture was extracted four times with 100-ml portions of chloroform. The combined extracts were dried over MgSO₄ and the solvent was removed in a vacuum. The residue was dissolved in 30 ml of dimethylformamide and allowed to react with 5.2 g of p-nitrophenyl N-carbobenzyoxy-L-phenylalaninate (2, 18) for 2 days at room temperature. Then 300 ml of ethyl acetate were added, and the solution was extracted with triethylamine-saturated water until the aqueous phase was colorless. The organic phase was then extracted five times with 0.5 M HCl, washed repeatedly with NaCl solution, and dried over MgSO₄. The solvent was removed by evaporation, and the residue was dissolved in boiling ethyl acetate. Petroleum ether was added until the solution became cloudy and, after 1 day at 5°C, the precipitating residue was collected, washed with ether, and dried to yield 9.5 g; m.p. 155-156°C; [α]D²⁰ +40.7° (c 1, in dimethylformamide). For analysis a sample was precipitated from the mixture of dimethylformamide, ethyl acetate, and cyclohexane. The melting point was 171-176°C (sintering at 164°C).

\[ \text{C}_2\text{H}_4\text{N}_2\text{O}_4 \text{S}_2 \]
Calculated: C 57.8, H 6.27, N 11.5
Found: C 58.1, H 6.43, N 11.5

**N-Carbobenzyoxy-L-glutaminyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-N'-tosyl-L-lysylglycinamide**—A solution of 6.8 g of the preceding protected pentapeptide in 30 ml of glacial acetic acid was treated with 40 ml of a solution of HBr in glacial acetic acid (40%, w/w). After 1.5 hours the hydrobromide of the free base was precipitated with 600 ml of ether, washed several times with ether, and dissolved in 125 ml of dry methanol. The solution was passed through a Rexyn RGI (OH⁻) column, which was then washed with 200 ml of methanol. After removal of the solvent from the combined eluate and washing, the residue was dissolved in 6 ml of dimethylformamide, and 3.3 g of p-nitrophenyl N-carbobenzoxy-L-glutaminate (20) were added. After 2 days, 100 ml of ethyl acetate were added to the reaction mixture and the precipitate was collected. Since treatment of the gelatinous material with ethanol and ethyl acetate did not remove the p-nitrophenyl, the material was dissolved in 40 ml of dimethylformamide, precipitated by addition of 400 ml of ethyl acetate, collected, and washed repeatedly with ethanol and ethyl acetate. After being dried over P₂O₅ in a vacuum the compound weighed 5.1 g; m.p. 164-167°C; [α]D²⁰ +40.8° (c 1, in dimethylformamide).

\[ \text{C}_2\text{H}_4\text{N}_2\text{O}_4 \text{S}_2 \]
Calculated: C 55.4, H 6.27, N 12.9
Found: C 56.2, H 6.35, N 19.7

**N-Carbobenzyoxy-L-phenylalanyl-L-glutaminyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-N'-tosyl-L-lysylglycinamide**—A solution of 1.1 g of the preceding protected hexapeptide in 11 ml of glacial acetic acid was treated with 11 ml of a solution of HBr in glacial acetic acid (40%, w/w). After 1 hour, 200 ml of dry ether were added. The precipitate was washed with ether, dried, and then dissolved in 40 ml of dry methanol and passed through a column of Rexyn RGI (OH⁻), which was then washed with 60 ml of methanol. After removal of the methanol from the combined eluate and washing, the residue was dissolved in 2.5 ml of dimethylformamide and allowed to react with 0.55 g of p-nitrophenyl N-carbobenzoxy-L-phenylalaninate. After 1 week, 80 ml of ethyl acetate were added to the reaction mixture and the precipitate was washed in the usual manner with ethanol and ethyl acetate. After being dried over P₂O₅ in a vacuum the compound weighed 0.9 g; m.p. 226-227°C (sintering at 172°C); [α]D²⁰ +40.5° (c 1, in dimethylformamide).

\[ \text{C}_2\text{H}_4\text{N}_2\text{O}_4 \text{S}_2 \]
Calculated: C 58.0, H 6.26, N 12.4
Found: C 58.5, H 6.26, N 12.4

**N-Carbobenzyoxy-O-benzyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-N'-tosyl-L-lysylglycinamide**—Two grams of the protected heptapeptide described in the preceding section were dissolved in 15 ml of warm glacial acetic acid, cooled, and treated with a solution of HBr in glacial acetic acid (40%, w/w). After 1 hour the hydrobromide of the free base was isolated in the manner already described and dissolved in 100 ml of dry methanol. The hydrobromic acid was removed in the usual manner with Rexyn RGI (OH⁻) and the residue obtained after evaporation of the methanol was dissolved in 4 ml of dimethylformamide and allowed to react with 1.0 g of p-nitrophenyl N-carbobenzyoxy-O-benzyl-L-tyrosinate. After 2 days, 100 ml of ethyl acetate were added to the semisolid reaction mixture. The solid was collected, washed with ethyl acetate and ethanol, and dried over P₂O₅ in a vacuum; weight, 2.05 g; m.p. 221-223°C; [α]D²⁰ +42.1° (c 1, in dimethylformamide).

\[ \text{C}_2\text{H}_4\text{N}_2\text{O}_4 \text{S}_2 \]
Calculated: C 61.8, H 6.20, N 11.2
Found: C 61.7, H 6.22, N 11.0

**N-Carbobenzyoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-N'-tosyl-L-lysylglycinamide**—A stream of HBr was bubbled for 4 hours through a suspension of 0.9 g of the preceding protected octapeptide in 25 ml of trifluoroethanol. After an additional 4 hour, the solvent was removed in a vacuum and the residue was dissolved in 25 ml of methanol, precipitated with ether, washed repeatedly by decantation with ether, separated by filtration, dried, and dissolved in 3 ml of dimethylformamide. The solution was cooled to 0°C before the pH was adjusted to 8.5 with triethylamine. Then 0.35 g of p-nitrophenyl N-carbobenzyoxy-S-benzyl-L-cysteininate was added. After 2 days the reaction solution was poured into a mixture of 35 ml of water and 15 ml of ethanol. The solid was collected and washed twice with 25-ml portions of ethanol and three times with 25-ml portions of ethyl acetate. After being dried over P₂O₅ in a vacuum the compound weighed 0.75 g; m.p. 200-202°C (sintering at 192°C); [α]D²⁰ +42.0° (c 1, in dimethylformamide).

\[ \text{C}_2\text{H}_4\text{N}_2\text{O}_4 \text{S}_2 \]
Calculated: C 60.9, H 6.11, N 11.3
Found: C 60.1, H 6.13, N 11.0

**5-Decarboxamido-L-lysine-vasopressin (5-L-alanine)-L-lysine-vasopressin**—This compound was prepared from the preceding protected polypeptide by treatment with sodium in liquid ammonia and subsequent oxidation according to the procedures already described. The crude material prepared from 303 mg of protected polypeptide was applied to a Sephadex G-25 column (2.15 × 120 cm) that had been equilibrated with the aqueous phase of the solvent system butanol-pyridine-0.2 N acetic acid.
The chromatogram was developed with the organic phase at a flow rate of 16.5 ml per hour, and 5-ml fractions were collected. The desired material was eluted in a broad peak with a maximum at Fraction 56. The amount of 5-decarboxamido-8-lysine-vasopressin isolated from the contents of Fractions 44 through 68 was 138.4 mg. This material was then submitted to chromatography in the solvent system butanol-ethanol-pyridine-0.2 N acetic acid (4:1:1:7) on a Sephadex column (2.83 X 58 cm) that had been equilibrated with both phases of this system. The analogue emerged from this column with an Rf value of 0.24. The material weighed 72.6 mg; [α]25° = −9.8° (c, 0.5, in 1 N acetic acid). A sample of this material traveled as a single spot when subjected to electrophoresis and thin layer chromatography under the conditions already described. It also emerged as a single peak when subjected to gel filtration in 0.2 N acetic acid.

When the analogue was dried over P2O5 in a vacuum for analysis a loss of weight of 12.47, was observed. The material weighed 64.1 mg; [α]25° = −9.8° (c, 0.5, in 1 N acetic acid). A sample of this material traveled as a single spot when subjected to electrophoresis and thin layer chromatography and found to travel as a single spot. Only one peak was observed when an aliquot of the compound was subjected to gel filtration in 0.2 N acetic acid. When a sample was dried over P2O5 in a vacuum at 100° a loss of weight of 10.3% was observed.

C42H40N9O12S2-C2H4O2
Calculated: C 53.0, H 6.39, N 15.7
Found: C 53.0, H 6.43, N 15.5

A sample was hydrolyzed in the usual manner and analyzed in the 50°-50° system on a Beckman/Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained, with the value for glycine taken as 1.0: glutamic acid, 1.0; proline, 1.0; glycine, 1.0; alanine, 1.0; cystine, 1.0; tyrosine, 0.9; phenylalanine, 1.0; lysine, 1.0; ammonia, 2.0.

S-Benzyl-β-mercaptopyropionyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-N′-tosyl-β-lysylglycinamide—The hydrobromide of the free octapeptide was prepared from 1.0 g of N-carbobenzoxy-O-benzyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-alanyl-L-benzyl-L-cysteinyl-L-prolyl-N′-tosyl-β-lysylglycinamide—The hydrobromide of the free octapeptide was prepared from 1.0 g of N-carbobenzoxy-O-benzyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-alanyl-L-benzyl-L-cysteinyl-L-prolyl-N′-tosyl-β-lysylglycinamide as already described. After the salt had been dried overnight in a desiccator over NaOH and CaCl2 it was dissolved in 3 ml of dimethylformamide. The pH was adjusted to 8.5 with triethylamine, and 0.25 g of p-nitrophenyl S-benzyl-β-mercaptopyropionate was added. After 2 days, the product was precipitated by addition of a mixture of 25 ml of ethanol and 25 ml of water. The solid was collected, washed with ethanol and ethyl acetate, and dried over P4O5 in a vacuum; weight, 0.65 g; m.p. 170°. For analysis a sample was precipitated from a mixture of dimethylformamide and ethyl acetate; m.p. 181°-184° (sintering at 155°); [α]25° = −41.3° (c, 1, in dimethylformamide).

C42H40N9O12S2
Calculated: C 59.4, H 6.37, N 11.5
Found: C 59.8, H 6.31, N 11.2

1-Deamino-5-decarboxamido-8-lysine-vasopressin (1-β-Mercaptopyropionic acid, 5-L-alanine)-8-lysine-vasopressin—The preparation of this analogue from the preceding protected polypeptide followed the procedures already described. The crude material prepared from 106 mg of protected polypeptide was applied to a Sephadex G-25 column (2.15 X 120 cm) that had been equilibrated with the aqueous phase of the solvent system 1-butanol-pyridine-0.2 N acetic acid (6:1:8). The chromatogram was developed with the organic phase of this system, and 5-ml fractions were collected at a flow rate of 12 ml per hour. The desired compound was eluted in a symmetrical peak with a maximum at Fraction 63. From the contents of Fractions 57 through 68, 34.8 mg of 1-deamino-5-decarboxamido-8-lysine-vasopressin were isolated. This material emerged with an Rf value of 0.46 when submitted to chromatography in the solvent system butanol-ethanol-pyridine-0.2 N acetic acid (9:1:1:8) on a Sephadex column (2.83 X 58 cm) that had been equilibrated with both phases of this system. The 1-deamino-5-decarboxamido-8-lysine-vasopressin weighed 53.2 mg; [α]25° = −39.5° (c, 0.5, in 1 N acetic acid). A sample of this material was subjected to electrophoresis and thin layer chromatography and found to travel as a single spot. When a sample was dried over P2O5 in a vacuum at 100° a loss of weight of 10.3% was observed.

Comparison of the antidiuretic,pressor, oxytocic, and avian vasodepressor potencies of 4-decarboxamido-8-lysine-vasopressin recorded in Table I with the practically negligible values obtained on assay of 5-decarboxamido-8-lysine-vasopressin reveals striking differences in the contribution of the carboxamide groups at positions 4 and 5 of lysine-vasopressin to the exhibition of activities. Similar differences were observed in the case of the carboxamide groups at positions 4 and 5 of oxytocin (2). Thus, the presence of the carboxamide group of the glutamine residue at position 4 of 8-lysine-vasopressin is not essential for the exhibition of an appreciable degree of these pharmacological activities, whereas the presence of the carboxamide group of the asparagine residue at position 5 is essential.

Replacement of the carboxamide group at position 4 of 8-lysine-vasopressin by hydrogen results in a pronounced decrease in pressor activity and substantial decreases in oxytocic and avian vasodepressor activities, as was noted in the case of oxytocin. However, a marked distinction between these two posterior pituitary hormones is apparent in regard to the effect of this replacement on the antidiuretic activity. In the case of oxytocin, the slight antidiuretic activity is nearly abolished by the structural change at position 4, whereas the same change causes an approximately 3-fold increase in the antidiuretic activity in the case of 8-lysine-vasopressin (210 units per mg) and thus makes 4-decarboxamido-8-lysine-vasopressin (700 units per mg) an extremely potent antidiuretic agent. On the other hand, the pressor potency of the 4 decarboxamido-8 lysine vasopressin (10 units per mg) is less than 4% of that of the hormone (206 units per mg).

The 1-deamino-4-decarboxamido-8-lysine-vasopressin is also highly potent with respect to antidiuretic activity (730 units per mg), whereas it possesses only about 3 units per mg of pressor activity. It may be noted from the data in Table I that the antidiuretic potency of 1-deamino-4-decarboxamido-8-lysine-vasopressin is more than twice that of 1-deamino-8-lysine-vasopressin. This material emerged with an Rf value of 0.56 when submitted to chromatography in the solvent system butanol-ethanol-pyridine-0.2 N acetic acid (9:1:1:8) on a Sephadex column (2.83 X 58 cm) that had been equilibrated with both phases of this system. The 1-deamino-4-decarboxamido-8-lysine-vasopressin weighed 85.2 mg; [α]25° = −49.3° (c, 0.5, in 1 N acetic acid). A sample of this material was subjected to electrophoresis and thin layer chromatography and found to travel as a single spot. When a sample was dried over P2O5 in a vacuum at 100° a loss of weight of 10.3% was observed.
vasopressin, whereas the pressor potency of the former compound is less than 3.3% of that of the latter. 1-Deamino-5-decarboxamido-8-lysine-vasopressin has practically no antidiuretic, pressor, oxytocic, or avian vasodepressor activity.

The successive replacement of the carboxyamide and amino groups at positions 4 and 1 of 8-lysine-vasopressin with hydrogen causes a striking enhancement of antidiuretic activity coupled with a marked decrease in pressor activity. The ratio between antidiuretic and pressor potencies is approximately 1:1 for lysine-vasopressin, 70:1 for 4-decarboxamido-8-lysine-vasopressin, and 200:1 for 1-deamino-4-decarboxamido-8-lysine-vasopressin.

Other vasopressin analogues possessing antidiuretic potencies considerably higher than their pressor potencies have been reported by other investigators. The most striking are 1-deamino-8-arginine-vasopressin, which possesses 1300 antidiuretic and 370 pressor units per mg (26, 27); 1-deamino-2-phenylalanine-8-arginine-vasopressin, which possesses 800 antidiuretic and 29 pressor units per mg (27, 28); I-deamino-8-γ-aminobutyryl-vasopressin, which possesses approximately 670 antidiuretic and 11 pressor units per mg (29).

The work on the decarboxamido-lysine-vasopressins reported in the present paper is being extended to the arginine-vasopressin series.

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The Synthesis and Pharmacological Properties of 4-Decarboxamido-8-lysine-vasopressin, 5-Decarboxamido-8-lysine-vasopressin, and Their 1-Deamino Analogues

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