Acknowledgment. The chemical investigations and X-ray studies reported herein were supported by the National Institutes of Health and the U. S. Air Force Office of Scientific Research, respectively.

The Synthesis of Deamino-oxytocinoic Acid and Acetone-oxytocinoic Acid and Their Use in the Preparation of Deamino-oxytocinoyloxytocin and Oxytocinoyloxytocin[1,2]

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Abstract: Deamino-oxytocinoic acid and acetone-oxytocinoic acid have been synthesized. Deamino-oxytocinoic acid possessed approximately 8.6 units/mg of avian vasodepressor activity and less than 0.2 units/mg of oxytocic activity. Acetone-oxytocinoic acid possessed no detectable avian vasodepressor or oxytocic activity, whereas oxytocinoic acid possesses approximately 3.8 units/mg of oxytocic activity and no avian vasodepressor activity. Deamino-oxytocinoic acid and acetone-oxytocinoic acid were each condensed with oxytocin to give deamino-oxytocinoyloxytocin and acetone-oxytocinoyloxytocin. Heating of the latter compound with 0.25% acetic acid liberated oxytocinoyloxytocin. Deamino-oxytocinoyloxytocin and oxytocinoyloxytocin exhibited extremely low levels of oxytocic and avian vasodepressor activity.

In studies on the relationship of structure to the pharmacological activity of oxytocin, a number of peptide derivatives of oxytocin have been synthesized by addition of one or more amino acid residues to the free amino group at position 1 of the hormone.[4-9] It has also become of interest to study the pharmacological properties of compounds in which the oxytocin molecule is extended at position 9, and it occurred to us that the acetone derivative of oxytocinoic acid should prove useful as an intermediate in the synthesis of analogs of this type. In this derivative the free amino group of oxytocinoic acid[10] would be protected by acetone and, by analogy to the behavior of acetone-oxytocin,[11-13] the structure of which is shown in Figure 1, the acetone should be readily removable under the mild conditions used in the regeneration of oxytocin from its acetone derivative. For lengthening of the peptide side chain of deamino-oxytocin,[14-17] deamino-oxytocinoic acid should prove useful. Deamino-oxytocinoic acid and acetone-oxytocinoic acid were therefore synthesized and used in the preparation of deamino-oxytocinoyloxytocin and oxytocinoyloxytocin, respectively.

Deamino-oxytocinoic acid was synthesized by use of the solid phase method as described for the synthesis of deamino-oxytocin,[18] except that unnitrated chloromethylcopolystyrene–2% divinylbenzene was used and cleavage of the protected polypeptide from the resin was effected by HBr in trifluoroacetic acid.[19] The analog was purified by partition chromatography[20] and gel filtration[21] on Sephadex G-25, and was crystallized from water. Deamino-oxytocinoic acid possessed approximately 8.6 units/mg of avian vasodepressor activity[22] and less than 0.2 units/mg of oxytocic activity.[22] Crystalline deamino-oxytocin possesses approximately 975 units/mg of avian vasodepressor activity and 800 units/mg of oxytocic activity.[17]

(1) This work was supported in part by Grant No. HE-11680 from the National Heart Institute, U. S. Public Health Service.

(2) All optically active amino acid residues are of the L variety.

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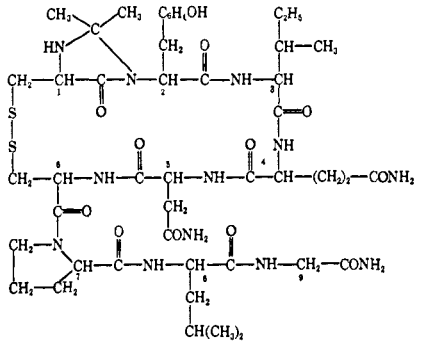

Figure 1. Structure of acetone-oxytocin with numbers indicating the position of the individual amino acid residues.

For the synthesis of acetone-oxytocinoic acid, oxytocinoic acid was first prepared and purified in the same manner as deamino-oxytocinoic acid. Its behavior on thin layer chromatography agreed with that of a sample of oxytocinoic acid synthesized earlier by classical methods.[23] The oxytocinoic acid prepared by the solid phase method possessed approximately 3.8 units/mg of oxytocic activity and no detectable avian vasodepressor activity. Ferrier and du Vigneaud[23] had reported 1.3 units/mg of oxytocic activity and approximately 0.2 units/mg of avian vasodepressor activity for their sample of oxytocinoic acid and 1.8 units/mg of oxytocic activity and no avian vasodepressor activity for a sample received from Klostermeyer.[24] A partially protected nonapeptide precursor of oxytocinoic acid has been synthesized by the solid phase method by Beyerman, *et al.*,[25] employing active ester coupling and using 1,2,4-triazole.

Comparison of the pharmacological characteristics of oxytocinoic acid and deamino-oxytocinoic acid shows that the replacement of the free amino group at position 1 of oxytocinoic acid by hydrogen decreases the oxytocic potency and increases the avian vasodepressor potency.

Oxytocinoic acid was converted to the acetone derivative by treatment with 80% aqueous acetone as in the synthesis of acetone-oxytocin[11] except that pyridine was added to the solution. Acetone-oxytocinoic acid was purified by partition chromatography and found to possess no detectable avian vasodepressor or oxytocic activity. Oxytocinoic acid was regenerated from acetone-oxytocinoic acid under the same conditions used for the regeneration of oxytocin from acetone-oxytocin.[11]

Deamino-oxytocinoic acid and acetone-oxytocinoic acid were each coupled with oxytocin, with the use of N-ethyl-5-phenylisoxazolium-3′-sulfonate (Woodward's Reagent K),[26] to give deamino-oxytocinoyloxytocin and acetone-oxytocinoyloxytocin, respectively. Oxytocinoyloxytocin was generated from acetone-oxytocinoyloxytocin by a previously described procedure for the removal of the acetone blocking group from acetone-oxytocin.[11] Purification of deamino-oxytocinoyloxytocin and oxytocinoyloxytocin was effected by gel filtration on Sephadex G-25.

Deamino-oxytocinoyloxytocin possessed approximately 2.45 units/mg of oxytocic activity and less than 0.28 units/mg of avian vasodepressor activity. Oxytocinoyloxytocin exhibited approximately 1.0 units/mg of oxytocic activity and less than 0.44 units/mg of avian vasodepressor activity. It should be noted that the ratios of oxytocic to avian vasodepressor potency for the two compounds are quite different from that for oxytocin, which is approximately 1:1.

Experimental Section[27]

Boc-glycyl Resin. A solution of 1.67 g (9.4 mmol) of Boc-glycine and 1.18 ml (9.4 mmol) of triethylamine in 30 ml of absolute ethanol was added to 10 g of chloromethylcopolystyrene–2% divinylbenzene (Bio-Beads S.X-2, 200–400 mesh, capacity 1.5 mequiv/g). The reaction mixture was stirred at 75° for 24 hr. The esterified resin was filtered off and washed with a solvent system that was changed gradually from absolute ethanol to water and then progressively to absolute methanol. The esterified resin was then dried *in vacuo* over KOH pellets; yield 10.28 g. Amino acid analysis of an acid hydrolysate (dioxane–12 *N* HCl, 1:1) showed the product to contain 0.25 mmol of glycine/g of esterified resin.

S-Benzyl-β-mercaptopropionyl-O-benzyltyrosylisoleucylglutaminylasparaginyl-S-benzylcysteinylprolylleucylglycyl Resin. Boc-glycyl resin (5 g) was placed in the reaction vessel. The procedure described in an earlier communication[18] for the introduction of each new amino acid residue was followed.

For the washing steps 25-ml portions of the appropriate solvent were used. In the deprotection step 25 ml of 1 *N* HCl in glacial acetic acid was used and the hydrochloride was neutralized with 2 ml of triethylamine in 25 ml of dimethylformamide. In the coupling steps 2 mmol of the appropriate Boc-amino acid in 20 ml of methylene chloride and 2.1 mmol of N,N′-dicyclohexylcarbodiimide in 5 ml of methylene chloride were used. The coupling reactions involving Boc-asparagine and Boc-glutamine were carried out *via* their *p*-nitrophenyl esters (2 mmol in 25 ml of distilled dimethylformamide) with a reaction time of 20 hr.

At the stage of incorporation of Boc-isoleucine into the peptide chain the following modifications were involved: (1) cleavage of the Boc group of the glutamine residue[18] by treatment with 25 ml of trifluoroacetic acid for 15 min at 25°; (2) neutralization of the trifluoroacetate with 1 ml of triethylamine in 24 ml of dimethylformamide for 5 min.

Following the incorporation of S-benzyl-β-mercaptopropionic acid, the protected polypeptide resin compound was further washed with 25-ml portions of glacial acetic acid (three times), absolute ethanol (three times), and methylene chloride (three times). The product was dried *in vacuo* over KOH pellets.

S-Benzyl-β-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-S-benzylcysteinylprolylleucylglycine. Trifluoroacetic acid (25 ml) was added to the preceding protected polypeptide–resin in the Merrifield reaction flask. Hydrogen bromide gas, which had been passed through a calcium chloride column and a naphthalene column, was bubbled through the suspension of the peptide–resin in trifluoroacetic acid for 90 min. The resin was filtered off and washed two times with 20-ml portions of trifluoroacetic acid.

The combined trifluoroacetic acid filtrate and washings was evaporated on a rotary evaporator. Trituration of the yellow viscous residue with 100 ml of absolute ethanol yielded a white solid, which was filtered off, washed three times with absolute ethanol, and dried *in vacuo* over KOH pellets: yield 574 mg; mp 220–224°. This product was dissolved in 20 ml of dimethylformamide, and water was slowly added until the solution was slightly turbid. The solution was then allowed to stand overnight in the refrigerator. The precipitate was filtered off, washed with water, and dried *in vacuo* over KOH pellets: yield 400 mg; mp 222–224°.

An analytical sample was prepared by dissolving 93 mg in dimethylformamide, filtering the resulting solution through a sintered-glass funnel, and adding water until the solution became turbid.

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This turbid solution was allowed to stand in the refrigerator overnight. The white precipitate was filtered off, washed three times with water, and dried *in vacuo* over KOH pellets; yield 84 mg; mp 223–225°; $[\alpha]^{22}D$ −53° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{57}H_{78}N_{10}O_{13}S_2$: C, 58.2; H, 6.69; N, 11.9. Found: C, 58.1; H, 6.68; N, 12.0.

Deamino-oxytocinoic Acid. S-Benzyl-β-mercaptopropionyltyrosylisoleucylglutaminylasparaginyl-S-benzylcysteinylprolylleucylglycine (280 mg) was dissolved in 250 ml of stirred boiling liquid ammonia (distilled from sodium in an all-glass apparatus). A fresh sodium stick was momentarily introduced intermittently until the blue color persisted for 20 sec.[28] The ammonia was evaporated at the water pump and the last 30 ml was removed by lyophilization. The residue was dissolved in 700 ml of 0.05% trifluoroacetic acid and the pH was adjusted to 8.0 with dilute ammonium hydroxide. An excess of 0.1 *N* potassium ferricyanide (8 ml) was added to the stirred solution.[15] After 15 min AG 3-X4 resin (trifluoroacetate form) (Bio-Rad Laboratories) was added, and stirring was continued for 15 min to remove ferrocyanide and excess ferricyanide ions. The resin was removed by filtration and the solution was lyophilized. The residue was dissolved in 15 ml of the upper phase of the solvent system 1-butanol–benzene–3.5% aqueous acetic acid (containing 1.5% pyridine) (2:1:3) and applied to a Sephadex G-25 (100–200 mesh) column (2.85 × 58 cm) that had been equilibrated with the lower and upper phases. The column was eluted with the upper phase and 70 fractions of 10 ml each were collected. The chromatogram obtained by plotting the Folin–Lowry color values[29] of the fractions showed a small minor peak with R_f 0.76 and a major symmetrical peak with a maximum at fraction 26 (R_f 0.32). The fractions corresponding to this major peak were pooled, twice the volume of water was added, and the resulting mixture was concentrated to about 30 ml under reduced pressure and lyophilized.

The lyophilized powder (146 mg) was dissolved in 7 ml of 0.2 *N* acetic acid and subjected to gel filtration on a Sephadex G-25 (200–270 mesh) column (2.82 × 68 cm) that had been equilibrated with 0.2 *N* acetic acid. The column was eluted with 0.2 *N* acetic acid and 100 fractions of 6.5 ml each were collected. A plot of the Folin–Lowry color values of the various fractions showed a single symmetrical peak with a maximum at fraction 53. The fractions corresponding to this peak were pooled and lyophilized to give a white powder.

The lyophilized powder (128 mg) was dissolved in 3.3 ml of water at a water-bath temperature of 80–90°. The solution was filtered through a sintered-glass funnel. An additional 0.8 ml of water was passed through the funnel. The filtrate was allowed to stand in the refrigerator for 7 days. The crystals of deamino-oxytocinoic acid were filtered off, washed with cold water, and dried *in vacuo;* yield 83 mg; mp 179–180°, uncorrected (Fisher-Johns melting point apparatus); $[\alpha]^{23}D$ −113.3° (*c* 0.58, 1 *N* acetic acid). Thin layer chromatography on silica gel G (E. Merck AG-Darmstadt) in the solvent system 1-butanol–pyridine–acetic acid–water (15:10:3:12) gave a single spot (R_f 0.67).

Anal. Calcd for $C_{43}H_{64}N_{10}O_{13}S_2$: C, 52.0; H, 6.50; N, 14.1. Found: C, 51.7; H, 6.57; N, 14.3.

A sample was hydrolyzed in 6 *N* HCl at 110° for 24 hr and analyzed[30] in the Beckman-Spinco amino acid analyzer. The following molar ratios were obtained: aspartic acid, 1.0; glutamic acid, 1.1; proline, 1.0; glycine, 1.0; half-cystine, 0.44; mixed disulfide[15] of β-mercaptopropionic acid and cysteine, 0.54; isoleucine, 0.9; leucine, 1.1; tyrosine, 1.0; and ammonia, 2.1.

S-Benzylcysteinyltyrosylisoleucylglutaminylasparaginyl-S-benzylcysteinylprolylleucylglycine Hydrobromide. Boc-glycyl resin (5 g) was placed in the reaction vessel. The procedure and quantities of reagents used were identical with those used for the synthesis of S-benzyl-β-mercaptopropionyl-O-benzyltyrosylisoleucylglutaminylasparaginyl-S-benzylcysteinylprolylleucylglycyl resin, except that Boc-S-benzylcysteine was used instead of S-benzyl-β-mercaptopropionic acid in the final cycle. The protected nonapeptide–resin compound was treated with HBr in trifluoroacetic acid. The combined trifluoroacetic acid filtrate and washings were evaporated to dryness on a rotary evaporator. The residue was triturated with about 200 ml of absolute ethanol. The insoluble material was filtered off and dried *in vacuo* over KOH pellets: yield 617 mg; mp 212–215°. This solid (617 mg) was ground to a powder in 15 ml of absolute ethanol, filtered, washed with absolute ethanol, and dried *in vacuo* over KOH pellets: yield 525 mg; mp 214–216°; $[\alpha]^{22}D$ −35° (*c* 1, dimethylformamide) (lit.[25] mp 200–210°; $[\alpha]D$ −38° (temperature not reported) (*c* 1.1, dimethylformamide)).

Oxytocinoic Acid. The preceding protected nonapeptide hydrobromide (350 mg) was converted to oxytocinoic acid by the procedures used for the preparation of deamino-oxytocinoic acid. After removal of the AG 3-X4 resin and lyophilization of the resulting solution, the residue was dissolved in 10 ml of the upper phase of the solvent system 1-butanol–3.5% aqueous acetic acid (containing 1.5% pyridine) (1:1) and placed on a Sephadex G-25 (100–200 mesh) column (2.81 × 65 cm) that had been equilibrated with the lower and upper phases. The column was eluted with upper phase and 85 fractions of 9.6 ml each were collected. A plot of the Folin–Lowry color values of the fractions showed a major symmetrical peak with a maximum at fraction 44 (R_f 0.24). The fractions corresponding to this major peak were pooled, twice the volume of water was added, and the resulting mixture was concentrated to about 25 ml under reduced pressure and lyophilized.

The lyophilized powder (151 mg) was dissolved in 7 ml of 0.2 *N* acetic acid and placed on a Sephadex G-25 (200–270 mesh) column (2.82 × 68 cm) that had been equilibrated with 0.2 *N* acetic acid. The column was eluted with 0.2 *N* acetic acid and 100 fractions of 6.2 ml each were collected. The chromatogram obtained by plotting the absorbance of the fractions at 275 mμ showed a single symmetrical peak with a maximum at fraction 52. The fractions corresponding to this peak were pooled and lyophilized to give a white powder: yield 117 mg; $[\alpha]^{23}D$ −30° (*c* 0.5, 1 *N* acetic acid) (lit.[23] $[\alpha]^{19}D$ −31.7° (*c* 0.6, 1 *N* acetic acid)).

Thin layer chromatography on silica gel G in the solvent system 1-butanol–pyridine–acetic acid–water (15:10:3:12) showed this material (R_f 0.60) to be identical in behavior with a sample of oxytocinoic acid made by classical methods.[23]

A sample was hydrolyzed in 6 *N* HCl at 110° for 24 hr and analyzed in the Beckman-Spinco amino acid analyzer. The following molar ratios were obtained: aspartic acid, 1.0; glutamic acid, 1.1; proline, 0.9; glycine, 1.0; cystine, 1.0; isoleucine, 1.0; leucine, 1.0; tyrosine, 0.9; and ammonia, 1.8.

Acetone-oxytocinoic Acid. Oxytocinoic acid (39 mg) was dissolved in 10 ml of 80% aqueous acetone. The pH of the solution (as measured by a pH meter) was adjusted from 5.8 to 7.0 by the addition of pyridine, and the solution was stirred at room temperature for 48 hr. Water (50 ml) was added, and the acetone was removed under reduced pressure. The resulting aqueous solution was lyophilized, and the residue was dissolved in 3.5 ml of cold (3°) upper phase of the solvent system 1-butanol–3.5% aqueous acetic acid (containing 1.5% pyridine) (1:1) and placed on a Sephadex G-25 (100–200 mesh) column (2.20 × 52 cm) which had been equilibrated at 3° with the upper and lower phases. Elution of the column with the upper phase was carried out at 3°, and 70 fractions of 6.2 ml each were collected. The chromatogram obtained by plotting of the Folin–Lowry color values of the fractions gave a single symmetrical peak with R_f 0.58. The fractions corresponding to this peak were pooled, twice the volume of water was added, and the resulting mixture was concentrated to about 20 ml under reduced pressure and lyophilized: yield 39 mg; $[\alpha]^{25}D$ +10° (*c* 0.5, 80% aqueous acetone).

Anal. Calcd for $C_{46}H_{69}N_{11}O_{13}S_2$: C, 52.7; H, 6.63; N, 14.7. Found: C, 52.8; H, 6.63; N, 14.8.

Amino acid analysis of an acid hydrolysate (6 *N* HCl at 110° for 24 hr) of the compound gave the following molar ratios: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; cystine, 1.0; isoleucine, 1.0; leucine,1.0; tyrosine, 0.9; and ammonia, 1.9.

For quantitative acetone determination, a sample (1.4 mg) was boiled in 5 ml of 0.1 *N* acetic acid with simultaneous distillation. The distillate (~3 ml) was collected at 0° in a 5-ml volumetric flask containing 2 ml of water. Appropriate aliquots of the distillate were analyzed by the salicylaldehyde method.[31] The sample liberated 94% of the theoretical amount of acetone.

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Regeneration of Oxytocinoic Acid from Acetone-oxytocinoic Acid. A sample (4.41 mg) of the lyophilized powder from the preceding section was dissolved in 4.5 ml of 0.25% acetic acid and heated at 90° for 30 min.[32] The solution was then lyophilized and the resulting material was compared with oxytocinoic acid and acetone-oxytocinoic acid by thin layer chromatography on MN-Polygram Silica Gel S-HR/UV_{254} (Macherey-Nagel and Co., Düren) in the solvent system 1-butanol–pyridine–acetic acid–water (15:10:3:12). Its behavior was found to be identical with that of oxytocinoic acid (R_f 0.70). Acetone-oxytocinoic acid had an R_f of 0.74. The regenerated oxytocinoic acid possessed the same level of oxytocic activity as the original oxytocinoic acid.

Deamino-oxytocinoyloxytocin. N-Ethyl-5-phenylisoxazolium-3′-sulfonate (Woodward's Reagent K) (10.06 mg) was added to a stirred solution of deamino-oxytocinoic acid (39.18 mg) and triethylamine (4.9 mg) in distilled dimethylformamide (0.66 ml). The solution was stirred at room temperature for 1 hr until most of the Woodward's Reagent K had dissolved. A solution of oxytocin (39.74 mg) and triethylamine (4.0 mg) in distilled dimethylformamide (0.46 ml) was added and the reaction mixture was stirred at room temperature for 48 hr. Water (30 ml) was added to the reaction mixture and this aqueous solution was lyophilized.

The residue was dissolved in 3 ml of 25% aqueous acetic acid and subjected to gel filtration on a Sephadex G-25 (200–270 mesh) column (2.82 × 68 cm) that had been equilibrated with 1 *N* acetic acid. The column was eluted with 1 *N* acetic acid and 100 fractions of 5.5 ml each were collected. A plot of the absorbance of the various fractions at 275 mμ showed two symmetrical peaks of peptide material with maxima at fraction 52 and fraction 59. The peak with maximum at fraction 59 was shown to be a mixture of deamino-oxytocinoic acid and oxytocin by thin layer chromatography.

The fractions corresponding to the peak with maximum at fraction 52 were pooled and lyophilized. The residue (30.5 mg) was dissolved in 1.0 ml of 25% aqueous acetic acid and again subjected to gel filtration on a Sephadex G-25 (200–270 mesh) column (1.24 × 165 cm) that had been equilibrated with 1 *N* acetic acid. The column was eluted with 1 *N* acetic acid and 100 fractions of 2.65 ml each were collected. A plot of the absorbance of the various fractions at 275 mμ showed a single peak with a maximum at fraction 54. The fractions corresponding to this peak were pooled and lyophilized to give a white powder: yield 27.4 mg; $[\alpha]^{24.5}D$ −68° (*c* 0.5, 25% aqueous acetic acid).

Thin layer chromatography of the product on MN Silica Gel S-HR/UV_{254} was carried out in two solvent systems. The solvent system 1-propanol–water (7:3) gave only one spot (R_f 0.69) and the solvent system 1-butanol–acetic acid–water (4:1:1) also gave only one spot (R_f 0.17).

(32) This is the procedure used to regenerate oxytocin from acetone-oxytocin; see ref 11.

Anal. Calcd for $C_{86}H_{128}N_{22}O_{24}S_4$: C, 52.1; H, 6.51; N, 15.6. Found: C, 52.0; H, 6.78; N, 15.3.

A sample was hydrolyzed for 24 hr in 6 *N* HCl at 110° and analyzed on a Beckman-Spinco amino acid analyzer. The following molar ratios were obtained: aspartic acid, 1.0; glutamic acid, 1.1; proline, 1.1; glycine, 1.0; half-cystine, 1.0; mixed disulfide[15] of β-mercaptopropionic acid and cysteine, 0.4; isoleucine, 1.0; leucine, 1.0; tyrosine, 0.9; and ammonia 2.6.

Oxytocinoyloxytocin. Acetone-oxytocinoic acid (39.88 mg) was condensed with oxytocin in the manner described for the synthesis of deamino-oxytocinoyloxytocin. The moles of reagents, volume of solvents, reaction times and temperature, and the procedure used for gel filtration of the condensation product were identical with those used for the preparation of deamino-oxytocinoyloxytocin.

A total of 100 fractions of 5.5 ml each were collected during the gel filtration of the condensation product. The chromatogram obtained by plotting the absorbance of the various fractions at 275 mμ showed two peptide-containing peaks with maxima at fraction 50 and fraction 59. The peak with maximum at fraction 59 represented a mixture of acetone-oxytocinoic acid, oxytocinoic acid, and oxytocin.

The fractions corresponding to the peak with maximum at fraction 50 were pooled and lyophilized. The lyophilized residue (20.15 mg) was dissolved in 1.0 ml of 25% aqueous acetic acid and again subjected to gel filtration on a Sephadex G-25 (200–270 mesh) column (1.24 × 165 cm). One hundred fractions of 2.65 ml each were collected. A plot of the absorbance of the various fractions at 275 mμ showed a single peak with a maximum at fraction 52. The fractions corresponding to this peak were pooled and lyophilized. The residue (16.22 mg) was dissolved in 160 ml of 0.25% acetic acid and heated at 90° for 30 min in order to remove the acetone blocking group.[11] The solution was then lyophilized to give a white powder: yield 15 mg; $[\alpha]^{24}D$ −44.2° (*c* 0.5, 25% aqueous acetic acid). A quantitative acetone determination showed that no detectable amount of acetone was present in the compound.[31]

Thin layer chromatograms on MN Silica Gel S-HR/UV_{254} developed in the solvent systems 1-propanol–water (7:3) and 1-butanol–acetic acid–water (4:1:1) each showed only one spot with R_f 0.61 and R_f 0.17, respectively.

Anal. Calcd for $C_{86}H_{129}N_{23}O_{24}S_4 \cdot C_2H_4O_2$: C, 51.4; H, 6.52; N, 15.7. Found: C, 51.1; H, 6.38; N, 15.7.

A sample was hydrolyzed for 24 hr in 6 *N* HCl at 110° and was analyzed on a Beckman-Spinco amino acid analyzer. The following molar ratios were obtained: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; cystine, 0.9; isoleucine, 1.0; leucine, 1.0; tyrosine 0.9; and ammonia, 2.6.

Acknowledgments. The authors are indebted to Miss Paula Glose for the bioassays performed under the direction of Dr. L. Nangeroni, New York State Veterinary College, Cornell University.