Anal. Calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.76; H, 5.80; N, 11.47.

2-(L-*arabino*-1′,2′,3′-Triacetoxybutyl)quinoxaline (Enantiomer of 6c). A suspension of 18.0 g (0.1 mol) of rhamnose hydrate, 11.0 g (0.1 mol) of *o*-phenylenediamine, 10 ml (0.21 mol) of anhydrous hydrazine, and 6.0 ml (0.1 mol) of acetic acid in 200 ml of pyridine was stirred at 100° for 4 hr. The clear red solution was concentrated *in vacuo*. The residue was extracted with 200 ml of propanol. The alcoholic extract was decolorized with charcoal and concentrated. Treatment of the oily, amber residue with 120 ml of pyridine–acetic acid, 1:1, gave 5.37 g of crystalline N,N′-diacetyl-*o*-phenylenediamine, which was removed by filtration. Concentration of the filtrate and trituration of the residue with 150 ml of benzene gave an additional 4.63 g (61% total recovery) of N,N′-diacetyl-*o*-phenylenediamine. The benzene filtrate was extracted three times with 25-ml portions of 6 *N* sulfuric acid, extracted with water, and decolorized with charcoal. Removal of the benzene gave 24.3 g of red-brown oil.

A portion of the oil (8.03 g) was further purified by elution from a 250-g column of alumina with carbon tetrachloride–chloroform solvent. One liter of solvent eluted 2.48 g of liquid 2-(L-*arabino*-1′,2′,3′-triacetoxybutyl)quinoxaline which was freed of solvent by heating in a rotary evaporator under vacuum until constant pressure readings were obtained; uv spectrum λ_{max}^{MeOH} 236 mμ (ε 31,500), 309.5 mμ (shoulder, ε 5600), and 317.5 mμ (ε 6500).

Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.91; H, 5.74; N, 7.65.

Acknowledgment. This investigation was supported by U. S. Public Health Service Research Grant GM-11966 from the National Institute of General Medical Sciences. The Cary 60 spectropolarimeter was purchased with funds from the Public Health Service grant and from National Science Foundation Grant GP-2125. We thank Mr. Robert Butenko for assistance in preparing some of the compounds.

The Synthesis of Deamino-oxytocin by the Solid Phase Method[1]

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Abstract: Deamino-oxytocin, a highly potent analog of oxytocin, has been synthesized by the solid phase method. The fully protected polypeptide-nitrated resin compound, S-benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl nitrated resin, was prepared and cleavage of the peptide chain from the resin was effected by ammonolysis. Debenzylation of the protected polypeptide followed by oxidative cyclization of the resulting deamino-oxytoceine yielded deamino-oxytocin. The purified deamino-oxytocin crystallized readily from water. This crystalline analog possessed full biological activity.

The solid phase method[3–5] has facilitated the synthesis of peptides since it offers speed and simplicity as well as good yields. We therefore desired to apply this solid phase technique to the synthesis of analogs of the posterior pituitary hormones oxytocin and vasopressin. To this end the synthesis of deamino-oxytocin,[6–9] a highly potent crystalline analog of oxytocin, was undertaken.

The synthesis of deamino-oxytocin by the resin method essentially followed the procedure outlined previously for the synthesis of angiotensins by this technique,[5] except for the fact that the polymer support was used in the nitrated form[3] in order to facilitate the subsequent cleavage of the peptide by ammonolysis.[10] *t*-Butyloxycarbonyl (Boc) amino acids were used and N,N′-dicyclohexylcarbodiimide[11] was the coupling reagent except for the coupling of the asparagine and glutamine residues. The latter were incorporated into the growing peptide chain by means of their nitrophenyl esters[12] since nitrile formation has been observed in coupling reactions involving these amino acids when N,N′-dicyclohexylcarbodiimide was used as the coupling reagent.[13] However, once the glutamine and asparagine residues have been incorporated into the peptide chain, carbodiimide may be used for subsequent couplings without danger of nitrile formation.[14,15]

Boc-glycine was esterified to nitrated chloromethylcopolystyrene–2% divinylbenzene, and the stepwise synthesis was carried through eight cycles[5] to give the fully protected polypeptide-nitrated resin compound, S-benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl nitrated resin.

In preliminary runs incomplete coupling was encountered in extending the chain beyond the glutamine

(1) This work was supported in part by Grant HE-01675 to Cornell University Medical College and by Grant HE-11680 to Cornell University from the National Heart Institute, U. S. Public Health Service.

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residue. This problem was circumvented by deblocking with trifluoroacetic acid[16,17] (instead of 1 *N* HCl in glacial acetic acid) and by increasing the coupling time. The stepwise synthesis of the protected polypeptide-nitrated resin compound was then effectively carried out.

Cleavage of the protected peptide chain from the nitrated resin was effected by ammonolysis of the benzyl ester linkage by which the peptide is attached to the polymer support[10] to give the protected polypeptide, S-benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. This peptide intermediate differs from that used in the synthesis of deamino-oxytocin by the stepwise nitrophenyl ester method[6,7,9] in that the O-benzyl protecting group of the tyrosine residue is still intact and has not been cleaved during the deprotection step.

Debenzylation of the protected polypeptide was performed with sodium and liquid ammonia by the method of Sifferd and du Vigneaud[18] as used in the synthesis of oxytocin,[19] and oxidative cyclization of the resulting deamino-oxytoceine with potassium ferricyanide yielded deamino-oxytocin.[7] The deamino-oxytocin was purified by partition chromatography on Sephadex G-25[20] in the solvent system 1-butanol–benzene–3.5% aqueous acetic acid (containing 1.5% pyridine) (1:1:2).[9] The R_f of the deamino-oxytocin was identical with that of deamino-oxytocin prepared by the stepwise nitrophenyl ester method.[9] The lyophilized powder from partition chromatography was subjected to gel filtration on Sephadex G-25 (200–270 mesh)[21] equilibrated with 0.2 *N* acetic acid. This purified deamino-oxytocin prepared by the solid phase method crystallized readily from water.[9] The crystalline analog possessed within the limits of experimental error the avian vasodepressor activity of 975 units/mg reported by Ferrier, Jarvis, and du Vigneaud.[9]

The solid phase method has thus been successfully applied to the synthesis of deamino-oxytocin, a highly potent analog of the posterior pituitary hormone oxytocin. The deamino-oxytocin prepared by this method was obtained in good over-all yields and possessed full biological activity. It should be mentioned that Beyerman[22] has carried out the solid phase synthesis of the partially protected nonapeptide leading to 9-deamido-oxytocin[23] using the active ester coupling method with 1,2,4-triazole catalysis, and Manning[24] has utilized the solid phase technique for the synthesis of oxytocin.

Experimental Section

Nitrated Chloromethylcopolystyrene–2% Divinylbenzene.[3] Dry chloromethylated copolystyrene–2% divinylbenzene (40 g) (Bio-Beads S·X-2, 200–400 mesh, capacity 1.5 mequiv/g) was added slowly with stirring to 400 ml of fuming nitric acid that had been cooled to 0°. The mixture was stirred for 1 hr at 0° and then poured onto crushed ice. The light tan nitrated resin was filtered and washed with solutions that changed gradually from water to pure dioxane and then progressively to 100% methanol. The nitrated resin was dried *in vacuo* over KOH pellets; yield, 52.9 g.

Boc-glycyl Nitrated Resin. A solution of 0.83 g (4.7 mmol) of Boc-glycine and 0.59 ml (4.7 mmol) of triethylamine in 20 ml of ethyl acetate was added to 5.04 g of nitrated chloromethylcopolystyrene–2% divinylbenzene. The reaction mixture was stirred at reflux temperature for 24 hr. The esterified resin was filtered off and washed with ethyl acetate (three times), methanol (three times), water (three times), and again with methanol (three times). The esterified resin was then dried *in vacuo* over KOH pellets; yield, 5.24 g. Amino acid analysis of an acid hydrolysate (dioxane–12 *N* HCl, 1:1) showed the product to contain 0.22 mmol of glycine/g of esterified resin.

Boc-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl Nitrated Resin. The Boc-glycyl nitrated resin (5 g) was placed in the reaction vessel. The following cycle of deprotection, neutralization, and coupling was carried out for the introduction of each new residue: (1) three washings with 25-ml portions of glacial acetic acid; (2) cleavage of the Boc group by treatment with 1 *N* HCl in glacial acetic acid (25 ml) for 30 min at 25°; (3) three washings with 25-ml portions of glacial acetic acid; (4) three washings with 25-ml portions of absolute ethanol; (5) three washings with 25-ml portions of dimethylformamide; (6) neutralization of the hydrochloride with 3 ml of triethylamine in 25 ml of dimethylformamide for 10 min; (7) three washings with 25-ml portions of dimethylformamide; (8) three washings with 25-ml portions of methylene chloride; (9) addition of 3.3 mmoles of the appropriate Boc-amino acid in 20 ml of methylene chloride and mixing for 10 min; (10) addition of 3.3 mmol of N,N′-dicyclohexylcarbodiimide in 5 ml of methylene chloride, followed by a reaction period of 3 hr at 25°; (11) three washings with 25-ml portions of methylene chloride; (12) three washings with 25-ml portions of absolute ethanol.

The coupling reactions involving Boc-L-glutamine and Boc-L-asparagine were carried out *via* their nitrophenyl esters. After steps 1–7 of the cycle were carried out, a solution of 3.3 mmol of the *p*-nitrophenyl ester of the appropriate Boc-amino acid in 15 ml of freshly distilled dimethylformamide was added, and the reaction was allowed to proceed overnight. The polypeptide-nitrated resin was washed three times with 25-ml portions of dimethylformamide followed by three washings with 25-ml portions of absolute ethanol and drying *in vacuo* over KOH pellets; yield, 5.89 g (0.2 mmol of peptide/g).

Boc-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl Nitrated Resin. The Boc-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl nitrated resin (3.5 g, equivalent to 0.7 mmol) was placed in the reaction flask. Steps 1–12 of the reaction cycle were carried out with the following modifications and with the use of 15-ml portions of solvents in place of the 25-ml portions used in the prior couplings: step 2, cleavage of the Boc group of the glutamine residue by treatment with 15 ml of trifluoroacetic acid for 15 min at 25°; step 6, neutralization of the trifluoroacetate with 0.6 ml of triethylamine in 15 ml of dimethylformamide for 5 min; step 9, addition of 0.45 g (1.98 mmol) of Boc-L-isoleucine in 12 ml of methylene chloride; step 10, addition of 0.42 g (1.98 mmol) of N,N′-dicyclohexylcarbodiimide in 3 ml of methylene chloride, followed by a reaction period of 16 hr.

S-Benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl Nitrated Resin. The O-benzyl-L-tyrosine and S-benzyl-β-mercaptopropionic acid residues were incorporated onto the Boc-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl nitrated resin utilizing steps 1–12 of the reaction cycle previously described and using a 15-ml portion of the appropriate solvent for each washing. Solutions of 1.98 mmol of the protected amino acid in 12 ml of methylene chloride and of 1.98 mmol of N,N′-cyclohexylcarbodiimide in 3 ml of methylene chloride were used in steps 9 and 10, respectively. The coupling reactions were allowed to proceed overnight.

After step 12 following the incorporation of the S-benzyl-β-mercaptopropionic acid, the protected polypeptide-nitrated resin compound was further washed with 15-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 overnight.

S-Benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucyl-

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glycinamide. Dry ammonia was bubbled into a stirred suspension of 3.54 g of the S-benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycyl nitrated resin in 150 ml of anhydrous methanol at 0° for 1.5–2 hr (until the solution was saturated with ammonia). The reaction mixture was stirred overnight at 0–4°.[25] The methanol and ammonia were removed under aspirator vacuum. Dimethylformamide (200 ml) was added to the dry residue and the suspension was stirred vigorously for 3 hr. The resin was filtered off and washed three times with dimethylformamide.

The solvent was removed from the combined filtrate and washings on a rotary evaporator at below 40°. The residue was dissolved in 20 ml of dimethylformamide and 15 ml of distilled water was added gradually with stirring. The turbid solution was allowed to stand in the refrigerator overnight. The precipitate was filtered off, washed with ethanol, and dried *in vacuo* over KOH pellets; yield, 197 mg, mp 234–236°.

A sample was prepared for analysis by reprecipitating twice from a dimethylformamide–water mixture. The compound was filtered off, washed with ethanol, and dried *in vacuo*, over KOH pellets; mp 238–240°, $[\alpha]^{21}D$ −37.3° (*c* 0.6, dimethylformamide).

Anal. Calcd for $C_{64}H_{85}O_{12}N_{11}S_2$: C, 60.8; H, 6.78; N, 12.2. Found: C, 60.9; H, 6.78; N, 11.9.

Deamino-oxytocin. S-Benzyl-β-mercaptopropionyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (155 mg) was dissolved in 125 ml of stirred boiling liquid ammonia (distilled from sodium in an all-glass apparatus). A fresh sodium stick was introduced and withdrawn when the blue color pervaded the solution. The sodium stick was momentarily introduced intermittently until the blue color persisted for 30 sec. The ammonia was removed by lyophilization at the water pump and the residue was dissolved in 300 ml of 0.03% trifluoroacetic acid. The pH of the solution was adjusted to 8.0 with ammonium hydroxide and an excess of 0.1 *N* potassium ferricyanide (4.5 ml) was added to the stirred solution. After 15 min AG 3-X4 resin (chloride form) 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 lyophilized residue was dissolved in 20 ml of the upper phase of the solvent system 1-butanol–benzene–3.5% aqueous acetic acid (containing 1.5% pyridine) (1:1:2) and applied to a Sephadex G-25 (100–200 mesh) column (2.16 × 110 cm) that had been equilibrated with the lower phase. The column was eluted with the upper phase and 96 fractions of 9.5 ml each were collected. The chromatogram obtained by plotting the Folin–Lowry color values[26] of the fractions showed a single peak with a maximum at fraction 52 (R_f 0.19; lit.[9] 0.19). The fractions corresponding to this peak were pooled, twice the volume of water was added, and the resulting mixture was concentrated under reduced pressure and lyophilized; yield, 49 mg (41%).

The lyophilized powder was dissolved in 6 ml of 0.2 *N* acetic acid and subjected to gel filtration[21] on a Sephadex G-25 (200–270 mesh) column (2.82 × 64 cm) that had been equilibrated with 0.2 *N* acetic acid. The column was eluted with 0.2 *N* acetic acid and 120 fractions of 4.9 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 65. The fractions corresponding to this peak were pooled and lyophilized to give a white powder; yield, 39.4 mg; $[\alpha]^{25}D$ −95.1° (*c* 0.5, 1 *N* acetic acid), lit. $[\alpha]^{20}D$ −88.3° (*c* 0.5, 1 *N* acetic acid)[9] and $[\alpha]^{21}D$ −107° (*c* 0.5, 1 *N* acetic acid).[7]

This lyophilized powder (25 mg) was dissolved in 0.8 ml of water in a water bath at 80–90°. The solution was filtered through a sintered-glass funnel and was allowed to stand at room temperature for 5 hr. Crystals began to form within 1 hr and the bulk of the deamino-oxytocin had crystallized out after 5 hr. The solution was allowed to stand overnight in the refrigerator. The deamino-oxytocin crystals were filtered off, washed with water, and dried *in vacuo*; yield, 14.8 mg; mp 182–183°, uncorrected (Fisher-Johns melting point apparatus; electrically heated aluminum block), lit.[9] mp 179° (modified Kofler block, corrected) and 178–184° (capillary, corrected); $[\alpha]^{21}D$ −103.3° (*c* 0.46, 1 *N* acetic acid), lit.[9] $[\alpha]^{20}D$ −90.4° (*c* 0.5, 1 *N* acetic acid).

Acknowledgments. The authors are indebted to the following: Mr. Joseph Albert for the elemental analyses; Mr. Roger Sebbane for the amino acid analyses; and Miss Margitta Wahrenburg and Mrs. Jessie Lawrence for the bioassays, under the direction of Dr. W. Y. Chan.

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The Structure of Frenolicin[1]

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Received September 14, 1967

Abstract: Evidence is put forward which describes the structure and stereochemistry of frenolicin as I.

The isolation and preliminary characterization of frenolicin, a pale yellow crystalline antibiotic from *Streptomyces fradiae*, were described by Van Meter, Dann, and Bohonos.[2] They suggested the molecular formula to be $C_{13}H_{14}O_5$ and indicated the presence of two acidic functions assigned to a phenolic or enolic grouping and a carboxylic acid. In addition, they observed that frenolicin readily absorbed 2 mol of hydrogen and contained a C-methyl group. We wish to report in detail work which has led to the structure and relative stereochemistry of frenolicin as that formulated by the novel naphthoquinone epoxide I.[3]

Repetition of the elemental analyses gave figures more indicative of the formula $C_{18}H_{18}O_7$, rather than that previously proposed,[2] and the molecular weight (346) required by this formula was confirmed by mass spec-

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