of methanol gave the analytical sample of cyclonucleoside **15** with mp 238–239° dec; $\lambda_{max}^{pH\ 1.7}$ 271 mμ (ε 14,700); $\lambda_{max}^{pH\ 13}$ 273 mμ (ε 8500).

Anal. Calcd for $C_{17}H_{19}N_5O_5S$: C, 50.4; H, 4.70; N, 17.3. Found: C, 50.9; H, 4.80; N, 17.2.

Clark, Todd, and Zussman[15] report $\lambda_{max}^{0.05\ N\ HCl}$ 272 mμ (ε 16,310) for the analogous cyclonucleoside of adenosine.

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Isomeric Dimers of Oxytocin[1]

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Abstract: Two isomeric dimers of oxytocin have been obtained as by-products in the oxidation of oxytoceine to oxytocin, the last step in the synthesis of this posterior pituitary hormone. The dimeric material was isolated by partition chromatography on Sephadex and was separated by gel filtration into two components designated as α dimer and β dimer. Determination of molecular weights gave a value of 2006 for the α dimer and 1995 for the β dimer; the calculated value for the molecular weight of a dimer of oxytocin is 2014. Both dimers exhibit low but definite oxytocic activity of the order of 1 unit/mg, compared to approximately 500 units/mg for oxytocin. Both dimeric forms are readily converted to oxytocin with full biological activity by reduction with sodium in liquid ammonia and subsequent oxidation. The reduction liberates sulfhydryl groups in an amount consistent with a dimeric structure containing two disulfide linkages in a 40-membered ring. The α dimer and the β dimer have also been obtained through disulfide interchange by treatment of oxytocin with triethylamine.

In the synthesis of oxytocin,[3] the cyclic disulfide is obtained in the last step by oxidation of the linear disulfhydryl intermediate, oxytoceine,[4] the structure of which is shown in Figure 1. When the products of oxidation of the oxytoceine are subjected to partition chromatography on Sephadex G-25 in the solvent system 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (6:2:1:9) (solvent system A), the hormone emerges with an R_f of about 0.24, and some peptide material which travels slightly faster (R_f 0.32) is also obtained.[5]

In preliminary studies on the nature of this peptide material, it was submitted to countercurrent distribution[6] in 1-butanol–1-propanol–0.5% acetic acid in 0.1% aqueous pyridine (6:1:8). The material represented by the major peak with partition coefficient 0.19 was isolated and subjected to partition chromatography on Sephadex G-25 in 1-butanol–ethanol–pyridine–0.2 *N* aqueous acetic acid (8:1:1:10) (solvent system B). A single sharp symmetrical peak was obtained with an R_f of about 0.55. The isolated material possessed a molecular weight of about 2030; the calculated value for oxytocin is 1007. An acid hydrolysate of the material had an amino acid and ammonia composition identical with that of an hydrolysate of the hormone.

Although the dimeric material had behaved like a single compound in the partition chromatography employed, further efforts were made to establish whether or not it was truly homogeneous. It was finally found that gel filtration of the material on Sephadex G-25 in 0.2 *N* acetic acid resulted in the appearance of two very closely spaced peaks, provided that a long column (1.24 × 159 cm) of suitable efficiency was employed. We have designated the substance corresponding to the faster traveling peak as the α dimer and that corresponding to the slower traveling peak as the β dimer.

In order to obtain sufficient amounts of these two dimers for more complete characterization, studies were undertaken on the preparation and isolation of these compounds on a larger scale. N-Carbobenzoxy-S,S′-dibenzyloxytoceine, prepared by the stepwise *p*-nitrophenyl ester method,[7] served as the starting material. This protected nonapeptide derivative was treated with sodium in liquid ammonia for removal of the N-carbobenzoxy and S-benzyl protecting groups according to the method of Sifferd and du Vigneaud[8] as used in the synthesis of oxytocin.[3] The oxytoceine so obtained was oxidized by aeration in aqueous solution near pH 8 at a peptide concentration of about 0.75 mg/ml. After evaporation and lyophilization of the solution, the products were subjected to partition chromatography on Sephadex G-25 in solvent system A. Plotting of Folin–Lowry color values[9] gave a chromatogram containing a large peak representing oxytocin (R_f 0.24) and

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(3) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, and S. Gordon, *J. Am. Chem. Soc.*, **75**, 4879 (1953); V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, and P. G. Katsoyannis, *ibid.*, **76**, 3115 (1954).

(4) D. Yamashiro, D. Gillessen, and V. du Vigneaud, *Biochemistry*, **5**, 3711 (1966).

(5) D. Yamashiro, *Nature*, 201, 76 (1964).

(6) L. C. Craig, W. Hausmann, E. H. Ahrens, Jr., and E. J. Harfenist, *Anal. Chem.*, **23**, 1236 (1951).

(7) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(8) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(9) O. H. Lowry, N. J. Rosebrough, A. L. Barr, and R. J. Randall, *ibid.*, **193**, 265 (1951).

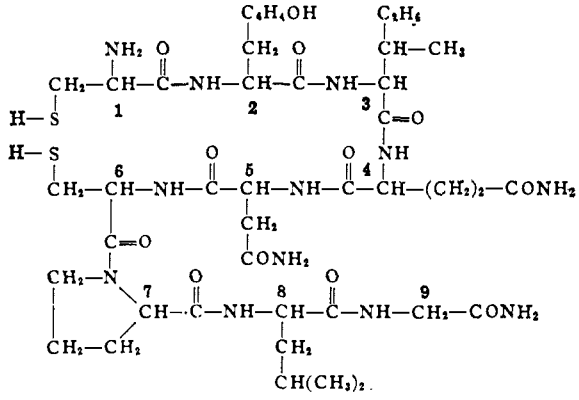

Figure 1. Structure of oxytoceine with numbers indicating the position of the individual amino acid residues. Oxytocin is the corresponding cyclic disulfide containing a cystine residue in place of the two cysteine residues in oxytoceine.

a small peak representing the mixture of dimers (R_f 0.30). The weight ratio of oxytocin to the mixture of dimers was 4:1.

The mixture of dimers was subjected to gel filtration on a short Sephadex G-25 column (2.8 × 62 cm) in 0.2 *N* acetic acid and to partition chromatography on Sephadex G-25 in solvent system B, neither procedure effecting separation of the two forms. The α dimer was then separated from the β dimer by gel filtration on a long Sephadex G-25 column (1.24 × 159 cm). Analysis with the Ellman reagent[10] showed that neither dimer peak contained sulfhydryl materials. Approximately equal amounts of the two dimers were isolated. The dimers were then subjected individually to gel filtration in the same manner. A sample of about 1 mg of either α dimer or β dimer on the 1.24 × 159 cm column gave a single, symmetrical peak, whereas gel filtration of samples of 5 mg or more of either substance on this column resulted in unsymmetrical peaks with maxima corresponding to larger elution volumes. The chromatogram obtained for a mixture containing about 1 mg each of α dimer and β dimer showed two closely spaced but well-separated symmetrical peaks.

Elemental analysis of both the α dimer and the β dimer gave values in agreement with those calculated for a dimer of oxytocin. Upon amino acid analysis, each dimer was found to contain the same constituents in the same molar ratios as oxytocin. The specific rotations of the α dimer and the β dimer were -106 and $-78°$, respectively, as compared to a value of $-23°$ for oxytocin[11] under comparable conditions. Determination of molecular weight by short-column equilibrium centrifugation[12] gave values of 2006 for the α dimer and 1995 for the β dimer as compared to a value of 2014 calculated for a dimer of oxytocin.

On paper chromatography in 1-butanol–acetic acid–water (5:1:5) the two dimers travel at about the same rate but move slower than oxytocin. On paper electrophoresis in sodium acetate buffer of pH 5.5 both dimers migrate toward the cathode at the same rate as oxytocin. In the oxytocic assay on the isolated rat uterus[13] both

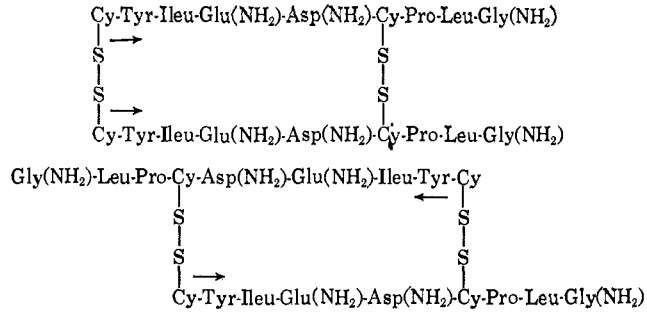

Figure 2. Upper: cyclic parallel dimer of oxytocin. Lower: cyclic antiparallel dimer of oxytocin. The arrows indicate the direction of peptide linkages.

dimers showed very low but definite activity of the order of 1 unit/mg, as compared to about 500 units/mg for oxytocin.[14]

If these dimers represent intermolecular oxidation products between pairs of oxytoceine molecules, then parallel and antiparallel structures are possible, each containing a 40-membered ring with two disulfide bridges as shown in Figure 2. Since previous work has shown that the disulfide bridge in oxytocin is reduced by sodium in liquid ammonia to give oxytoceine,[15] the same treatment of either parallel or antiparallel dimer should also give oxytoceine which could then be converted to oxytocin by oxidation. Therefore, the α dimer and the β dimer were each treated with sodium in liquid ammonia and the sulfhydryl contents of aqueous solutions of the reduced materials were measured by the method of Ellman. The results showed that the α dimer and the β dimer liberated about 86 and 88%, respectively, of the amount of sulfhydryl groups theoretically obtainable from a structure containing two disulfide linkages. Both solutions were aerated near pH 7 until the tests for sulfhydryl groups were negative. The products isolated by lyophilization of these solutions were subjected to partition chromatography on Sephadex G-25 in solvent system A, which is the solvent system used previously for the isolation of highly purified oxytocin by partition chromatography.[5] Both chromatograms contained a major peak corresponding to oxytocin, and the materials isolated from the eluates represented by these peaks each exhibited an activity of about 500 units/mg in the avian vasodepressor assay,[16] in close agreement with the activity reported for highly purified oxytocin.[14] Hence reduction of both α dimer and β dimer liberates oxytoceine, since subsequent oxidation in both cases gave oxytocin possessing the full biological activity of the hormone.

The evidence obtained on reduction and subsequent oxidation, along with all of the other data, is consistent with a 40-membered ring structure containing two disulfide linkages for each dimer. Thus one dimer would represent the parallel structure and the other dimer the antiparallel structure. We hope that further work now under way will establish which structure corresponds to the α dimer and which to the β dimer.[17]

(10) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(11) C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **79**, 4511 (1957).
(12) D. A. Yphantis, *Ann. N. Y. Acad. Sci.*, **88**, 586 (1960).
(13) Oxytocic assays were performed on isolated rat uteri from rats in natural estrus according to the method of P. Holton, *Brit. J. Pharmacol.*, **3**, 328 (1948), as modified by R. A. Munsick, *Endocrinology*, **66**, 451 (1960), with the use of magnesium-free van Dyke–Hastings solution.
(14) W. Y. Chan and V. du Vigneaud, *ibid.*, 71, 977 (1962).
(15) S. Gordon and V. du Vigneaud, *Proc. Soc. Exptl. Biol. Med.*, **84**, 723 (1953).
(16) R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *Endocrinology*, **66**, 860 (1960).

It has been reported that treatment of oxytocin with aqueous sodium bicarbonate at pH 8.3 gives a dimer or other higher molecular weight form of the hormone, presumably by disulfide interchange.[18] In some of our preliminary experiments treatment of oxytocin with triethylamine in aqueous solution had given a product similar in chromatographic behavior to the mixture of dimers already described herein. With the isolation of the α and β dimers and the establishment of some of their properties, a reinvestigation of the triethylamine reaction was undertaken. A 2% aqueous solution of oxytocin containing triethylamine in a molar ratio of about 6:1 of triethylamine to oxytocin was allowed to stand at 25° for 4.5 hr and the products were subjected to gel filtration on the 1.24 × 159 cm Sephadex G-25 column in 0.2 *N* acetic acid. Four peaks corresponding to the positions of α dimer, β dimer, triethylamine, and oxytocin were obtained as shown in Figure 3. Treatment of oxytocin with triethylamine was then carried out on a larger scale and, after subjection of the reaction mixture to partition chromatography on Sephadex in solvent system A, the α dimer and β dimer were separated by gel filtration on the 1.24 × 159 cm Sephadex G-25 column in 0.2 *N* acetic acid. The weight ratio of α dimer to β dimer was about 2:1. α Dimer prepared in this manner was mixed with α dimer isolated as a by-product in the synthesis of oxytocin and subjected to gel filtration. One sharp symmetrical peak was obtained. The same result was obtained with a mixture of β dimers from the two sources. Furthermore, the optical rotations of the α and β dimers obtained by the triethylamine treatment were in agreement with those established for the respective isomers isolated as by-products in the synthesis of oxytocin.

Experimental Section

Methods and Materials. Sephadex G-25 medium block polymerizate (Pharmacia Fine Chemicals, Inc., New Market, N. J.) was used for both partition chromatography and gel filtration. The sieve fraction 100–200 mesh (U. S. Standard) was used for partition columns as described previously.[5,19] The solvent system 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (6:2:1:9) is referred to as solvent system A and 1-butanol–ethanol–pyridine–0.2 *N* aqueous acetic acid (8:1:1:10) as solvent system B. The sieve fraction 200–270 mesh was used in the gel filtration experiments in 0.2 *N* acetic acid. Peptides were detected in aliquots of column fractions by the Folin–Lowry procedure,[9] and the color values so obtained were used to delineate the chromatograms. The method of Ellman was used for the determination of sulfhydryl groups.[10] Products were isolated after partition chromatography by mixture of selected fractions with twice their volume of water, evaporation to a low volume, and lyophilization. After gel filtration the selected fractions were lyophilized.

For molecular weight determination a sample (6.3 mg) was dissolved in 0.6 ml of 0.01 *N* HCl–0.16 *M* KCl, and short-column equilibrium centrifugation was employed.[12]

The Mixture of Isomeric Dimers. N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[7] (N-carbobenzoxy-S,S′-dibenzyloxytoceine) (2 g) was dissolved in about 500 ml of liquid ammonia (distilled from sodium) in an apparatus protected from moisture by a sodium hydroxide trap. The solution was brought to the boiling point and treated with sodium until a blue color persisted throughout the solution for about 3 min. The solution was concentrated *in vacuo* to a volume of about 50 ml and then lyophilized. The products obtained from two such runs were dissolved in deaerated water (4 l.) containing trifluoroacetic acid (1.5 ml). The solution (pH 8) was aerated for 24 hr until the thiol content was negligible. The pH of the solution was then adjusted to about 4 with glacial acetic acid, and the solution was concentrated *in vacuo* to a volume of about 1 l. and lyophilized.

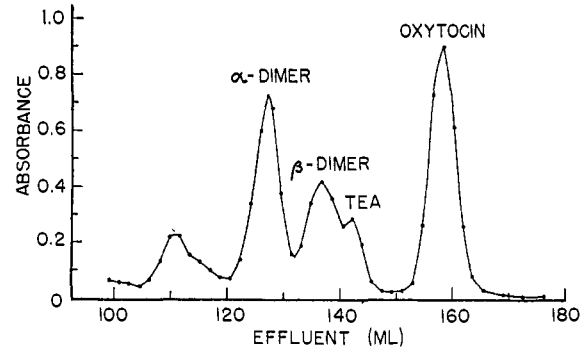

Figure 3. Gel filtration of oxytocin after treatment with triethylamine (TEA) for 4.5 hr. Chromatography was performed on Sephadex G-25 in 0.2 *N* acetic acid; Folin–Lowry color values, ——.

The crude material was dissolved in 20 ml of the organic phase of solvent system A and subjected to partition chromatography on Sephadex G-25 in solvent system A under the following conditions: column size, 4.68 × 114 cm; hold-up volume, 485 ml; fraction volume, 10.8 ml; flow rate, 100 ml/hr; regenerating solvent, pyridine–0.1% aqueous acetic acid (3:5). The chromatogram contained two peaks: a small one with R_f 0.30 (mixture of dimers) and a large one with R_f 0.24 (oxytocin). Isolation of the materials from the eluates corresponding to these two peaks gave 306 mg of the mixture of dimers and 1240 mg of oxytocin.

The mixture of dimers was dissolved in 0.2 *N* acetic acid (5.0 ml) and subjected to gel filtration on a 2.82 × 62.2 cm Sephadex G-25 column in 0.2 *N* acetic acid. A flow rate of 27 ml/hr was maintained while the eluates were collected in fractions of 4.45 ml. The chromatogram contained one major peak with a maximum at effluent volume 307 ml, and isolation of material from the eluates represented by the central portion of this peak gave 191 mg. This material was dissolved in 4 ml of the organic phase of solvent system B and subjected to partition chromatography in solvent system B under the following conditions: column size, 2.83 × 53.4 cm; hold-up volume, 88 ml; fraction volume, 4.9 ml; flow rate, 22 ml/hr; regenerating solvent, pyridine–0.1% aqueous acetic acid (1:4). The chromatogram showed one sharp peak with R_f 0.52, and isolation of the material from the eluates represented by this peak gave 176 mg of the mixture of isomeric dimers.

Separation of α and β Dimers of Oxytocin by Gel Filtration. A sample (51.3 mg) of the mixture of dimers was dissolved in 0.2 *N* acetic acid (1 ml) and subjected to gel filtration on a 1.24 × 159 cm column in 0.2 *N* acetic acid. A flow rate of about 5.7 ml/hr was maintained while the eluate was collected in 0.84-ml fractions after a forerun of 118.6 ml. Two peaks were obtained with maxima corresponding to effluent volumes of 137 ml (α dimer) and 141 ml (β dimer). No sulfhydryl substances were detected in the eluates corresponding to these peaks. Isolation of materials from the eluates corresponding to the two peaks gave 20.8 mg of α dimer and 24.8 mg of β dimer.

A. α Dimer. When the sample of α dimer (20.8 mg) was again subjected to the same gel filtration procedure, one peak was obtained with a maximum corresponding to effluent volume 137 ml. Isolation of α dimer gave 14.2 mg, $[\alpha]^{27}D$ −98° (*c* 0.5, 1 *N* acetic acid) and $[\alpha]^{20}D$ −106° (*c* 0.5, 1 *N* acetic acid). For analysis a sample was dried at 100° over phosphorus pentoxide *in vacuo* with a loss in weight of 6.5%.

Anal. Calcd for $C_{86}H_{132}N_{24}O_{24}S_4$: C, 51.3; H, 6.60; N, 16.7. Found: C, 51.2; H, 6.67; N, 16.4.

The α dimer was hydrolyzed in 6 *N* HCl at 110° for 24 hr and analyzed[20] in a Beckman-Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were found, with the value of leucine taken as 1.0; aspartic acid, 1.0; glutamic acid,

(17) A. V. Schally and J. F. Barrett, *J. Am. Chem. Soc.*, **87**, 2497 (1965), have obtained evidence that the dimer preparation of lysine-vasopressin which they have studied possesses the antiparallel 40-membered ring structure.

(18) C. Ressler, *Science*, **128**, 1281 (1958).

(19) D. Yamashiro, D. Gillessen, and V. du Vigneaud, *J. Am. Chem. Soc.*, **88**, 1310 (1966).

(20) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

1.0; proline, 1.0; glycine, 1.0; cystine, 1.0; leucine, 1.0; isoleucine, 1.0; tyrosine, 0.9; and ammonia, 3.0.

The molecular weight of a sample of α dimer was found to be 2006; the calculated value for a dimer of oxytocin is 2014. Low but definite oxytocic activity on the isolated rat uterus[13] of the order of 1 unit/mg was observed.

Paper chromatography of the α dimer on Whatman No. 1 paper in 1-butanol–acetic acid–water (5:1:5) gave one spot, R_f 0.28, when color was developed with the Pauly reagent. Under the same conditions oxytocin travels with R_f 0.50. On paper electrophoresis in 0.1 *M* sodium acetate buffer of pH 5.5 (18 hr at 4° and 300 V) the α dimer migrated as one spot toward the cathode. Color was developed with the Pauly reagent. Under the same conditions oxytocin migrates the same distance toward the cathode.

A sample (0.96 mg) of α dimer was dissolved in 0.2 *N* acetic acid (0.5 ml) and subjected to gel filtration on the 1.24 × 159 cm column in 0.2 *N* acetic acid at a flow rate of about 5.7 ml/hr. The eluates were collected in 0.90-ml fractions after a forerun of 104.1 ml. The chromatogram showed one sharp symmetrical peak with a maximum at effluent volume 127 ml.

B. β Dimer. When the sample of β dimer (24.8 mg) was again subjected to gel filtration, one small peak with a maximum corresponding to effluent volume 127 ml (α dimer) and a major peak with a maximum corresponding to effluent volume 144.5 ml (β dimer) were obtained. Isolation of β dimer gave 18.6 mg, $[\alpha]^{27}D$ −77° (*c* 0.5, 1 *N* acetic acid) and $[\alpha]^{20}D$ −78° (*c* 0.5, 1 *N* acetic acid). For analysis a sample was dried at 100° over phosphorus pentoxide *in vacuo* with a loss in weight of 7.9%.

Anal. Calcd for $C_{86}H_{132}N_{24}O_{24}S_4$: C, 51.3; H, 6.60; N, 16.7. Found: C, 51.2; H, 6.72; N, 16.6.

Amino acid analysis[20] of an hydrolysate of the β dimer gave the following molar ratios of amino acids and ammonia, with the value of leucine taken as 1.0: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.1; glycine, 1.0; cystine, 1.0; leucine, 1.0; isoleucine, 1.0; tyrosine, 0.9; and ammonia, 2.9. The β dimer was not soluble in 6 *N* HCl at room temperature, whereas the α dimer was readily soluble.

The molecular weight of a sample of β dimer was found to be 1995. A low but definite oxytocic activity of the order of 1 unit/mg was observed.

Paper chromatography of the β dimer gave one spot, R_f 0.23, compared to R_f values of 0.28 for the α dimer and 0.50 for oxytocin. On paper electrophoresis the β dimer migrated as one spot toward the cathode and traveled the same distance as oxytocin and the α dimer.

A small sample (0.89 mg) of β dimer was subjected to gel filtration in the same manner described for the α dimer, and the resulting chromatogram showed one sharp symmetrical peak with a maximum at effluent volume 137 ml. A mixture of α dimer (0.94 mg) and β dimer (0.88 mg) was subjected to gel filtration in the same manner, and the resulting chromatogram showed two well-separated peaks with maxima at effluent volumes 127 and 137 ml.

Reduction of α Dimer with Sodium in Liquid Ammonia Followed by Oxidation and the Isolation of Oxytocin. α Dimer (6.01 mg) was dissolved in about 25 ml of liquid ammonia (distilled from sodium) and treated at the boiling point with sodium until a blue color persisted throughout the solution for 2 min. The solution was evaporated *in vacuo* to low volume and lyophilized. The residue was dissolved in deaerated water (30 ml) containing 0.1 *N* trifluoroacetic acid (0.2 ml). The sulfhydryl content of the solution was 9.7 μmol. The pH of the solution was adjusted to 7 with 0.1 *N* trifluoroacetic acid, and the solution was aerated for 2.5 hr until the thiol content was negligible. The solution was acidified with glacial acetic acid (0.05 ml) and lyophilized.

The product was dissolved in 2 ml of the organic phase of solvent system A and subjected to partition chromatography in solvent system A under the following conditions: column size, 1.05 × 52.0 cm; hold-up volume, 11.3 ml; fraction volume, 0.96 ml; flow rate, 3.6 ml/hr; regenerating solvent, pyridine–0.1% aqueous acetic acid (3:5). The chromatogram showed a major peak with R_f 0.28 corresponding to the position of oxytocin under these conditions. Isolation of the oxytocin gave 2.73 mg which possessed an avian vasodepressor potency of about 500 units/mg.

Reduction of β Dimer with Sodium in Liquid Ammonia Followed by Oxidation and the Isolation of Oxytocin. β Dimer (6.14 mg) was dissolved in 25 ml of liquid ammonia (distilled from sodium) and treated at the boiling point with sodium until a blue color persisted throughout the solution for 1 min. The solution was evaporated *in vacuo* to low volume and lyophilized. The residue was dissolved in deaerated water (30 ml) containing 0.1 *N* trifluoroacetic acid (0.2 ml). The sulfhydryl content of the solution was 10.1 μmol. The pH of the solution was adjusted to 7 with 0.1 *N* trifluoroacetic acid and 0.1 *N* NH_4OH. The solution was aerated for 2.5 hr until the thiol content was negligible. The solution was acidified with glacial acetic acid (0.05 ml) and lyophilized.

The product was dissolved in 2 ml of the organic phase of solvent system A and subjected to partition chromatography in solvent system A under the following conditions: column size, 0.95 × 55.3 cm; hold-up volume, 10.3 ml; fraction volume, 0.91 ml; flow rate, 3.3 ml/hr; regenerating solvent, pyridine–0.1% aqueous acetic acid (3:5). The chromatogram showed a major peak with R_f 0.27 and isolation of the oxytocin gave 3.46 mg which possessed an avian vasodepressor potency of about 500 units/mg.

Reaction of Oxytocin with Triethylamine and Isolation of α and β Dimers. Oxytocin (3.9 mg) was dissolved in water (0.15 ml) and mixed with 0.05 ml of 6% (v/v) aqueous triethylamine. The molar ratio of triethylamine to oxytocin was about 6:1. The solution was allowed to stand at 25° for 4.5 hr and then lyophilized. The product was dissolved in 0.2 *N* acetic acid (0.5 ml) and subjected to gel filtration on the 1.24 × 159 cm Sephadex G-25 column in 0.2 *N* acetic acid. A flow rate of about 5.7 ml/hr was maintained while the eluates were collected in 0.90-ml fractions after a forerun of 98.2 ml. The chromatogram (Figure 3) showed peaks with maxima at effluent volumes 127, 137, 142, and 159 ml which correspond to α dimer, β dimer, triethylamine, and oxytocin, respectively.

For isolation of α and β dimers a larger sample of oxytocin (114.7 mg) was dissolved in water (4.4 ml) and mixed with 1.47 ml of 6% (v/v) aqueous triethylamine. The molar ratio of triethylamine to oxytocin was about 6:1. The solution was allowed to stand at 25° for 6.3 hr and then lyophilized. The product was stirred in 10 ml of the organic phase of solvent system A, and after separation of some insoluble material by centrifugation the solution was subjected to partition chromatography in solvent system A under the following conditions: column size, 2.83 × 53.9 cm; hold-up volume, 85.5 ml; fraction volume, 7.65 ml; flow rate, 37 ml/hr; regenerating solvent, pyridine–0.1% aqueous acetic acid (3:5). The chromatogram showed two peaks of virtually equal size with R_f values of 0.24 (oxytocin) and 0.32 (mixture of dimers). Isolation of the materials corresponding to these peaks gave 31.4 mg of oxytocin and 22.1 mg of the mixture of dimers. The latter material was subjected to gel filtration in the manner described in the section on the isolation of the α and β dimers. Two peaks were obtained with maxima at effluent volumes 140 and 145 ml.

Isolation of the material corresponding to the faster moving peak (effluent volume 140 ml) gave 10.9 mg, $[\alpha]^{20}D$ −104° (*c* 0.5, 1 *N* acetic acid), which is in agreement with the value for the α dimer. A sample (0.96 mg) subjected to gel filtration on the 1.24 × 159 cm Sephadex G-25 column in 0.2 *N* acetic acid at a flow rate of 5.7 ml/hr gave a chromatogram showing one sharp symmetrical peak with a maximum at effluent volume 127 ml, this behavior being identical with that of the α dimer. Gel filtration of a mixture of this material (0.61 mg) and an authentic sample (0.71 mg) of α dimer gave a chromatogram showing one sharp symmetrical peak with a maximum at the same effluent volume (127 ml).

Isolation of the material corresponding to the slower moving peak (effluent volume 145 ml) gave 5.9 mg, $[\alpha]^{22}D$ −79° (*c* 0.4, 1 *N* acetic acid), which is in agreement with the value for the β dimer. A sample (0.92 mg) subjected to the gel filtration procedure just described gave a chromatogram showing one sharp symmetrical peak with a maximum at effluent volume 137 ml, this behavior being identical with that of the β dimer. Gel filtration of a mixture of this material (0.47 mg) and an authentic sample (0.44 mg) of β dimer gave a chromatogram showing one sharp symmetrical peak with a maximum at the same effluent volume (137 ml).

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