g, 84%), and washed with dilute sulfuric acid, saturated sodium carbonate solution, and water. After drying, evaporation to dryness yielded a syrupy residue (3.08 g). This residue (2.85 g) was dissolved in 30 ml of hexane and hydrogenated over a period of 24 hr using 0.6 g of palladium black catalyst. The solution was filtered and evaporated to dryness, and the residue was dissolved in 30 ml of hot, dry acetone. On cooling 1.65 g of crystals was deposited, mp 68°. Tlc indicated at least four components. The crystals (1 g) were dissolved in chloroform and chromatographed on 75 g of silicic acid. Fractions of 25 ml were collected. Chloroform–methanol (99:1) eluted Ia (0.51 g) identified by melting point and tlc. Chloroform–methanol (96:4) eluted material tentatively identified as bis(1,2-di-O-stearoyl-L-glycerol) phosphate (IVa, 0.18 g); it had mp 69–70° (lit.[31] mp 69.5–70.5°) and phosphorus:fatty acid:glycerol content ratios of 1.0:4.1:2.3 (theoretical, 1:4:2). Chloroform–methanol (94:6) eluted material tentatively identified as 1,2-di-O-stearoyl-L-glycerol 3-phosphate (Ig, 0.022 g); it had phosphorus:fatty acid:glycerol content ratios of 1.0:2.0:1.2 (theoretical, 1:2:1). Chloroform–methanol (9:1) eluted the product IIIa (0.19 g). It was dissolved in warm acetone and neutralized with barium hydroxide. On cooling, crystals were deposited, mp 166°, $[\alpha]^{26}D$ +9.2° (*c* 1, pyridine). The over-all yield was 11%. From an average of three determinations in each case it had phosphorus:fatty acid:glycerol content ratios of 1.0:1.9:1.9 (theoretical, 1:2:2). The yield of formaldehyde released per mole of material oxidized with periodic acid[14] was 0.86, Ic being employed as a standard.

*Anal.* Calcd for $C_{42}H_{82}O_{10}P_1Ba_{0.5}$ (845): C, 59.6; H, 9.7; P, 3.7. Found: C, 59.0; H, 10.0; P, 3.6.

**Alkaline Hydrolysis.** The synthetic IIa and IIIa and ox heart cardiolipin were deacylated by the method described by Dawson and co-workers[20] for the identification of phospholipids in biological specimens. Samples from the three hydrolysates were subjected to paper chromatography and ionophoresis as described by the same workers. The hydrolysates from IIa and ox heart cardiolipin were identical in behavior. The hydrolysate from IIIa gave an identical picture with that of glycerophosphorylglycerol.[17]

**Acknowledgment.** We wish to thank Dr. A. A. Benson for a generous gift of glycerophosphorylglycerol, and Miss Lorraine Dreisbach for excellent technical assistance.

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# 7-D-Proline-oxytocin and Its Deamino Analog. Diastereoisomers of Oxytocin and Deamino-oxytocin[1]

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**Abstract:** 7-D-Proline-oxytocin, an analog differing from the posterior pituitary hormone only in the configuration of the amino acid residue in the 7 position, has been synthesized and tested for oxytocic and avian vasodepressor activities. The required synthetic nonapeptide intermediate was prepared from D-prolyl-L-leucylglycinamide by the stepwise *p*-nitrophenyl ester method. Reduction with sodium in liquid ammonia and subsequent oxidation of the sulfhydryl form to the cyclic disulfide yielded 7-D-proline-oxytocin. The analog exhibited 13 units/mg of oxytocic activity but the avian vasodepressor was nil. 1-Deamino-7-D-proline-oxytocin was also synthesized and found to possess 45 units/mg of oxytocic activity and no avian vasodepressor activity. For the preparation of D-prolyl-L-leucylglycinamide, DL-proline was used as the starting material. Resolution of the diasteroisomeric tripeptides resulting from the reaction of *p*-nitrophenyl carbobenzoxy-DL-prolinate with ethyl L-leucylglycinate was accomplished and the ethyl carbobenzoxy-D-prolyl-L-leucyglycinate so obtained was converted to the amide.

As a further contribution to the study of the relationship of the stereostructure of oxytocin to manifestation of pharmacological activity, 7-D-proline-oxytocin has been synthesized. This diastereoisomer of oxytocin differs from oxytocin (Figure 1) only in the configuration of the proline residue in the 7 position. The required intermediate nonapeptide for the synthesis of 7-D-proline-oxytocin, namely N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide, was obtained by the stepwide *p*-nitrophenyl ester method employed by Bodanszky and du Vigneaud for the synthesis of oxytocin,[2] but with D-prolyl-L-leucylglycinamide used as the starting material.

At the time the synthesis was undertaken D-proline was not available commercially. For the preparation of the desired ethyl D-prolyl-L-leucylglycinate intermediate the diastereoisomeric tripeptides resulting from the reaction of *p*-nitrophenyl carbobenzoxy-DL-prolinate with ethyl L-leucylglycinate were separated by fractional crystallization and thus the resolution of the DL-proline and the preparation of the desired tripeptide ester was accomplished in one step.

The ethyl D-prolyl-L-leucylglycinate was then converted to the amide and used for the subsequent synthetic steps for the preparation of the protected nonapeptide. The latter compound was treated with sodium in liquid ammonia to remove the protecting groups by the method of Sifferd and du Vigneaud[3] as used in the original synthesis of oxytocin.[4] The dithiol so obtained was oxidized to the cyclic disulfide by aeration in aqueous solution at pH 6.8. Purification of the D-proline analog was accomplished by countercurrent distribution[5] and by partition chromatography on Sephadex

(1) This work was supported in part by Grant HE-01675 from the National Heart Institute, U. S. Public Health Service.

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Table I. Biological Activities of 7-D-Proline-oxytocin and Oxytocin[a]

| Compound | Oxytocic (rat) | Vasodepressor (fowl) | Antidiuretic (rat) | Milk ejecting (rabbit) | Pressor (rat) |
|---|---|---|---|---|---|
| Oxytocin | 486 ± 5 | 507 ± 23 | 2.7 ± 0.2 | 410 ± 16 | 3.1 ± 0.1 |
| 7-D-Proline-oxytocin[b] | 13 ± 0.5 | Nil <0.005 | ~1.0 | 3.9 ± 0.1 | Nil <0.005 |

[a] Mean activities in units per milligram with standard error. [b] The values for 7-D-proline-oxytocin were obtained by the following methods: oxytocic, assays 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; vasodepressor, according to the method of R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *ibid.*, **66**, 860 (1960); antidiuretic, by the method of W. A. Jeffers, M. M. Livezey, and J. H. Austin, *Proc. Soc. Exptl. Biol. Med.*, **50**, 184 (1942), as modified by W. H. Sawyer, *Endocrinology*, **63**, 694 (1958); milk-ejecting, by the method of B. A. Cross and G. W. Harris, *J. Endocrinol.*, **8**, 148 (1952), as modified by H. B. van Dyke, K. Adamsons, Jr., and S. L. Engel, *Recent Progr. Hormone Res.*, **11**, 1 (1955), and by W. Y. Chan, *J. Pharmacol.*, **147**, 48 (1965); pressor, as described in "The Pharmacopeia of the United States of America," 16th rev, Mack Publishing Co., Easton, Pa., 1960, p 793. All the assays were carried out against the U.S.P. Posterior Pituitary Reference Standard.

G-25.[6,7] 7-D-Proline-oxytocin was distributed in the solvent system 1-butanol–1-propanol–water containing 0.5% acetic acid and 0.1% pyridine (6:1:8) and exhibited a partition coefficient ($K$) of 0.82 whereas oxytocin possesses a $K$ of 0.43 under these conditions. Further purification was achieved on Sephadex G-25 in the solvent system 1-butanol–water containing 3.5% acetic acid and 1.5% pyridine (1:1) in which the analog exhibited $R_f$ 0.37 whereas oxytocin traveled more slowly ($R_f$ 0.24) under the same conditions.

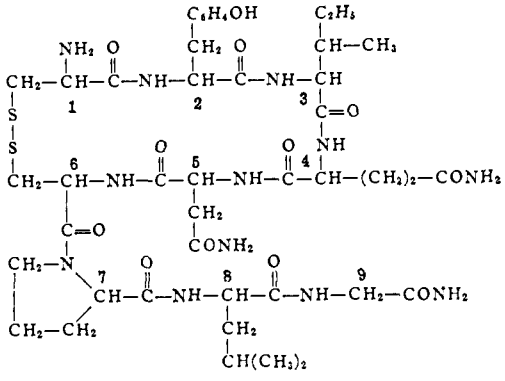

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues.

The highly purified 7-D-proline-oxytocin was tested for some of the pharmacological properties associated with oxytocin. These results are tabulated in Table I along with the values previously established for oxytocin.[8] Although the D-proline-oxytocin possesses 13 units/mg of oxytocic activity, the avian vasodepressor activity is nil as shown in Table I. Since oxytocin possesses approximately 500 units/mg of avian vasodepressor activity as well as about 500 units/mg of oxytocic activity, it is evident that the activity exhibited by D-proline-oxytocin is not due to contamination with oxytocin.

It may be recalled that deamino-oxytocin is a highly active analog of oxytocin, being more potent, for example, than the parent hormone in its oxytocic and avian vasodepressor activities.[9] It was of interest, therefore, to determine the effect of the replacement of the free amino group of 7-D-proline-oxytocin by hydrogen. For the preparation of 1-deamino-7-D-proline-oxytocin, the free octapeptide used for the preparation of the 7-D-proline-oxytocin was coupled with *p*-nitrophenyl S-benzyl-β-mercaptopropionate.[9] The resulting protected polypeptide was freed of protecting groups by treatment with sodium in liquid ammonia and the resulting dithiol was oxidized with potassium ferricyanide.[9] Purification was achieved by partition chromatography on Sephadex G-25 in the system 1-butanol–benzene–water containing 3.5% acetic acid and 1.5% pyridine (1:1:2). The $R_f$ of the 1-deamino-7-D-proline-oxytocin in this system is 0.51, whereas deamino-oxytocin exhibits $R_f$ 0.20 in the same system. The 1-deamino-7-D-proline-oxytocin possesses ~45 units/mg of oxytocic activity and no detectable vasodepressor activity. Thus, the formal replacement of the amino group by hydrogen in the D-proline analog does enhance the oxytocic activity, whereas the avian vasodepressor activity remains nil. The deamino-D-proline-oxytocin was also found to possess approximately 8 units/mg of milk-ejecting activity, a potency higher than that exhibited by D-proline-oxytocin. The deamino compound shows no pressor activity.

Further exploration of the effect of replacement of an individual L-amino acid residue by the corresponding D-amino acid residue is underway. Preliminary results indicate that 4-D-glutamine- and 5-D-asparagine-oxytocin are practically inactive when tested for oxytocic and avian vasodepressor activity.[10] It might be mentioned that the synthesis of D-oxytocin, the mirror image of the natural oxytocin, was reported recently from this laboratory and this enantiomer of the posterior pituitary hormone was found to be devoid of oxytocic and avian vasodepressor activity.[11]

## Experimental Section[12]

*p*-Nitrophenyl Carbobenzoxy-DL-prolinate. A solution of 46 g of DL-proline hydrochloride,[13] in 300 ml of 4 $N$ sodium hydroxide, was treated with 66 g of carbobenzoxy chloride at 0° and stirred overnight at room temperature. After extraction of the aqueous suspension with ether, the aqueous layer was acidified and the resulting

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(9) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *J. Biol. Chem.*, **235**, PC64 (1960); D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962); D. Jarvis and V. du Vigneaud, *Science*, **143**, 545 (1964); B. M. Ferrier, D. Jarvis, and V. du Vigneaud, *J. Biol. Chem.*, **240**, 4264 (1965).
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(11) G. Flouret and V. du Vigneaud, *J. Am. Chem. Soc.*, **87**, 3775 (1965).
(12) All melting points were done in capillary tubes and are corrected.
(13) D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, **193**, 81 (1951).

oil was taken up in ethyl acetate. The ethyl acetate solution was washed with 1 *N* hydrochloric acid and water, and finally dried over anhydrous sodium sulfate. After filtration, the solution was concentrated *in vacuo* to an oil which was dissolved in 1 l. of ethyl acetate. *p*-Nitrophenol (46 g) was added, the solution was chilled to 0°, and 68 g of dicyclohexylcarbodiimide was added. After the mixture was stirred for 0.5 hr at 0° and 1 hr at room temperature, acetic acid (1 ml) was added, and after 15 min the mixture was filtered and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in tetrahydrofuran and crystallized by the addition of hexane, 79 g, mp 71–73°. *Anal.* Calcd for $C_{19}H_{18}O_6N_2$: C, 61.6; H, 4.90; N, 7.57. Found: C, 61.8; H, 4.88; N, 7.60.

**Ethyl Carbobenzoxy-D-prolyl-L-leucylglycinate.** Ethyl carbobenzoxy-L-leucylglycinate (14.0 g), prepared according to the method of Bodanszky and du Vigneaud,[2] was decarbobenzoxylated by passing hydrogen gas through a suspension of 3.7 g of palladium chloride in 500 ml of an ethanolic solution of the ester. When carbon dioxide evolution stopped, the solution was filtered and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in 100 ml of chloroform and 15.2 g of *p*-nitrophenyl carbobenzoxy-DL-prolinate was added. Triethylamine (10 ml) was then added and the solution was stirred at room temperature for 2 days. After concentration to dryness *in vacuo*, the residue was suspended in a mixture of ethyl acetate and water, the water layer was separated and discarded, and the ethyl acetate layer was washed successively with 1 *N* ammonium hydroxide, water, 1 *N* hydrochloric acid, and water, and finally was dried over anhydrous sodium sulfate. After filtration, the solution was seeded with ethyl carbobenzoxy-L-prolyl-L-leucylglycinate,[14] and left overnight at 0°. The crystalline solid was filtered and dried, 7.4 g, mp 143–146°. A portion was recrystallized from aqueous ethanol, mp 150–151°, $[\alpha]^{20}D$ −81.6° (*c* 1, ethanol) (lit.[14] mp 150–152°, $[\alpha]^{21}D$ −83.2° (*c* 2.5, ethanol)). A mixture melting point of an authentic sample of ethyl carbobenzoxy-L-prolyl-L-leucylglycinate with the protected tripeptide ester obtained showed no depression.

To obtain the diastereoisomeric protected tripeptide ester the ethyl acetate mother liquor from the above filtration was diluted with hexane until cloudy and left at room temperature for 1 day and at 0° for 2 days. The crystalline precipitate was filtered off and dried, 5.70 g, mp 121–123°, $[\alpha]^{20}D$ −12.2° (*c* 1, ethanol). A portion was recrystallized from 50% aqueous ethanol, mp 122–124°, $[\alpha]^{20}D$ −10.5° (*c* 1, ethanol). The mixture melting point with the L isomer (mp 150–152°) was 95–130°. Further recrystallization did not change the rotation. *Anal.* Calcd for $C_{34}H_{33}O_6N_3$: C, 61.7; H, 7.43; N, 9.39. Found: C, 61.8; H, 7.30; N, 9.38.

**Carbobenzoxy-D-prolyl-L-leucylglycinamide.** The ethyl carbobenzoxy-D-prolyl-L-leucylglycinate (11.8 g) was dissolved in 200 ml of methanol and the solution was saturated with ammonia at 0°. After being allowed to stand overnight, the solution was concentrated to dryness *in vacuo* and triturated with ethyl acetate, 8.90 g, mp 164–166°. Recrystallization from hot water gave material with mp 166.5–168°, $[\alpha]^{20}D$ +6.6° (*c* 1, 95% ethanol). The rotation of carbobenzoxy-L-prolyl-L-leucylglycinamide is −73.3°.[15] *Anal.* Calcd for $C_{21}H_{30}O_5N_4$: C, 60.3; H, 7.23; N, 13.4. Found: C, 60.4; H, 7.35; N, 13.4.

**N-Carbobenzoxy-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The protected tripeptide amide (4.2 g) was decarbobenzoxylated by the procedure already described with the use of 0.90 g of palladium chloride and hydrogen gas. After filtration and concentration of the filtrate, the residue was dissolved in methanol (50 ml) and Amberlite IRA-400 (in OH cycle) was added until the solution was chloride free. The resin was filtered off and the filtrate was concentrated to dryness *in vacuo*. Dimethylformamide (DMF, 4 ml) was added followed by 4.5 g of *p*-nitrophenyl N-carbobenzoxy-S-benzyl-L-cysteinate.[16] After 3 days at room temperature, 100 ml of ethyl acetate was added along with 10 ml of 0.1 *N* ammonium hydroxide. After the mixture was stirred for 0.5 hr, the aqueous layer was discarded and the organic layer was washed repeatedly with 1 *N* ammonium hydroxide and then successively with water, 1 *N* hydrochloric acid, and finally with water. After being dried over anhydrous sodium sulfate the solution was filtered and hexane was added to the filtrate. The granular solid that deposited was filtered off and dried, wt 3.90 g, mp 126–127°, $[\alpha]^{20}D$ −48° (*c* 1, DMF).[17] *Anal.* Calcd for $C_{31}H_{41}O_6N_5S$: C, 60.9; H, 6.75; N, 11.5. Found: C, 60.9; H, 6.80; N, 11.5.

**S-Benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The carbobenzoxy tetrapeptide amide (1.00 g) was dissolved in acetic acid (4 ml) and treated with 4 ml of 4 *N* HBr in acetic acid. After 1 hr at room temperature, the solution was poured into 100 ml of dry ether and washed several times with dry ether by decantation. After being dried over $CaCl_2$ and NaOH, the residue was dissolved in 20 ml of methanol and treated with Amberlite IRA-400 (in the OH cycle). After removal of the methanol, the residue was dissolved in ethanol and ether was added to give a crystalline solid, 0.70 g, mp 169–170°.° Recrystallization from absolute ethanol gave material with the same melting point, $[\alpha]^{20}D$ +49° (*c* 1, acetic acid). *Anal.* Calcd for $C_{23}H_{34}O_4N_5S$: C, 58.0; H, 7.19; N, 14.7. Found: C, 57.8; H, 7.44; N, 14.5.

**Carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The tetrapeptide amide (2 g) was dissolved in 4 ml of DMF and 0.8 g of *p*-nitrophenyl carbobenzoxy-L-asparaginate[2] was added. The next day, a mixture of 0.1 *N* ammonium hydroxide and ethyl acetate was added and stirred for 15 min. The aqueous layer was discarded and the organic layer was washed successively with 1 *N* ammonium hydroxide, water, 1 *N* hydrochloric acid, and finally with water. After the solution was dried over anhydrous sodium sulfate and filtered, ether was added to give a solid which was further purified by dissolving it in hot ethyl acetate and allowing the solution to cool, 2.45 g, mp 145°, with sintering at 100°, $[\alpha]^{20}D$ −41.2° (*c* 1, DMF). *Anal.* Calcd for $C_{35}H_{47}O_8N_7S$: C, 57.9; H, 6.53; N, 13.5. Found: C, 57.6; H, 6.65; N, 13.4.

**Carbobenzoxy-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The protected pentapeptide amide (1.60 g) was treated with HBr in acetic acid according to the procedure already described. After removal of the methanol, 4 ml of DMF and 0.88 g of *p*-nitrophenyl carbobenzoxy-L-glutaminate[2] were added. The next day, ethyl acetate was added, and the solid was filtered and washed with ethyl acetate and ethanol, 1.74 g, mp 175–178°, $[\alpha]^{20}D$ −31.9° (*c* 1, DMF). *Anal.* Calcd for $C_{40}H_{55}O_{10}N_9S$: C, 56.3; H, 6.49; N, 14.8. Found: C, 55.9; H, 6.47; N, 14.6.

**Carbobenzoxy-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The protected hexapeptide (5.7 g) was decarbobenzoxylated with HBr in acetic acid and freed of HBr by the method already described. The residue obtained after evaporation of the methanol was dissolved in 4 ml of DMF and treated with 2.75 g of *p*-nitrophenyl carbobenzoxy-L-isoleucinate.[2] The next day, the product was isolated as described in the previous section, 5.5 g, mp 214–217°, $[\alpha]^{20}D$ −30.3° (*c* 1, DMF). *Anal.* Calcd for $C_{46}H_{66}O_{11}N_{10}S$: C, 57.1; H, 6.88; N, 14.5. Found: C, 56.8; H, 6.93; N, 14.3.

**N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The protected heptapeptide (5.00 g) was decarbobenzoxylated and freed of HBr in the usual manner. The residue obtained from the methanol solution was dissolved in 4 ml of DMF and treated with 2.90 g of *p*-nitrophenyl N-carbobenzoxy-O-benzyl-L-tyrosinate.[2] Isolation in the usual manner gave 5.40 g, mp 222–224°, $[\alpha]^{20}D$ −24.2° (*c* 1, DMF).

**N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The protected octapeptide (3.0 g) was suspended in 2,2,2-trifluoroethanol (75 ml) and saturated with anhydrous hydrogen bromide at 0°. After being stirred at room temperature for 1 hr, the solution was concentrated to dryness *in vacuo* and the residue was triturated with dry ether. The dry powder was then dissolved in methanol (100 ml) and treated with the anion-exchange resin described in previous sections. After removal of the methanol *in vacuo*, a residue weighing 1.78 g was obtained. This was dissolved in 2 ml of DMF and treated with 0.84 g of *p*-nitrophenyl N-carbobenzoxy-S-benzyl-L-cysteinate. After 2 days, the product was isolated in the usual manner to give 1.73 g of solid, mp 228–230°, $[\alpha]^{20}D$ −35.3° (*c* 1, DMF). A portion (100 mg) was dissolved in DMF and precipitated with water to give 80 mg of ma-

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(15) C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **76**, 3107 (1954).
(16) M. Bodanszky and V. du Vigneaud, *ibid.*, **81**, 2504 (1959).

(17) Occasionally this material crystallized in the form of needles, mp 90–92°, $[\alpha]^{20}D$ −45° (*c* 1 DMF). This material gave satisfactory analytical values for the protected tetrapeptide amide (Found: C, 60.6; H, 6.76; N, 11.5) but on standing became amorphous and when dissolved in ethyl acetate and precipitated by the addition of hexane the higher melting point was obtained.

terial with the same physical constants. *Anal.* Calcd for $C_{65}H_{86}O_{14}N_{12}S_2$: C, 59.0; H, 6.55; N, 12.7. Found: C, 58.8; H, 6.55; N, 12.6.

**7-D-Proline-oxytocin.** The protected nonapeptide (200 mg) was dissolved in 60 ml of anhydrous liquid ammonia at its boiling point. The reduction was carried out at the boiling point of the solution by dipping a glass tube containing sodium below the surface of the solution until a blue color remained for 30 sec. The ammonia was then removed *in vacuo* from the frozen state. Water (100 ml) containing 0.07 ml of acetic acid was then added to the dry residue. The pH of the solution was then adjusted to 6.8 and the solution was aerated overnight. After concentration to a volume of 50 ml *in vacuo*, the solution was placed in the first five tubes of an all-glass 200-tube Craig countercurrent distribution apparatus[5] in the system 1-butanol–1-propanol–water containing 0.5% acetic acid and 0.1% pyridine (6:1:8). After 200 distributions, Folin–Lowry color[18] determinations revealed the presence of one major peak with a $K$ of 0.82 and two very small slower moving peaks well separated from the major peak. Tubes 70 to 110 containing material ($K$ = 0.82) were pooled, concentrated *in vacuo*, and lyophilized to give 78 mg of white, fluffy powder. This was further purified by partition chromatography on Sephadex G-25[6,7] in the system 1-butanol–water containing 3.5% acetic acid and 1.5% pyridine (1:1). Folin–Lowry color determinations revealed a major peak ($R_f$ 0.37), which was isolated by pooling of the fractions, mixture with twice the volume of water, evaporation to a small volume, and lyophilization to yield 45 mg of a white, fluffy powder. For elemental analysis a sample of the material was dried *in vacuo* over $P_2O_5$ at 100° for 8 hr and a loss in weight of 9.11% was observed. *Anal.* Calcd for $C_{43}H_{66}O_{12}N_{12}S_2$: C, 51.3; H, 6.60; N, 16.7. Found: C, 51.8; H, 6.80; N, 16.5; $[\alpha]^{20}D$ +99° (*c* 0.50, 1 *N* acetic acid).

A sample was hydrolyzed in 6 *N* HCl *in vacuo* at 110° for 17 hr and analyzed in the Beckman-Spinco amino acid analyzer according to the procedure of Spackman, Stein, and Moore.[19] The following molar ratios were obtained, glycine being taken as 1.0: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; cystine, 1.0; isoleucine, 1.0; leucine, 1.0; tyrosine, 1.0; and ammonia, 3.1.

**S-Benzyl-β-mercaptopropionyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-D-prolyl-L-leucylglycinamide.** The protected octapeptide amide (1.00 g) was freed from its O and N protecting groups with HBr in 2,2,2-trifluoroethanol. The dried hydrobromide was dissolved in 25 ml of methanol and treated with triethylamine dropwise until 1 drop of the methanol solution in 3 ml of water gave a pH of 8. The solution was then concentrated to dryness *in vacuo* and suspended in 5 ml of DMF. *p*-Nitrophenyl S-benzyl-β-mercaptopropionate[9] (300 mg) was then added and the mixture was stirred at room temperature for 3 days. Ethyl acetate (50 ml) was then added and the solid material was isolated in the usual way, 0.75 g, mp 223–226°, $[\alpha]^{20}D$ −27.5° (*c* 1, DMF). *Anal.* Calcd for $C_{57}H_{79}O_{12}N_{11}S_2$: C, 58.3; H, 6.78; N, 13.1. Found: C, 57.7; H, 6.71; N, 13.0.

A sample was hydrolyzed in 6 *N* HCl *in vacuo* at 110° for 17 hr and analyzed in the Beckman-Spinco amino acid analyzer. The following molar ratios were obtained, glycine being taken as 1.0: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; S-benzylcysteine, 1.0; isoleucine, 1.0; leucine, 1.0; tyrosine, 0.92; and ammonia, 3.0.

**1-Deamino-7-D-proline-oxytocin.** The compound from the preceding section (200 mg) was dissolved in redistilled liquid ammonia (60 ml) and treated with sodium in the usual way. After removal of the ammonia, 100 ml of water containing 0.07 ml of acetic acid was added to the residue and potassium ferricyanide (35 ml, 0.01 *N*) was added dropwise with stirring. After 15 min the solution was passed through a column of AG3X4 resin (in chloride form). The column was washed with 100 ml of 0.1% acetic acid and combined with the solution. This mixture was concentrated *in vacuo* to about 10 ml and placed on a column of Sephadex G-25 which had previously been equilibrated with the lower phase of the solvent system 1-butanol–benzene–water containing 3.5% acetic acid and 1.5% pyridine (1:1:2). The column was then eluted with the upper phase. Folin–Lowry color determinations[18] on the eluate revealed three peaks, $R_f$'s 1 (minor), 0.51 (major), and 0.31 (minor). The major peak, $R_f$ 0.51, was isolated by the procedure described for 7-D-proline-oxytocin; lyophilized powder, 75 mg. For elemental analysis a sample of the material was dried *in vacuo* over $P_2O_5$ at 100° for 8 hr and a loss in weight of 7.55% was observed; $[\alpha]^{20}D$ −42° (*c* 0.5, 1 *N* acetic acid). *Anal.* Calcd for $C_{43}H_{63}O_{12}N_{11}S_2$: C, 52.0; H, 6.60; N, 15.5. Found: C, 52.2; H, 6.69; N, 15.2.

**Acknowledgments.** The authors are indebted to the following members of this laboratory: Mr. Joseph Albert for the elemental analyses; Mr. Roger Sebbane for the amino acid analyses; and Miss Margitta Wahrenburg, Mrs. Frances Richman, and Mrs. Maxine Goldberg for the bioassays, under the direction of Dr. W. Y. Chan.

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