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Analogs of Oxytocin Containing Glycine in place of Tyrosine, Isoleucine, or Glutamine[1]

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Three analogs of oxytocin in which the tyrosine, isoleucine, and glutamine residues in positions 2, 3, and 4 of the peptide chain are replaced, respectively, by a glycine residue have been synthesized and tested for some of the pharmacological properties characteristic of oxytocin. The required protected nonapeptide intermediates were prepared by use of the azide method and in the case of 2-glycine-oxytocin and 4-glycine-oxytocin the nonapeptides were also prepared by the stepwise p-nitrophenyl ester method. After removal of the protecting groups from the nonapeptides by means of sodium in liquid ammonia, the dithiols thus obtained were oxidized to 2-glycine-oxytocin, 3-glycine-oxytocin, and 4-glycine-oxytocin 4-Glycine-oxytocin possessed approximately 5 units per mg. of avian depressor activity, 3 units per mg. of oxytocic activity, 17 units per mg. of milk-ejecting activity, and negligible pressor and antidiuretic activities. The other two analogs were practically inactive.

In connection with a series of st dies in this laboratory on the significance of the chemical functional groups of oxytocin (Fig. 1) to its biological activities,[3] an analog of the hormone was prepared in which the carboxamide group of the glutamine residue in the 4-position of the molecule was replaced by hydrogen, namely 4-decarboxamido-oxytocin.[4] In this compound an L-α-aminobutyric acid residue replaces the L-glutamine residue present in the hormone. Thus in effect an ethyl group is attached to the carbon of the glycine residue in position 4 of the backbone structure of oxytocin. The 4-decarboxamido-oxytocin was found to possess approximately one-fifth of the avian depressor and one-sixth of the oxytocic activity of oxytocin, while its pressor and antidiuretic activities were extremely low. In the light of the relatively high avian depressor and oxytocic potencies of this analog, it was decided to investigate the effect of replacing the ethyl grouping by hydrogen. The present paper reports the synthesis and pharmacological properties of this 4-glycine-oxytocin, as well as two other analogs of oxytocin, 3-glycine-oxytocin and 2-glycine-oxytocin, in which the glycine residue replaces an isoleucine and a tyrosine residue, respectively.

For the preparation of the protected nonapeptide for the synthesis of 4-glycine-oxytocin the protected pentapeptide amide, carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide,[5] served as starting material. After removal of the carbobenzoxy group by treatment with HBr in glacial acetic acid, the peptide chain was lengthened stepwise by successive reactions with the appropriate protected p-nitrophenyl amino acid esters in a manner similar to that used by Bodanszky and du Vigneaud for the synthesis of oxytocin.[5] The nitrophenyl esters of carbobenzoxyglycine,[6] carbobenzoxy-L-isoleucine,[5] O-benzyl-N-carbobenzoxy-L-tyrosine,[5] and S-benzyl-N-carbobenzoxy-L-cysteine[5] were employed successively to obtain the required protected nonapeptide, S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucylglycyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The free heptapeptide with isoleucine at the amino end of the peptide chain, obtained during the course of this synthesis, was also coupled with the azide prepared from S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosine hydrazide[7] according to the procedure described by Honzl and Rudinger[8] to give the same protected nonapeptide.

For preparation of the protected nonapeptide for the synthesis of 3-glycine-oxytocin the S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosine azide was allowed to react with glycyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The required heptapeptide was itself prepared from L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[9,10] by reaction with p-nitrophenyl carbobenzoxyglycinate followed by removal of the carbobenzoxy group.

L-Isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[11] served as starting material for preparation of the protected nonapeptide intermediate for the synthesis of 2-glycine-oxytocin. The glycine and cysteine residues were linked to the heptapeptide by the stepwise p-nitrophenyl ester method in one route, and in the second S-benzyl-N-carbobenzoxy-L-cysteinylglycine hydrazide[12] was converted to the azide and allowed to react with the heptapeptide and form the required protected nonapeptide intermediate, S-benzyl-N-carbobenzoxy-L-cysteinylglycyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. It should be noted that the free octapeptide and protected nonapeptide intermediates in the synthesis of 2-glycine-oxytocin did not dissolve but formed a thick gel in dimethylformamide.

The protecting groups of the nonapeptide intermediates were removed by treatment with sodium in liquid ammonia by the method of Sifferd and du Vigneaud[13]

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

(2) Acknowledgment is made of a grant under the United States Government International Exchange Program and of a Fulbright Travel Grant.

(3) The biological activities of a highly purified sample of synthetic oxytocin have been recently reported by Chan and du Vigneaud [*Endocrinology*, **71**, 977 (1962)]. The values obtained are given in Table I in the present paper.

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(5) M. Bodanszky and V. du Vigneaud, *Nature*, **183**, 1324 (1959); *J. Am. Chem. Soc.*, **81**, 5688 (1959).

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(11) D. B. Hope and V. du Vigneaud, *J. Biol. Chem.*, **237**, 3146 (1962).

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

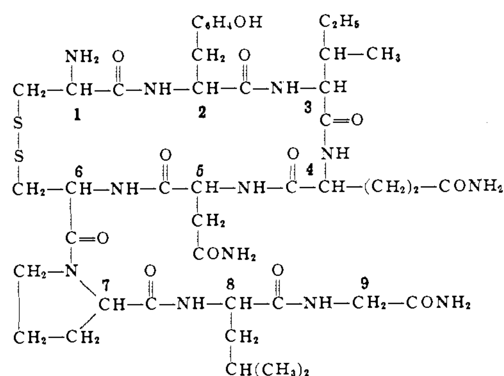

Fig. 1.—Structure of oxytocin with numbers indicating the position of the individual amino acid residues.

as used in the synthesis of oxytocin.[14] The dithiols so obtained were oxidized in neutral solution by aeration with carbon dioxide-free air followed by titration with a solution of potassium ferricyanide.[15] After removal of ferrous and ferric ions by means of resin the oxidized products were subjected to countercurrent distribution in the solvent system 1-butanol-1-propanol-0.5% acetic acid containing 0.1% pyridine (6:1:8)[16] and then isolated by concentration and lyophilization. 4-Glycine-oxytocin had a partition coefficient (K) of approximately 0.8, whereas the partition coefficients of 3-glycine-oxytocin and 2-glycine-oxytocin were very low, approximately 0.11 and 0.18, respectively. The latter two analogs of oxytocin were much less soluble in the organic phase than the 4-glycine-oxytocin and hence moved very slowly on countercurrent distribution. The three analogs were subjected to partition chromatography on Sephadex G-25 by a procedure patterned after that devised by Yamashiro for the purification of oxytocin.[17] The highly purified samples of the three analogs gave the expected amino acid and elemental analyses.

The glycine analogs were assayed for pharmacological activity under the direction of Dr. W. Y. Chan of this laboratory. The 2-glycine-oxytocin and 3-glycine-oxytocin had no appreciable avain depressor,[18] rat uterine-contracting,[19] or rat pressor[20] activity. The 4-glycine-oxytocin, however, was found to possess approximately 5 units per mg. of avian depressor activity, 3 units per mg. of rat uterine-contracting activity, 17 units per mg. of rabbit milk-ejecting activity,[21] and negligible rat pressor and antidiuretic[22] activities.

(14) 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).

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(16) The use of solvent systems containing pyridine in the countercurrent distribution of oxytocin and related compounds was found by Dr. Derek Jarvis of this laboratory to be advantageous in avoiding emulsification and consequent time loss.

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Experimental[23]

Carbobenzoxyglycyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—A suspension of 5 g. of finely powdered carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[5] in 17.5 ml. of glacial acetic acid was treated with 17.5 ml. of HBr in glacial acetic acid (25% w./w.). After 2 hr. at room temperature the solution was poured into 150 ml. of cold dry ether. The precipitated hydrobromide of the free base was washed by decantation with three 100-ml. portions of ether. After being dried *in vacuo* over potassium hydroxide and calcium chloride for several hours the hydrobromide was dissolved in 75 ml. of dry methanol and passed through a column of ion-exchange resin IRA-410 (OH form). The column was washed with 50 ml. of methanol. The solid obtained after evaporation of the solvent from the eluate and washing was dissolved in 20 ml. of dimethylformamide and 1.99 g. of p-nitrophenyl carbobenzoxyglycinate was added. After 2 days at room temperature the product was precipitated with 200 ml. of ethyl acetate, collected, washed with 100 ml. of ethyl acetate, 50 ml. of ethanol, and 30 ml. of ether, and dried to constant weight over P_2O_5 *in vacuo* at 56°, giving 3.5 g., m.p. 203–205°, $[\alpha]^{19.5}D$ $-55.8°$ (c 1, dimethylformamide).

Anal. Calcd. for $C_{37}H_{50}N_8O_9S$: C, 56.8; H, 6.44; N, 14.3. Found: C, 56.9; H, 6.63; N, 14.4.

Carbobenzoxy-L-isoleucylglycyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—The protected hexapeptide described in the preceding section, 2.9 g., was suspended in 15 ml. of glacial acetic acid and treated with 14 ml. of 25% HBr in glacial acetic acid according to the procedure already described. After 1.5 hr. the hydrobromide of the free hexapeptide was isolated, dried, and dissolved in 100 ml. of dry methanol. The solution was passed through a column of IRA-410 resin (OH cycle). The residue after removal of the solvent from the eluate was taken up in 15 ml. of dimethylformamide and 1.57 g. of p-nitrophenyl carbobenzoxy-L-isoleucinate was added. After 20 hr. at room temperature the product was precipitated with 200 ml. of ethyl acetate and washed with ethanol (50 ml.) and ether (50 ml.). After being dried *in vacuo* over P_2O_5 at 56° the product weighed 3.05 g., m.p. 213–215°, $[\alpha]^{19}D$ $-52.3°$ (c 1, dimethylformamide).

Anal. Calcd. for $C_{43}H_{61}N_9O_{10}S$: C, 57.6; H, 6.86; N, 14.1. Found: C, 57.8; H, 6.94; N, 14.1.

L-Isoleucylglycyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—Protected heptapeptide (6.5 g.) was suspended in 35 ml. of glacial acetic acid and 35 ml. of 25% HBr in glacial acetic acid was added. After 1.5 hr. the hydrobromide of the free base was precipitated with ether, dried, and taken up in 200 ml. of dry methanol. This solution was passed down a column of resin IRA-410 (OH form) and the residue after removal of the solvent (5.9 g.) was crystallized twice from water, giving 2.3 g., m.p. 181–182°, $[\alpha]^{19}D$ $-57.5°$ (c 1, dimethylformamide), R_f 0.68 (paper chromatography). On electrophoresis the product traveled as a single spot to the cathode.

Anal. Calcd. for $C_{35}H_{55}N_9O_8S$: C, 55.2; H, 7.28; N, 16.5. Found: C, 55.1; H, 7.14; N, 16.8.

O-Benzyl-N-carbobenzoxy-L-tyrosyl-L-isoleucylglycyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—Free heptapeptide (2.4 g.) dissolved in 15 ml. of dimethylformamide was allowed to react with 1.7 g. of p-nitrophenyl O-benzyl-N-carbobenzoxy-L-tyrosinate. After 2 days 200 ml. of ethyl acetate was added and the resulting precipitate was collected and triturated with 100 ml. of ethyl acetate, 100 ml. of ethanol, and 50 ml. of ether. After being dried *in vacuo* at 56° for a few hours, the compound weighed 2.8 g., m.p. 227–229°, $[\alpha]^{19.5}D$ $-40.9°$ (c 1, dimethylformamide).

Anal. Calcd. for $C_{59}H_{76}N_{10}O_{12}S$: C, 61.7; H, 6.67; N, 12.2. Found: C, 61.6; H, 6.78; N, 12.2.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucylglycyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. A. By Nitrophenyl Ester Method.—The protected octapeptide, 2.2 g., in 15 ml. of dimethylformamide

(23) Capillary melting points were determined for all compounds and are corrected. Paper chromatography was performed on Whatman No. 1 paper in 1-butanol–acetic acid–water (4:1:5, descending), and ninhydrin and platinum reagent [G. Toennies and J. J. Kolb, *Anal. Chem.*, **23**, 823 (1951)] were used for development. Paper electrophoresis was carried out at 4° for 18 hr. in pyridine acetate buffer of pH 5.6 at 300 v. and brom phenol blue–mercuric chloride reagent [E. L. Durrum, *J. Am. Chem. Soc.*, **72**, 2943 (1950)] was used for development.

was treated with 15 ml. of 25% HBr in glacial acetic acid. After 1.5 hr. the hydrobromide was precipitated with ether, dried, and dissolved in 15 ml. of dimethylformamide. The solution was cooled to 0° and the pH was adjusted to 8 by addition of triethylamine. The triethylamine hydrobromide was removed by filtration and 0.98 g. of *p*-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate was added to the filtrate. After 2 days at room temperature 200 ml. of ethyl acetate was added. The oily precipitate was kept for 2 hr. at −20° and the solidified material was collected and washed with ethanol (30 ml.) and ether (30 ml.). For further purification the material was dissolved in 30 ml. of dimethylformamide and allowed to stand for 40 hr. at 4° after the addition of 100 ml. of 0.1% acetic acid. The solid was collected, washed with methanol (50 ml.), ethanol (50 ml.), ethyl acetate (50 ml.), and ether (20 ml.), and dried *in vacuo* at 64°, giving 1.2 g., m.p. 225–227°, $[\alpha]^{18.5}D$ −60.6° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{62}H_{81}N_{11}O_{13}S_2$: C, 59.5; H, 6.47; N, 12.3. Found: C, 59.8; H, 6.64; N, 12.4.

B. By Azide Method.—S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosine hydrazide[7] (0.4 g.) was dissolved in 4.8 ml. of 95% tetrahydrofuran containing 4.5 *N* HCl and treated at −20° dropwise under stirring with a cold solution of 0.06 g. of sodium nitrite in 0.3 ml. of water. The reaction mixture was stirred at the same temperature for 10 min., diluted with 30 ml. of ethyl acetate, washed three times with 10-ml. portions of a precooled saturated solution of sodium bicarbonate containing a small amount of sodium chloride, and dried over magnesium sulfate before being added to a cold solution of 0.64 g. of L-isoleucylglycyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide in 20 ml. of dimethylformamide. The mixture was stirred for 12 hr. at 4° and then for 2 hr. at room temperature. Ethyl acetate (100 ml.) was added and the precipitate was collected and digested with ethanol (20 ml.), methanol (20 ml.), and ether (20 ml.), giving 0.7 g., m.p. 219–222°. The product was precipitated from glacial acetic acid with water, collected, washed with methanol, and dried at 100° *in vacuo;* m.p. 220–222°, $[\alpha]^{18.5}D$ −60.6° (*c* 1, dimethylformamide).

4-Glycine-oxytocin.—The procedure for the reduction of the protected nonapeptide intermediate with sodium in liquid ammonia and conversion to 4-glycine-oxytocin was essentially the same as that described for the synthesis of oxytocin.[14] The protected nonapeptide (230 mg.) was dissolved in 250 ml. of liquid ammonia (freshly distilled from sodium) and treated at the boiling point with sodium until a faint blue color enveloped the solution. Glacial acetic acid (0.1 ml.) was added and the residue obtained after evaporation of the ammonia *in vacuo* was dissolved in 200 ml. of 0.1% acetic acid. After adjustment of the pH to 6.8 the solution was oxidized by aeration with CO_2-free air for 1.5 hr. and then by titration with 0.02 *N* potassium ferricyanide. Ferrocyanide and ferricyanide ions were removed by passing the oxidized solution down a column of AG 3X4 resin in the chloride form. This solution was found to possess a total of 554 units of avian depressor activity. After concentration to a volume of 50 ml. it was placed in the first five tubes of a Craig countercurrent distribution machine and subjected to a total of 500 transfers in the solvent system 1-butanol–1-propanol–0.5% acetic acid containing 0.1% pyridine (6:1:8). A main peak with a *K* value of approximately 0.8 was obtained as determined by the Folin–Lowry[24] color values. The avian depressor activity was associated with the material of this peak. Concentration and lyophilization of the solutions from the central portion of the peak yielded 102 mg. of 4-glycine-oxytocin. For partition chromatography on Sephadex, the material (85 mg.) was dissolved in 7 ml. of the upper phase of the solvent system 1-butanol–1-propanol–3.5% acetic acid containing 1.5% pyridine (6:1:8, pH of lower phase 4.4) and applied to a Sephadex G-25 column (2.16 × 120 cm.) which had been equilibrated with both phases. Elution with the upper phase was performed and 4.3-ml. fractions were collected at a flow rate of about 17 ml. per hr. The Folin–Lowry color values showed one peak having an R_f (Sephadex) of 0.52 accompanied by a small amount of faster-moving material. The recovery of 4-glycine-oxytocin from this peak was 63 mg. The rotation of the compound was negligible in 1 *N* acetic acid, but in dimethylformamide it had $[\alpha]^{19}D$ +16° (*c* 0.5), R_f 0.58 (paper chromatography). On paper electrophoresis the product traveled as a single spot to the cathode.

For analysis a sample was dried at 100° over P_2O_5 *in vacuo* and a loss in weight of 8.2% was observed.

Anal. Calcd. for $C_{40}H_{61}N_{11}O_{11}S_2$: C, 51.3; H, 6.53; N, 16.5. Found: C, 51.4; H, 6.72; N, 16.2.

A sample was hydrolyzed in 6 *N* hydrochloric acid at 110° for 17 hr. and then analyzed by the method of Spackman, Stein, and Moore[25] in the 30–50° system on a Beckman–Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were obtained, with the value of aspartic acid taken as 1.0: aspartic acid 1.0, proline 1.0, glycine 2.0, cystine 1.0, isoleucine 1.0, leucine 1.0, tyrosine 0.9, ammonia 2.0.

Carbobenzoxyglycyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—To a solution of 3.6 g. of L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[9,10] in 20 ml. of dimethylformamide 1.8 g. of *p*-nitrophenyl carbobenzoxyglycinate was added. After 40 hr. 200 ml. of ethyl acetate was added and the precipitate was collected and washed with ethanol (50 ml.) and ether (20 ml.), giving 3.7 g., m.p. 202–204°, $[\alpha]^{19}D$ −50.9° (*c* 1, dimethylformamide). No change in melting point was observed when the product was dissolved several times in hot 80% ethanol and allowed to separate.

Anal. Calcd. for $C_{46}H_{58}N_{11}O_{10}S$: C, 55.4; H, 6.42; N, 15.4. Found: C, 55.2; H, 6.45; N, 15.1.

Glycyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—Protected heptapeptide (6.4 g.) was suspended in acetic acid (30 ml.) and treated with HBr in acetic acid (37%, 20 ml.). After 1.5 hr. at room temperature the hydrobromide was precipitated with ether, dried, taken up in 150 ml. of methanol, and passed through IRA-410 resin (OH form). Removal of the solvent from the eluate left 4.3 g. of residue, m.p. 206–211°, which was dissolved in 40 ml. of boiling water and then cooled. Since no separation of product occurred, the solution was concentrated to half-volume whereupon 2.6 g. of gelatinous solid, m.p. 214–216°, separated; 0.7 g. of this material was subjected to countercurrent distribution for a total of 500 transfers in the solvent system 1-butanol–1-propanol–benzene–1% acetic acid containing 0.15% pyridine (2:1:1:4). A main peak with a *K*-value of approximately 0.11 was found, accompanied by a small slower-moving peak. The recovery of the material from the central part of the main peak was 0.43 g., $[\alpha]^{20}D$ −51.3° (*c* 1, dimethylformamide), R_f 0.25 (paper chromatography).

Anal. Calcd. for $C_{34}H_{52}N_{10}O_9S$: C, 52.6; H, 6.75; N, 18.0. Found: C, 52.4; H, 6.79; N, 18.0.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosylglycyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—A solution of S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosine azide in ethyl acetate, obtained from 0.8 g. of the corresponding hydrazide as already described, was added to a cooled solution of 1.28 g. of glycyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide in 40 ml. of dimethylformamide. After the mixture was stirred for 12 hr. at 4° and 3 hr. at room temperature, 200 ml. of ethyl acetate was added to the milky solution. The resulting precipitate was collected and washed with 100 ml. of ethyl acetate, heated with 20 ml. of methanol, washed with ether, and dried *in vacuo* at 56° for 5 hr., giving 1.2 g., m.p. 210–212°, $[\alpha]^{20}D$ −50.3° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{61}H_{78}N_{12}O_{14}S_2$: C, 57.8; H, 6.20; N, 13.3. Found: C, 57.5; H, 6.75; N, 13.4.

3-Glycine-oxytocin.—The procedures for the reduction with sodium in liquid ammonia of 300 mg. of protected nonapeptide and for the subsequent oxidation by aeration and titration with potassium ferricyanide were the same as those used in the preparation of 4-glycine-oxytocin. The oxidized solution after removal of ferrocyanide and ferricyanide ions by means of resin AG 3X4 was concentrated to 50 ml., placed in the first five tubes of the countercurrent distribution machine, and submitted to a total of 700 transfers in 1-butanol–1-propanol–0.5% acetic acid containing 0.1% pyridine (6:1:8). One peak with a *K*-value of 0.11 was detected upon determination of the Folin–Lowry color values. Concentration and lyophilization of the contents of 40 tubes from the central portion of this peak gave 209 mg. of 3-glycine-oxytocin. On redistribution for a total of

(24) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

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

500 transfers in 1-butanol–1-propanol–1.5% acetic acid containing 3.5% pyridine (2:1:3) the 3-glycine-oxytocin traveled as one peak with a K-value of 0.38. From the contents of 45 tubes from the central portion of this peak 178 mg. of 3-glycine-oxytocin was isolated by concentration and lyophilization.

3-Glycine-oxytocin (90 mg.) was dissolved in 2 ml. of the upper phase of the solvent system 1-butanol–1-ethanol–pyridine–1 N acetic acid (pH of lower phase 4.9, 4:1:1:7) and submitted to partition chromatography on a Sephadex G-25 column (21.6 × 120 cm.). The sample was eluted with the upper phase and 6.6-ml. fractions were collected at a flow rate of about 20 ml. per hr. One peak with R_f 0.26 (Sephadex) was detected by measurement of Folin–Lowry color values. Concentration and lyophilization of the contents of this peak gave 65 mg. of 3-glycine-oxytocin, $[\alpha]^{20}D$ −62.0° (c 0.5, 1 N acetic acid), R_f 0.36 (paper chromatography). On electrophoresis the product traveled as one spot to the cathode.

For elemental analysis the product was dried at 100° over P_2O_5 *in vacuo* and a weight loss of 7.8% was observed.

Anal. Calcd. for $C_{39}H_{58}N_{12}O_{12}S_2$: C, 49.2; H, 6.15; N, 17.7. Found: C, 49.0; H, 6.11; N, 17.6.

The amino acid analysis gave the following expected molar ratios of amino acids and ammonia with the value of glutamic acid taken as 1: aspartic acid 1.0, glutamic acid 1.0, proline 1.0, glycine 2.0, cystine 1.0, leucine 1.1, tyrosine 1.0, ammonia 2.9.

Carbobenzoxyglycyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.—L-Isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[11] (1.57 g.) was dissolved by gentle heating in 30 ml. of dimethylformamide and cooled before the addition of 0.72 g. of p-nitrophenyl carbobenzoxyglycinate. The reaction was allowed to proceed for 2 days at room temperature and the product was then triturated with 200 ml. of ethyl acetate, collected, washed with ethanol (100 ml.) and ether (50 ml.), and dried *in vacuo* at 56° for 5 hr., giving 1.8 g., m.p. 229–231°, $[\alpha]^{20.5}D$ −49.4° (c 1, dimethylformamide).

Anal. Calcd. for $C_{48}H_{69}O_{12}N_{11}S$: C, 56.3; H, 6.80; N, 15.0. Found: C, 56.3; H, 6.95; N, 14.9.

S-Benzyl-N-carbobenzoxy-L-cysteinylglycyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. A. By Nitrophenyl Ester Method.—A suspension of 1.5 g. of protected octapeptide in 10 ml. of glacial acetic acid was treated with 10 ml. of 32% HBr in glacial acetic acid. After 1.5 hr. the hydrobromide was precipitated with ether, dried, dissolved in 200 ml. of methanol, and passed down a column of resin IRA-410 (OH form). The residue after removal of solvent from the eluate was suspended in 50 ml. of dimethylformamide. To the resulting thick gel 0.82 g. of p-nitrophenyl S-benzyl-N-carbobenzoxy-L-cysteinate was added and the mixture was allowed to stand for 8 days during which time the gel disappeared. Ethyl acetate (200 ml.) was then added and the resulting gel was kept at −22° for 4 hr. The solid was collected, and washed with ethyl acetate (100 ml.), ethanol (100 ml.), and ether (50 ml.). The slightly yellow product was reprecipitated from glacial acetic acid (10 ml.) with water and dried at 64° *in vacuo* for 8 hr., giving 0.8 g., m.p. 229–232°, $[\alpha]^{19}D$ −71.1° (c 1, glacial acetic acid).

Anal. Calcd. for $C_{58}H_{80}N_{12}O_{13}S_2$: C, 57.2; H, 6.62; N, 13.8. Found: C, 57.5; H, 6.86; N, 13.7.

B. By Azide Method.—A solution of 0.58 g. of S-benzyl-N-carbobenzoxy-L-cysteinylglycine hydrazide[12] in 7 ml. of 50% acetic acid containing 0.6 ml. of concentrated hydrochloric acid was treated at 0° with 1.2 ml. of a 10% solution of sodium nitrite. The precipitated azide was extracted with cold ethyl acetate (25 ml.) and the extract was washed three times with a 10% solution of sodium bicarbonate and dried over sodium sulfate. This solution of the azide was then added to a cold solution of 1.28 g. of L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide in 40 ml. of dimethylformamide. The mixture was stirred for 14 hr. at 4° and then for 2 hr. at room temperature. The product precipitated by addition of ethyl acetate (100 ml.) was collected, washed with ethanol (50 ml.), triturated with hot methanol (50 ml.), collected, and dried *in vacuo* at 64°, giving 1.3 g., m.p. 231–234°, $[\alpha]^{19}D$ −70.9° (c 1, glacial acetic acid).

2-Glycine-oxytocin.—The conditions already described were used for reduction of 344 mg. of the protected nonapeptide with sodium in liquid ammonia and subsequent oxidation. The oxidized solution after concentration to a volume of 50 ml. was placed in the first five tubes of the countercurrent distribution machine and subjected to a total of 700 transfers in the solvent system 1-butanol–1-propanol–0.5% acetic acid containing 0.1% pyridine (6:1:8). The main peak as determined by Folin–Lowry color values had a partition coefficient of approximately 0.18 and was accompanied by a small slower-moving peak with a K of 0.11. From the tubes in the central part of the main peak 180 mg. of material was obtained after concentration and lyophilization. The partition chromatography of this material on a Sephadex G-25 column (2.16 × 120 cm.) in the solvent system 1-butanol–1-propanol–3.5% acetic acid containing 1.5% pyridine (2:1:3, pH of lower phase 4.1) with the collection of 6.5-ml. fractions resulted in one peak with R_f 0.19 (Sephadex). Concentration and lyophilization of the contents of this peak gave 108 mg. of 2-glycine-oxytocin, $[\alpha]^{20}D$ −41.5° (c 0.5, 1 N acetic acid), R_f 0.38 (paper chromatography). On electrophoresis the product moved to the cathode as a single spot.

For analysis the sample was dried at 100° over P_2O_5 *in vacuo* and the loss in weight was 9.88%.

Anal. Calcd. for $C_{35}H_{60}N_{12}O_{11}S_2$: C, 48.0; H, 6.67; N, 18.7. Found: C, 47.9; H, 6.67; N, 18.5.

Amino acid analysis gave the following molar ratios of amino acids and ammonia with the value of glutamic acid taken as 1.0: aspartic acid 1.0, glutamic acid 1.0, proline 1.0, glycine 2.0, cystine 1.0, isoleucine 1.1, leucine 1.0, ammonia 3.0.

Discussion

The results on the 4-glycine analog are of particular interest in connection not only with the 4-L-α-aminobutyric acid-oxytocin (4-decarboxamido-oxytocin)[4] but also with 4-L-serine-oxytocin,[26] 4-L-alanine-oxytocin,[26] and the naturally occurring posterior pituitary hormone, isotocin (4-L-serine-8-L-isoleucine-oxytocin).[27] A comparison of the activities of these compounds is given in Table I. As noted in the table, the 4-alanine analog is less active in general than the 4-α-aminobutyric acid analog, but far more active than 4-glycine-oxytocin. The milk-ejecting potency of the alanine analog is almost the same as that of the α-aminobutyric acid analog. The structural difference between 4-glycine-oxytocin and 4-alanine-oxytocin is simply the substitution of a methyl group for a hydrogen on the carbon of the glycine residue in position 4 of the backbone structure of oxytocin. The absence of this methyl group results in a drastic loss in potency. It should be noted, however, that in 4-glycine-oxytocin a nonasymmetric amino acid residue replaces the amino acid residue of the L-configuration occurring in 4-alanine-oxytocin. Loss of asymmetry at this location could be significant with reference to conformation. It would be interesting to compare the glycine analog with 4-D-alanine-oxytocin. The addition of an hydroxyl group to the side chain of 4-L-alanine-oxytocin, as in 4-L-serine-oxytocin, enhances the potency many fold. In fact, the activities of the 4-L-serine-oxytocin compare favorably with those of isotocin.

Although the 4-glycine-oxytocin has low but appreciable activity, the 2-glycine-oxytocin and 3-glycine-oxytocin are practically inactive. Substitution of the tyrosine residue in the 2-position of oxytocin by phenylalanine gives deoxyoxytocin (2-phenylalanine-oxytocin), which possesses considerable activity.[28–31]

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TABLE I

EFFECT OF STRUCTURAL CHANGES IN POSITION 4 ON THE PHARMACOLOGICAL ACTIVITIES OF OXYTOCIN

Compound	Activities, units/mg. Depressor (fowl)	Oxytocic (rat)	Milk-ejecting (rabbit)	Pressor (rat)	Antidiuretic (rat)
Oxytocin[3]	507 ± 15	486 ± 5	410 ± 16	3.1 ± 0.1	2.7 ± 0.2
4-Decarboxamido-oxytocin[4] (4-L-α-aminobutyric acid-oxytocin)	108 ± 5	72 ± 2	225 ± 7	~0.1	0.2–0.3
4-L-Alanine-oxytocin[26]	65 ± 3	36 ± 6	240 ± 55	<0.01	<0.01
4-Glycine-oxytocin	5.5 ± 0.2	2.8 ± 0.1	17 ± 1	<0.002	<0.005
4-L-Serine-oxytocin[26]	230 ± 20	195 ± 30	255 ± 45	<0.1	0.06 ± 0.01
Isotocin[27] (4-L-serine-8-L-isoleucine-oxytocin)	320 ± 15	150 ± 12	300 ± 15	0.06 ± 0.01	0.18 ± 0.03

On the other hand, 2-leucine-oxytocin[31] has extremely low activity and 2-serine-oxytocin[32] has practically none. It is therefore not surprising that the 2-glycine-oxytocin was practically inactive in the bioassays performed.

With regard to the 3-position, substitution of the isoleucine residue in this position by a 5- or 6-carbon aliphatic amino acid[30,33,34] and even by the aromatic amino acid phenylalanine (oxypressin)[33,35] gives rise to analogs showing appreciable activity. The lack of appreciable activity in 3-glycine-oxytocin is thus of interest.

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COMMUNICATIONS TO THE EDITOR

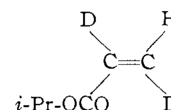
Stereospecificity in the Anionic Polymerization of Isopropyl Acrylate

Sir:

Natta, *et al.*,[1] have observed that the polarized infrared spectra of polymers of *cis*-1-d_1-propene and of *trans*-1-d_1-propene are quite different. Miyazawa and Ideguchi[2] have shown by infrared studies that *cis*-1-d_1-propene yields the *erythro*-diisotactic polymer (D on the opposite side of the planar zigzag from the methyl group) while the *trans*-1-d_1-propene yields the *threo*-diisotactic polymer (D on the same side). These results were interpreted as indicating that with the Ziegler–Natta catalyst employed, *cis* opening of the double bond occurs, as concluded by Natta, *et al.*,[3] for *trans*-propenyl isobutyl ether.

No studies have been reported of the stereospecificity, with respect to the methylene group, of the polymerization of any vinyl or vinylidene monomer other than propene or with any catalyst other than the Ziegler–Natta type. In this communication, we report an investigation by n.m.r. of the stereospecificity of the Grignard-initiated polymerization of isopropyl acrylate.

Isopropyl α-*cis*-β-d_2-acrylate was prepared by *trans* addition of deuterium to isopropyl propiolate.[4] The



structure was confirmed by n.m.r. (7.5% (w./v.) solution in toluene; Varian DP-60 spectrometer) which showed in the vinyl region a 1:1:1 triplet at τ 4.71, the spacing of which (*ca.* 1.6 c.p.s.) corresponds to *cis*-H^1–H^1 coupling (10.4 c.p.s.); the geminal coupling was, as expected, too small to observe. There were indications of some residual α- and *cis*-β-protons. Polymerization was carried out at *ca.* −78° (seven volumes of toluene, one volume of monomer, 8 mole % of phenylmagnesium bromide), the Grignard reagent being added under high vacuum; no ether was present. The polymer was purified by reprecipitation with petroleum ether and ethanol and freeze-drying from benzene. Nondeuterated polymer was prepared in the same manner.

The polymer prepared under these conditions is believed to be highly isotactic.[5] This was confirmed by the n.m.r. spectra, obtained on 14.0% (w./v.) solutions in chlorobenzene at 150°. In Fig. 1, the septet of the carbinyl proton (τ 4.96) and the doublet of the methyl protons (τ 8.76, J = 6.14) of the side chain are not shown. The observed backbone proton

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