filtrate was evaporated under pressure. The residue was at once refluxed for 2 hr. with concentrated HCl (180 ml.) and water (180 ml.). After cooling, recovered acid (30 g.) was removed by filtration and the filtrate was basified and extracted with ether. Evaporation of the ether left the **amine** as an oil, the **hydrochloride** (12 g.) separating from 2-propanol–ether as colorless needles, m.p. 228–229°.

Anal. Calcd. for $C_{11}H_{15}N \cdot HCl$: C, 66.82; H, 8.16; N, 7.09. Found: C, 66.97; H, 8.07; N, 7.20.

Several attempts to oxidize the amine as described for *t*-butylamine[17] failed to give identifiable products.

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9-Deamidooxytocin, an Analog of the Hormone Containing a Glycine Residue in Place of the Glycinamide Residue[1]

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The synthesis of 9-deamidooxytocin, in which the glycinamide residue at position 9 in oxytocin has been replaced by a glycine residue, is described. The synthetic intermediate of this analog was the protected nonapeptide benzyl ester, benzyl N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinate. From it the reduced form of the analog was prepared by the action of sodium in liquid ammonia, and the analog itself was subsequently obtained by oxidative cyclization. The biological properties of the compound, in terms of the pharmacological activities exhibited by oxytocin, have been determined and are reported.

The present communication reports the synthesis of 9-deamidooxytocin, an analog of oxytocin in which the glycinamide residue in the terminal position of the side chain of the molecule has been replaced by that of glycine, and includes the comparison of various biological activities of this analog with the hormone itself, the structure of which is shown in Figure 1. In a previous communication such a comparison was made between the pharmacological behavior of the analog in which the glutamine residue at position 4 was replaced by that of glutamic acid.[2] This 4-deamidooxytocin (4-glutamic acid oxytocin) was found to possess approximately 1/1000 of the avian depressor, 1/300 of the oxytocic, and 1/50 of the milk-ejecting activities of oxytocin. Thus, these activities, characteristic of oxytocin, were drastically reduced by replacement of the carboxamide group at position 4 by a carboxyl group, and we therefore became interested in determining whether replacement of a carboxamide group by a carboxyl at position 9 would have a comparable effect.

For the synthesis of 9-deamidooxytocin, a protected nonapeptide benzyl ester intermediate was synthesized, the benzyl ester function being at the position where the free carboxyl group was ultimately desired. Treatment of the protected nonapeptide ester with sodium in liquid ammonia by the method of Sifferd and du Vigneaud,[3] as used in the synthesis of oxytocin,[4] cleaved the protecting benzyl groups from the cysteine sulfhydryl groups and the carbobenzoxy group from the 1-cysteine amino group, and at the same time cleaved the benzyl ester to liberate the free carboxylic acid. The analog itself was obtained by subsequent oxidation and was purified by countercurrent distribution. The protected nonapeptide ester, benzyl N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinate, was prepared by a dicyclohexylcarbodiimide coupling[5] of the protected pentapeptide, N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine,[6] and the tetrapeptide benzyl ester, benzyl S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinate. The latter compound was obtained by a sequence of reactions starting with the coupling, by the mixed-anhydride method,[7] of N-carbobenzoxy-L-prolyl-L-leucine[8] and benzyl glycinate[9] to give benzyl N-carbobenzoxy-L-prolyl-L-leucylglycinate. Hydrogen bromide in acetic acid was used to remove the carbobenzoxy group from this protected tripeptide and the resultant product was coupled with N-carbobenzoxy-S-benzyl-L-cysteine[10] to give benzyl N-carbobenzoxy-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinate, from which the carbobenzoxy group was removed by hydrogen bromide in acetic acid.

The protecting groups were removed from the protected nonapeptide ester by the action of sodium in liquid ammonia, and oxidative ring closure was accomplished by aeration[4] followed by treatment with aqueous potassium ferricyanide solution.[11] The ferrocyanide and excess ferricyanide ions were removed

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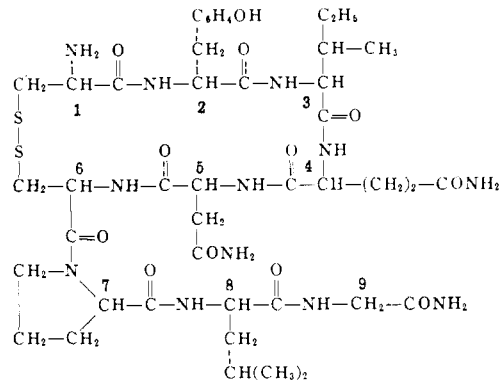

Figure 1.—Oxytocin, with numbers indicating the positions of the individual amino acid residues.

by passage through a column of AG 3-X4 resin, and the solution was concentrated and submitted to countercurrent distribution[12] in the solvent system: water (containing 0.5% acetic acid and 0.1% pyridine)–1-propanol–1-butanol (8:1:6).[13] In this system 9-deamidooxytocin had a partition coefficient (K) of 0.67.

Avian depressor assays[14] of the 9-deamidooxytocin, so obtained, indicated a potency of approximately 0.8 unit/mg. This did not account for the activity detected in the crude oxidized solution and the possibility was considered that there existed contamination by oxytocin, which may have been produced by amidation of the benzyl ester during the treatment in liquid ammonia. In the solvent system described, oxytocin has a K of 0.45,[13] which should mean that it would travel separately from, but close to, 9-deamidooxytocin, if there is no interaction between the two species. In another preparation using 200 mg. of the protected nonapeptide, the total avian depressor activity detected prior to countercurrent distribution was 55 units. This would be produced by an oxytocin contamination of 0.1 mg., a quantity which could not readily be detected in a mixture by available chemical means. After countercurrent distribution, the contents of the tubes in which oxytocin would be expected, although none had been detected by the Folin–Lowry color reaction,[15] were pooled, concentrated, and assayed and were found to possess 44 units of avian depressor activity. The peak representing the analog was then halved and the solid materials obtained from the two halves were assayed separately. The half nearer to the oxytocin position was found to have approximately 0.4 unit/mg. of avian depressor activity, while the half distant from oxytocin possessed approximately 0.2 unit/mg. These results indicate that 9-deamidooxytocin may have some weak intrinsic avian depressor activity, but that the higher activity initially detected was due to slight contamination with oxytocin.

The 9-deamidooxytocin having an apparent avian depressor activity of 0.2 unit/mg. was assayed for its oxytocic,[16] milk-ejecting,[17] antidiuretic,[18] and pressor activities.[19] The results of these assays are shown in Table I, along with the corresponding values, already reported from this laboratory, for oxytocin[20] and 4-deamidooxytocin.[2] It should be noted that the ratios of the potencies found in the different assays are quite different from those of oxytocin.

TABLE I

BIOLOGICAL POTENCIES OF OXYTOCIN,[a] 4-DEAMIDOOXYTOCIN,[b] AND 9-DEAMIDOOXYTOCIN[c]

Compd.	Depressor (fowl)	Oxytocic (rat)	Milk-ejecting (rabbit)	Antidiuretic (rat)	Pressor (rat)
Oxytocin	505	515[d,e]	410	2.7	3.1
4-Deamidooxytocin	~0.5	~1.5[d]	~11	Nil	Nil
9-Deamidooxytocin	~0.2	1.3[d]	1.2	~0.02	Nil

[a] See ref. 20. [b] See ref. 2. [c] Values are given in units per milligram. [d] See ref. 16. [e] See ref. 20b.

9-Deamidooxytocin has also been synthesized by Klostermeyer,[21] a sample of whose preparation was sent to us by Professor H. Zahn for bioassay in this laboratory. It was found to have no avian depressor activity and an oxytocic activity of 1.8 units/mg.

Experimental Section[22]

Benzyl N-Carbobenzoxy-L-prolyl-L-leucylglycinate.—To triethylamine (1.4 ml.) and 2.9 g. of N-carbobenzoxy-L-prolyl-L-leucine[8] in 40 ml. of freshly distilled tetrahydrofuran, cooled to −10°, was added 1.1 g. of isobutyl chloroformate in 20 ml. of tetrahydrofuran. The mixture was stirred at this temperature for 20 min., then treated with 3.24 g. of glycine benzyl ester *p*-toluenesulfonate[9] in 10 ml. of water containing 1.4 ml. of triethylamine. Stirring was continued at room temperature for 90 min., and the mixture was acidified (cooling) by the addition of concentrated HCl. The tetrahydrofuran was removed by evaporation in a rotary evaporator under reduced pressure. This caused separation of an oily product, which partially solidified when it was kept at 4° overnight. It was dissolved in 10 ml. of ethanol, reprecipitated by the addition of water, and kept overnight at 4°. The solution and reprecipitation were repeated until, after the fourth time, the product solidified well on being kept overnight at room temperature. It was filtered and dried; yield 3.6 g., m.p. 76–78°. A Lassaigne test showed that it contained no sulfur. A sample was crystallized successively from ethyl acetate–petroleum ether (30–75°), benzene–petroleum ether, tetrahydrofuran–water, and then repeatedly from large volumes of ethanol–water until it had constant properties of m.p. 93–95°, $[\alpha]^{21}D$ −75.0° (*c* 1, 95% ethanol).

Anal. Calcd. for $C_{28}H_{35}N_3O_6$: C, 66.0; H, 6.92; N, 8.27. Found: C, 65.5; H, 6.86; N, 8.25.

Benzyl N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-

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

(13) This solvent system is one of a series containing pyridine, developed by Dr. Derek Jarvis in this laboratory. Their use for the countercurrent distribution of oxytocin and related compounds was found to reduce emulsification greatly.

(14) Avian depressor assays were carried out according to the method of R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *Endocrinology*, **66**, 860 (1960).

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(17) Milk-ejecting assays were performed according to H. B. van Dyke, K. Adamsons, Jr., and S. L. Engel, *Recent Progr. Hormone Res.*, **11**, 1 (1955), modified by W. Y. Chan, *J. Pharmacol. Exptl. Therap.*, **147**, 48 (1965).

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(19) Rat pressor assays were carried out as described in "The Pharmacopeia of the United States of America," 11th Rev., Mack Publishing Co., Easton, Pa., 1960, p. 793.

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L-leucylglycinate.—Benzyl N-carbobenzoxy-L-prolyl-L-leucylglycinate (0.85 g.) was dissolved in 2 ml. of acetic acid and stirred with 4 ml. of HBr in acetic acid (31% w./w.) until the evolution of CO_2 had stopped (15 min.). This solution was poured into 100 ml. of stirred, dry ether, and the solid tripeptide ester hydrobromide which separated was washed several times by decantation of dry ether. It was kept under ether for 1 hr., filtered, washed thoroughly with ether, and dried *in vacuo* over $CaCl_2$. It was dissolved in 5 ml. of acetic acid, reprecipitated by pouring into ether, then washed, isolated, and dissolved in 5 ml. of anhydrous methanol. The precipitation and washing with ether were repeated to give a solid which was dissolved in 15 ml. of methanol and treated with Dowex IRA-410 resin (OH form), with stirring, until the pH of the solution was approximately 8. The resin was filtered and washed with methanol. The combined filtrate and washings were evaporated under reduced pressure to give a pale yellow oil (0.46 g.). It was dissolved with 0.46 g. of N-carbobenzoxy-S-benzyl-L-cysteine[10] in 2 ml. of freshly distilled tetrahydrofuran. The solution was cooled in ice–water and to it was added 0.29 g. of dicyclohexylcarbodiimide in 1 ml. of tetrahydrofuran. The mixture was kept at room temperature overnight and diluted with 15 ml. of ethyl acetate. The insoluble dicyclohexylurea was filtered and washed with 5 ml. of ethyl acetate. The combined filtrate and washings were evaporated to give a colorless oil, which was dissolved in 20 ml. of ethyl acetate and washed successively with 1 *N* HCl, water, 0.5 *N* $NaHCO_3$, water, and saturated saline. The solution was dried ($MgSO_4$), filtered, and evaporated to give 0.73 g. of a colorless oil. Of this, 0.6 g. was dissolved in 2 ml. of acetic acid and stirred with 3 ml. of HBr in acetic acid (31% w./w.) until the evolution of CO_2 had stopped (12 min.), when the solution was poured into 100 ml. of dry ether. The solid S-benzyl tetrapeptide benzyl ester hydrobromide which separated was purified and the free base was isolated by the method already described for the preparation of the tripeptide ester. The product was obtained as a pale yellow oil (0.39 g.). N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine (0.57 g.)[6] was suspended in 4 ml. of dimethylformamide at 0° and to it was added with stirring, 0.44 g. of dicyclohexylcarbodiimide. After 5 min. at 0°, a solution of the S-benzyl tetrapeptide benzyl ester (0.39 g.) in 2 ml. of dimethylformamide was added to the mixture which was then stirred at room temperature for 2 hr. and kept at 3° for 48 hr. To it were then added 0.8 ml. of acetic acid and 70 ml. of water. This caused a white solid to separate, and after being stirred for 0.5 hr. it was filtered, washed thoroughly with water, and dried *in vacuo* over P_2O_5 to give 1.22 g. of product which was dissolved in 7 ml. of dimethylformamide. The solution was filtered from dicyclohexylurea, and water was added to the filtrate until precipitation was complete. The separated solid was filtered, washed with water, and dried *in vacuo* over P_2O_5 to give 0.76 g. of off-white amorphous material, which was dissolved in 5 ml. of dimethylformamide. The peptide material was reprecipitated by the addition of 10 ml. of 1-propanol followed by hexane to saturation. The separated solid was filtered, ground in a mortar under 30 ml. of methanol, filtered again, and washed with methanol, then ether. The white product was dried *in vacuo;* yield 0.3 g., m.p. 228–230°, $[\alpha]^{20}D$ −24.0° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{72}H_{91}N_{11}O_{15}S_2$: C, 61.1; H, 6.48; N, 10.9. Found: C, 60.7; H, 6.49; N, 10.8.

9-Deamidooxytocin.—The preceding compound (200 mg.) was dissolved in 150 ml. of boiling, redistilled liquid ammonia. A freshly prepared sodium stick was dipped into the stirred solution and was removed whenever the whole solution became blue. When this color faded (12 sec.) the stick was momentarily reintroduced into the solution. After the color so produced had faded, the ammonia was removed by lyophilization (water pump) and the solid residue was dissolved in 200 ml. of 0.25% acetic acid. The pH of this solution was adjusted to 6.5 and a slow stream of air (CO_2-free) was bubbled through it for 5 hr. Potassium ferricyanide (0.02 *N*, 2 ml.) was then added and was not consumed. Any ferrocyanide and excess ferricyanide ions were removed by passage of the solution through a column of AG 3-X4 resin (4 × 4 cm.). The column was washed with water and the volume of the combined eluate and washings was made up to 250 ml. Of this, 1 ml. was diluted to 5 ml. and assayed for avian depressor activity. The volume of the solution was reduced to 50 ml. by evaporation in a rotary evaporator under reduced pressure, with the temperature not exceeding 25°, and it was then submitted to countercurrent distribution in the solvent system 1-butanol–1-propanol–water containing 0.5% acetic acid and 0.1% pyridine (6:1:8).[13] After 300 transfers the distribution was visualized by the development of the Folin–Lowry color[15] of samples of lower phase. Three peaks of partition coefficients 0.11, 0.67, and 1.4 were seen. On examination, the materials of K = 0.11 and K = 1.4 were obviously by-products. The contents of the tubes representing the peak of K = 0.67 were concentrated and lyophilized. The average yield of lyophilized powder in three preparations was 28 mg., $[\alpha]^{19}D$ −31.7° (*c* 0.6, 1 *N* acetic acid).

Anal. Calcd. for $C_{43}H_{65}N_{11}O_{13}S_2$: C, 51.2; H, 6.50; N, 15.3. Found: C, 51.4; H, 6.57; N, 15.2.

A sample was hydrolyzed in 6 *N* HCl at 110° for 22 hr. in an evacuated tube and analyzed for amino acids on a Beckman-Spinco analyzer.[23] The following molar ratios were obtained, with the value of aspartic acid being taken as 1: aspartic acid 1.0, proline 1.0, glycine 0.9, glutamic acid 1.0, cystine 1.0, isoleucine 1.0, leucine 1.0, tyrosine 0.9, and ammonia 2.2.

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Synthesis of β-(4-Hydroxy-1-naphthyl)-DL-alanine[1]

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The synthesis of β-(4-hydroxy-1-naphthyl)-DL-alanine by hydrolysis of 5-(4-hydroxy-1-naphthyl)methylene-hydantoin or -thiohydantoin has been carried out. This new synthetic amino acid, an analog of tyrosine, is useful for histochemical demonstration of tyrosinase activity.

Our interest in the preparation of β-(4-hydroxy-1-naphthyl)-DL-alanine (XII) was initiated because of its possible use as a chromogenic substrate for histochemical demonstration of protein or enzyme synthesis. This amino acid, by virtue of its structural similarity to tyrosine, could be a substrate for tyrosinase or a good competitive amino acid to undergo peptide synthesis where tyrosine is involved.[2a] Histochemical visualiza-

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