The Synthesis and Some Pharmacological Activities of [4-L-Norvaline]-oxytocin and [4-L-Norleucine]-oxytocin and Their Deamino Analogs[1a,b]

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[4-Norvaline]-oxytocin, [1-deamino,4-norvaline]-oxytocin, [4-norleucine]-oxytocin, and [1-deamino,4-norleucine]-oxytocin have been synthesized from the requisite protected polypeptide precursors, which were prepared by the stepwise *p*-nitrophenyl ester method. The compounds were isolated by countercurrent distribution followed by partition chromatography on Sephadex G-25 and then tested for a number of pharmacological activities. The avian vasodepressor potencies were found to be 99, 128, 51, and 53 units/mg, respectively; the oxytocic potencies 61, 56, 20.5, and 12.8 units/mg, respectively; and the milk-ejecting potencies 182, 134, 87, and 50 units/mg, respectively. The antidiuretic and pressor activities of all four analogs were extremely low or negligible.

The present communication is an extension of our work on the replacement of the glutamine residue at position 4 of oxytocin (Figure 1) by aliphatic amino acid residues. We present here the synthesis and pharmacological activities of [4-norvaline]-oxytocin, [4-norleucine]-oxytocin, and their deamino analogs. For the synthesis of these compounds the methods used were patterned after those employed for the preparation of [4-valine]-oxytocin and [1-deamino,4-valine]-oxytocin.[2] The present analogs were isolated by countercurrent distribution[3] and were further purified by partition chromatography[4] on Sephadex G-25.

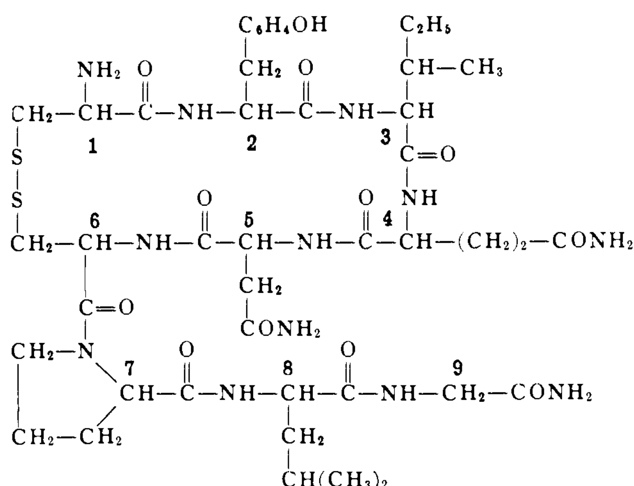

Figure 1.—Structure of oxytocin with numbers indicating the position of the individual amino acid residues.

The four-point assay design[5] was used for measurement of the pharmacological activities against the USP posterior pituitary reference standard. Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by Munsick, *et al.*[6] Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of Holton,[7] as modified by Munsick,[8] with the use of magnesium-free van Dyke–Hastings solution as the bathing fluid. Milk-ejecting activity was measured on anesthetized rabbits by the method of Cross and Harris,[9] as modified by van Dyke, *et al.*,[10] and by Chan.[11] Rat pressor assays were carried out on anesthetized male rats as described in the U. S. Pharmacopeia.[12] Assays for antidiuretic activity were performed on anesthetized male rats according to the method of Jeffers, *et al.*,[13] as modified by Sawyer.[14] The potencies so obtained for [4-norvaline]-oxytocin, [4-norleucine]-oxytocin, [1-deamino,4-norvaline]-oxytocin, and [1-deamino,4-norleucine]-oxytocin are presented in Table I along with corresponding data for related analogs of oxytocin and deaminooxytocin.

As can be seen from Table I, as the length of the straight-chain amino acid substituent increases from glycine to α-aminobutyric acid, the avian vasodepressor, oxytocic, and milk-ejecting potencies of the analogs also increase. Further increases in chain length result in decreased potencies. The [4-norleucine]-oxytocin is even less potent than [4-alanine]-oxytocin. It will also be noted that in the corresponding deamino series of analogs there is a progressive decrease in potencies as the chain length is increased from that of α-aminobutyric acid to that of norleucine.

Of the three oxytocin analogs in Table I with branched-chain amino acids in position 4, the [4-valine]-oxytocin is far more potent than [4-isoleucine]-oxytocin and [4-leucine]-oxytocin with respect to avian vasodepressor, oxytocic, and milk-ejecting activities, with [4-leucine]-oxytocin being the least potent. [4-Valine]-oxytocin is also considerably more potent than the straight-chain analog [4-norvaline]-oxytocin. On

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(14) W. H. Sawyer, *Endocrinology*, **63**, 694 (1958).

TABLE I

PHARMACOLOGICAL POTENCIES[a] OF A SERIES OF OXYTOCIN AND DEAMINO-OXYTOCIN ANALOGS WITH MODIFICATIONS AT POSITION 4

Oxytocin compd	Vasodepressor (fowl)	Oxytocic (rat)	Milk-ejecting (rabbit)	Antidiuretic (rat)	Pressor (rat)
Oxytocin	507 ± 23[b]	546 ± 18[c]	410 ± 16[b]	2.7 ± 0.2[b]	3.1 ± 0.1[b]
4-Glycine-	5.5 ± 0.2[d]	2.8 ± 0.1[d]	17 ± 1[d]	<0.002[d]	<0.005[d]
4-Alanine-	65 ± 3[e]	36 ± 6[e]	240 ± 55[e]	<0.01[e]	<0.01[e]
4-α-Aminobutyric acid-	108 ± 5[f]	72 ± 2[f]	225 ± 7[g]	0.2–0.3[f]	~0.1[f]
4-Norvaline-	99 ± 4	61 ± 3	182 ± 3	0.04	<0.001
4-Norleucine-	51 ± 3	20.5 ± 0.5	87 ± 4	<0.04	0.015
4-Valine-	230 ± 14[g]	139 ± 5[h]	~419[h]	~0.5[h]	<0.005[h]
4-Isoleucine-	81 ± 2[i]	~37[i]	~184[i]	~0.02[i]	<0.05[i]
4-Leucine-	44 ± 1[i]	13 ± 1[i]	66 ± 3[i]	Diuretic[i]	Weak depressor[i]
1-Deamino-	975 ± 24[j]	803 ± 36[j]	541 ± 13[j]	~19[j]	1.44 ± 0.06[j]
1-Deamino-4-α-aminobutyric acid-	193 ± 9[k]	93 ± 2[k]	266 ± 2[k]	~2[k]	nil[k]
1-Deamino-4-norvaline-	128 ± 9	56 ± 3	134 ± 7	0.03	<0.001
1-Deamino-4-norleucine-	53 ± 1.4	12.8 ± 0.4	50 ± 3	<0.005	0.025
1-Deamino-4-valine-	770 ± 25[h]	322 ± 16[h]	338 ± 2[h]	4.5 ± 0.4[h]	<0.005[h]
1-Deamino-4-isoleucine-	307 ± 24[l]	67 ± 1[l]	~156[l]	<0.05[l]	~0.06[l]
1-Deamino-4-leucine-	141 ± 9[l]	37 ± 1[l]	143 ± 4[l]	~0.015[l]	<0.05[l]

[a] Expressed in units per milligram as mean potencies ± standard error. [b] W. Y. Chan and V. du Vigneaud, *Endocrinology*, **71**, 977 (1962). [c] W. Y. Chan, M. O'Connell, and S. R. Pomeroy, *ibid.*, **72**, 279 (1963). [d] S. Drabarek, *J. Amer. Chem. Soc.*, **86**, 4477 (1964). [e] S. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, **46**, 1626 (1963). [f] V. du Vigneaud, G. S. Denning, Jr., S. Drabarek, and W. Y. Chan, *J. Biol. Chem.*, **238**, PC1560 (1963); **239**, 472 (1964). [g] W. Y. Chan and V. du Vigneaud, as reported in L. A. Branda and V. du Vigneaud, *J. Med. Chem.*, **9**, 169 (1966). [h] W. Y. Chan and V. du Vigneaud, unpublished data. Approximate values were reported in ref 2. [i] V. J. Hruby, G. Flouret, and V. du Vigneaud, *J. Biol. Chem.*, in press. [j] B. M. Ferrier, D. Jarvis, and V. du Vigneaud, *ibid.*, **240**, 4264 (1965). [k] L. Branda, S. Drabarek, and V. du Vigneaud, *ibid.*, **241**, 2572 (1966). [l] H. Takashima, V. J. Hruby, and V. du Vigneaud, unpublished data.

the other hand, there is not much difference in the degree of these activities exhibited by [4-leucine]-oxytocin and [4-norleucine]-oxytocin.

As shown in Table I, when the amino group of [4-valine]-oxytocin is replaced by hydrogen ([1-deamino,4-valine]-oxytocin), the avian vasodepressor and oxytocic potencies show a dramatic increase similar to the increase from oxytocin to deamino-oxytocin. This difference in potency is also shown between the [4-isoleucine]- and [4-leucine]-oxytocin and their deamino analogs. However, in the case of the norvaline and norleucine compounds there is no significant difference in potency between the oxytocin and deamino-oxytocin analogs.

As shown in Table I, the norleucine and norvaline analogs of oxytocin and deamino-oxytocin possess very low or negligible pressor and antidiuretic potencies.

It is to be noted that [4-leucine]-oxytocin (Table I) did not show any antidiuretic activity but, surprisingly, exhibited a diuretic effect instead. As reported by Chan, Hruby, Flouret, and du Vigneaud,[15] [4-leucine]-oxytocin was found to possess not ony a potent diuretic effect but also a potent natriuretic effect. Furthermore, it antagonized the antidiuretic activity of arginine-vasopressin.

Experimental Section[16]

***p*-Nitrophenyl N-Benzyloxycarbonylnorvalinate.**—A stirred solution of 3.87 g of N-benzyloxycarbonylnorvaline[17] and 2.52 g of *p*-nitrophenol in 54 ml of EtOAc was cooled in ice, and 3.4 g of DCI was added. After 4.5 hr AcOH (5 drops) was added, and after 15 min the dicyclohexylurea formed was filtered off and washed with EtOAc. The filtrate and washings were combined and concentrated, and the oil obtained was crystallized from 10 ml of EtOH by the addition of petroleum ether (bp 60–70°); yield 4.6 g, mp 69–70°, $[\alpha]^{20}D$ −31.2° (*c* 2, DMF). *Anal.* ($C_{19}H_{20}N_2O_6$) C, H, N.

N-Benzyloxycarbonylnorvalylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of 0.65 g of N-benzyloxycarbonylasparaginyl-S-benzylcysteinylprolylleucylglycinamide[18] in 4 ml of anhydrous AcOH was treated with 4 ml of HBr in AcOH (32%, w/w) for 1.5 hr at room temperature. The resulting solution was added to 300 ml of Et_2O and the precipitate was washed by decantation with four 400-ml portions of Et_2O. After being dried *in vacuo* over KOH and P_2O_5, the solid residue was dissolved in 120 ml of MeOH and the solution was passed through a column of ion-exchange resin IRA-410 (OH). The resin was washed with 100 ml of MeOH. The combined filtrate was slightly basic and it gave a negative test for bromide ion with $AgNO_3$. The white solid obtained after evaporation of the solvent under reduced pressure was dried for 2 hr *in vacuo*, dissolved in 4 ml of DMF, and treated with 0.34 g of *p*-nitrophenyl N-benzyloxycarbonylnorvalinate. The reaction mixture, which solidified overnight, was triturated with 100 ml of EtOAc and filtered. The solid was washed with EtOH and finally Et_2O; yield 0.62 g, mp 252–253°, $[\alpha]^{20}D$ −57° (*c* 1, DMF). *Anal.* ($C_{40}H_{56}N_8O_9S$) C, H, N.

N-Benzyloxycarbonylisoleucylnorvalylasparaginyl-S-benzylcysteninylprolylleucylglycinamide.—A suspension of 2.38 g of the preceding protected hexapeptide in 10 ml of anhydrous AcOH was treated with 10 ml of 32% HBr in AcOH. The peptide hydrobromide was isolated as before, dried, dissolved in 80 ml of MeOH, and passed through a column containing Rexyn RG1 (OH). The eluate was evaporated *in vacuo* to give a residue which was dissolved in 5 ml of DMF and treated with 1.34 g of *p*-nitrophenyl N-benzyloxycarbonylisoleucinate.[18] The reaction mixture solidified overnight and was triturated with EtOAc. The solid residue was collected, dissolved in hot DMF, and then precipitated by adding the solution to 400 ml of Et_2O. The resulting precipitate was filtered off and washed with EtOH and finally with Et_2O; yield 2.27 g, mp 243–245°, $[\alpha]^{20}D$ −54.7° (*c* 1, DMF). *Anal.* ($C_{46}H_{67}N_9O_{10}S$) C, H, N.

(15) W. Y. Chan, V. J. Hruby, G. Flouret, and V. du Vigneaud, *Science*, **161**, 280 (1968).

(16) All melting points are corrected capillary melting points and were taken on a Thomas-Hoover melting point apparatus. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values. All protected peptides were dried to constant weight over P_2O_5 under vacuum at 100° before yields were determined.

(17) D. Uchino, *Nippon Kagaku Zasshi*, **82**, 109 (1961); *Chem. Abstr.*, **56**, 10268*h* (1962).

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

N-Benzyloxycarbonyl-O-benzyltyrosylisoleucylnorvalylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of 2.24 g of the preceding protected heptapeptide in 13 ml of anhydrous AcOH was treated with 13 ml of 32% HBr in AcOH. The heptapeptide hydrobromide was isolated and converted to the free heptapeptide by the usual procedure. The white powder obtained was dissolved in 8 ml of DMF and treated with 1.51 g of *p*-nitrophenyl N-benzyloxycarbonyl-O-benzyltyrosinate.[18] The solid mass formed overnight was broken up with 100 ml of EtOAc, filtered off, and washed with EtOH and finally with Et_2O; yield 2.43 g, mp 253–255°, $[\alpha]^{19}D$ −49° (*c* 2, DMF). *Anal.* ($C_{62}H_{82}N_{10}O_{12}S$) C, H, N.

N-Benzyloxycarbonyl-S-benzylcysteinyltyrosylisoleucylnorvalylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of 0.5 g of the preceding protected octapeptide in 25 ml of ice-cooled trifluoroethanol was saturated at 0° with HBr.[4] The resulting solution was allowed to stand at room temperature for 1 hr and then was evaporated to dryness under vacuum. The residue was washed with Et_2O and dried *in vacuo* over P_2O_5. The octapeptide hydrobromide was dissolved in 100 ml of MeOH and neutralized with ion-exchange resin IRA-410 (OH). The suspension was filtered, evaporated to dryness, and dried over P_2O_5 *in vacuo*. The free peptide was dissolved in 3 ml of DMF and 235 mg of *p*-nitrophenyl N-benzyloxycarbonyl-S-benzylcysteinate[18] was added. The reaction mixture, which solidified overnight, was triturated with 20 ml of EtOAc and filtered. The solid material was washed with EtOH and finally with EtOAc; yield 412 mg, mp 242–244°, $[\alpha]^{20}D$ −53.8° (*c* 1, DMF). *Anal.* ($C_{65}H_{87}N_{11}O_{13}S_2$) C, H, N.

[4-Norvaline]-oxytocin.—A solution of 145 mg of the preceding protected nonapeptide in 400 ml of boiling anhydrous NH_3 was treated with Na[19] until a blue coloration persisted for about 1 min. The NH_3 was removed by evaporation *in vacuo* and lyophilization. The resulting residue was dissolved in 200 ml of 0.25% AcOH, the pH was adjusted to 6.8 with 1 *N* NH_4OH, and the resulting solution was aerated with CO_2-free air overnight. The oxidation was completed by treatment with 0.011 *N* $K_3Fe(CN)_6$ solution.[20] The ferro- and ferricyanide ions were removed by means of AG3-X4 resin (chloride form). The solution was concentrated to approximately 20 ml, placed in the first five tubes of a 400-tube countercurrent distribution apparatus, and subjected to 500 transfers in the solvent system *n*-BuOH–*n*-PrOH–0.5% AcOH containing 0.1% pyridine (6:1:8). The Folin–Lowry color values[21] defined a main peak (K = 2.1). The solutions corresponding to this peak were combined, concentrated to a small volume, and lyophilized. This material (24 mg) was subjected to further purification by dissolving it in 5 ml of the upper phase of the solvent system *n*-BuOH–C_6H_6–3.5% AcOH containing 1.5% pyridine (3:1:4) and subjecting it to partition chromatography[4] on a 2.15 × 110 cm column of Sephadex G-25. The fractions corresponding to the major peak, as determined from Folin–Lowry color values, were pooled and lyophilized; yield 16 mg, $[\alpha]^{22}D$ −22° (*c* 0.5, 1 *N* AcOH). *Anal.* ($C_{43}H_{67}N_{11}O_{11}S_2$) C, H, N.

A sample was hydrolyzed for 22 hr in 6 *N* HCl at 110° and subjected to amino acid analysis[22] on a Beckman-Spinco amino acid analyzer. The following molar ratios were obtained, with the value of glycine taken as 1.0: aspartic acid 1.0, proline 1.0, glycine 1.0, cystine 1.0, isoleucine 0.9, leucine 1.0, tyrosine 0.87, norvaline 1.0, and NH_3 2.0.

Paper electrophoresis with pyridine–AcOH buffer pH 5.6 (19 hr) showed [4-norvaline]-oxytocin to have approximately the same electrophoretic mobility as that of oxytocin and to travel as a single spot. Descending paper chromatography in two different solvent systems, *n*-BuOH–AcOH–H_2O (4:1:5) and pyridine–AcOH–H_2O (10:7:3), showed [4-norvaline]-oxytocin to travel as a single spot (R_f 0.70 and 0.81, respectively).

[1-Deamino,4-norvaline]-oxytocin.—A suspension of 0.64 g of the protected octapeptide N-benzyloxycarbonyl-O-benzyltyrosylisoleucylnorvalylasparaginyl-S-benzylcysteinylprolylleucylglycinamide in 4 ml of anhydrous AcOH was treated with 4 ml of 32% HBr in AcOH. The free octapeptide, isolated as usual, was dissolved in 3 ml of DMF and treated with 187 mg of *p*-nitrophenyl S-benzyl-β-mercaptopropionate.[20] After 2 days the solution was treated with EtOAc, and the resulting precipitate was collected and washed with EtOH and Et_2O; yield 440 mg, mp 243–245°, $[\alpha]^{20}D$ −51.2° (*c* 1, DMF).

A solution of 200 mg of this material was reduced with sodium in liquid ammonia by the usual method and converted to [1-deamino,4-norvaline]-oxytocin by oxidation with 0.011 *N* $K_3Fe(CN)_6$. After removal of ferri- and ferrocyanide ions, the resulting solution gave a negative Ellman test.[23] The solution was concentrated to about 15 ml, placed in the first five tubes of a 400-tube countercurrent distribution apparatus, and subjected to 400 transfers in the solvent system *n*-BuOH–C_6H_6–0.5% AcOH containing 0.1% pyridine (1:3:4). The distribution profile, determined by the Folin–Lowry color reaction, revealed only one peak (K = 0.91). The contents of the tubes from the central portion of the peak were combined, concentrated to a small volume, and lyophilized to give 66 mg of product. For further purification, 47 mg of this material was dissolved in 5 drops of *n*-BuOH, 5 ml of lower phase, and 5 ml of upper phase of the solvent system *n*-BuOH–C_6H_6–3.5% AcOH containing 1.5% pyridine (1:3:4), and the resulting solution was subjected to partition chromatography on a 2.15 × 110 cm column of Sephadex G-25 which had been equilibrated with lower phase. Lyophilization of the contents of tubes represented by the central portion of the main peak of Folin–Lowry color values yielded 33 mg of product, $[\alpha]^{22}D$ −118° (*c* 0.5, 1 *N* AcOH). *Anal.* ($C_{43}H_{66}N_{10}O_{11}S_2$) C, H, N.

The analog was hydrolyzed in 6 *N* HCl at 110° for 22 hr and the following molar ratios of amino acids and ammonia were found, with leucine taken as 1.0: aspartic acid 1.0, proline 1.0, glycine 0.95, norvaline 1.0, isoleucine 1.0, leucine 1.0, tyrosine 1.0, NH_3 2.17, cystine 0.23, and the mixed disulfide[20] of cysteine and β-mercaptopropionic acid 0.79.

Paper chromatography with the solvent systems *n*-BuOH–C_6H_6–pyridine–0.1% AcOH (1:3:1:5) and *n*-BuOH–C_6H_6–3.5% AcOH (1:3:4) showed [1-deamino,4-norvaline]-oxytocin to have only one major component with R_f 0.76 and 0.78, respectively.

***p*-Nitrophenyl N-benzyloxycarbonylnorleucinate.**—A solution of 6.46 g of N-benzyloxycarbonylnorleucine[24] and 4.16 g of *p*-nitrophenol in 60 ml of EtOAc was cooled in ice. A solution of 5.04 g of DCI in 30 ml of EtOAc was added to the stirred reaction mixture. After 3 hr, AcOH (20 drops) was added, and after 30 min the dicyclohexylurea was filtered off and washed with EtOAc The filtrate and washings were combined and evaporated *in vacuo*, and the residual oil was crystallized from 15 ml of EtOH by the addition of hexane to incipient cloudiness; yield 8.0 g, mp 70.5–72°, $[\alpha]^{20}D$ −30.8° (*c* 2, DMF). *Anal.* ($C_{20}H_{22}N_2O_6$) C, H, N.

N-Benzyloxycarbonylnorleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of 3.04 g of N-benzyloxycarbonylasparaginyl-S-benzylcysteinylprolylleucylglycinamide in 15 ml of dry AcOH was treated with 15 ml of 32% HBr in AcOH. The pentapeptide hydrobromide was isolated and converted as usual to the free peptapeptide, which was dissolved in 9 ml of DMF and treated with 1.78 g of *p*-nitrophenyl N-benzyloxycarbonylnorleucinate. The waxy material which formed overnight was broken up with 100 ml of EtOAc and filtered. Trituration with EtOH and finally with Et_2O yielded 2.8 g, mp 246–247°, $[\alpha]^{19}D$ −61.8° (*c* 1, DMF). *Anal.* ($C_{41}H_{58}N_8O_9S$) C, H, N.

N-Benzyloxycarbonylisoleucylnorleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of 1.85 g of the preceding protected hexapeptide in 12 ml of AcOH was converted to the free hexapeptide in the usual manner. The product was dissolved in 6 ml of DMF and treated with 0.94 g of *p*-nitrophenyl N-benzyloxycarbonylisoleucinate. After 2 days the waxy material formed was broken up with 100 ml of EtOAc and filtered. The material collected was washed with EtOH and finally with Et_2O; yield 1.85 g, mp 255–259°. This material was dissolved in 80% EtOH and reprecipitated by addition to EtOAc, filtered off, and washed with Et_2O; yield 1.35 g, mp 260–262°, $[\alpha]^{19}D$ −56.3° (*c* 1, DMF). *Anal.* ($C_{47}H_{69}N_9O_{10}S$) C, H, N.

N-Benzyloxycarbonyl-O-benzyltyrosylisoleucylnorleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of

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(23) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

(24) N. Izumiya, H. Uchino, and T. Yamashita, *Nippon Kagaku Zasshi*, **79**, 420 (1958); *Chem. Abstr.*, **52**, 14539*b* (1958).

1.18 g of the protected heptapeptide in 7 ml of AcOH was converted to the free heptapeptide by the usual methods. The product was dissolved in 5 ml of DMF and treated with 0.72 g of *p*-nitrophenyl N-benzyloxycarbonyl-O-benzyltyrosinate. The solidified reaction mixture was broken up with 50 ml of EtOAc and filtered, and the solid material was washed with EtOH–THF and finally with Et_2O; yield 1.1 g, mp 250–252°, $[\alpha]^{20}D$ −45.4° (*c* 1, DMF). *Anal.* ($C_{63}H_{84}N_{10}O_{12}S$) C, H, N.

N-Benzyloxycarbonyl-S-benzylcysteinyltyrosylisoleucylnorleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of 0.6 g of the preceding octapeptide in 25 ml of trifluoroethanol was saturated at 0° with HBr. After 1 hr at room temperature, the resulting solution was evaporated *in vacuo*. The residue was washed with Et_2O and dried. The product was dissolved in MeOH and neutralized with TEA. After evaporation of this solution to dryness *in vacuo*, the residue was suspended in $CHCl_3$ and filtered off. The filtered solid was dissolved in 1 ml of DMF and 257 mg of *p*-nitrophenyl N-benzyloxycarbonyl-S-benzylcysteinate was added. After 5 hr, 1 ml of AcOH was added followed by 50 ml of EtOAc. The mixture was allowed to stand for 1.5 hr and filtered. The solid material collected was washed with EtOH and finally with petroleum ether; yield 310 mg, mp 240–242°, $[\alpha]^{20}D$ −55.4° (*c* 1, DMF). *Anal.* ($C_{66}H_{85}N_{11}O_{13}S_2$) C, H, N.

[4-Norleucine]-oxytocin was prepared from 100 mg of the preceding protected nonapeptide, using conditions similar to those described for [4-norvaline]-oxytocin and its deamino analog. After removal of ferro- and ferricyanide ions, the clear solution obtained gave a negative test to nitroprusside and to Ellman's reagent. This solution was concentrated to about 30 ml, placed in the first four tubes of a 200-tube countercurrent distribution machine, and subjected to 200 transfers in the solvent system *n*-BuOH–C_6H_6–0.5% AcOH containing 0.1% pyridine (2:1:3). The Folin–Lowry color values showed one major peak (K = 0.43). The contents of tubes corresponding to this peak were combined, concentrated to a small volume, and lyophilized to give 31 mg of product, which was combined with 35 mg of similarly prepared material and subjected to further purification. The combined material was dissolved in 5 ml of upper phase of the solvent system *n*-BuOH–C_6H_6–3.5% AcOH containing 1.5% pyridine (2:1:3) and subjected to partition chromatography on a Sephadex G-25 column (2.15 × 110 cm). Fractions corresponding to the major peak (R_f 0.42) of Folin–Lowry color values were pooled and lyophilized; yield 45 mg, $[\alpha]^{22}D$ −15.05° (*c* 0.5, 1 *N* AcOH). *Anal.* ($C_{44}H_{69}N_{11}O_{11}S_2$) C, H, N.

The analog was hydrolyzed in 6 *N* HCl at 110° for 22 hr and the following molar ratios of amino acids and NH_3 were found, with glycine taken as 1.0: aspartic acid 1.0, proline 1.0, glycine 1.0, cystine 1.0, isoleucine 1.0, leucine 1.0, tyrosine 0.9, norleucine 1.0, and NH_3 2.0.

Paper electrophoresis with pyridine–AcOH buffer pH 5.6 (18 hr) showed [4-norleucine]-oxytocin to have a mobility similar to that of oxytocin and to travel as a single spot. On descending paper chromatography in *n*-BuOH–AcOH–H_2O (4:1:5) and pyridine–AcOH–H_2O (10:7:3), [4-norleucine]-oxytocin traveled as a single spot with R_f 0.75 and 0.86, respectively.

S-Benzyl-β-mercaptopropionyltyrosylisoleucylnorleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide.—A suspension of 0.7 g of N-benzyloxycarbonyl-O-benzyltyrosylisoleucylnorleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide in 6 ml of AcOH was treated with 7 ml of 30% HBr in AcOH. The octapeptide hydrobromide was isolated by the usual method, dissolved in 3 ml of DMF, and neutralized by the addition of TEA. To the clear solution was added 221 mg of *p*-nitrophenyl S-benzyl-β-mercaptopropionate. After 1 hr the reaction mixture had solidified. After 3 additional hr a mixture of 1 ml of AcOH and 10 ml of EtOH was added and the resulting mixture was filtered. The solid product was washed with EtOH, EtOAc, and finally Et_2O; yield 0.48 g, mp 252–253°, $[\alpha]^{20}D$ −50.7° (*c* 1, DMF). *Anal.* ($C_{58}H_{82}N_{10}O_{11}S_2$) H, N; C: calcd, 60.1; found, 59.6.

[1-Deamino,4-norleucine]-oxytocin.—The protected octapeptide, S-benzyl-β-mercaptopropionyltyrosylisoleucylnorleucylasparaginyl-S-benzylcysteinylprolylleucylglycinamide (100 mg), was converted to [1-deamino,4-norleucine]-oxytocin by the usual procedures. After removal of ferro- and ferricyanide ions, the resulting solution and another solution prepared identically were combined, concentrated to about 30 ml, treated with a few drops of *n*-BuOH, saturated with upper phase of the solvent system *n*-BuOH–C_6H_6–0.5% AcOH containing 0.1% pyridine (1:3:4), and placed in the first three tubes of a 200-tube countercurrent distribution machine. After 200 transfers, the Folin–Lowry color reaction revealed a main peak (K = 2.34). The contents of tubes corresponding to this peak were combined and lyophilized to give 30 mg of product. For further purification, 25 mg of this material and an additional 52 mg similarly obtained were combined and dissolved in 5 drops of AcOH, 5 drops of *n*-BuOH, and 5 ml of upper phase of the solvent system *n*-BuOH–C_6H_6–3.5% AcOH containing 1.5% pyridine (1:4:5), and subjected to partition chromatography on a Sephadex G-25 column (2.15 × 110 cm). The fractions corresponding to the central portion of the peak of Folin–Lowry color values (R_f 0.42) yielded after lyophilization 60 mg, $[\alpha]^{20}D$ −43° (*c* 0.5, 1 *N* AcOH).

The analog was hydrolyzed in 6 *N* HCl at 110° for 22 hr and the following molar ratios of amino acids and NH_3 were found, with glycine taken as 1.0: aspartic acid 1.0, proline 1.0, glycine 1.0, isoleucine 1.0, leucine 1.0, norleucine 1.0, tyrosine 0.95, NH_3 2.1, cystine 0.25, mixed disulfide of cysteine and β-mercaptopropionic acid 0.84.

Paper chromatography showed [1-deamino,4-norleucine]-oxytocin to travel as a single spot, with an R_f of 0.85 in *n*-BuOH–C_6H_6–pyridine–0.1% AcOH (1:3:1:5) and an R_f of 0.84 in *n*-BuOH–C_6H_6–3.5% AcOH (1:3:4).

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