L(−)-butyramide·HCl[2] was synthesized for testing as an inhibitor of L-asparagine synthetase.

The conversion of L-asparagine to its *N*-Cbz derivative and subsequent esterification were achieved by adaptations of known procedures. Reduction of the latter blocked L-asparagine methyl ester was effected by $Ca(BH_4)_2$,[3] a less commonly known reagent. Attempts to reduce either L-asparagine, *N*-carbobenzoxy-L-asparagine, or *N*-carbobenzoxy-L-methyl ester with LAH were unsuccessful.

Experimental Section[4]

***N*-Carbobenzoxy-L-asparagine.**[5]—To a mixt of 39.6 g (0.30 mole) of L-Asp and 25.8 g (0.64 mole) of MgO in 300 ml of H_2O was added in 4 portions at 5° 51.0 g (0.30 mole) of carbobenzoxy chloride. After stirring 15 min longer, the thick reaction mixt was stirred for 3 hr at room temp, acidified with 2 *N* HCl, and filtered, and the solid was washed with H_2O, giving 66.2 g of air-dried product, mp 158–161°. The entire amt was recrystd from 700 ml of MeOH to yield 36.7 g, mp 164–165° (lit.[5] mp 165°). Recrystn in the same manner of the residue obtained from concn of the mother liquors to dryness gave 9.4 g, mp 165°. Again concn of the mother liquors and recrystn of the solid thereof led to 8.3 g, mp 164–166°, for a total yield of 54.5 g (74.5%).

***N*-Carbobenzoxy-L-asparagine Methyl Ester.**—A suspension of 30 g (0.113 mole) of *N*-Cbz-L-Asp in 800 ml of 0.1 *N* MeOH–HCl was stirred overnight at room temp. The resulting soln was concd to dryness, the residue was triturated with Et_2O, and the ester was filtered off and air-dried to yield 31.6 g (100%), mp 150° (lit.[5] mp 150°).

3-Carbobenzoxyamino-4-hydroxy-L-butyramide.—A mixt of anhyd $CaCl_2$, 55.5 g (0.5 mole), and $NaBH_4$, 76 g (1.0 mole), in 1000 ml of abs EtOH was stirred for 1 hr at −10° to −20°. *N*-Cbz-L-Asp-OMe, 25 g (0.1 mole), was added and stirring was contd at the same temp for 4 hr after which 100 ml of H_2O was added dropwise at 0–5°. The mixt was stirred for 30 min, acidified with concd HCl to congo red, and concd to dryness *in vacuo*. The residue was stirred vigorously on the steam bath with 1000 ml of H_2O, and the mixt was filtered. From the cooled filtrate 15.6 g (69.3%) of 3-carbobenzoxyamino-4-hydroxy-L-butyramide was obtd, mp 142–144°. A mmp of the product with starting material showed a depression of 50°: nmr (60 MHz, DMSO-d_6), δ 2.33 ppm (d, 2, CH_3CO), 3.41 (t, CCH_2O), 3.92 (sextet, 1, CCNHC), 4.80 (t, 1, OH), 5.07 (s, 2 $PhCH_2O$), ~6.9 (broad s, $CONH_2$), ~7.0 (d, CONHC), 7.40 (s, Ph).

3-Amino-4-hydroxy-L(−)butyramide·HCl.—A soln of 15.6 g (0.0618 mole) of 3-carbobenzoxyamino-4-hydroxy-L-butyramide in 780 ml of MeOH was hydrogenolyzed for 4 hr at room temp using 3.12 g of 10% Pd/C. The filtered soln was concd *in vacuo* to about 250 ml and again filtered to remove trace impurities. The clear filtrate was further concd to an oil which was dissolved in 10 ml of H_2O, acidified with concd HCl, and filtered. An addl 10 ml of H_2O was used for the transfer. Me_2CO (400 ml) was added and the cloudy soln stored at 5° overnight. After decantn of the supernatant phase, the semisolid residue was stirred with 400 ml of Me_2CO for 2 hr at 5°. The filtered crude product was very hygroscopic. It was recrystd from 200 ml of MeOH, stored at 5° overnight, and filtered, and the product was washed with cold MeOH and Et_2O. After drying over P_2O_5 (0.1 mm) (25°, 18 hr), the yield was 2 g. *Anal.* ($C_4H_{11}N_2O_2Cl$) C, H, N, Cl. The residue from the concn of the mother liquors and washes to dryness was recrystd in the same manner from 200 ml of EtOH to give an addl 2.5 g. *Anal.* ($C_4H_{11}N_2O_2Cl$) C, H, N, Cl. The total yield of pure material was 4.5 g (47.1%): mp 134–135°; $[\alpha]^{25}D$ −7.6° (*c* 1.0, H_2O); ir spectrum supported the proposed structure; nmr (60 MHz, D_2O) δ 2.6–2.8 (m, 2, CH_2CO) and 3.5–4.0 ppm (m, 3, OCH_2 and CH), remaining active proton sites of the molecule are deuterated in this medium.

(2) During the prepn of the manuscript, M. R. Harnden and T. O. Yellin reported the synthesis of L(−)-3-amino-4-hydroxybutyramide trifluoroacetate by a different route as one of a series of agents for evaluation as inhibitors of L-asparaginyl-tRNA lygase. The compd was not fully characterized. *J. Med. Chem.*, **13**, 1095 (1970).

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(4) Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncor. Analyses indicated only by the symbols of the elements were within ±0.3% of the theor values. The ir spectra were obtained using a Perkin-Elmer Model 621 recording spectrophotometer, and a Varian A60 spectrometer was used to obtain the nmr spectra. The authors are grateful to Dr. A. W. Douglas for the ir and nmr spectra and to Mr. R. N. Boos and associates for the elemental anal.

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Solid Phase Synthesis of [4-Proline]oxytocin, [4-Proline]mesotocin, [4-Proline]glumitocin, and [4-Lysine]mesotocin

M. Manning,* J. W. M. Baxter, T. C. Wuu, V. Smart-Abbey, K. Morton, E. J. Coy, and W. H. Sawyer

Department of Biochemistry, Medical College of Ohio at Toledo, Toledo, Ohio 43614, Department of Biochemistry, McGill University, Montreal, Quebec, Canada, and Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York 10032

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In an attempt to establish the identity of a postulated evolutionary intermediate between the 4-serine-containing and 4-glutamine-containing neurohypophysial hormones, the following 4-substituted analogs, [4-proline]oxytocin, [4-proline]mesotocin, [4-proline]glumitocin, and [4-lysine]mesotocin were synthesized. The pharmacological properties of these analogs have been presented elsewhere.[1,2] This communication describes the solid phase synthesis of these analogs *via* their respective protected nonapeptide intermediates. The methods used were essentially the same as those described previously for the synthesis of oxytocin[3] and [4-threonine]oxytocin.[4] The physical characteristics of all of these compds together with the individual yields obtained are presented in Tables I and II.

Experimental Section[5]

***N*-BOC-*S*-benzyl-L-Cys-*O*-benzyl-L-Tyr-L-Ile-L-Pro-L-Asp-*S*-benzyl-L-Cys-L-Pro-L-LeuGly-NH_2 (1).**—BOC-glycyl resin (5.0 g, 0.805 mmole of glycine) was treated in an 8-cycle procedure as described for the synthesis of (4-threonine)oxytocin,[4] except that BOC-L-proline was used in the sixth incorporation step to give the protected nonapeptide resin, weight 6.0 g. Ammonolytic cleavage of the protected nonapeptide resin (3.0 g) was carried out as described for [4-threonine]oxytocin and the protected peptide was extd with DMF and MeOH. Solvents were removed *in vacuo*, and the residue was purified by trituration with 95% EtOH (30 ml) to give **1** as an amorphous white powder, weight 550 mg (Table I). Amino acid analysis gave Asp, 1.00; Leu, 1.00; Gly, 1.08; Bzl-Cys, 2.08; Ile, 0.99; Tyr, 0.82; Pro, 2.00; NH_2, 2.30.

[4-Proline]oxytocin (2).—1 (142 mg) was reduced, reoxidized, and purified by the procedure used in the synthesis of [4-threonine]oxytocin. **2** was obtained as a fluffy white powder, weight 41.5 mg, shown to be homogeneous by tlc and paper electrophoresis at different pH values as described for [4-threonine]-oxytocin (Table II). Amino acid analysis gave: Asp, 1.00; Gly, 1.00; Pro, 2.16; Cys, 1.96; Ile, 0.95; Tyr, 0.94; Leu, 1.05; NH_3, 2.12.

***N*-BOC-*S*-benzyl-L-Cys-*O*-benzyl-L-Tyr-L-Ile-L-Pro-L-Asp-*S*-benzyl-L-Cys-L-Pro-L-IleGly-NH_2 (3).**—BOC-glycyl resin (5.0 g,

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TABLE I

PROTECTED NONAPEPTIDES OF [4-PROLINE]OXYTOCIN, [4-PROLINE]MESOTOCIN, [4-PROLINE]GLUMITOCIN, AND [4-LYSINE]MESOTOCIN

Bzl OBzl Bzl

Z—Cys·Tyr·Ile·(X)·Asn·Cys·Pro·(X)·Gly—NH_2

1 2 3 4 5 6 7 8 9

No.	Amino acids in positions 4	8	Formula[a]	Mp,[b] °C	Yield on resin,[c] %	Yield on ammonolysis,[c] %	$[\alpha]^{T\,d}D$, deg
1	Pro	Leu	$C_{77}H_{91}N_{11}O_{13}S_2$	177–178	99.5	83.5	−62.0
3	Pro	Ile	$C_{72}H_{91}N_{11}O_{13}S_2$	193–195	100	87	−52.0
5	Pro	Gln	$C_{71}H_{88}N_{12}O_{14}S_2$	225–227	70	36	−46.8
7	N^{ϵ}-Z-Lys	Ile	$C_{81}H_{102}N_{12}O_{15}S_2$	251–253	100	82	−39.0

[a] Elemental anal. were performed by Galbraith Laboratories, Knoxville, Tenn. The anal. results were within ±0.4% of the theor values, all compounds were anal. for C, H, and N. [b] Melting points were taken in an open capillary and are uncor. [c] Yields are based on the initial glycine incorporation in the resin. [d] In DMF (*c* 1.0), *T* = 20°, 20°, 22.5°, and 21°, resp.

TABLE II

[4-PROLINE]OXYTOCIN, [4-PROLINE]MESOTOCIN, [4-PROLINE]GLUMITOCIN, AND [4-LYSINE]MESOTOCIN

CysTyrIle(X)AsnCysPro(X)GlyNH$_2$

1 2 3 4 5 6 7 8 9

No.	Amino acids in position 4	8	Formula[a]	$[\alpha]^{T\,b}D$, deg	R_f[c]	Yield from protected nonapeptide, %	Yield overall, %[d]
2	Pro	Leu	$C_{43}H_{65}N_{11}O_{11}S_2 \cdot CH_3COOH \cdot 3H_2O$	−16.3	0.29	59.0	49.5
4	Pro	Ile	$C_{43}H_{65}N_{11}O_{11}S_2 \cdot CH_3COOH \cdot 3H_2O$	−21.6	0.30	41.5	38.5
6	Pro	Gln	$C_{42}H_{62}N_{12}O_{12}S_2$	−15.5	0.25	50.0	18.0
8	Lys	Ile	$C_{44}H_{70}N_{12}O_{11}S_2$	−19.6	0.16	27.7	22.7

[a] See footnote *a*, Table I. [b] In 1 *N* AcOH (*C* 0.5), *T* = 19.0°, 23.0°, 21.5°, and 24.0°, resp. [c] Samples run on silica gel G plates in the upper phase of the solvent system *n*-BuOH–AcOH–H_2O (4:1:5, v/v, ascending). [d] Based on the initial glycine incorporation on the resin.

0.985 mmole of glycine) was treated by the 8-cycle procedure as described for the synthesis of [4-threonine]oxytocin[4] except that BOC-L-isoleucine and BOC-L-proline were used in the first and sixth incorporation steps, resp, to give the protected nonapeptide resin (6.21 g). Ammonolytic cleavage[3,4] of the protected nonapeptide resin (3.0 g) gave **3** as a white amorphous powder, weight 570 mg (Table I). Amino acid analysis give: Asp, 1.01; Pro, 2.05; Gly, 1.00; Ile, 1.95; Tyr, 0.80; Bzl-Cys, 1.90; and NH_3, 2.20.

[4-Proline]mesotocin (4).—**3** (150 mg) was reduced, reoxidized, deionized, and lyophilized as previously described,[4] weight 24.75 mg. It has shown to be homogeneous by tlc and paper electrophoresis (Table II). Amino acid analysis gave: Asp, 1.05; Pro, 2.06; Gly, 1.10; Cys, 1.98; Ile, 2.12; Tyr, 0.93; and NH_3, 2.11.

***N*-BOC-*S*-benzyl-L-Cys-*O*-benzyl-L-Tyr-L-Ile-L-Pro-L-Asp-*S*-benzyl-L-Cys-L-Pro-L-GluGly-NH_2 (5).**—The protected nonapeptide resin was prepd from BOC-Gly resin (4.57 g, 0.899 mmole of glycine) by the method used for the synthesis of [4-threonine]-oxytocin[4] except that BOC-L-glutamine *p*-nitrophenyl ester in DMF was used in the first incorporation step and BOC-L-proline was used in the sixth incorporation step. The BOC-glutaminyl residue was deprotected with F_3CCO_2H[3] (5.36 g). Ammonolytic cleavage[3,4] of this nonapeptide resin (3.0 g) yielded **5**, weight 360 mg (Table I). Amino acid analysis gave: Asp, 1.00; Glu, 1.03; Gly, 1.08; Bzl-Cys, 2.08; Ile, 0.99; Tyr, 0.82; Pro, 1.81; NH_3, 3.30.

[4-Proline-8-glutamine]oxytocin (6).—**5** (125 mg) was reduced, reoxidized, and purified in the usual manner,[4] weight 45 mg (Table II). It was shown to be homogeneous by the usual methods.[4] Amino acid analysis gave: Asp, 1.00; Glu, 0.99; Gly, 1.10; Pro 1.91; Cys 1.89; Ile, 1.14; Tyr, 0.99; NH_3, 3.20.

***N*-BOC-*S*-benzyl-L-Cys-*O*-benzyl-L-Tyr-L-Ile-N^{ϵ}-BOC-L-Lys-L-Asp-*S*-benzyl-L-Cys-L-Pro-L-IleGly-NH_2 (7).**—BOC-glycyl resin (4.02 g, 0.648 mmole of glycine) was treated by the 8-cycle procedure used for the synthesis of [4-threonine]oxytocin[4] with BOC-L-isoleucine and N^{α}-BOC-N^{ϵ}-Z-lysine being incorporated in the second and sixth steps, resp, to give the protected nonapeptide resin, weight 5.02 g. Ammonolysis of the protected nonapeptide resin (2.5 g) gave **7** as an amorphous powder (410 mg) (Table I). Amino acid analysis gave: Asp, 1.00; Gly, 1.08; Bzl-Cys, 2.08; Ile, 1.99; Tyr, 0.82; Pro, 1.00; Lys, 1.06; NH_3, 2.10.

[4-Lysine]mesotocin (8).—Reduction, reoxidation, and purification[4] of **7** (150 mg) gave **8** as a white fluffy powder, weight 27 mg, shown to be homogeneous by tlc and by electrophoresis at pH 3.5. Electrophoresis at pH 6.42 showed a second component in the direction of the cathode. **8** (0.5 mg) was incubated in pH 6.42 buffer (0.1 ml) at room temperature for 3 hr. Tlc showed the slow formation of an unidentified side product, R_f 0.08; thus indicating that the second component observed after electrophoresis at the higher pH was an artifact of the electrophoresis conditions rather than an inherent inhomogeneity of this synthetic peptide. Amino acid analysis of **8** gave: Asp, 1.00; Gly, 1.05; Pro, 0.98; Cys, 2.08; Ile, 2.10; Tyr, 0.97; Lys, 1.04; NH_3, 2.05.

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