Oxytocin Analogues Containing No Disulfide Bond. III. The Synthesis of Three Analogues of 1,6-Aminosuberic Acid-Oxytocin

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[Asu^{1,6}]-oxytocin, an analogue of deamino-oxytocin in which the disulfide bond is replaced by an ethylene linkage, is completely resistant to deterioration during storage under concentrated conditions. In the present study, three new analogues of this material, [Phe^3, Asu^{1,6}]-oxytocin, [Val^4, Asu^{1,6}]-oxytocin, and [Gly^7, Asu^{1,6}]-oxytocin, were synthesized to find out a hormonal compound possessing a specifically higher uterotonic activity. Among them, the [Gly^7]-analogue was found to be the most promising compound; it showed a relatively higher uterotonic activity (245 IU/mg) in the rat, with a faint antidiuretic activity (0.02 IU/mg) and a weak depressor activity.

Deamino-oxytocin, which has a β-mercaptopropionyl residue in place of the cysteinyl residue in the 1 position of oxytocin, exhibits a higher oxytocic activity than the original oxytocin.[2] This fact indicates that the free amino group of oxytocin is not essential for the hormonal function. One or both sulfur atoms of the disulfide bridge in the deamino-oxytocin can also be replaced by one or two methylene groups respectively without any conspicuous loss of the hormonal activities.[3-5] In the first paper of this series,[5] we introduced an efficient procedure for the synthesis of such an analogue of deamino-oxytocin, [Asu^{1,6}]-oxytocin, in which the disulfide bond is replaced by an ethylene linkage, as is shown in Fig. 1.[5] The compound synthesized by the new procedure showed a much higher oxytocic potency than the reported value[4] for the compound. Then, the procedure was applied to the syntheses of [Asu^{1,6}]-vasopressins and vasotocins. These new derivatives were also confirmed to be biologically active, as had been expected.[6-8] Thus, it was concluded that the disulfide bridge itself in neurohypophyseal hormones in not required, in general, for the exhibition of their hormonal activities.[8] Later, the [Asu^{1,6}]-oxytocin synthesized by our procedure was found to be crystallized from water,[9] and the crystallized compound showed a higher uterotonic activity (160 IU/mg) than the highest value (134 IU/mg),[9] so far reported for the amorphous compounds.

Both natural and synthetic neurohypophyseal hormones are known to be reduced in their potency in the course of such common procedures for isolation as concentration and lyophilization. The Asu-derivatives, however, are completely resistant to inactivation during those procedures, even in the presence of triethylamine.[8,9]

Therefore, if we can find an analog of the Asu-oxytocin, which has a sufficiently higher potency in oxytocic activity, with negligibly lower potencies in pressor and antidiuretic activities, the use of such a compound as a drug for labor induction should be much more advantageous than that of natural oxytocin.

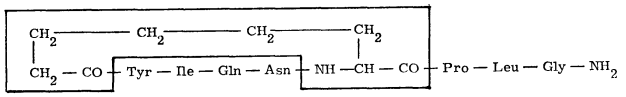

Fig. 1. Structure of [Asu^{1,6}]-Oxytocin.

In the present study, we have synthesized three new analogues of the Asu-oxytocin, [Phe^3, Asu^{1,6}]-oxytocin, [Val^4, Asu^{1,6}]-oxytocin, and [Gly^7, Asu^{1,6}]-oxytocin, and their hormonal activities have been compared in order to find a promising compound for use as an oxytocic hormone. Their parent compounds, [Phe^3]-oxytocin,[10] [Val^4]-oxytocin,[11] and [Gly^7]-oxytocin,[12] are already known to exhibit specific spectra in their biological activities (See Table 1).

The syntheses were carried out by the stepwise elongation technique,[13] as is shown in Charts 1, 2, and 3. Two different procedures, which had previously

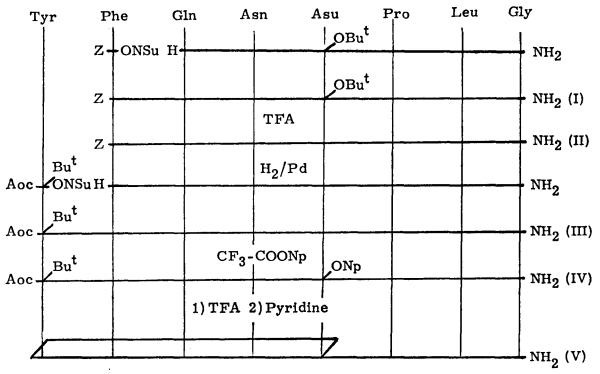

Chart 1. Synthesis of [Phe^3, Asu^{1,6}]-Oxytocin.

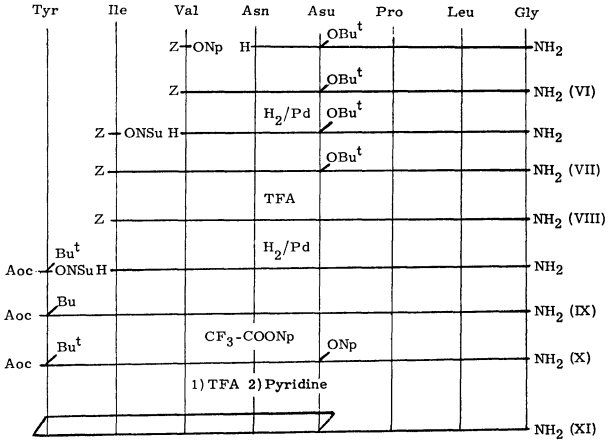

Chart 2. Synthesis of [Val^4, Asu^{1,6}]-Oxytocin.

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Table 1. Biological potencies of oxytocin analogues

Compound	Oxytocic (rat)	Depressor (fowl)	Pressor (rat)	Antidiuretic (rat)
[Phe^3, Asu^{1,6}]-Oxytocin(V)	21.6	—	<0.1	—
[Val^4, Asu^{1,6}]-Oxytocin(XI)	14.9	—	<0.25	—
[Gly^7, Asu^{1,6}]-Oxytocin(XX)	244.7	4.6	Weak depressor	0.02
[Phe^3]-Oxytocin[b]	20	45	3	—
[Val^4]-Oxytocin[c]	139±5	230 ±14	<0.005	0.5
[Gly^7]-Oxytocin[d]	330	—	—	0.01
Oxytocin[e]	450±30	450 ±30	5±1	5±1
Deaminooxytocin[f]	803±36	975 ±24	1.44±0.06	19
[Asu^{1,6}]-Oxytocin[g]	160±4.4	44 ±1.7	0.10±0.02	4.7±0.3

a) Expressed in IU/mg. Determined by T. Okada *et al.* of Yoshitomi Pharmaceutical Industries, Ltd. Details will be published elsewhere. b) Cited from Ref. 10. c) Cited from Ref. 11. d) Cited from Ref. 12. e) R. A. Boissonnas, S. Guttmann, B. Berde, and H. Konzett, *Experientia*, **17**, 377 (1961). f) B. M. Ferrier, D. Jarvis, and V. du Vigneaud, *J. Biol. Chem.*, **240**, 4264 (1965). g) Cited from Ref. 9.

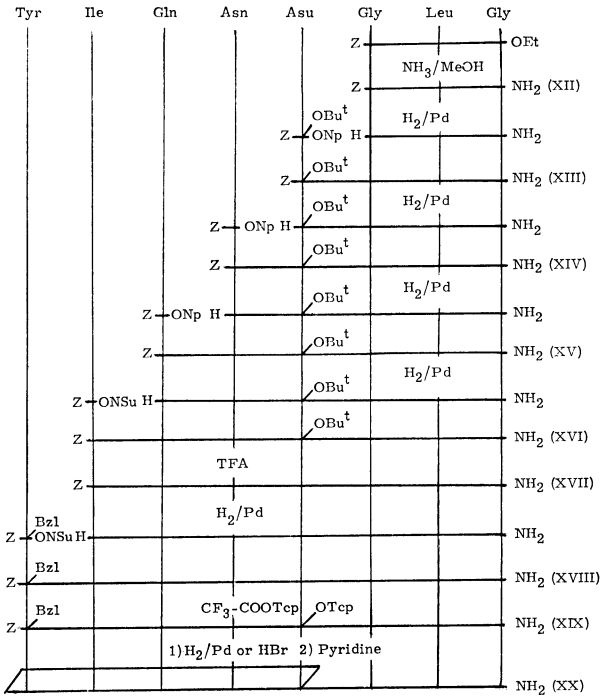

Chart 3. Synthesis of [Gly^7, Asu^{1,6}]-oxytocin.

been used for the synthesis of Asu-oxytocin[5] and Asu-vasopressins,[8] were applied to the cyclization of these linear peptides in order to compare the efficiency of the respective procedures. Consequently, no particular difference was found between these two procedures in terms of the yield.

The biological activities of the products were determined by the Yoshitomi Pharmaceutical Industry; the data are shown in Table 1. For comparison, the reported values for the original compounds are also included in the Table. In the cases of Phe- and Val-analogues, no significant changes were observed in their biological activities. However, the Gly-analogue was considered to be one of the promising compounds as an oxytocic hormone; its uterotonic activity was much more potent than that of the original Asu-oxytocin, and the ratios of the pressor and antidiuretic activity to the uterotonic activity were both sufficiently lower than those of the original compounds.

Experimental[14]

All the melting points were determined by the capillary method, and are given as uncorrected values. The products were dried over phosphorus pentoxide *in vacuo* at room temperature, unless otherwise noted. The solvent systems used for chromatography were: I, Chloroform: methanol: acetic acid (95: 5: 3 v/v); II, *n*-butanol: acetic acid: water (4: 1: 1 v/v); III, *n*-butanol: pyridine: water (4: 1: 1 v/v).

General Procedure for the Removal of Benzyloxycarbonyl or Benzyl Groups by Catalytic Hydrogenolysis. Hydrogen was gently bubbled through a solution or suspension of a benzyloxycarbonyl or benzyl derivative at room temperature, in which palladium black or palladium charcoal had previously been added as the catalyst. The progress of the reaction was monitored by thin-layer chromatography using Merck's Silica-gel G. After the completion of the reaction had been confirmed by thin-layer chromatography, the catalyst was removed by filtration, the filtrate was concentrated under reduced pressure, and the residue was subjected to the next step.

Benzyloxycarbonyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-ω-t-butyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide (I).

Benzyloxycarbonyl-L-glutaminyl-L-asparaginyl-ω-*t*-butyl-L-α-aminosubery-L-prolyl-L-leucylglycine amide[5] (4.0 g, 4.41 mmol) was subjected to catalytic hydrogenolysis in a mixture of dioxane–water (3: 1 v/v, 80 ml), and the product was dissolved in DMF (20 ml). The benzyloxycarbonyl-L-phenylalanine *N*-hydroxysuccinimide ester[15] (2.8 g, 7.06 mmol) was then added to the solution, and the mixture was allowed to react for 3 days at room temperature. Ethyl acetate was added to the reaction mixture to precipitate the product, which was collected by filtration and recrystallized from 90% aqueous ethanol; wt. 3.79 g (81.5%); mp 184—186 °C, $[\alpha]_D^{17}$ −42.7° (*c* 0.95, DMF).

Found: C, 57.95; H, 7.28; N, 13.47%. Calcd for $C_{51}H_{74}O_{13}N_{10}\cdot H_2O$: C, 58.16; H, 7.27; N, 13.30%.

Benzyloxycarbonyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide (II). Compound I (3.71 g, 3.5 mmol) was dissolved in TFA (50 ml), and the solution was kept for an hour at room temperature. Then, the TFA was removed under reduced pressure and the oily residue was solidified by trituration with ether. The product was reprecipitated from 90% ethanol, washed with ethanol, and dried; wt. 2.85 g (80.7%); mp 182.5—185 °C (decomp.), $[\alpha]_D^{18}$ −45.9° (*c* 0.91, DMF).

Found: C, 56.14; H, 6.90; N, 13.57%. Calcd for $C_{47}H_{66}O_{13}N_{10}\cdot 1.5H_2O$: C, 56.10; H, 6.91; N, 13.92%.

t-*Amyloxycarbonyl*-O-t-*butyl*-L-*tyrosyl*-L-*phenylalanyl*-L-*glutaminyl*-L-*asparaginyl*-L-α-*aminosuberyl*-L-*prolyl*-L-*leucylglycine Amide (III)*. Compound II (4.5 g, 4.4 mmol) was subjected to catalytic hydrogenolysis in a mixture of dioxane–water (4: 1 v/v, 100 ml). Then the heptapeptide amide thus formed was treated with the *t*-amyloxycarbonyl-*O*-*t*-butyl-L-tyrosine *N*-hydroxysuccinimide ester[8] (4.0 g, 8.9 mmol) in DMF (20 ml) at room temperature for 24 hr. The product was precipitated by the addition of ethyl acetate, and the precipitates were collected by filtration, washed well with hot ethyl acetate, and then reprecipitated from 33% aqueous ethanol; wt. 2.17 g (41.3%); mp 197.5—198.5 °C (decomp.), $[\alpha]_D^{23}$ −37.7° (*c* 1.0, DMF).

Found: C, 58.09; H, 7.40; N, 12.74%. Calcd for $C_{58}H_{87}O_{15}N_{11}\cdot H_2O$: C, 58.22; H, 7.50; N, 12.88%.

t-*Amyloxycarbonyl*-O-t-*butyl*-L-*tyrosyl*-L-*phenylalanyl*-L-*glutaminyl*-L-*asparaginyl*-ω-p-*nitrophenyl*-L-α-*aminosuberyl*-L-*prolyl*-L-*leucylglycine Amide (IV)*. Compound III (1.33 g, 1.11 mmol) was treated with *p*-nitrophenyl trifluoroacetate[5,16] (2.8 g, 11.9 mmol) in a mixture of DMF (25 ml) and pyridine (6.2 ml) for 3 hr at 50 °C. The product was precipitated with ether, collected by filtration, and washed well with ether. The washed material was reprecipitated from 40% aqueous ethanol; wt. 1.08 g (74%); mp 198—199 °C (decomp.), $[\alpha]_D^{23}$ −32.9° (*c* 0.75, DMF).

Found: C, 58.34; H, 7.19; N, 12.42%. Calcd for $C_{64}H_{90}O_{17}N_{12}$–H_2O: C, 58.34; H, 7.04; N, 12.76%.

Lactam of L-*Tyrosyl*-L-*phenylalanyl*-L-*glutaminyl*-L-*asparaginyl*-L-α-*aminosuberyl*-L-*prolyl*-L-*leucylglycine Amide (V)*: [*Phe*[3], *Asu*[1,6]]-*Oxytocin*. Compound IV (616 mg, 0.47 mmol) was dissolved in TFA (6 ml), and the solution was allowed to react for 1.5 hr at room temperature. Then, the TFA was evaporated out under reduced pressure, and the residue was dissolved in DMF (10 ml). The solution was stirred into pyridine (300 ml), drop by drop, at 40 °C. After the addition, the mixture was kept overnight at room temperature, and then concentrated to a residue under reduced pressure, and the residue was treated with ether to obtain the product as a powder. The powdered material was dissolved in a minimum amount of methanol and charged on the top of a column of Sephadex LH-20 (1.5×45.5 cm). The column was eluted with methanol, 1.5 g fractions were collected, and peptides were detected by means of the UV absorption at 280 nm. The effluents in tubes No. 21 to 27 were pooled and concentrated. The product was applied to 7 thin-layer plates of Silica-gel GF 254 (5×16 cm each) and developed by the solvent system II. The locations of the separated materials were detected by UV-irradiation, and the major band on each plate was collected by scraping. The material adsorbed on the pooled silica-gel was extracted with methanol and further applied to a column of CM-Sephadex C-25 (H^+ form) (1.5×27 cm). The column was eluted with 50% aqueous methanol, and 3 g fractions were collected. The effluents in tubes No. 15 to 24 were pooled and concentrated, and the residue was lyophilized from water; wt. 47 mg (overall 18%), $[\alpha]_D^{17}$ −68.1° (*c* 0.36, methanol).

Ratio of amino acids in an acid hydrolyzate (6 M HCl, 48 hr at 105 °C): Tyr, 1.00; Phe, 1.00; Glu, 1.03; Asp, 1.00; Asu, 0.97; Pro, 0.94; Leu, 0.97; Gly, 0.97. The average recovery was 93%.

Found: C, 51.58; H, 7.01; N, 13.08%. Calcd for $C_{48}H_{67}O_{12}N_{11}\cdot 7H_2O$: C, 51.65; H, 7.31; N, 13.08%.

This material was confirmed to be homogeneous by thin-layer chromatography (Silica-gel G, Solvent II) and by paper electrophoresis (Toyo, No. 514, pH 4.8 pyridine-acetic acid buffer). The R_f-values on paper chromatography (Toyo, No. 51) were 0.74 for Solvent II and 0.55 for Solvent III. These chromatograms were located with Pauly's reagent.

Benzyloxycarbonyl-L-*valyl*-L-*asparaginyl*-ω-t-*butyl*-L-α-*aminosuberyl*-L-*prolyl*-L-*leucylglycine Amide (VI)*. Benzyloxycarbonyl-L-asparaginyl-ω-*t*-butyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine amide[5] (3.5 g, 4.6 mmol) was subjected to catalytic hydrogenolysis in a dioxane–water mixture (3: 1 v/v, 80 ml), and the resulting material was allowed to react with the benzyloxycarbonyl-L-valine *p*-nitrophenyl ester[17] (2.25 g, 6.0 mmol) in DMF (16 ml) for 2 days at room temperature. Excess ethyl acetate was added to the reaction mixture to precipitate the product, which was then collected by filtration and washed with ethyl acetate. The filtrate was recrystallized from 90% aqueous ethanol; wt. 3.4 g (86%); mp 222—223 °C (decomp.), $[\alpha]_D^{26}$ −46.5° (*c* 0.79, DMF).

Found: C, 59.02; H, 7.94; N, 12.61%. Calcd for $C_{42}H_{66}O_{11}N_8$: C, 58.72; H, 7.75; N, 13.02%.

Benzyloxycarbonyl-L-*valyl*-L-*asparaginyl*-ω-t-*butyl*-L-α-*aminosuberyl*-L-*leucylglycine Amide (VII)*. Compound VI (3.15 g, 3.67 mmol) was suspended in a mixture of dioxane and water (2: 1 v/v, 75 ml), and the mixture was subjected to catalytic hydrogenolysis as has been shown above. During the course of the hydrogenolysis, the insoluble material went into a solution. After the removal of the catalyst, the solution was concentrated under reduced pressure, the residue was dissolved in 30 ml of DMF, and then the benzyloxycarbonyl-L-isoleucine *N*-hydroxysuccinimide ester[15] (2.0 g, 5.5 mmol) was added to the solution. The mixture was allowed to react for one day at room temperature. Then, ethyl acetate was added to the reaction mixture, the precipitated product was collected by filtration, and it was reprecipitated from 90% aqueous ethanol; wt. 2.45 g (67.5%); mp 240—240.5 °C (decomp.), $[\alpha]_D^{17}$ −40.6° (*c* 0.85, DMF).

Found: C, 58.18; H, 8.06; N, 12.48%. Calcd for $C_{48}H_{77}O_{12}N_9\cdot H_2O$: C, 58.22; H, 8.04; N, 12.73%.

Benzyloxycarbonyl-L-*isoleucyl*-L-*valyl*-L-*asparaginyl*-L-α-*aminosuberyl*-L-*prolyl*-L-*leucylglycine Amide (VIII)*. Compound (VII) (2.17 g, 2.16 mmol) was dissolved in TFA (25 ml) and the solution was kept for one hour at room temperature. Then, the TFA was removed under reduced pressure, and an oily residue was solidified by trituration with ether. The product was reprecipitated from 90% aqueous ethanol, collected by filtration, washed with ethanol, and dried; wt. 1.63 g (80.1%); mp 224—226 °C (sintered) and 236—238 °C (decomp.), $[\alpha]_D^{23}$ −44.9° (*c* 0.84, DMF).

Found: C, 55.88; H, 7.46; N, 13.08%. Calcd for $C_{44}H_{59}O_{12}N_9\cdot 1.5H_2O$: C, 56.03; H, 7.70; N, 13.37%.

t-*Amyloxycarbonyl*-O-t-*butyl*-L-*tyrosyl*-L-*isoleucyl*-L-*valyl*-L-*asparaginyl*-L-α-*aminosuberyl*-L-*prolyl*-L-*leucylglycine Amide (IX)*.

Compound VIII (1.57 g, 1.68 mmol) was subjected to catalytic hydrogenolysis in dioxane–water (3: 1 v/v) as has been described above. The catalyst was filtered off, the filtrate was concentrated under reduced pressure, and the resulting peptide amide was dissolved in DMF (60 ml). The *t*-amyloxycarbonyl-*O*-*t*-butyl-L-tyrosine *N*-hydroxysuccinimide ester[8] (1.29 g, 2.88 mmol) was then added to the solution, and the mixture was allowed to react for 2 days at room temperature; the pH of the solution was kept at 7—8 throughout the period using *N*-ethylmorpholine. Then, the product was precipitated by the addition of ethyl acetate, collected by filtration, and washed well with ethyl acetate. Finally, the washed product was reprecipitated from 90% aqueous ethanol; wt. 0.93 g (49%); mp 229—230 °C (decomp.), $[\alpha]_D^{25}$ −43.1° (*c* 0.77, DMF). Ratio of amino acids in an acid hydrolyzate (6 M HCl, 48 hr at 105 °C): Tyr, 1.29; Ile+Asu, 2.17; Val, 0.84; Asp, 0.97; Pro, 1.00; Leu, 1.19;

Gly, 0.97.

Found: C, 58.27; H, 8.36; N, 12.08%. Calcd for $C_{55}H_{90}O_{14}N_{10}\cdot H_2O$: C, 58.28; H, 8.18; N, 12.36%.

t-Amyloxycarbonyl-O-t-butyl-L-tyrosyl-L-isoleucyl-L-valyl-L-asparaginyl-ω-p-nitrophenyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide (X). The protected octapeptide amide (IX) (871.7 mg, 0.77 mmol) was treated with *p*-nitrophenyl trifluoroacetate[5,16] (2.8 g, 11.9 mmol) for 3 hr at 50 °C in a mixture of DMF (25 ml) and pyridine (6.2 ml). Then, the product was precipitated with ether, collected by filtration, and washed well with ether. The product was purified by reprecipitation from DMF–ether; wt. 0.60 g (62%); mp 251—252 °C (decomp.), $[\alpha]_D^{27}$ −37.0° (*c* 0.73, DMF).

Found: C, 58.67; H, 7.78; N, 12.09%. Calcd for $C_{61}H_{93}O_{16}N_{11}\cdot H_2O$: C, 58.40; H, 7.63; N, 12.28%.

Lactam of L-Tyrosyl-L-isoleucyl-L-valyl-L-asparaginyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide (XI): [Val⁴, Asu¹,⁶]-Oxytocin. Compound X (0.67 g, 0.53 mmol) was dissolved in TFA (6 ml), and the mixture was allowed to react for 1.5 hr at room temperature. Then, the TFA was evaporated out under reduced pressure and the residue was dissolved in DMF (10 ml). The solution was stirred into pyridine (300 ml), drop by drop, at 40 °C. After standing overnight at room temperature, the pyridine solution was concentrated to a residue under reduced pressure, the residue was triturated with ether, and the separated material was collected. The product was dissolved in water (15 ml), and the solution was passed through a column of Amberlite IR-45 (OH^- form, 1×10 cm) and a column of CM-Sephadex C-25 (H^+ form, 1×10 cm) successively. The effluents were monitored by means of the UV absorption at 280 nm, and the fractions containing a major peak were pooled and lyophilized to obtain the final product; wt. 99.5 mg (24.8%); $[\alpha]_D^{20}$ −101° (*c* 0.47, water). The homogeneity of the product was confirmed by thin-layer chromatography on Silica gel G using the solvent system II (single spot, with R_f 0.58 as located by exposure to iodine vapor) and by paper electrophoresis (Toyo No. 514), using a pyridine–acetic acid buffer pH 4.8 (50V/cm, 60 min, located by a Pauly reagent). Ratio of amino acids in an acid hydrolyzate (6 M HCl, 48 hr at 105 °C): Tyr, 0.95; Ile, 1.00; Val, 0.98; Asp, 1.04; Asu, 1.05; Pro, 1.04; Leu, 1.03; Gly, 0.98. The average recovery was 85%.

Found: C, 53.89; H, 8.00; N, 13.86%. Calcd for $C_{45}H_{70}O_{11}N_{10}\cdot 4H_2O$: C, 54.09; H, 7.87; N, 14.02%.

Benzyloxycarbonyl-glycyl-L-leucylglycine Amide (XII). Benzyloxycarbonyl-glycyl-L-leucylglycine ethyl ester[18] (26.0 g) was dissolved in methanol (340 ml), and the solution was saturated with ammonia at 0 °C. After having been kept for 72 hr at room temperature, the solution was concentrated under reduced pressure and the residue was recrystallized from ethanol –*n*-hexane; wt. 19.6 g (81.3%); mp 123—125 °C, $[\alpha]_D^{16}$ −15.2° (*c* 1.1, DMF). lit[12], mp 113—115°C, $[\alpha]_D^{28}$ −15.3° (*c* 1, DMF).

Found: C, 56.49; H, 7.11; N, 14.69%. Calcd for $C_{18}H_{26}O_5N_4\cdot 1/4H_2O$: C, 56.46; H, 6.98; N, 14.63%.

Benzyloxycarbonyl-ω-t-butyl-L-α-aminosuberylglycyl-L-leucylglycine Amide (XIII). Compound XII (8.2 g, 22 mmol), was subjected to catalytic hydrogenolysis in ethanol, and the glycyl-L-leucylglycine amide thus obtained was coupled with the benzyloxycarbonyl-ω-*t*-butyl-L-α-aminosuberic acid α-*p*-nitrophenyl ester[5] (8.4 g, 16.8 mmol) in DMF (12 ml) for 24 hr at room temperature. Water was added to the reaction mixture to precipitate the product, which was then taken up with ethyl acetate. The solution was washed thoroughly with 1 M aqueous ammonia until no yellow color was detectable in the washing; then the solution was washed once with 0.6 M hydrochloric acid and dried over sodium sulfate. The dried solution was concentrated under reduced pressure, and the residue was recrystallized from ethyl acetate–ethanol; wt. 9.1 g (88.1%); mp 144—146 °C, $[\alpha]_D^{22}$ −10.8° (*c* 0.98, DMF).

Found: C, 59.01; H, 8.13; N, 11.11%. Calcd for $C_{30}H_{47}O_8N_5\cdot 1/4H_2O$: C, 59.04; H, 7.85; N, 11.48%.

Benzyloxycarbonyl-L-asparaginyl-ω-t-butyl-L-α-aminosuberyglycyl-L-leucylglycine Amide (XIV). Compound XIII (9.3 g, 15.4 mmol) was subjected to hydrogenolysis in ethanol, and the product was allowed to react with the benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester[13] (6.3 g, 16.2 mmol) in DMF (20 ml) for 2 days at room temperature. The product was precipitated by dilution with ethyl acetate and collected by filtration; the collected material was washed with ethyl acetate and acetone, and recrystallized from 90% aqueous ethanol; wt. 9.8 g (86.3%); mp 223—224.5 °C (decomp.), $[\alpha]_D^{22}$ −13.1° (*c* 0.96, DMF).

Found: C, 55.59; H, 7.52; N, 13.09%. Calcd for $C_{34}H_{53}O_{10}N_7\cdot H_2O$: C, 55.34; H, 7.51; N, 13.29%.

Benzyloxycarbonyl-L-glutaminyl-L-asparaginyl-ω-t-butyl-L-α-aminosuberylglycyl-L-leucylglycine Amide (XV). The benzyloxycarbonyl group of Compound XIV (9.8 g, 13.3 mmol) was removed by catalytic hydrogenolysis, and the product was coupled with the benzyloxycarbonyl-L-glutamine *p*-nitrophenyl ester[13] (5.6 g, 14 mmol) as above. The product was precipitated from the reaction mixture, collected by filtration, and boiled in 90% aqueous ethanol to extract the soluble materials. The remaining insoluble material was collected by filtration and dried; wt. 9.8 g (85.9%); mp 244.5—245 °C (decomp.), $[\alpha]_D^{25}$ −30.7° (*c* 0.75, acetic acid). Ratio of amino acids in an acid hydrolyzate (6 M HCl, 48 hr at 105 °C): Glu, 1.08; Asp, 1.00; Asu, 0.94; Gly, 2.03; Leu, 1.00; The average recovery was 97%.

Found: C, 54.76; H, 7.35; N, 14.92%. Calcd for $C_{39}H_{61}O_{12}N_9\cdot 1/2H_2O$: C, 54.66; H, 7.29; N, 14.71%.

Benzyloxycarbonyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-ω-t-butyl-L-α-aminosuberylglycyl-L-leucylglycine Amide (XVI). The benzyloxycarbonyl group of Compound XV (9.8 g, 11.4 mmol) was removed by catalytic hydrogenolysis, and the product was coupled with the benzyloxycarbonyl-L-isoleucine *N*-hydroxysuccinimide ester[15] (5.5 g, 15.2 mmol) in DMF (90 ml), as in the case of Compound VII. Ethyl acetate was added to the reaction mixture, and the precipitate was reprecipitated from DMF–ethyl acetate; wt. 8.1 g (73.3%); mp 248—249 °C (decomp.), $[\alpha]_D^{25}$ −39.4° (*c* 0.83, acetic acid).

Found: C, 55.69; H, 7.72; N, 14.50%. Calcd for $C_{45}H_{72}O_{13}H_{10}\cdot 1/2H_2O$: C, 55.71; H, 7.58; N, 14.44%.

Benzyloxycarbonyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberylglycyl-L-leucylglycine Amide (XVII). Compound XVI (7.9 g, 8.14 mmol) was treated with TFA (80 ml) as in the case of the synthesis of compound VIII. The product was reprecipitated from 90% aqueous ethanol; wt. 7.55 g (98.6%); mp 239—240 °C (decomp.), $[\alpha]_D^{23}$ −33.3° (*c* 1.0, acetic acid).

Found: C, 52.57; H, 7.22; N, 14.88%. Calcd for $C_{41}H_{64}O_{13}N_{10}\cdot 2H_2O$: C, 52.33; H, 7.28; N, 14.89%.

Benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberylglycyl-L-leucylglycine Amide (XVIII). Compound XVII (7.45 g, 7.9 mmol) was subjected to hydrogenolysis in an ethanol–water–acetic acid mixture (5: 5: 2 v/v, 100 ml), and the free heptapeptide amide thus obtained was treated with the benzyloxycarbonyl-*O*-benzyl-L-tyrosine *N*-hydroxysuccinimide ester[19] (7.0 g, 13.9 mmol) in a mixture of dimethyl sulfoxide (250 ml) and DMF (50 ml) for 2 days at room temperature. The reaction mixture was kept at pH 6—7 with *N*-ethylmorpholine throughout the period. Excess ethyl acetate was added to the reaction mixture, and the precipitate was collected by filtration and boiled in 80%

aqueous ethanol (400 ml). The remaining material was collected by filtration at room temperature and washed well with ethanol; wt. 8.0 g, (86.5%); mp 251—252 °C (decomp.), $[\alpha]_D^{25}$ −15.2° (*c* 0.63, dimethylsulfoxide). Ratio of amino acids in an acid hydrolyzate (6 M HCl, 48 hr at 105 °C): Tyr, 0.90; Ile, 1.10; Glu, 1.00; Asp, 0.98; Asu, 1.03; Gly, 1.90; Leu, 0.98. The average recovery was 98%.

Found: C, 58.67; H, 6.97; N, 13.15%. Calcd for $C_{57}H_{79}O_{15}N_{11}\cdot 1/2H_2O$: C, 58.65; H, 6.91; N, 13.20%.

Benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-ω-2,4,5-trichlorophenyl-L-α-aminosuberylglycyl-L-leucylglycine Amide (XIX). Compound XVIII (4.3 g, 3.68 mmol) was suspended as a fine powder in a mixture of DMF (80 ml) and pyridine (29 ml), and 2,4,5-trichlorophenyl trifluoroacetate[5,16] (10.7 g, 36.4 mmol) was added to the suspension. The mixture was stirred for 7 hr at 50 °C, and then the product was precipitated by the addition of ether, collected by filtration, and dried; wt. 4.64 g (93.7%); mp 253—254 °C (decomp.), $[\alpha]_D^{25}$ −21.4° (*c* 0.42, dimethyl sulfoxide).

Found: C, 56.20; H, 6.37; N, 11.63; Cl, 8.37%. Calcd for $C_{63}H_{80}O_{15}N_{11}Cl_3\cdot 1/2H_2O$: C, 56.18; H, 6.06; N, 11.44; Cl, 7.90%.

Lactam of L-Tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberylglycyl-L-leucylglycine Amide (XX): [Gly,[7] Asu[1,6]]-Oxytocin. a) Compound XIX (1.31 g, 0.97 mmol) was subjected to catalytic hydrogenolysis using palladium black in DMF (350 ml). After 40 hr at room temperature, the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was triturated with ether to obtain a hygroscopic powder, which was then extracted with water (15 ml×2). The extract was passed through a column of Amberlite IR-45 (OH^- form, 3×11.5 cm), which was then eluted with water. The fractions of the effluents which showed an optical density of >2.0 at 280 nm were collected and passed through another column of CM-Sephadex C-25 (H^+ form, 3×12.5 cm). The effluent and washing of the latter column were pooled and lyophilized to obtain a crude product; wt. 504 mg (52.5%). A portion (240 mg) of this material was further purified by silica-gel column chromatography (Merck's Silica-gel for column chro matography, 70—325 mesh, 2.0×22.5 cm) using the solvent system. II Fractions of 6 g were collected, and the presence of peptides was monitored by means of the UV absorption at 280 nm. The effluents in tubes No. 57 to 63 were collected and concentrated under reduced pressure. The residue was passed through a column of CM-Sephadex C-25 (H^+ form, 3×8.5 cm). The effluents in fractions corresponding to the volume from 75 to 110 ml were pooled and lyophilized to obtain the final product; wt. 164 mg (recovery 68.3%); $[\alpha]_D^{25}$ −44.0° (*c* 0.55, water). The homogeneity of the purified product was confirmed by thin-layer chromatography, as in the case of compound XI. The R_f-values on paper chromatography (Toyo No. 51) were 0.65 for the solvent system II, 0.59 for the solvent system, III and 0.76 for a solvent system of *n*-butanol: acetic acid: pyridine: water (15: 3: 10: 6 v/v). This peptide was Pauly-positive and ninhydrin-negative. Ratio of amino acids in an acid hydrolyzate (6 M HCl, 48 hr at 105 °C): Tyr, 1.00; Ile+Asu, 2.00; Glu, 1.02; Asp, 1.00; Gly, 1.98; Leu, 0.96. The average recovery was almost quantitative.

Found: C, 51.22; H, 7.31; N, 15.31%. Calcd for $C_{42}H_{65}O_{12}N_{11}\cdot 4H_2O$: C, 51.05; H, 7.45; N, 15.60%.

b) Compound XIX (20.0 g, 14.8 mmol) was dissolved in TFA (150 ml) together with anisole (50 ml), and HBr gas was introduced gently into the solution for 90 min at room temperature. Then, the mixture was concentrated under reduced pressure, and the residue was washed with ether and dried. The dried compound was dissolved in 150 ml of DMF, and the solution was stirred into pyridine (31), drop by drop, at 50 °C over a 4 hr period. After standing overnight at room temperature, the solution was concentrated under reduced pressures (lower than 45 °C), the residue was treated with columns of Amberlite IR-45 and CM-Sephadex C-25 successively, as in the case of a), and the crude lactam was obtained as an amorphous powder; wt. 3.0 g (21%). This material was subjected to further purification as has been shown above to obtain the final product; wt. 2.2 g (recovery 73%).

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References

1) A part of this work was presented at the 7th Symposium on Peptide Chemistry, Tokyo, November 21, (1969). Part II in this series is Ref. 7.

2) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *J. Biol. Chem.*, **235**, PC 64 (1960); D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962).

3) J. Rudinger and K. Jošt, *Experientia*, **20**, 570 (1964).

4) K. Jošt and J. Rudinger, *Collect. Czech. Chem. Commun.*, **32**, 1229 (1967).

5) A. Kobayashi, S. Hase, R. Kiyoi, and S. Sakakibara, This Bulletin, **42**, 3491 (1969).

6) S. Sakakibara and S. Hase, *ibid.*, **41**, 2816 (1968).

7) S. Hase, T. Morikawa, and S. Sakakibara, *Experientia*, **25**, 1239 (1969).

8) S. Hase, S. Sakakibara, M. Wahrenburg, M. Kirchberger, I. L. Schwartz, and R. Walter, *J. Amer. Chem. Soc.*, **94**, 3590 (1972).

9) T. Yamanaka, S. Hase, S. Sakakibara, I. L. Schwartz, B. M. Dubois, and R. Walter, *Mol. Pharmacol.*, **6**, 474 (1970).

10) R. A. Boissonnas, S. Guttmann, P. A. Jaquenoud, and J. P. Waller, *Helv. Chim. Acta*, **39**, 1421 (1956); P. G. Katsoyannis, *J. Amer. Chem. Soc.*, **79**, 109 (1957).

11) V. du Vigneaud, G. Flouret, and R. Walter, *J. Biol. Chem.*, **241**, 2093 (1966); G. Flouret and V. du Vigneaud, *J. Med. Chem.*, **12**, 1035 (1969).

12) M. Bodanszky and R. J. Bath, *Chem. Commun.*, **1968**, 766.

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

14) The special abbreviations used in the text are: Asu, α-aminosuberic acid residue; HONSu, *N*-hydroxysuccinimide; HOTcp, 2,4,5-trichlorophenol; Aoc, *t*-amyloxycarbonyl; TFA, trifluoroacetic acid; DMF, *N,N*-dimethylformamide.

15) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **86**, 1839 (1964).

16) S. Sakakibara and N. Inukai, This Bulletin, **38**, 1979 (1965); *ibid.*, **39**, 1567 (1966).

17) B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).

18) R. W. Young, K. H. Wood, R. J. Joyce, and G. W. Anderson, *J. Amer. Chem. Soc.*, **78**, 2126 (1956).

19) M. Löw and L. Kisfaludy, *Acta Chim. Hung.*, **44**, 61 (1965).